

## Effect of Prophylactic Supplementation of Vitamin E and Se on Antioxidant Enzymes during Endotoxic Shock in Buffalo Calves

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**ABSTRACT :** This study was conducted to determine the effects of prophylactic supplementation of vitamin E and Se on oxidative damage and antioxidant status. Fifteen healthy male buffalo (*Bubalus bubalis*) calves between the age of 6 to 12 months were divided into three groups of five animals each: Group I-control, group II-endotoxic shock group infused with lyophilized *E coli* endotoxin @ 5 µg/kg body wt, and group III-supplemented with vitamin E @ 250 mg and Se @ 7.5 mg, one month prior to induction of endotoxic shock. All the animals in group II and group III exhibited signs of endotoxic shock. When the endotoxic shock was induced, there was significant ( $p < 0.05$ ) increase in the circulating levels of malonyl dialdehyde MDA (an indicator of lipid peroxidation). In the supplemented group III the magnitude of formation of MDA was also less as compared to group II at every stage of study. There was significant ( $p < 0.05$ ) decrease in circulating levels of SOD, GSH-Px, Catalase and G-6-PD activity from the normal (0 h) value with passage of time. As a result of endotoxic shock, these values reached a lowest value, and then showed a tendency towards the 0 h value. Prophylactic supplementation with vitamin E and Se was successful in reducing the quantum of oxidative damage due to formation of free radicals because of endotoxic shock. (*Asian-Aust. J. Anim. Sci.* 2003. Vol 16, No. 11 : 1577-1582)

**Key Words :** Buffalo Calves, Vitamin E,  $\alpha$ -tocopherol, Se, Lipid Peroxidation, Endotoxic Shock, SOD, GSH-Px, G-6-PD, Catalase

### INTRODUCTION

Free radicals can cause damage when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs. Unsaturated fatty acids, particularly the polyunsaturated fatty acids (PUFA) are the components of tissue membranes including erythrocytic membranes. PUFA are susceptible to oxidation and the resulting products in the form of reactive oxygen species (ROS) may be toxic to the cell (Halliwell and Chirico, 1993). This process tends to produce a chain reaction called lipid peroxidation. To prevent free radical damage the body has a defense system of antioxidants.

Antioxidants act as scavengers, helping to prevent cell and tissue damage that could lead to cellular damage and various diseases. The vitamins E and C are thought to protect the body against the destructive effects of free radicals. Vitamin C is the most abundant water-soluble antioxidant in the body. Vitamin E is the most important lipid soluble chain breaking antioxidant in tissues, red cells and plasma (Burton and Traber, 1990).

Although there are several enzyme systems within the body that scavenge free radicals, the principle enzymes are superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase and glucose-6-phosphate dehydrogenase (Halliwell, 1999). Reduced glutathione can chemically detoxify hydrogen peroxide. This reaction is catalysed by

glutathione peroxidase (Pamela et al., 1994), a Se containing enzyme, which destroys lipid peroxidase before they can damage cell membranes. After being oxidized and before being decomposed, vitamin E can be re-reduced by ascorbic acid and glutathione. This reaction is dependent on the concentration of these substances and/or the enzymes that maintain them in their reduced form. So selenium, a trace metal is also required for proper function of one of the body's antioxidant enzyme systems. The present study was undertaken to evaluate the effect of prophylactic supplementation of vitamin E and Se on level of antioxidant enzymes during endotoxic shock.

### MATERIALS AND METHODS

Fifteen healthy male Murrah buffalo (*Bubalus bubalis*) calves between 4 to 6 month of age were selected for this present investigation. All the animals were kept under same managerial conditions as practiced at Punjab Agricultural University dairy farm, Ludhiana, India. These animals were kept under semi-loose housing conditions with half walled open sheds and pucca floors. Throughout the experimental period, the animals were given adequate amount of chaffed/unchaffed green fodder (Barseem) depending upon availability along with wheat bhusa and water *ad libitum*. All the animals were apparently healthy and were vaccinated against various diseases and dewormed as per the schedule followed at the university dairy farm. These animals were randomly divided into 3 groups of five animals each. The animals of group I served as control. They were neither given any endotoxin nor vitamin E and Se and were administered only normal saline solution

