Protective Effectes of Antioxidants Against Rheumatoid Arthritis-Induces Lipid Peroxidation and Glutathione Dpletion

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ABSTRACT

The effects of rheumatoid arthritis (RA) and antioxidant therapy for 10 days with vitamin E, Vitamin C and vitamin A on serum lipid peroxidation (MDA content), glutathione (GSH) content, glutathione S-transferase (GST) activity, glutathione reductase (GSR) activity, both total and selenium dependent glutathione peroxidase (GSH-Px) activities were tested in 18 rheumatic patients. RA patients had increased MDA content, and index of lipid peroxidation, depleted GSH levels, elevated GST activity and inhibited GSR and GSH-GSH-Px activities, as compared to 20 healthy subjects.

Supplementation with vitamin E decreased MDA content, elevated GSH levels, GSR and GSH-Px activities after 5 days of therapy. Vitamin C supplementation did so only after 10 days of therapy, while treatment with vitamin A showed no significant changes in these parameters compared to pretreatment levels.

The results suggest that antioxidant therapy may afford some

protection against oxidative stress associated with RA.

Introduction:

Several lines of evidence suggest that reactive oxygen species are involved in the pathogenesis of rheumatoid arthritis (RA) (McCord 1974; Nurcombe et al 1991). Elevated levels of serum and synovial lipid peroxidation products have been observed in patients with RA (Rowley et al 1984; Merry et al 1991).

Various other indices of oxidative stress in RA have also been reported. Defective antioxidant activity with a decrease in reduced glutathione (GSH) content and glutathione peroxidase (GSH-Px) activity (Hassan et al 1997), vitamin C (Lunec & Blake 1985), riboflavin, (Mulherin et al 1996) and degradation of hyaluronic acid (Grootveld et al 1991) are also observed in RA patients. Therefore, and alteration in the prooxidant-antioxidant balance in favor of the prooxidant side is evident in these patients which may contribute to the increased peroxidative damage.

If the toxicity of RA is due at least in part to reactive oxygen species-mediated lipid peroxidation and other tissue damaging effects, then decreasing the damage could be feasible by increasing the antioxidant concentration in tissues (Harman 1993). One approach may be to use vitamins such as E, C and A to combat the normal or diminished antioxidant defences against free-radical reaction. Therefore, we have studied the effects of these vitamins on serum lipid peroxidation (MDA content), reduced glutathione (GSH) content, glutathions S-transferase (GST) activity. glutathione reductase (GSR) activity and on glutathione peroxidase (GSH-Px) activities in RA patients.

Sujects and Methods

Eighteen patients diagnosed as rheumatic patients according to the criteria established by the American Rheumatism Association (Popes et al 1958) were divided into 3 groups. Each group was given either vitamin E (200mg/day) or vitamin C (500mg/day) or vitamin A (20000u/day) for 10 days. Venous blood samples (10 ml) were drawn every 5 days thereafter. Serum was prepared by standard methods and assays were conducted within several hours of blood collection.

Twenty healthy subjects, age and sex-matched with the patients, served as controls. None of the control subjects or patients had clinical or laboratory evidence of a disease that may affect the parameters to be measured and none had been taking these vitamins as supplements prior to the study.

Lipid peroxidation was determined using the thiobarbituric acid method of Buege & Aust (1978), with malondialdehyde as the standard. Serum glutathione (GSH(content was determined according to the method of Ellman (1959). Glutathione S-transferase (GST) activity was estimated according to the spectrophotometric procedure of Habig et al (1974). Glutathione reductase (GSR) activity was measured according to the procedure of beutler (1969). Total selenium dependent glutathione peroxidase (GSH-Px) activities were determined by the coupled-assay procedure of paglia and Valentine (1967) as modified by lee et al (1987). Protein was determined by the method of Lwery et al (1951) using bovine serum albumin as a standard. Multiple group comparisons were made using analysis of variance (ANOVA). Two-way comparisons of data utilized student test.

