

Effect of vitamin E isoforms on the primary intention skin wound healing of diabetic rats

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ABSTRACT

Introduction: Impaired wound healing events is a common complication in diabetes. One of the effective nutritional antioxidant on skin wound healing is vitamin E which contains saturated tocopherol and unsaturated tocotrienol forms. This present study is designed to explore the effect of different vitamin E isoforms on stitched skin wound in both healthy and diabetic rats. **Materials and Methods:** Forty eight albino rats were divided into eight groups; healthy control, diabetic control, healthy treated (d- α -tocopherol, d- δ -TRF and co-administrated) and diabetic treated (d- α -tocopherol, d- δ -TRF and co-administrated). Diabetes was induced through single subcutaneous injection of alloxan at the dose of 100 mg/kg. Treated groups were administered d-a-tocopherol (200 mg/kg), d- δ -TRF (200 mg/kg) and co-administration (100 mg/kg of these two compounds each) orally and daily for three weeks. A horizontal skin incision was made on right mid-thigh region at 2.95 ± 0.17 cm in length and wound was closed with an absorbable suture. **Results:** Histopathological and histomorphological results at the end of 3rd week revealed that the d- δ -TRF treated groups promote the regeneration and reorganization of epidermal and dermal components in healing of primary intention more effectively than the d- α -tocopherol and co-administrated groups. **Conclusion:** It is concluded that among different vitamin E isoforms the d- δ -TRF appears to be a more effective nutritional antioxidant on skin wound healing in both healthy and diabetics.

Key words: Antioxidant; d-a-tocopherol; d- δ -TRF; Diabetes; Rats; Skin; Wound

INTRODUCTION

In diabetes the free radicals impair the normal wound healing by damaging keratinocyte, endothelial cells, capillary permeability and collagen metabolism [1]. Oxidative stress induces cellular dysfunction and retards angiogenesis and the healing process [2]. Thus, elimination of reactive oxygen species (ROS) is an important strategy to improve the healing of wounds in diabetes mellitus patients [3].

The unsaturated tocotrienol forms of vitamin E are more potent antioxidants [4] and suppress ROS production more efficiently than most active saturated tocopherol forms [5,6]. Each isoforms of vitamin E have different biological activities towards free radicals [7]. In addition tocotrienol possess antidiabetic

and anticancer properties as well [8]. Interestingly, the antitumor activity of tocotrienols is not dependent on its antioxidant activity [9,10]. The highly biopotent γ and δ - tocotrienols may play a physiological role in modulating normal cell growth, function and remodeling. These compounds inhibit tumor growth without harming normal tissues [11-14].

Sutures enhance wound closure and promote healing. Sutures initially provide the mechanical strength to seal the wound and protect it from pathogens [15]. Available studies [16,17] revealed that topically applied vitamin E does not help in improving the cosmetic appearance of scars or its failure to reduce postoperative scar formation. In a 10 days study [18] it has shown that topical tocopherol treatment enhances the rate of secondary skin wound closure in streptozotocin-induced diabetic rat.

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According to Zaini et al [19], the tocotrienol-rich fraction (TRF) treatment accelerate the wound contraction rate, enhance the reepithelialization, the regeneration process and stimulate the granulation tissue formation in deep partial-thickness burn wounds. In another study [20] it was revealed that the supplementation of TRF at 200 mg/kg was able to improve wound healing in type I induced diabetic rat.

Our previous studies [21-23] explain the effect of different vitamin E isoforms via single and co-administrations on secondary skin wound healing and from these studies it was concluded that the d- δ -TRF treated group showed comparatively faster recovery and regeneration of epidermal and dermal components than other treated and control groups.

At present all available data on vitamin E application on skin wound healing either topical application on primary intention of healing or oral administration on secondary intention of healing. To our knowledge, so far no information is available on oral administration of vitamin E isoforms on primary intention type of skin wound healing. Hence the present study was attempted to explore the effect of oral administration of different isoforms of vitamin E on the basis of histopathological characteristics and histomorphological measurements of incised skin wounds in both healthy and diabetic rats closed with absorbable suture in experimental surgery.