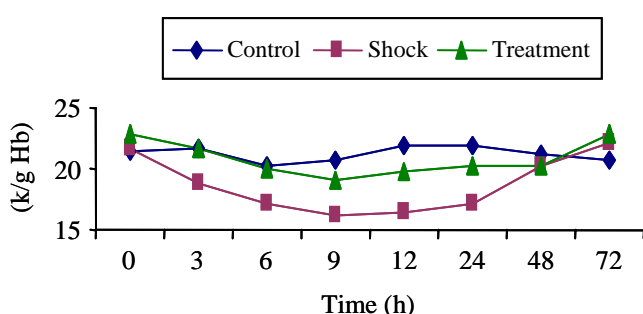
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**Table 1.** Levels of erythrocytic lipid peroxidation, plasma vitamin E and glutathione peroxidase activity (GSH-Px) levels in three groups

Time (h)	Lipid peroxidation (MDA levels)			Vitamin E ( $\mu\text{g/ml}$ )			Glutathione peroxidase activity (GSH-Px) (U/mg Hb)		
	Group I (Mean $\pm$ SE)	Group II (Mean $\pm$ SE)	Group III (Mean $\pm$ SE)	Group I (Mean $\pm$ SE)	Group II (Mean $\pm$ SE)	Group III (Mean $\pm$ SE)	Group I (Mean $\pm$ SE)	Group II (Mean $\pm$ SE)	Group III (Mean $\pm$ SE)
0	303.45 $\pm$ 3.56	284.51 $\pm$ 2.40	208.81 $\pm$ 2.87	0.846 $\pm$ 0.008	0.874 $\pm$ 0.007	2.212 $\pm$ 0.05	13.31 $\pm$ 0.121	13.19 $\pm$ 0.113	17.48 $\pm$ 0.206
1	330.81 $\pm$ 3.41	625.59 $\pm$ 2.87*	234.96 $\pm$ 2.89	0.816 $\pm$ 0.010	0.792 $\pm$ 0.008*	2.164 $\pm$ 0.06	13.39 $\pm$ 0.130	12.84 $\pm$ 0.127	17.07 $\pm$ 0.211
3	315.99 $\pm$ 3.27	575.01 $\pm$ 2.71*	256.51 $\pm$ 2.91*	0.854 $\pm$ 0.011	0.726 $\pm$ 0.009*	1.926 $\pm$ 0.05*	13.29 $\pm$ 0.118	12.22 $\pm$ 0.121*	16.29 $\pm$ 0.22*
6	306.05 $\pm$ 3.31	500.35 $\pm$ 2.54*	291.53 $\pm$ 2.81*	0.820 $\pm$ 0.007	0.632 $\pm$ 0.011*	1.800 $\pm$ 0.061*	13.34 $\pm$ 0.125	11.09 $\pm$ 0.131*	15.52 $\pm$ 0.223*
9	298.14 $\pm$ 3.66	561.89 $\pm$ 2.77*	296.31 $\pm$ 2.77*	0.858 $\pm$ 0.008	0.538 $\pm$ 0.009*	1.600 $\pm$ 0.041*	13.01 $\pm$ 0.201	10.15 $\pm$ 0.133*	15.18 $\pm$ 0.206*
12	327.03 $\pm$ 3.21	533.63 $\pm$ 2.50*	293.12 $\pm$ 2.71*	0.857 $\pm$ 0.011	0.494 $\pm$ 0.010*	1.52 $\pm$ 0.038*	13.38 $\pm$ 0.223	9.23 $\pm$ 0.11*	14.33 $\pm$ 0.211*
24	312.43 $\pm$ 3.19	470.76 $\pm$ 2.41*	266.10 $\pm$ 2.66*	0.822 $\pm$ 0.012	0.548 $\pm$ 0.011*	1.572 $\pm$ 0.04*	13.40 $\pm$ 0.131	10.81 $\pm$ 0.117*	14.83 $\pm$ 0.223*
48	309.59 $\pm$ 3.23	533.79 $\pm$ 2.53*	248.46 $\pm$ 2.71*	0.842 $\pm$ 0.007	0.602 $\pm$ 0.011*	2.116 $\pm$ 0.05	13.04 $\pm$ 0.119	11.75 $\pm$ 0.118*	16.74 $\pm$ 0.218
72	317.64 $\pm$ 3.41	468.12 $\pm$ 2.43*	231.76 $\pm$ 2.69	0.866 $\pm$ 0.012	0.696 $\pm$ 0.008*	2.156 $\pm$ 0.052	13.20 $\pm$ 0.128	12.09 $\pm$ 0.123	17.19 $\pm$ 0.231

Values in the columns having asterisk (\*) differ significantly ( $p < 0.05$ ) from the normal (0 h) values.



