

Original Article

Effect of vitamin E on plasma malondialdehyde, antioxidant enzyme levels and the rates of wound closures during wound healing in normal and diabetic rats

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Vitamin E is composed of various subfamilies that include tocopherols and tocotrienols. These compounds have antioxidant properties but differ in structure, dietary source and potency. In this study we evaluated the efficacy of α -tocopherol as an antioxidant and its role in wound closure in normal and streptozotocin-induced diabetic rats. The healing of 6 cm linear incisions created on the back of each male Sprague–Dawley rat (250–300 g) was monitored by measuring the length of the wounds daily. The rats were divided into two categories; normal and streptozotocin-induced diabetic rats. For each category, the animals were further divided into two groups; those untreated and those receiving 200 mg/kg bodyweight α -tocopherols daily by oral gavage. All rats were fed standard food and water ad libitum. Blood samples were taken at 0, 5 and 10 days after the wounds were created for the determination of malondialdehyde levels and red cell superoxide dismutase, catalase and glutathione peroxidase activities. The results showed that α -tocopherol reduced plasma malondialdehyde levels, increased glutathione peroxidase activity and accelerated the rate of wound closure in treated rats.

Key words: antioxidant enzymes, α -tocopherol, diabetic, malondialdehyde, vitamin E, wound closure, wound healing.

Introduction

Vitamin E is one of the most effective antioxidants in animals. It is composed of various subfamilies of which tocopherols and tocotrienols are the most studied. The structural difference between the two subfamilies is that tocotrienols possess three double bonds in their isoprenoid side chain,¹ and this structural difference results in differences in their efficacy and potency as antioxidants.

Oxidative stress has been implicated as playing a role in the development of diabetic complications such as atherosclerosis,² peripheral polyneuropathy³ and the increased incidence of abnormal embryonic development in pregnancies of diabetic mothers.⁴ The accumulating data suggest that in diabetes there is an increased production of reactive oxygen species⁵ and decreased antioxidant scavenging activities.⁶ This results in increased lipid peroxidation and DNA oxidation, as assessed by both elevated plasma levels of lipid and DNA oxidation products.⁷

Wounding is another condition that results in a decrease in antioxidants.⁸ It is not surprising, therefore, to find that the process of healing is especially delayed in diabetes mellitus.⁹ We have previously shown that palm vitamin E extract, which contains a mixture of 60% tocotrienol and 40% tocopherol, enhances wound healing in diabetic rats and increases the activity of the antioxidant enzyme glutathione

peroxidase (GPx).¹⁰ In this paper, we report our findings on the effects of α -tocopherol supplementation on plasma MDA levels, antioxidant enzyme activities and the rate of wound closure during wound healing in normal and diabetic rats.

Materials and methods

Male Sprague–Dawley rats (250 g–300 g) were divided into four groups consisting of six animals each – untreated and α -tocopherol-treated normal rats and untreated and α -tocopherol treated streptozotocin-induced diabetic rats.

Diabetes was induced by an intramuscular injection of streptozotocin (Sigma, Watford, UK) in saline (65 mg/kg bodyweight) to an overnight fasted animal. Fasting blood glucose was determined (using a glucometer and a reagent strip (Advantage; Boehringer Mannheim, Mannheim, Germany)) to confirm diabetes 3 days later. The experiment was carried out five days after the confirmation of diabetes. Mid-dorsal linear

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incisions of 6 cm in length were made on each animal and sutured immediately under sterile conditions.

The rats were then fed normal standard food and water ad libitum. The treated groups were given 200 mg/kg body-weight α -tocopherol by gavage daily. Untreated groups received an equivalent volume of olive oil. Wounds were observed and measured daily and photographs taken on days 0, 5 and 10 after wounding. Blood samples were taken at 0, 5 and 10 days after the introduction of the cuts for the determination of plasma malondialdehyde (MDA) level and superoxide dismutase (SOD), catalase and GPx activity in red cells, following the methods of Ledwozyw,¹¹ Beyer and Fridovich,¹² Aebi¹³ and Paglia and Valentine,¹⁴ respectively.

Results

Figure 1 shows the mean \pm SD of plasma MDA in untreated and treated normal and diabetic rats. The plasma MDA levels in untreated normal rats were decreased during wound repair. Normal rats supplemented with α -tocopherol showed a significantly greater reduction of MDA levels compared to the untreated normal group ($P < 0.001$). In the diabetic group, there was a progressive increase in MDA level during the period of wound healing. The MDA level on day 10 was significantly greater than its levels on day 0 for the untreated diabetic rats. Supplementation of α -tocopherol was shown to reduce plasma MDA levels significantly ($P < 0.001$).