MATERIALS AND METHODS

Forty eight albino rats of either sex each weighing 230-320g was obtained from central animal house of JN medical college, AMU, Aligarh. The study has been approved by Institutional Animal Ethical Committee (No. 8937/2014).

This present study followed the same method as described in our previous studies [21,22] of animal care, induction of diabetes, monitoring of blood sugar level, surgical procedure, tissue sample collections and fixation.

Experimental Groups, Route and Dosage of Treatment

Total forty eight animals were divided into eight groups having six rats in each group: (1) healthy control- HC; (2) diabetic control- DC; (3) healthy d- α -tocopherol treated- HPT and (4) diabetic d- α -tocopherol treated- DPT; (5) healthy d- δ -TRF treated- HTT and

(6) diabetic d- δ -TRF treated – DTT; (7) healthy d- α -tocopherol and d- δ -TRF treated- HXT and (8) diabetic d- α -tocopherol and d- δ -TRF treated- DXT.

The d- α -tocopherol treated groups were received 200mg/kg d- α -Tocopherol (Myra e capsule [Vitamin E] manufactured by PT Daya- Baria laboratoria Tbk, Indonesia; Imported and packed by United laboratories, Inc, 66 United St, Philippines). The d- δ -TRF treated groups were received 200mg/kg d- δ -TRF (Unique E Tocotrienol, tocopherol free, 90% δ and 10% γ tocotrienols, AC Grace Company, P.O Box 570, Big Sandy, TX 75755, USA). The co-administrated groups were received 100mg/kg of d- α -tocopherol and d- δ -TRF each.

All treated groups were supplemented vitamin E isoforms (d- α -tocopherol and d- δ -TRF) daily for three weeks by oral administration.

Surgical Procedure

All animals received general anesthesia via inhalation of ether. Horizontal skin incision was made on the shaved right mid-thigh region at 2.95 ± 0.17 cm in length. Skin were closed with 3-0 Vicryl (2metric–NW2401) absorbable sterilized surgical needled suture USP (synthetic; braided coated polyglactin 910 violet; from Ethicon, manufactured in India by Johnson and Johnson Ltd, Aurangabad). Povidone-iodine solution (antisepsis) was applied on the wound and 0.5 ml Voveran (analgesic) and 2 mg single shot of Gentamycin (antibiotic) were also injected simultaneously.

Sample Collection and Fixation of Tissue

On completion of three weeks animals were sacrificed under deep ether anesthesia and then excised the healed parts of skin with adjacent area. The excised tissues were immersion-fixed in 10% neutral buffered formalin

Macroscopic Examination

The macroscopic changes in the wound healing stages were observed and recorded photographically on 1st, 7th, 14th & 21st day of creation of wounds.

Histopathology and Histomorphometry

Fixed tissue samples were processed for light microscopical studies. The 5m thick paraffin sections were stained with Haematoxylin & Eosin (H&E),

Masson's Trichrome (MT) and Aldehyde Fuchsin with Fast Green (AF with FG).

In histomorphometry the measurements of epidermal and neopidermal thickness were performed on the H & E and MT stained sections by using software Motic images plus version 2.0.

Statistical Analysis

Histomorphological measurements were statistically evaluated and the significance calculated by using one way 'ANOVA' followed by Tukeys test. All the results were expressed as mean \pm SD and $P < 0.05$ was considered as statistically significant.

RESULTS

Body Weight and Blood Sugar Level

Weight and blood sugar levels of all animals in each group were monitored at weekly intervals and results were reported in previous studies [21-23].

Macroscopic Observations

On 3rd week scar were not formed in any groups by morphological examination on stitched skin wounds and better wound healing was observed in all treated groups especially in d- δ -TRF treated group showed almost complete healing as compared to control groups (Fig. 1).

Microscopic Observations

Histomorphometry

In treated groups the mean values of neopidermis were significantly thicker ($P < 0.01$) than the corresponding epidermal border thickness which in the order of d- δ -TRF > co-administrated > d- α -tocopherol groups as compared to control groups (Fig. 2).