**Figure 1.** Effect of prophylactic supplementation of vitamin E and Se on erythrocytic catalase levels during endotoxic shock in buffalo calves.

intravenously. In group II and III, endotoxic shock was induced by giving *E. coli* lyophilized endotoxin (Serotype 0111:B<sub>4</sub>) procured from Sigma Chemicals, USA. Endotoxin was given @ dose of 5  $\mu\text{g/kg}$  body wt. Total dose was dissolved in 100 ml normal saline solution (0.9% NaCl) and infused through intravenous route over a period of 30 minutes. In group III, the animals were supplemented prophylactically with 250 mg vitamin E ( $\alpha$ -tocopherol acetate) and 7.5 mg selenium (Se) (Inj. E-Care-Se<sup>®</sup> marketed by Vetcare, Bangalore, India) at weekly interval, one month prior to induction of shock through intramuscular route.

After induction of shock, blood samples were collected at 0, 1, 3, 6, 9, 12, 24, 48 and 72 h from jugular vein in dry heparinised capped plastic collection vials and were immediately placed over ice. Plasma samples were separated using a refrigerated centrifuge at 3,000 rpm for 15 minutes and stored at  $-20^{\circ}\text{C}$  in different aliquots for the analysis of various vitamins. Erythrocyte pellet was washed thrice with normal saline solution and then distilled water was added to prepare hemolysate. Lipid peroxidation was immediately assayed. All the chemicals used were of AR/GR grade. Lipid peroxidation was assayed by method of Placer et al. (1966). Vitamin E was assayed by the method of Kayden et al. (1973). Activities of SOD and

GSH-Px was determined by the methods of Nishikimi et al. (1972) and Hafeman et al. (1974) which was a modification of Mills procedure (Mills, 1958) respectively. Catalase and G-6-PD activities were estimated by the methods of Hugo Aebi (1984) and Deutsch (1978), respectively. The results were subjected to analysis of variance (ANOVA) on computer using Randomized Complete Block Design (RCB).

## RESULTS AND DISCUSSION

Within a few minutes of intravenous administration of endotoxin, hyperpnea and later dyspnea with markedly abdominal respiratory movements, profuse salivation and watery diarrhoea without blood was observed. The animals usually collapsed into lateral recumbancy within an hour and exhibited cyanotic mucous membranes. Rumen stasis was evidenced by bloat.

### Lipid Peroxidation

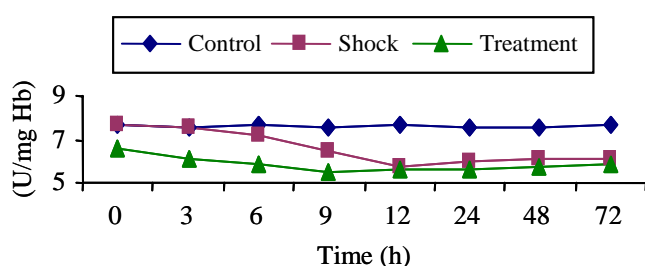
The mean ( $\pm$ SE) values of malonyl dialdehyde (MDA) levels in control (Group I), endotoxic shock (Group II) and treatment (Group III) are presented in Table 1. The mean normal concentration of malonyl dialdehyde MDA in group I didn't differ significantly throughout the period of study. All the three groups differed significantly from each other. The onset of significant rise of MDA in group II was earlier as compared to group III. Moreover the rise in MDA level was also less in group III as compared to group II at every stage of study. The rise in endogenous MDA levels (an indicator of lipid peroxidation) indicated cellular damage as well as increased susceptibility to stress due to production of free radicals.

