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Alpha Tocopherol May Reduce Endosulfan Induced Toxicity in Mice

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Abstract. - The objective of the study was to analyze the protective role of vitamin E as an antioxidant against endosulfan induced toxication. Three different experiments were executed involving low level exposure (intermittent inhalation of saturated vapors of 375 ppm for a total of 6 minutes in 3 hours), medium level exposure (inhalation of saturated vapors of 375 ppm for 10 minutes/week) and high level exposure (05mg/Kg body weight through intramuscular injection) of endosulfan with and without supplementation of vitamin E. In all experiments, Endosulfan intoxication led to the significant depletion in total protein, albumin, gamma globulin and a-gamma globulin, with elevation in albumin/globulin ratio. Vitamin treatment in low level toxicity experimental led to the reversion of total serum protein and gamma globulin levels to normal level in 30 days of treatment and serum globulin and a-gamma globulin after 15 days. Similar trend was observed in medium level toxicity. In this experiment, total protein, globulin and a-gamma globulin were recorded to be similar as in control group after 15 days vitamin treatment. Improvement in albumin and gamma globulin was obvious after 30 days. However, at high dose of endosulfan, the vitamin treatment failed to revert changes induced by toxicant. It is concluded that vitamin E treatment can minimize the lethal effect of low level toxicity.

Key words: Endosulfan, alpha tocopherol, globulin, albumin, total seral proteins.

INTRODUCTION

Mechanoindustrial setup in various fields of life including agriculture emerged to cope with need of increasing human population. This resulted in the formulation and application of a number of xenobiotics to control various crops pests. Practice of such chemicals undoubtedly increased the yield of various crops up to desired levels however; soon their entry in food chain was realized imparting, directly or indirectly lethal effects on human health at various levels (McKinlay *et al*., 2008; Sarkar *et al*., 2008; Xuemei *et al*., 2008).

Endosulfan is an organochlorine which is widely used as insecticide and acaricide. Its exposure happens through food chain, skin contact, breathing or drinking contaminated water. Severe damages have been reported in kidneys, liver, phagocytic cells, male reproductive system and major endocrine axes due to production of reactive oxygen species induced by the toxicant (Ali and Shakoori, 1999; Beckmen *et al.,* 2003; Pistl *et al.*, 2003; Sohn *et al*., 2004).

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For the protection of human health, it is important to eliminate the harmful effects of endosulfan. Alpha-tocopherol (Vitamin E) is an essential nutrient which is appropriately described as an antioxidant than a vitamin. It does not act as a co-factor for enzymatic reactions but serves to scavenge free radicals formed in redox reactions and lipid peroxidation chain reactions particularly in cellular and sub cellular membranes (González-Pérez *et al.*, 2008). Its potential to neutralize reactive oxygen species can make it ideal for the control of harmful effects of endosulfan. The present study was conducted to investigate the importance of vitamin E against the potential damages and abnormalities due to endosulfan exposure using mice as a mammalian model.

MATERIALS AND METHODS

Experimental animals and their maintenance

A group of healthy and adult male albino mice (*Mus musculus*) was kept at room temperature (25±3°C) and humidity conditions, for 12 hours photo-period. During this period animals were fed on chick broiler feed # 4 (Hi-Tech Shadman, Lahore), which was purchased from local market.

The feed and water were provided to the animals *ad libitum*. Animals were acclimatized for a period of 15 days before starting the experiment.

Basic experimental plan

Three independent experiments were performed to investigate the effects of vitamin E on (i) high level toxicity (5 mg/kg b.w), (ii) medium level toxicity (exposure to fumes for 10 min/week) and (iii) low level toxicity (exposure to fumes for 6 min/ 15 days). The basic experimental plan for all experiments was the same. Animals (n = 40 in each experiment) were equally divided into 2 groups, vitamin treated and vitamin nontreated. Each group was further splitted into two subgroups, Exposed (E) and Control (C) groups, (n = 10) with respect to insecticide exposure. A widely used insecticide, Endosulfan (35% EC) manufactured by Agrevo GMBH, Germany, was used in the study. Vitamin dose for all experiments was prepared by suspending 1 capsule of vitamin E (400 mg) in a total volume of 1 ml of liquid Paraffin. Vitamin E treatment was started 15 days prior to first exposure to Endosulfan. In all experiments vitamin E was administered to animals by forced drinking (160 mg/kg body weight per day).