Results

Figure 1 summarizes the effects of RA and antioxidant therapy on serum MDA content. RA patients had a 3-fold increase in MDA content over control subjects. Vitamin E treatment of the patients caused a significant decrease (31%) in serum MDA content after 5 days of therapy, while a 72% decrease was produced after 10 days which was equivalent to control levels (fig. 1A). Patients treated with vitamin C showed a significant decrease (31%) in MDA content only after 10 days of therapy compared to its levels before treatment. (fig. 1B). Treatment with vitamin A for 10 days showed no significant decrease in the levels of MDA as compared to pretreatment levels (fig. 1 C).

The effects of RA and antioxidant therapy on serum GSH levels are shown in Figure 2. RA patient had 50% decrease in serum GSH content. Serum GSH content from RA patients demonstrated a significant elevation 5 days after treatment with vitamin E (fig 2 A). Maximum increase in GSH content was achieved after 10 days (160%). Patients treated with vitamin C showed a significant increase in GSH content (31%) only after 10 days of treatment compared to its levels before treatment. (fig. 2B). Treatment of the patients with vitamin A caused 18% increase in serum GSH content after 10 days which was not significant ($P > 0.05$) compared to pretreatment level (fig. 2C).

Figure 3 is summary of RA and antioxidant therapy on serum GST activity. RA had caused 220% increase in GST activity over control subjects level. Treatment with each type of the vitamins caused no father increase in its activity over pretreatment levels. All types of antioxidants failed to reduce its activity to control levels (fig. 3).

The activity of GSR was lower in RA patient compared to

their corresponding control subjects values (fig. 4). The activity of this enzeme showed a 14% and 69% increase after 5 and 10 days treatment with vitamin E respectively. However, the increase in GSR activity after 10 days was not high enough to match its level in normal control subjects (fig. 4).

The activity of GSH-Px is shown in figure 5. A 50% reduction in the activity of this enzyme was observed in RA patients. Treatment of the patients with vitamin E showed greatest activity for the total enzyme (39%) after 10 days of therapy and 44% increase in the activity of selenium-dependent enzyme was observed at this time point. Both activities did not reach control levels (fig. 5).

Discussion

Several studies indicate that oxygen-free radicals may play a key role in the toxicity of RA. Increased production of oxygen-free radicals and/or impaired defence mechanisms result in an oxidant-antioxidant imbalance with consequent oxidative cellular damage (Yu 1994). Therefore, we examined the effects of antioxidant therapy with vitamin E, vitamin C and vitamin A on parameters that implicate oxidative cellular damage in RA patients.

The major defence mechanisms against oxygen-free radicals induced cellular damage include GSH and enzymes associated with its metabolism (Kehrer 1990). These mechanisms had been shown to be impaired in RA (Hassan et al 1997). The significant increase in serum GSH levels after antioxidant therapy may be attributed to either a direct effect of the antioxidants on the synthesis of GSH or to enhancement of phase II metabolism to eliminate the toxic metabolites (Benson et al 1979). In addition, the lack of effect on GST activity may be due to maximal

induction of this enzyme system in RA (fig. 3) as a result of accumulation of toxic metabolites and the decrease or malfunction of other components of the major cellular defence mechanisms (hassan et al 1997). Studies by Stone and Dartz (1980) have shown that when rats fed a diet deficient in vitamin E and selenium, increased activity of GST occurred in all tissue tested.

The observed increases in the activity of GST (fig. 4) are consistent with the role of this enzyme in maintaining a normal reduced glutathione: oxidized glutathione ratio (Beutler 1969). However, since a malfunction of this enzyme system is suggested in RA patients (Mulherin 1996) the antioxidant therapy may have resulted in correcting the underlying cause. The data also showed a highly significant increase in GSH-Px activity which may reflect the improvement in GSR activity or GSH levels. It has been proposed that glucose 6-phosphate dyhydrogenase, GSR and GSH-Px work in concert to combat oxidative cellular damage (Chow & Tappel 1972). Furthermore, the increased resistance of human endothelial cells against hyperoxia was attributed to an increase in GSH-Px activity (Michiels et al 1994).