Reepithelialization

Complete reepithelialization was noticed in all groups (controls and treated). On 3rd weeks, all groups were showed interdigitations but well defined interdigitations at dermoepidermal junction appeared on the entire length of neopidermis were seen only in treated groups (Fig. 3).

Cellular components

On 3rd week more cellularity were observed in control groups as compared to all treated groups. Among

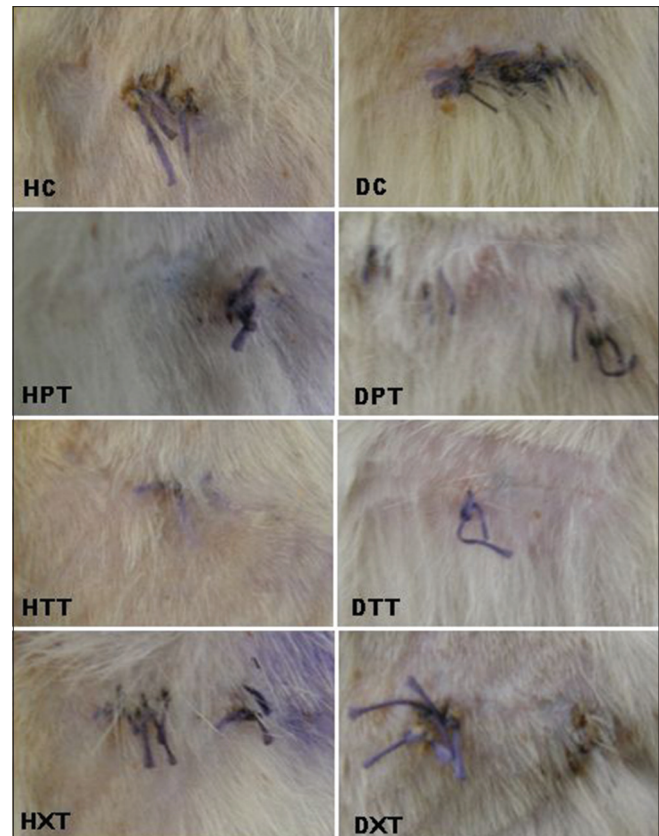


Figure 1: Photographs showing skin wounds of all groups on 3rd week. The d- δ -TRF treated group showed almost complete healing and all other treated groups have shown better healing as compared to control groups.

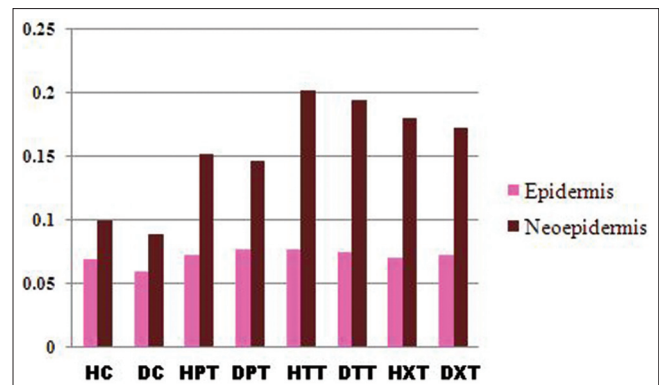


Figure 2: Border & neopidermal thickness (mm; Mean \pm SD) at the end of 3rd week. In all treated groups the mean values of neopidermis were significantly thicker ($P < 0.01$) than the corresponding epidermal border thickness as compared to control groups.

different treated groups reduced cellularity were seen in d- δ -TRF treated group than d- α -tocopherol and co-administrated treated groups (Fig. 4).

Neovascularization

Reduced vascularity was observed in all treated groups. In HC has shown more vertically oriented blood capillaries whereas in DC blood capillaries were swollen (Fig. 3).