In high level toxicity experiment, the intoxication was performed by injecting Endosulfan through intramuscular route. A dose of 5 mg/kg body weight was given once a week for a period of 4 weeks. In median level toxicity experiment the animals were kept in a chamber saturated with vapors of endosulfan (375 ppm) for 10 min/week. In low level toxicity experiment the exposure to endosulfan was made through inhalation by keeping animals in a chamber saturated with vapors of endosulfan (375ppm) for 2 min, and the process was repeated three times at an interval of one hour. In this way the total exposure time was 6 minutes. Whole process of intoxication was repeated after 15 days of 1st treatment. In each experiment blood samples were collected after 15 and 30 days of the beginning of experiment.

Biochemical procedures

After 15 and 30 days of first exposure to the toxicant the animals (n=5) were sacrificed and blood samples were collected. Serum was separated and used for the estimation of protein, albumin, globulin, gamma and A-gamma globulin. Serum protein was estimated following Biuret method while albumin was estimated by bromocresol green reagent's method using commercial Randox kits. Serum globulin was determined by subtracting the albumin from protein (Varley *et al*., 1980). Albumin/Globulin ratio was determined by dividing albumin contents from globulin. Gamma globulins of the plasma were estimated by precipitating with ammonium sulphate, sodium chloride reagent (Wolfson *et al.*, 1952), whereas A-gamma globulin was determined by subtracting the serum gamma-globulin levels from serum globulin levels.

Data was analyzed statistically using SPSS software for window version 10.0 (SPSS Inc, Chicago). Comparison was made with respective control groups using t-test. The $p \leq 0.05$ was considered as significant.

RESULTS

High level toxicity experiment indicates the reduction in the total serum protein, globulin, gamma globulin and A-gamma globulin which was recorded in intoxicated mice in both vitamin E treated as well as in vitamin E non treated group after 15 and 30 days. Serum albumin concentration also decreased only in vitamin non treated group after 15 days, but this reduction was not observed in vitamin E treated group (Table I).

In medium dose experiment a decrease in total protein, globulin, gamma and A-gamma globulin was recorded in vitamin E non treated mice. On the other hand vitamin E intoxicated group showed similar variation in serum proteins and gama-globulin, while other parameters remained in the normal range (Table II).

In low dose experiment the intoxicated animals without vitamin-E treatment showed depletion in total serum protein and A-gamma globulin (after 15 days), albumin and gamma globulin (after 30 days) and globulin level (15 and 30 days). On the other hand no change was observed in the vitamin E treated group (Table III).

Table I.- Effects of vitamin E treatment on mice exposed to endosulfan (5mg/kg body weight) through intramuscular route.

Treatment	Duration (Days)	Without vitamin		With vitamin	
		Control (n=5)	Exposed (n=5)	Control (n=5)	Exposed (n=5)
Total serum protein	15	12.52±0.49	8.37±0.95*	12.31±0.61	9.41±0.63*
	30	12.52±0.49	7.11±0.27*	12.57±0.61	9.80±0.29*
Total serum albumin	15	5.53±0.29	4.10±0.25*	5.07±0.72	5.00±0.85
	30	5.53±0.29	4.88±0.14	5.35±0.72	5.20±0.25
Serum globulin level	15	6.65±0.15	4.76±0.28*	6.52±0.34	4.66±0.14*
	30	7.15±0.27	5.26±0.42*	7.45±0.17	5.23±0.29*
Albumin / globulin level	15	0.79±0.02	0.92±0.01*	0.77±0.01	1.06±0.09*
	30	0.76±0.02	0.93±0.02*	0.87±0.01	0.98±0.02*
Gamma globulin	15	2.63±0.14	2.20±0.14*	3.01±0.21	1.60±0.22*
	30	2.77±0.12	1.90±0.07*	2.95±0.22	2.30±0.16
A – gamma globulin	15	4.45±0.15	2.67±0.04*	3.54±0.11	2.37±0.07*
	30	5.50±0.12	4.30±0.13*	5.59±0.18	3.34±0.15*

*P≤0.05

Table II.- Effects of vitamin E treatment on mice exposed to endosulfan (375 ppm) through inhalation for 10 minutes.