The highly protective effect provided by the antioxidant therapy is shown by the decrease in serum lipid peroxidation (fig. 1). Vitamin E, vitamin C and Vitamin A are major extracellular antioxidant defencese. Vitamin E and vitamin A are lipophilic in nature and they concentrate in lipophilic membrances (Krinsky 1988). The antioxidant activity of vitamin A has been suggested because of its quenching capacity for singlet oxygen (Foote & Denny 1968). Vitamin A in this study has little protective effect, which may be due to the insufficient dose of the vitamin given or that singlet oxygen may not play a role in the oxidative damage. Vitamin E is believed to exert its antioxidant action by halting the

propagation steps in lipid peroxidation (Burton & Ingold 1989). Vitamin C is water soluble and reacts with activated electrophils in the cytoplasm (frei et al 1989).

In summary, this study demonstrated the potentially beneficial effect of antioxidant therapy in RA patients. Vitamin E therapy enhanced major intra cellular defense mechanisms including GSH content, GSR and GSH-Px activities, resulting in decreased lipid peroxidation.

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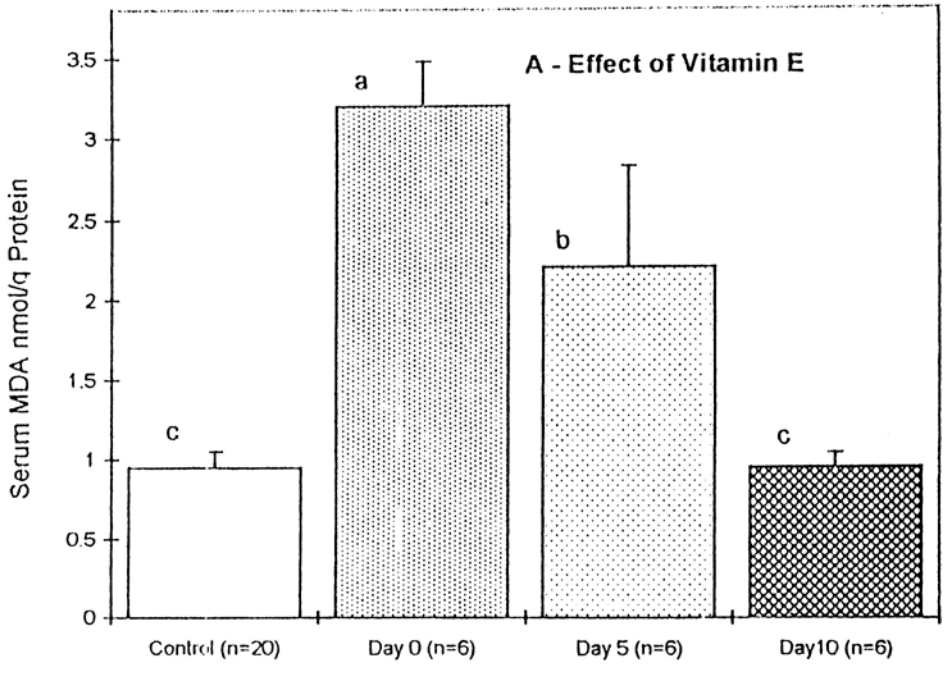
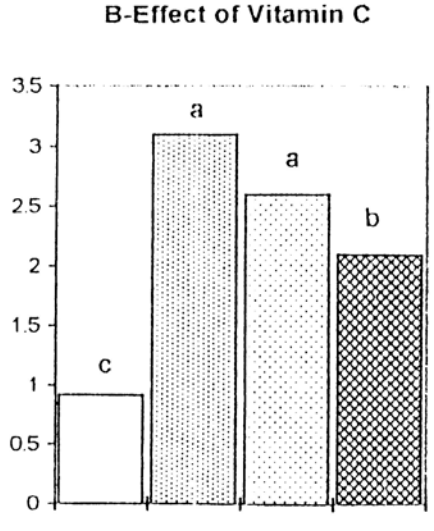
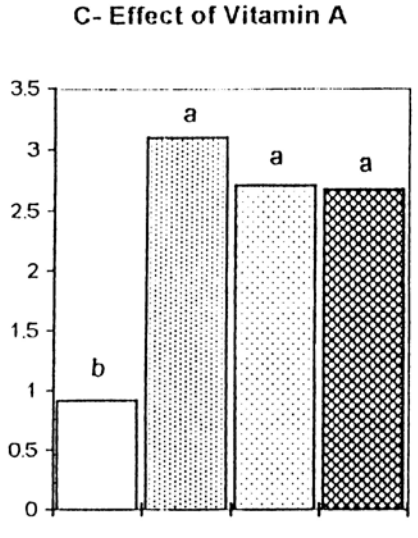
Figure 1. Effect of Antioxidants on Lipid Peroxidation (MDA content) in RA Patients