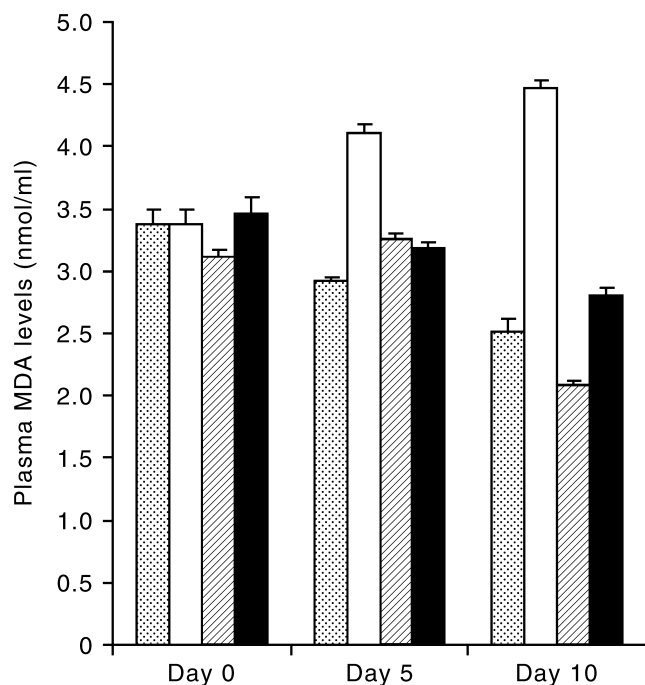


Figure 1. Malondialdehyde (MDA) levels in untreated and α -tocopherol-treated normal and diabetic rats. Untreated and treated normal rats had reduced plasma MDA levels during the healing period. Untreated diabetic rats showed increased levels while treated rats showed decreased plasma MDA levels. (◻), Untreated; (◻), untreated diabetic; (▨), normal rats treated with α -tocopherol; (■), diabetic rats treated with α -tocopherol. Values are mean \pm SD.

The mean \pm SD SOD activity in red cells of untreated and α -tocopherol-treated normal and diabetic rats is shown in Figure 2. There was no significant difference in the SOD activities of untreated and treated normal rats, which remained relatively constant throughout the healing period. An increase in SOD activity towards the later stage of the wound healing period was observed in the untreated and treated diabetic groups. These levels (taken on day 10) were significantly greater than the SOD activities at the start of the study (day 0) ($P < 0.05$).

The mean \pm SD GPx activity in red cells of untreated and α -tocopherol-treated normal and diabetic rats is given in Figure 3. Supplementation with α -tocopherol significantly increased the activity of GPx in both the normal and diabetic rats ($P < 0.001$). Untreated diabetic rats showed a marked decrease in the activity of this enzyme during the wound healing process.

Figure 4 shows the mean \pm SD catalase activity in red cells of untreated and treated normal and diabetic rats. There were no significant differences in catalase activity between the four experimental groups. Figure 5 shows that supplementation of α -tocopherol to both the normal and diabetic rats significantly increased the rate of wound closure ($P < 0.001$).

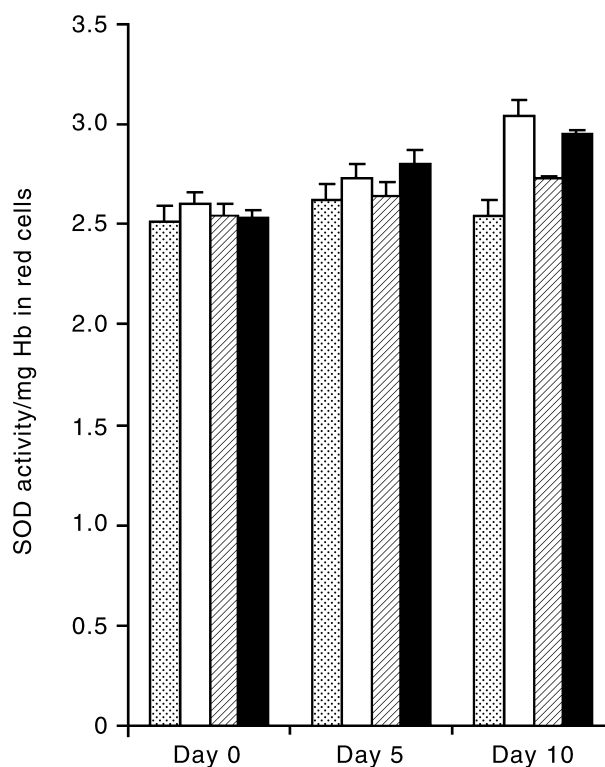


Figure 2. Red cell superoxide dismutase (SOD) levels in untreated and α -tocopherol-treated normal and diabetic rats. An increase in SOD activity toward the later stage of the wound healing period was observed in the untreated and treated diabetic groups. These levels (at day 10) were significantly greater than the SOD activities at the start of the study (day 0) ($P < 0.05$). Hb, haemoglobin. (◻), Untreated; (◻), untreated diabetic; (▨), normal rats treated with α -tocopherol; (■), diabetic rats treated with α -tocopherol. Values are mean \pm SD.