Treatment	Duration (Days)	Without vitamin		With vitamin	
		Control (n=5)	Exposed (n=5)	Control (n=5)	Exposed (n=5)
Total serum protein	15	12.50±0.49	9.49±0.52*	12.31±0.61	10.71±0.58
	30	12.50±0.49	11.75±0.59	12.57±0.62	12.03±1.52
Total serum albumin	15	5.53±0.29	5.26±0.04	5.07±0.72	4.66±0.22
	30	5.53±0.29	3.91±0.30*	5.35±0.72	5.10,±0.72
Serum globulin level	15	6.65±0.15	5.08±0.07*	5.80±0.28	5.24±0.21
	30	7.45±0.27	5.92±0.27*	7.14±0.29	6.89±0.38
Albumin / globulin Level	15	0.79±0.02	0.86±0.04	0.77±0.01	0.82±0.01
	30	0.76±0.02	0.71±0.02	0.87±0.01	0.79±0.02
Gamma globulin	15	2.63±0.14	1.90±0.07	2.76±0.13	2.33±0.22
	30	2.77±0.12	1.90±0.07*	2.95±0.18	2.54±0.12
A – gamma globulin	15	4.45±0.15	3.86±0.05*	3.54±0.11	3.43±0.09
	30	5.36±0.15	4.87±0.19	5.59±0.18	4.82±0.35

*P≤0.05

DISCUSSION

Vitamin E (α tocopherol) is a well known antioxidant and organochlorine insecticides are supposed to cause toxicity by inducing oxidative stress. This study was conducted to investigate whether the treatment of vitamin E could minimize the endosulfan induced damages. The effects were monitored in three independent experiments by administering the toxicant at three different levels (high, medium and low). The present study demonstrates that endosulfan adversely affects the serum protein profile. A decline in total serum proteins level was observed in endosulfan intoxicated animals. Such variation may occur either due to increased rate of proteolytic activity or reduced rate of transcription or translation due to enosulfan (Das and Mukherjee, 2003). It was observed that vitamin E failed to decrease the toxic effects in case of high level intoxication. However, inhalation experiments present the data showing beneficial role of vitamin E on total serum proteins. In the present study, different types of proteins including albumin, gamma and a-gamma globulin

Table III.- Effects of vitamin E treatment on mice exposed to endosulfan (375 ppm) through inhalation for 6 minutes.

Treatment	Duration (Days)	Without vitamin		With vitamin	
		Control (n=5)	Exposed (n=5)	Control (n=5)	Exposed (n=5)
Total serum protein	15	12.50±0.49	9.49±0.52*	12.31±0.61	10.71±0.58
	30	12.50±0.49	11.75±0.59	12.57±0.62	12.03±1.52
Total serum albumin	15	5.53±0.29	5.26±0.04	5.07±0.72	4.66±0.22
	30	5.53±0.29	3.91±0.30*	5.35±0.72	5.10,±0.72
Serum globulin level	15	6.65±0.15	5.08±0.07*	5.80±0.28	5.24±0.21
	30	7.45±0.27	5.92±0.27*	7.14±0.29	6.89±0.38
Albumin / globulin level	15	0.79±0.02	0.86±0.04	0.77±0.01	0.82±0.01
	30	0.76±0.02	0.71±0.02	0.87±0.01	0.79±0.02
Gamma globulin	15	2.63±0.14	1.90±0.07	2.76±0.13	2.33±0.22
	30	2.77±0.12	1.90±0.07*	2.95±0.18	2.54±0.12
A – gamma globulin	15	4.45±0.15	3.86±0.05*	3.54±0.11	3.43±0.09
	30	5.36±0.15	4.87±0.19	5.59±0.18	4.82±0.35

*: P≤0.05

fractions were estimated along with total serum proteins and albumin/globulin ratio.