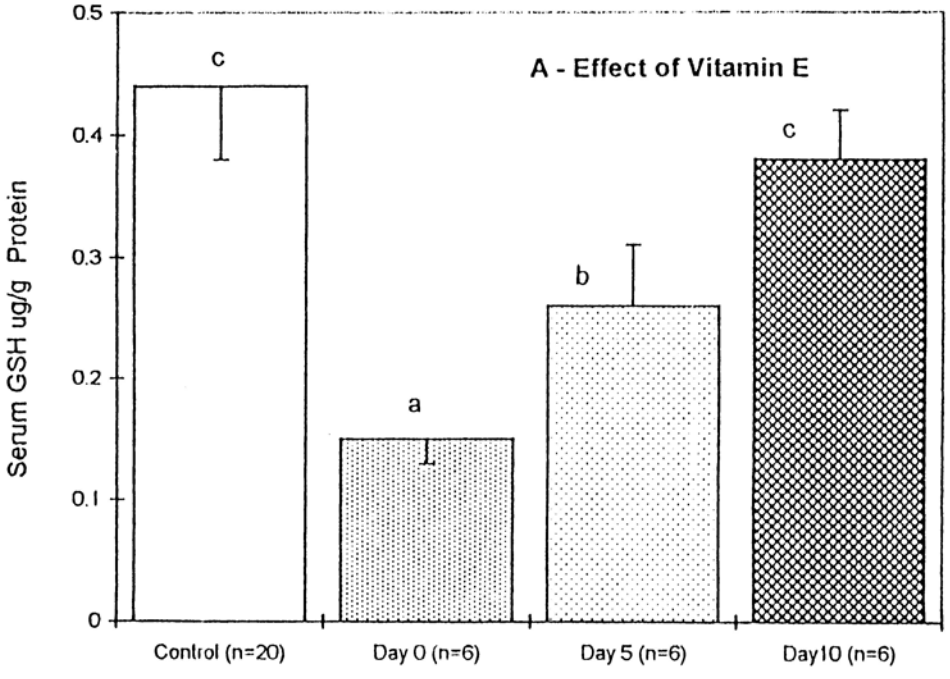


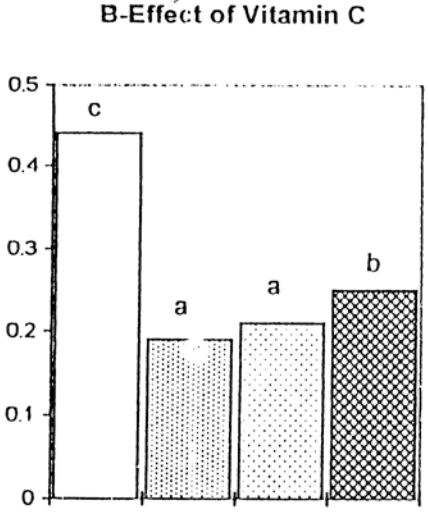




(a,b,c) superscripts are significantly different ($p < 0.05$)