This finding is supported by the work of Kovacheva et al. (1996) who has also concluded that lipid peroxidation increased by 120% in vitamin E non-supplemented rats after exposure to endotoxin while in vitamin E supplemented rats, this increase did not exceed 26%. Therefore decline in erythrocytic lipid peroxidation after

**Table 2.** Levels of erythrocytic catalase, glucose-6-phosphate dehydrogenase and superoxide dismutase activity (SOD) levels in three groups

Time (h)	Catalase (k/g Hb)			Glucose-6-phosphate dehydrogenase (U/g Hb)			Superoxide Dismutase (U/mg Hb)		
	Group I (Mean±SE)	Group II (Mean±SE)	Group III (Mean±SE)	Group I (Mean±SE)	Group II (Mean±SE)	Group III (Mean±SE)	Group I (Mean±SE)	Group II (Mean±SE)	Group III (Mean±SE)
0	21.45±0.45	21.78±0.41	22.94±0.51	5.50±0.052	5.75±0.060	3.30±0.056	7.618±0.020	7.702±0.023	6.600±0.020
1	22.08±0.40	20.038±0.39*	22.228±0.52	5.59±0.056	5.22±0.057*	3.16±0.050	7.688±0.022	7.680±0.027	6.430±0.025
3	21.614±0.49	18.71±0.38*	21.778±0.44*	5.44±0.048	4.31±0.050*	2.96±0.047*	7.598±0.019	7.590±0.029	6.100±0.021
6	20.18±0.51	17.22±0.42*	20.118±0.43*	5.31±0.050	4.03±0.047*	2.87±0.052*	7.648±0.023	7.200±0.030*	5.860±0.028
9	20.82±0.42	16.084±0.44*	19.03±0.51*	5.53±0.059	4.24±0.046*	2.99±0.041*	7.566±0.025	6.460±0.028*	5.480±0.038
12	21.91±0.44	16.32±0.47*	19.77±0.47*	5.65±0.060	5.10±0.044*	3.025±0.053*	7.678±0.020	5.770±0.032*	5.57±0.018*
24	21.86±0.52	17.13±0.42*	20.18±0.49*	5.47±0.057	5.25±0.041*	3.086±0.057*	7.556±0.024	5.940±0.035*	5.64±0.027*
48	21.11±0.40	20.176±0.49*	20.34±0.41	5.56±0.055	5.47±0.052*	3.17±0.051	7.588±0.018	6.150±0.027*	5.70±0.035*
72	20.79±0.44	22.198±0.39	22.81±0.45 <sup>a</sup>	5.43±0.051	5.71±0.058	3.21±0.048	7.658±0.021	6.06±0.031*	5.83±0.029*

Values in the columns having asterisk (\*) differ significantly ( $p < 0.05$ ) from the normal (0 h) values.

**Figure 2.** Effect of prophylactic supplementation of vitamin E and Se on erythrocytic SOD levels during endotoxic shock in buffalo calves.

supplementation of vitamin E and Se in the present study might be due to chain breaking antioxidant action of supplemented vitamin E which must have prevented membrane instability in endotoxin poisoning (Sakaguchi et al., 1981).

### Vitamin E

The mean ( $\pm$ SE) values of vitamin E in control (Group I), endotoxic shock (Group II) and treatment (Group III) are presented in Table 2. The increase in normal (0 h) value of treatment group (Group III) was due to prophylactic supplementation of vitamin E and Se in form of  $\alpha$ -tocopherol acetate, one month prior to induction of shock. From the comparison of the trends followed by vitamin E levels in (Group II) and treatment (Group III), it is evident that supplementation with vitamin E and Se was successful in significantly delaying the onset of decline in the circulating plasma levels of vitamin E and moreover levels of vitamin E returned toward normal earlier in group III as compared to group II.