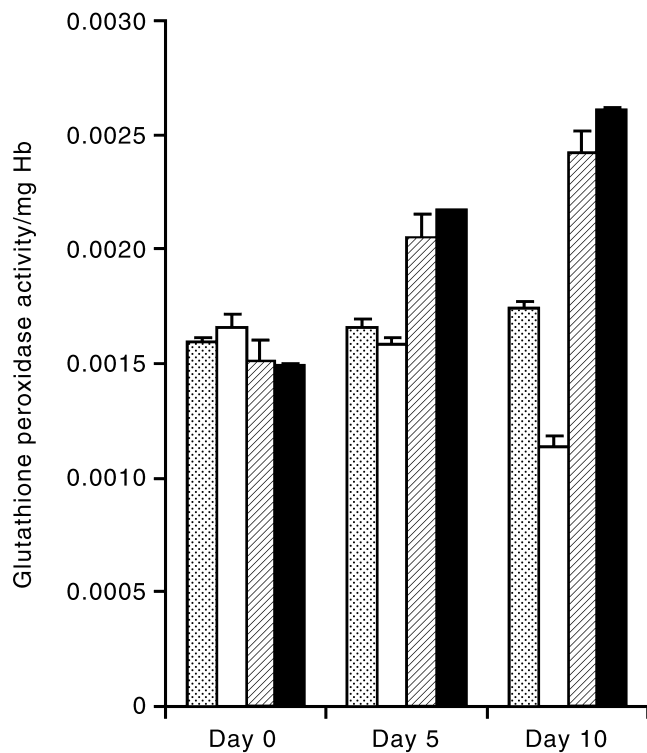


Figure 3. Red cell glutathione peroxidase (GPx) levels in untreated and α -tocopherol-treated normal and diabetic rats. Treatment with α -tocopherol significantly increased the activity of GPx in both the normal and diabetic rats ($P < 0.001$). Hb, haemoglobin. (▨), Untreated; (□), untreated diabetic; (▧), normal rats treated with α -tocopherol; (■), diabetic rats treated with α -tocopherol. Values are mean \pm SD.

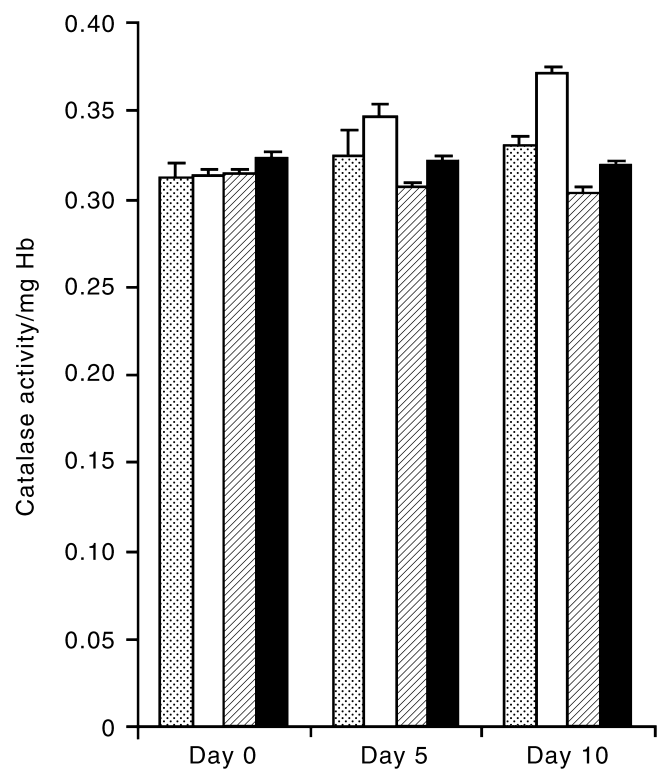


Figure 4. Red cell catalase levels in untreated and α -tocopherol-treated normal and diabetic rats. Only the untreated diabetic rats showed an increase in the activity of catalase while there were no significant differences in the catalase activities of the other groups. Hb, haemoglobin. (▨), Untreated; (□), untreated diabetic; (▧), normal rats treated with α -tocopherol; (■), diabetic rats treated with α -tocopherol. Values are mean \pm SD.

Discussion

The present study shows that α -tocopherol enhances the rate of wound closure in both normal and diabetic rats. From our previous study, palm vitamin E extract was observed to enhance wound healing only in diabetic rats. In this study, therefore, we showed that α -tocopherol is also effective in accelerating wound closure not only in diabetic rats, but also in normal rats.