Figure 2. Effect of Antioxidants on Glutathione (GSH) levels ir RA Patients





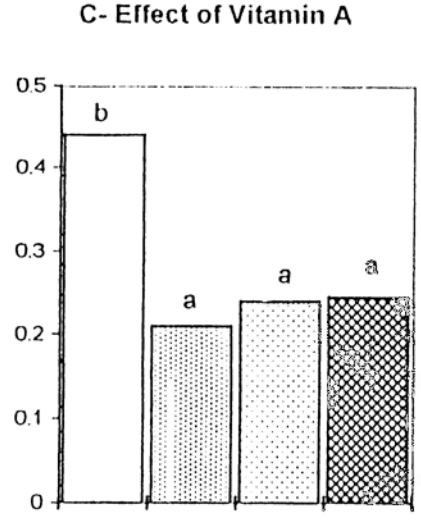


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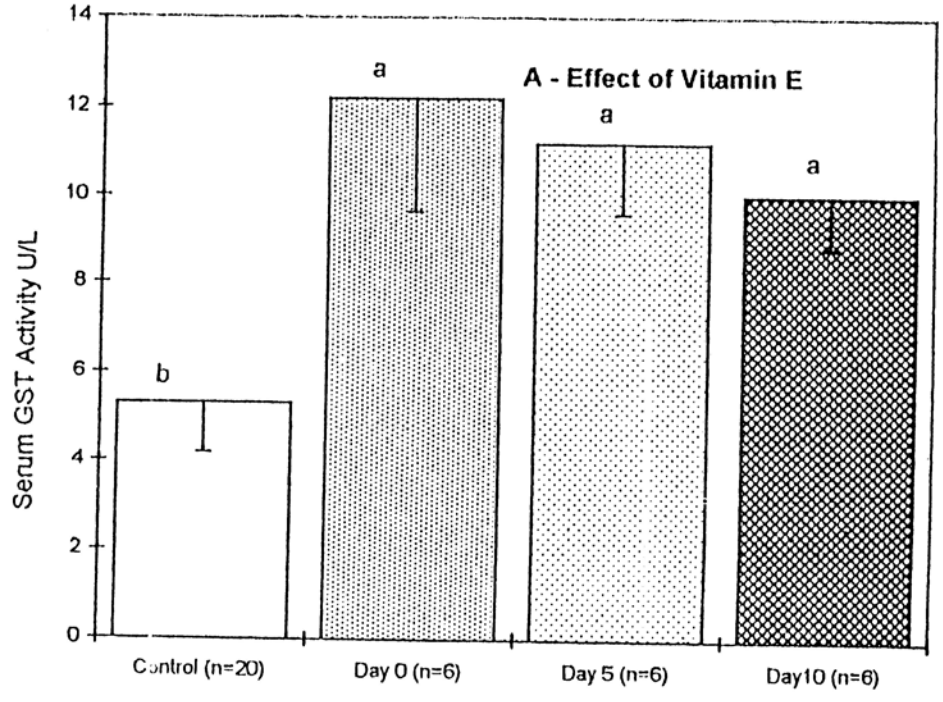
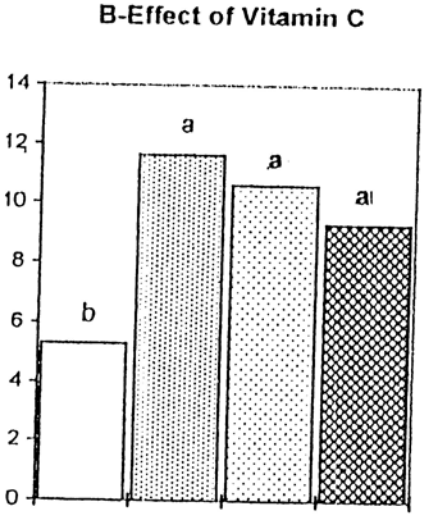
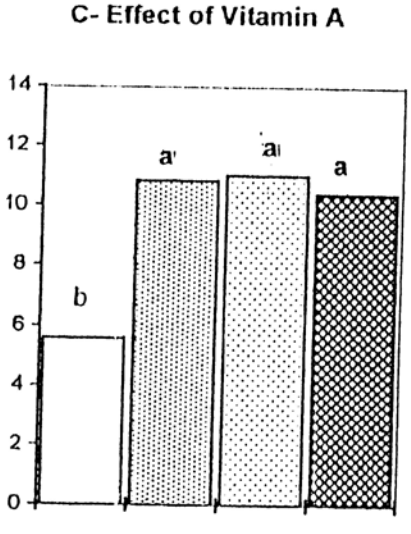
Figure 3 Effect of Antioxidants on Serum Glutathione S-Transferase (GST) Activity in RA Patients