Vitamin E interacts with Se containing GSH-Px to prevent the oxidative breakdown of tissue membranes associated with free radical production due to septic shock. Cadenas et al. (1995) studied the level of lipid peroxidation at various levels of vitamin E supplementation in guinea

pigs, an animal that can't synthesize ascorbate, and reported that the increase in vitamin E supplementation was accompanied by a reduction in sensitivity to enzymatic lipid peroxidation without depressing endogenous antioxidant defenses. Therefore depletion of vitamin E during endotoxic shock in the present study indicated that there was excess production of free radicals (Barja et al., 1996) and vitamin E was utilized in chain breaking intracellular antioxidant action, thereby offering protection from lipid peroxidation.

### Catalase

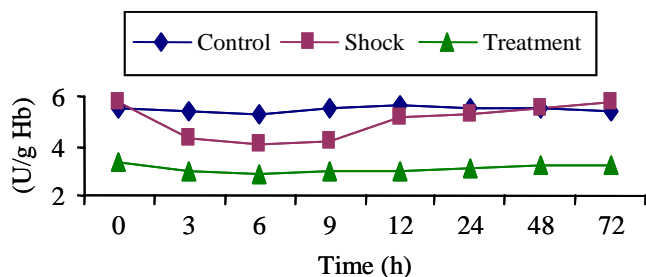
The mean ( $\pm$ SE) values of catalase activity in control (Group I), endotoxic shock (Group II) and treatment (Group III) are presented in Table 1 and Figure 1. The mean normal (0 h) catalase activity of control (Group I) did not change significantly throughout the period of study. The normal (0 h) mean erythrocytic catalase activity was recorded to be 21.78 $\pm$ 0.41 k/g Hb in group II. The enzyme activity remained significantly ( $p < 0.05$ ) low between 1 h to 48 h after induction of endotoxic shock.

In the group III the mean normal erythrocytic catalase activity was 22.94 $\pm$ 0.51 k/g Hb. It decreased significantly to a level of 22.778 $\pm$ 0.44 k/g Hb after 3 h of post infusion, reached lowest at 9 h and remained significantly low till 24 h.

As compared to group II where significant ( $p < 0.05$ ) decrease occurred after 1 h post infusion, the significant decrease in group III occurred after 3 h post infusion and continued till 48 h. Portholes et al. (1996) also observed marked decrease in the liver catalase activity during *E. coli* endotoxin shock in rats.

Kadota et al. (1989) studied the role of oxygen free radicals and catalase as free radical scavenger in experimentally induced septic and hemorrhagic shock in rats. Results of present study i.e. significant ( $p < 0.05$ ) decline in activity of catalase with onset of endotoxic shock are in accordance with above mentioned findings.

Catalase converts hydrogen peroxide ( $H_2O_2$ ) to oxygen



**Figure 3.** Effect of prophylactic supplementation of vitamin E and Se on erythrocytic G-6-PD levels during endotoxic shock in buffalo calves.

and water which are non injurious to tissues and thus protects cellular damage (Pamela et al., 1994). In the present study a significant ( $p < 0.05$ ) decrease in the activity of catalase with onset of endotoxic shock indicates that it involves generation of ROS eg.  $H_2O_2$  from partial reduction of molecular oxygen. Catalase was involved in the detoxification of hydrogen peroxide to  $O_2$  and water.

Turrens et al. (1984) studied the effect of injection of liposomes containing catalase into rats before giving endotoxic shock and found that there was increase in survival rates. It indicated the formation of  $H_2O_2$  and subsequent disposal by the catalase. Moreover catalase has exclusive role in the removal of hydrogen peroxide in normal human erythrocytes (Mueller et al., 1997).

### Superoxide Dismutase

The mean ( $\pm$ SE) values of superoxide dismutase activity in control (Group I), endotoxic shock (Group II) and treatment (Group III) are presented in Figure 2 and Table 2. The normal (0 h) mean activity of SOD in group I didn't differ significantly throughout the period of study. The SOD activity in Group III differed significantly ( $p < 0.05$ ) than that in the other two groups.

In group II (Endotoxic shock), on induction of shock, there was significant decline in the activity at 6 hours post infusion ( $7.20 \pm 0.03$  U/mg Hb.). The decline in activity was lowest at 12 h post infusion ( $5.77 \pm 0.032$  U/mg Hb). This significant ( $p < 0.05$ ) decrease in activity started at 6 h post infusion, reached a lowest level at 12 h post infusion and was still significantly lower at 72 h post infusion ( $6.06 \pm 0.031$  U/mg Hb).