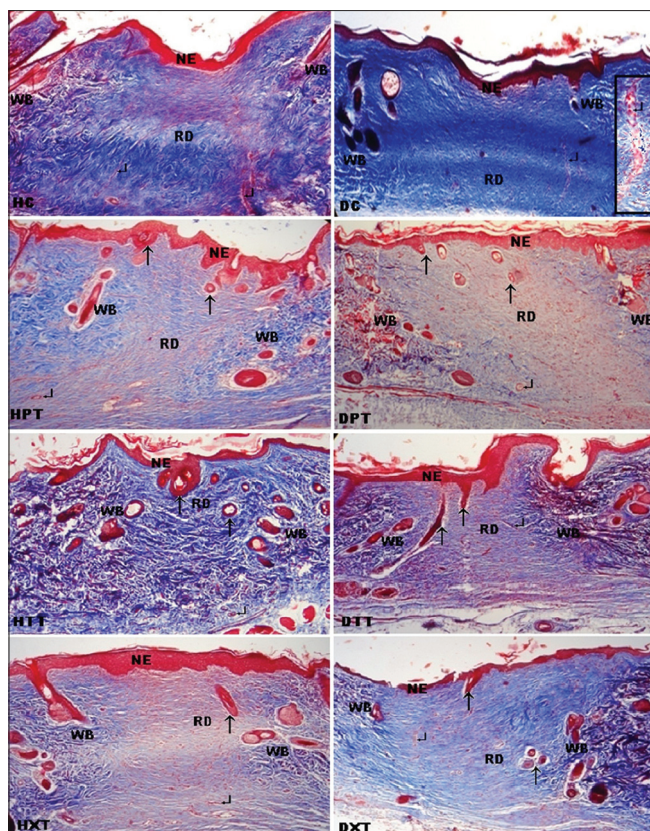


Figure 3: Representative images of MT stained sections on 3rd weeks, showing collagen fibres (Blue colour) in the regenerating dermis (RD), interdigitations, ↑: Hair follicles with hair and sebaceous glands, NE: Neopepidermis, WB: Wound Borders, ↓: Blood capillaries in all groups at initial magnification x100 and in DC inset ↓: Swollen blood capillary at initial magnification x400.

Matrix remodeling and skin appendages

On 3rd weeks, in all treated groups the collagen fibres in the regenerated dermis were mostly horizontally arranged and compactly interwoven especially in HTT most of the collagen fibres were matured but these fibres were more thickened which form fibrosis in control groups (Fig. 3).

Well defined elastin fibres were seen in the area of regenerating dermis and nearby this area in all treated groups and reappearance of these fibres in the order of d- δ -TRF > co-administrated > d- α -tocopherol groups. Whereas in control groups these fibres were seen only at wound margins (Fig. 5).

At the end of study period, more hair follicles with hairs and sebaceous glands were observed in regenerating dermis and neopepidermis of all treated groups which in the order of d- δ -TRF > d- α -tocopherol > co-administrated groups. But in control groups these features were restricted at the wound margins (Figs 3 and 4).