(a,b) superscripts are significantly different ($p < 0.05$)

Figure 4. Effect of Vitamin E on Glutathione Reductase (GSR) Activity in RA Patients

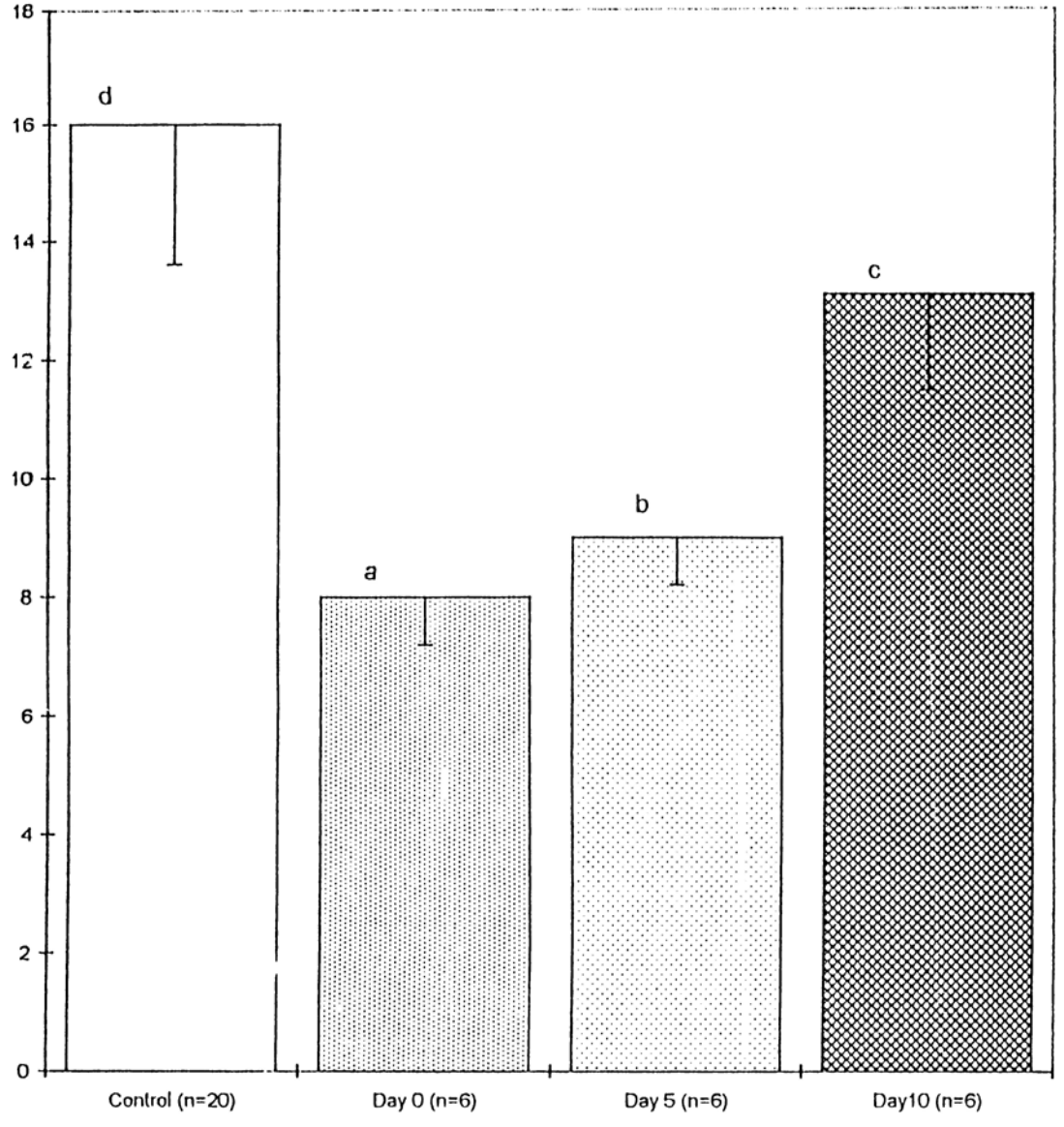