The mean (0 h) normal SOD activity in group III was  $6.60 \pm 0.02$  U/mg Hb. Which was significantly lower than that of other two groups i.e. group I ( $7.618 \pm 0.02$  U/mg Hb) and group II ( $7.702 \pm 0.023$  U/mg Hb). On induction of shock, there was significant decrease in activity at 9 h post infusion ( $5.48 \pm 0.038$  U/mg Hb) which was lowest, then it started rising toward normal value but was not able to attain the normal level even at 72 h. post infusion ( $5.83 \pm 0.029$  U/mg Hb).

SOD disproportionates superoxide to hydrogen peroxide, which is further metabolised in the intracellular compartments by Se dependent GSH-Px. Superoxide anion can react with hydrogen peroxide to form hydroxyl radical, which reacts with PUFA to generate lipid peroxide (Pironi et al., 1996). The endothelium is a key site of injury due to ROS but can be potentially protected by the antioxidant enzymes-SOD and catalase (Beckman et al., 1986).

Sakaguchi et al. (1981) studied lipid peroxide formation during endotoxic shock in mice and reported that SOD activity was decreased between 18-48 h after induction of shock and thereafter tend to increase. Similar trend was found in the present study.

### Glucose-6-Phosphase Dehydrogenase

The mean ( $\pm$ SE) values of G-6-PD activity in control (Group I), endotoxic shock (Group II) and treatment (Group III) are presented in Table 2 and Figure 3. The normal mean activity of G-6-PD of control (Group I) was  $1.80 \pm 0.015$  U/g Hb and it didn't show any significant ( $p < 0.05$ ) change throughout the period of study i.e. till 72 h.

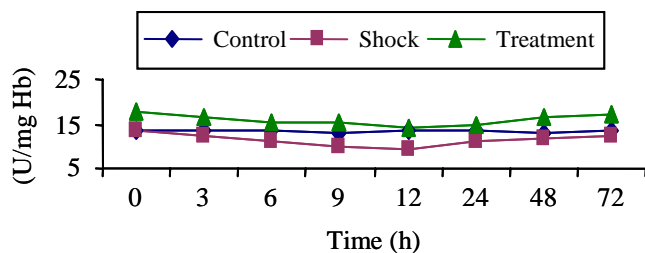
The normal mean activity of plasma G-6-PD was  $1.882 \pm 0.022$  U/g Hb in group II which declined significantly ( $p < 0.05$ ) 1 h post infusion of endotoxin, reached lowest activity at 6 h post infusion ( $1.32 \pm 0.021$  U/g Hb) and remained significantly low till 48 h.

The mean normal activity of G-6-PD in group III was observed to be  $1.08 \pm 0.0031$  U/g Hb which was significantly ( $p < 0.05$ ) lower than the normal (0 h) mean activity of group I and II. It showed a significant decrease from 3 h ( $0.97 \pm 0.0028$  U/g Hb) onwards, attained lowest circulating level at 6 h post infusion ( $0.94 \pm 0.0027$  U/g Hb) and remained significantly low till 24 h ( $1.01 \pm 0.0018$  U/g Hb).

All the three groups differed significantly ( $p < 0.05$ ) from each other. As compared to group II where significant decline in activity was observed between 1 h to 48 h of post infusion, the significant decrease in group III was observed only between 3 h to 24 h of post infusion. As evident from the Table 5, the onset of shock was delayed as well as recovery was earlier in group III as compared to group II.

G-6-PD catalyzes the first step in the glucose metabolism through HMP and generates NADPH from oxidation of glucose-6-Phosphate to 6-phospho gluconolactone. Therefore HMP plays a central role in the metabolic protection against ROS by generating NADPH, which serves as an electron donor in the reduction of GSSG to 2GSH in a reaction catalyzed by glutathione reductase. In turn GSH removes  $H_2O_2$  and ROOH from erythrocytes in a reaction catalyzed by GSH-Px. (Harvey, 1989). In the present study there was also decline in G-6-PD activity with onset of shock.