Serum albumin protein is essential due to its contribution in the maintenance of osmotic pressure of plasma. It is carrier of many vital substances like steroid hormones, hemin and fatty acids (Nicholson *et al.*, 2000). In present investigation, depletion in albumin level was observed in the groups exposed to endosulfan in all experiments. It may occur due to the excessive dehydration or decreased production of albumin by liver (Aldana *et al.,* 2001; Wang *et al.,* 2001). Vitamin E treatment helped in reducing this toxicity that was evident by the normal level of albumin in vitamin treated group. Similar results showing antioxidant nature of vitamin E in the management of hepatotoxicity due to oxidative stress were reported by Bestas *et al.* (2008).

Globulin fraction of protein comprises of enzymes, hormones and antibodies. In all experiments, vitamin non treated groups presented a decreasing pattern of globulin which reflects the state of the decreased immunity. Tryphonas *et al.* (2003) reported immunotoxicity of similar chlorinated compounds while studying the effects of organochlorines on a number of immunologic end points. Vitamin E treatment helped in reversing the toxic effects of endosulfan in low toxicity experiments. Such beneficial effects could not be observed in high dose experiment. The severity of damage at high level of toxicant might be the possible justification of least recovery by vitamin E treatment. For the treatment of such severe variation, vitamin E may be used with another antioxidant like lycopene or alpha-lipoic acid (Bestas *et al*., 2008; González-Pérez *et al*., 2008), however, further studies are required to support this view. The present study suggests that vitamin E can improve the globulin level following low level intoxication. These findings are in accordance to Aldana *et al.* (2001) who reported modulation of liver toxicity following alpha-tocopherol treatment. In contrast Uusitalo *et al.* (2008) could not find any association in the concentration of alpha-tocopherol and beta cell autoimmunity in young children.

Albumin/Globulin ratio is an important indicator of pathological alterations in protein metabolism and disproportionate variation in protein fractions (Varley *et al.*, 1980). Significantly higher levels of A/G ratios recorded in both vitamin treated and non-treated groups of high dose experiment reflect more depletion in globulin fraction as compared to the albumin fractions. As the globulin fraction contains enzymes, hormones and antibodies, which are synthesized at various places in the body. Their depletion marks the generalized toxicity of endosulfan. The alpha tocopherol could not alter the endosulfan induced toxicity at high concentration of toxicant.

Gamma globulin concentration is a reliable indicator of humoral immunity. Its principal

component is IgG but other isotypes of antibodies are also present in this fraction (Goldsby *et al.*, 2001). It was noted that endosulfan adversely affects the gamma globulin fraction both at high and low dose. The significant depletion in vitamin treated groups at 15 days and its normal levels after 30 days of intoxication points towards a beneficial role of vitamin E in the detoxification of endosulfan.

A-gamma globulin is fraction of globulin that contains hormones and enzymes, which are mostly synthesized by endocrine glands and liver. Endosulfan was found to affect A-gamma globulin in high dose experiments. No significant variation was observed in inhalation experiments. The normal levels at day 30 in inhalation experiments may be due to detoxification of low doses of toxicant at later stages. No repairing effects of vitamin E in high intoxication experiments may either be due to higher dose of toxicant or limited capability of anti-oxidant to help toxicity. In low dose experiments, no drastic change was observed in vitamin treated groups. It also indicates the limited protective role of vitamin E.

CONCLUSION

Results depict that mammalian system can degrade endosulfan or remove it from system at low concentrations. Antioxidant nature of alpha tocopherol can help in protection against oxidative damage induced by endosulfan but this protection level depends upon the concentration of endosulfan.

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