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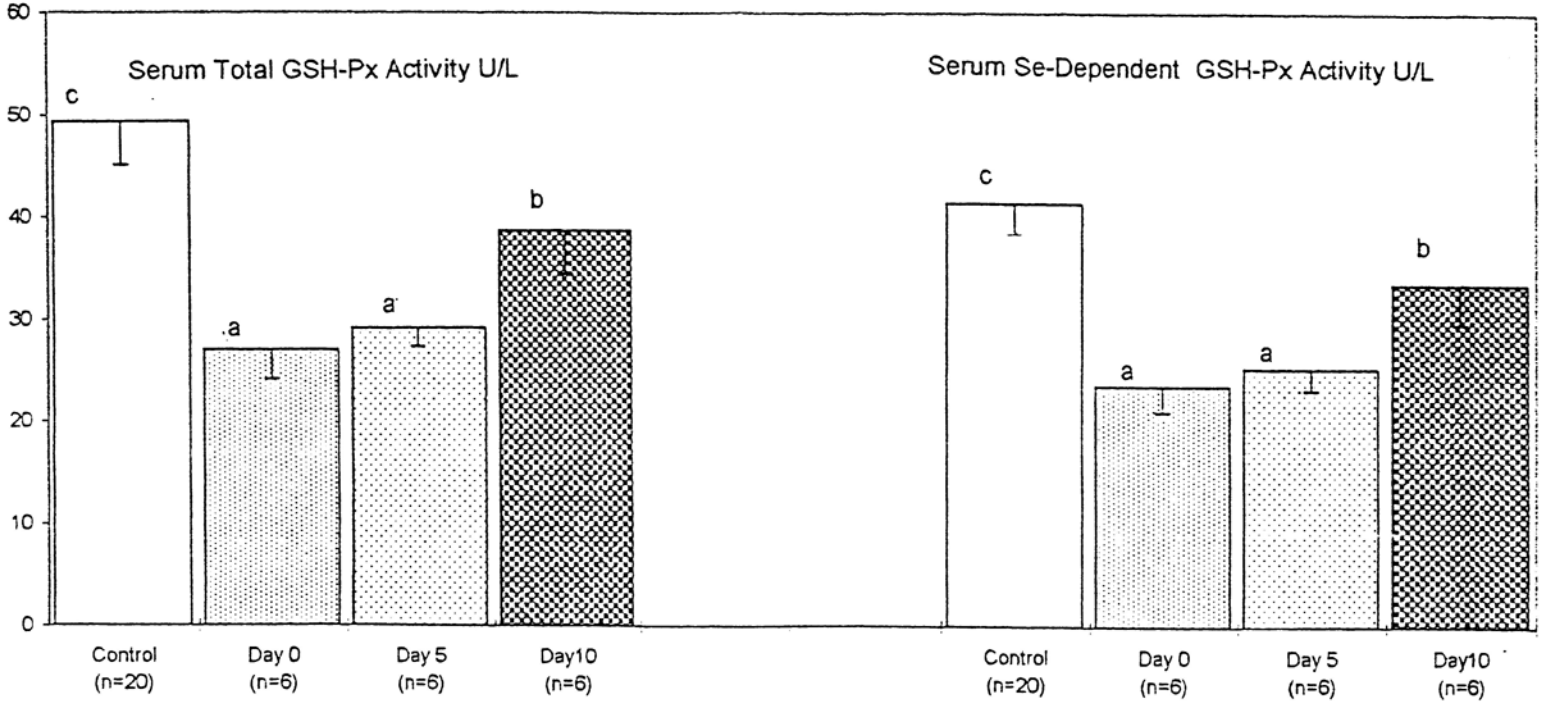
Figure 5. Effect of Vitamin E on Glutathione Peroxidases (GSH-Px) Activities in RA Patients



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