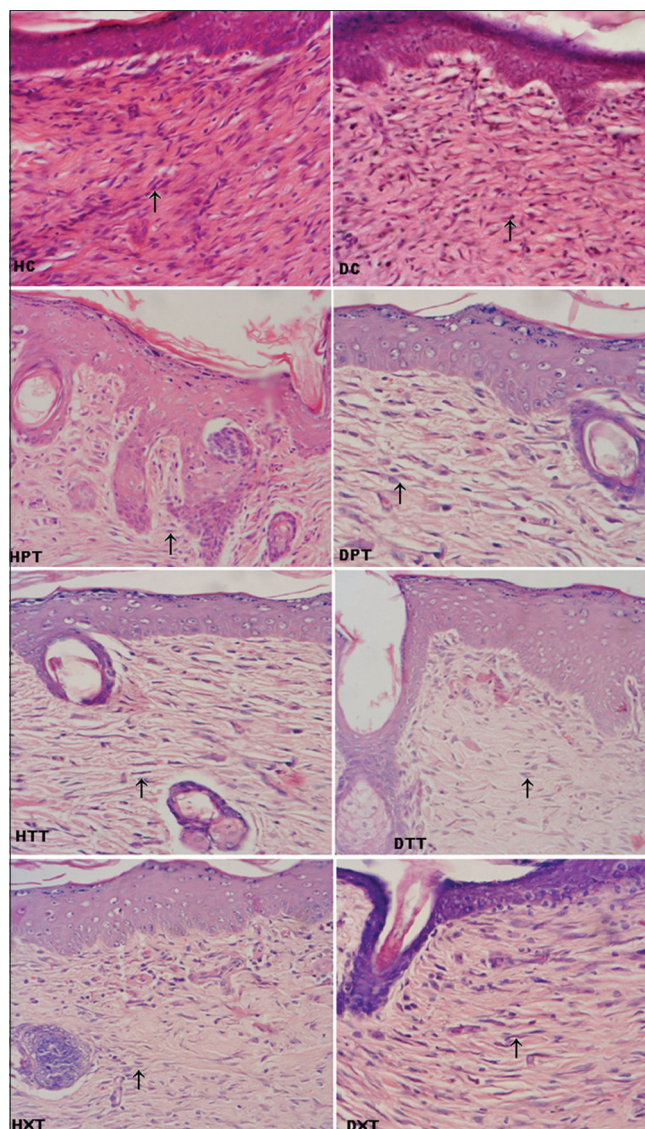


Figure 4: Representative images of H & E stained sections on 3rd weeks, ↑: Showing more cellularity in regenerating dermis of control groups than all treated groups and presence of hair follicles with hair and sebaceous glands in all treated groups at initial magnification x400.

DISCUSSION

Impeded wound healing is now a well-known phenomenon in both experimental and clinical diabetes [24]. Hyperglycemia is known to cause increased production of free radicals and insufficiencies in the antioxidant system [25]. The antioxidants have the ability to reduce the diabetic complications by arresting free radical-induced damage [26]. Vitamin E is a family of essential micronutrients [27] and its one of the most popular applications are in the treatment of burns, surgical scars and wounds [28].

Macroscopic examinations healing wounds on 3rd week revealed that scar was not formed in any

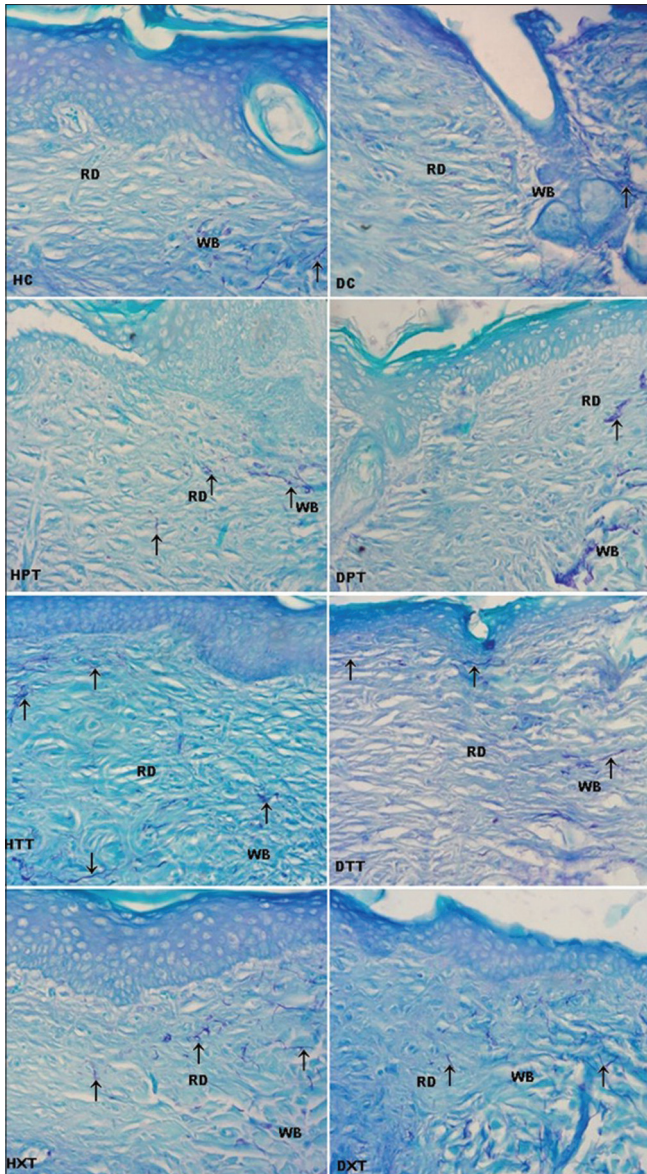


Figure 5: Representative images of AF with FG stained sections on 3rd weeks, showing elastin fibres arrangement. Arrows (→) pointing the presence of elastin fibres (violet colour) WB: wound borders; RD: regenerating dermis, initial magnification x400.

groups. However, a better and faster wound healing was observed in d- δ -TRF treated group followed by d- α -tocopherol treated and co-administrated groups as compared to control groups.