Plasma MDA levels (Fig. 1) were increased in untreated diabetic rats. This probably reflects the increase in lipid oxidation due to either increased production of free oxidative radicals⁵ or decreased antioxidant defence mechanisms,⁶ or both. The same observations have also been reported in humans⁷ and rats.¹⁰ Supplementation with α -tocopherol decreased the level of MDA in both the normal and diabetic groups, confirming the role of α -tocopherol as a powerful antioxidant. Similar observations were also noted with the use of palm vitamin E extract.¹⁰

Results from the antioxidant enzyme determinations (Figs 2–4) showed that basal GPx levels were lower in diabetic rats, confirming the earlier reports of Jones *et al.*,⁶ Cunningham *et al.*¹⁵ and Musalmah *et al.*¹⁰ Daily oral supplementation with α -tocopherol increased the level of SOD and GPx. These enzymes scavenge free radicals and prevent oxidative damage.¹⁶ This finding was slightly different to

what was observed earlier when we supplemented palm vitamin E extract to normal and diabetic rats recovering from wounds. In the earlier study, we observed an increase in GPx activity but decreased SOD levels.¹⁰ The difference in the response of this enzyme to the two subfamilies of vitamin E is unknown but may indicate differences in their mechanism of action. As with our previous study on supplementation of palm vitamin E extract, we found that the activity of catalase remained constant throughout the wound healing period in both the α -tocopherol-treated and untreated rats. Catalase is the slowest of the above antioxidant enzymes to respond to an increased level of free radicals in blood. Therefore, the 10-day period in which this experiment was carried out may be insufficient to witness any change in the activity of this enzyme.

Wounding results in the loss of different free radical scavengers, both enzymatic and non-enzymatic, that either partially or completely recover following healing.⁸ The increase in free radical production and diminished antioxidant activities may worsen the situation and account for the delay in healing in diabetic patients. The present study shows that supplementation of α -tocopherol enhances wound closure in normal and diabetic rats. This probably involves its action as an antioxidant, reducing the level of free radicals and, hence, free radical damage (as indicated in the reduced

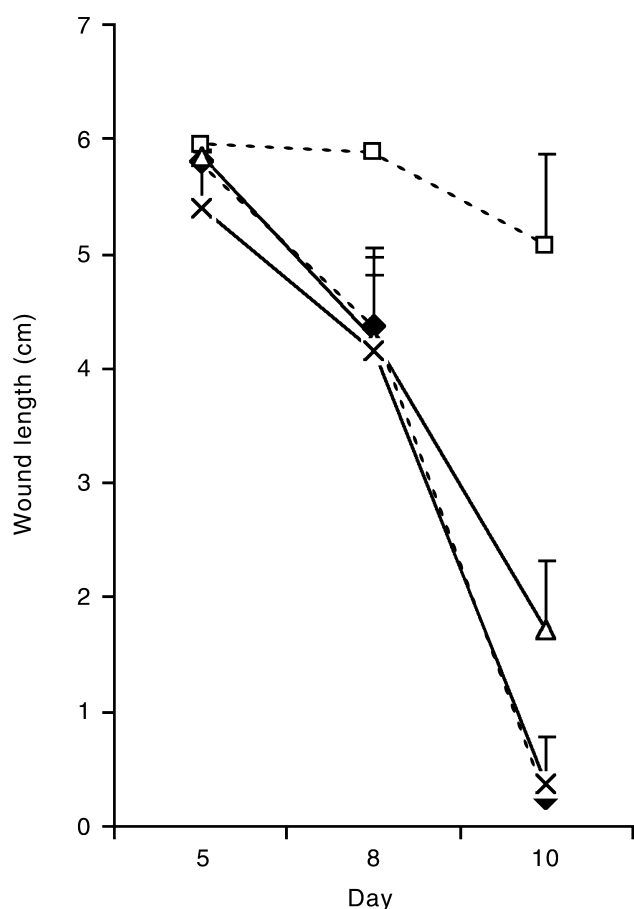


Figure 5. Rates of wound closure in untreated and α -tocopherol-treated normal and diabetic rats. Rats supplemented with α -tocopherol had a faster rate of wound closure than untreated rats. (--- \square ---), Untreated; (--- \diamond ---), untreated diabetic; (— \triangle —), normal rats treated with α -tocopherol; (— \times —), diabetic rats treated with α -tocopherol.

plasma MDA levels), and by increasing the activity of antioxidant enzymes, especially GPx. Other antioxidants such as ascorbic acid¹⁷ and trolox have also been shown to enhance wound healing in diabetic humans and rats, respectively.⁹

In conclusion, the present study shows that α -tocopherol enhances wound closure in normal and diabetic rats. Its mechanism may involve the reduction of free radicals and increased GPx.

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