With oxidative stress, NADPH is oxidized, that stimulates HMP as the activity of G-6-PD is directly related



**Figure 4.** Effect of prophylactic supplementation of vitamin E and Se on erythrocytic GPx levels during endotoxic shock in buffalo calves.

to the concentration of NADP<sup>+</sup> and inversely related to that of NADPH (Yoshida, 1973).

Supplementation of  $\alpha$ -tocopherol inhibited the erythrocytic SOD activities and lipid peroxidation which lead to the decreased generation of H<sub>2</sub>O<sub>2</sub> and ROOH. Therefore a decreased requirement of intracellular GSH during  $\alpha$ -tocopherol supplementation might have increased intra cellular NADPH, which resulted in inhibition of erythrocytic G-6-PD activity. It might be the reason for the decreased enzyme activity at 0 h in group III as compared to the enzyme activity in other two groups. (group I and II)

#### Glutathione Peroxidase

The mean ( $\pm$ SE) values of glutathione peroxidase activity in control (Group I), endotoxic shock (Group II) and treatment (Group III) are presented in Table 1 and Figure 4. The mean normal (0 h) activity of group I didn't differ significantly throughout the period of study. The normal (0 h) mean activity of glutathione peroxidase (GSH-Px) was 13.19 $\pm$ 0.113 U/mg Hb in group II (Shock group). Enzymatic activity showed a significant ( $p < 0.05$ ) decline at 3 h post infusion (12.22 $\pm$ 0.121 U/mg Hb), continued till 12 h post infusion where it recorded a lowest activity of 9.22 $\pm$ 0.110 U/mg Hb and after that it started rising but remained significantly low till 48 h.

In group III, the mean normal value of GSH-Px was 17.48 $\pm$ 0.206 U/mg Hb which was significantly higher ( $p < 0.05$ ) than the other two groups-group I and group II. On induction of shock, the activity showed significant fall after 3 h of endotoxic infusion (16.29 $\pm$ 0.22 U/mg Hb), continued to decline till 12 h (14.33 $\pm$ 0.211 U/mg Hb) post infusion and after that it started rising, but remained significantly ( $p < 0.05$ ) lower till 24 h (14.83 $\pm$ 0.223 U/mg Hb) of post infusion.

Mean normal value of GSH-Px in group III was higher than mean normal value of GSH-Px in group I and II after 4 weeks of supplementation. The GSH-Px activity of all the three groups differed significantly ( $p < 0.05$ ) from each other at every stage of the experiment.

Sakaguchi (1981) also reported decrease in activities of glutathione reductase and glutathione peroxidase in liver,

after 18 hours of endotoxin infusion in mice but prophylactic administration of  $\alpha$ -tocopherol prevented the lipid membrane damage that arose from endotoxin challenge. The result suggested that GSH-Px might be playing active role in tackling free radicals produced during endotoxic shock. Osame et al. (1992) also reported that supplementation with Se increased serum Se concentration and consequently blood GSH-Px activity.

During endotoxic shock, when there is a period of oxidative stress, GSH scavenges excess intra cellular free radicals and is oxidized to GSSG through the action of GSH-Px (Kurt et al., 1997). Vitamin E and Se act synergistically and reduce requirement of each other in their action against lipid peroxides (Mayes, 1996).

Vitamin E reduces requirement of Se by preventing loss of Se from body or maintaining it in an active form (Murray et al., 1996). Therefore increased erythrocytic GSH-Px activity observed after vitamin E and Se supplementation in the present study support the antioxidative action of vitamin E and Se.

Portoles (1996) in his study on hepatic response to the oxidative stress induced by *E. coli* endotoxin also reported a significant decline in liver GSH-Px activity after 5 hours of endotoxin administration. It also suggested that ROS may be important mediators of cellular damage during endotoxic shock.

#### IMPLICATIONS

It is concluded that prophylactic supplementation with vitamin E and Se was helpful in decreasing the oxidative damage due to free radical production during endotoxic shock. This prophylactic supplementation resulted in delaying the onset of oxidative damage due to endotoxic shock as well as speeding up the recovery from oxidative stress. Magnitude of oxidative stress was also less at every stage of period of study in antioxidant supplemented group as compared to shock group. So supplementation with vitamin E and Se is desirable in treating clinical conditions which are known to cause oxidative stress.

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