Good marker for superficial changes in the wound is the epidermal thickness [29]. The mean values of histomorphological measurement in the present study showed that on 3rd week in d- δ -TRF group the neoepidermal thickness was remarkably higher than the corresponding border epidermal thickness compared to all other groups. But in co-administrated groups it

was thicker than the respective border epidermis of d- α -tocopherol and control groups.

The histopathological observations have shown complete reepithelialization and interdigitations in all groups. But well defined interdigitations on the entire length of neoepidermis appeared only in treated groups. The interdigitations at dermoepidermal junction are known to provide both physical and trophic support. Therefore, the neoepidermis in treated groups has obviously more capacity to resist the possibility of desquamations [21-23].

Presence of more fibroblasts in the granulation tissues is an indicator of dermal regeneration [24] but reduced cellularity indicates that the dermal components are in advanced stages of remodeling [30]. On 3rd weeks cellular components were more in control groups whereas these features were reduced in d- δ -TRF group than d- α -tocopherol and co-administrated groups. These results suggest that the dermal regeneration process was slow in control groups but the three weeks d- δ -TRF supplementation boost the early dermal regeneration as compared to d- α -tocopherol supplementation and co-administration.

Well-structured capillary vessels with absence of hemorrhage are the characteristic feature of neovascularization [24]. More vertically oriented capillary vessels that run towards the epithelial surface were seen in HC whereas in DC blood capillaries were swollen. Reduced vascularity in the reparative tissue is an indicator of dermal remodeling [30]. This finding is supported by the present study as it indicated that the numbers of blood capillary vessels were reduced in all treated groups. These results were also in agreement with our previous studies [21-23].

The collagen fibres are mainly found in the papillary and reticular layers of the dermis and they provide both mechanical and structural integrity to the dermis [31]. At the end of study period, in both control groups the collagen fibres were thicker in the scar tissue. In all treated groups collagen fibres were horizontally placed and compactly interwoven and in addition to these features in HTT most of the collagen fibres were mature, a feature similar to our previous studies [21-23] on secondary skin wound healing. The horizontal alignment of collagen fibres suggests a better tissue remodeling [32].

Tough the elastin is a minor component of the dermis it has an important function in providing the elasticity of the skin [33]. Well defined elastin fibres were seen in the regenerating dermis and its nearby area in all treated groups, plenty of these fibres reappeared in d- δ -TRF treated group followed by co-administrated group compared to d- α -tocopherol treated group. But in control groups these fibres were observed only at wound margins. Same observations were found in our previous studies [21-23] of secondary skin wound healing. Presence of elastin fibres in the healing wound indicates final stages of matrix remodeling [34].

Presence of epidermal appendages such as hair follicles and sebaceous glands in the regenerating dermis and neoepidermis indicate the faster healing and quicker remodeling of wound matrix [32]. At the end of study period, more hair follicles with hairs and sebaceous glands were observed in all treated groups whereas these features were restricted at wound margins in control groups. This study is in agreement with our previous studies [21-23] that among different isoforms of vitamin E, the d- δ -TRF has potency to accelerate the matrix remodeling more effectively than d- α -tocopherol and co-administration of both these isoforms. In matrix remodeling the d- α -tocopherol treated group had shown better results than co-administrated groups.

CONCLUSION

Based on histopathological and histomorphological results it is concluded that the d- δ -TRF accelerates the regeneration and reorganization of epidermal and dermal components in healing of primary intention more effectively than the d- α -tocopherol and co-administration. Therefore among these different vitamin E isoforms the oral administration of d- δ -TRF is a most potent nutritional adjuvant on incisional skin wound healing in both healthy and diabetics.

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