Palm Tocotrienol: A Good Antioxidant for Skin Wound Healing

Zaizuhana Shahrim*, Zaliha Omar*, Zaida Zainal* and Zafarizal Aldrin Azizul Hasan*

INTRODUCTION

Skin, as the largest organ of the body, acts as a barrier against the surroundings. It plays an important protective as well as reparative function in the body. When an injury occurs, either acute or chronic, our body automatically sets in motion dynamic and multiple steps of processes at the injured site (Akbik *et al.*, 2014) as illustrated in *Figure 1*. Wound repair takes an intricate course, but there is distinctively concerted interaction among inflammatory cells and related growth, forming an important coordination of the intricate phases of wound repair (Öztürk and Ermertcan, 2011). Over the years, the stages of wound repair have been well-documented.

IMPORTANT ASPECTS OF SKIN WOUND HEALING

Over the years, there is now a better understanding of skin wound healing and this multifaceted process in wound repair has been simplified into three major phases, namely: (i) Inflammatory, (ii) Proliferative (re-epithelialisation, angiogenesis and granulation), and (iii) Remodelling of the extracellular matrix (ECM) (Abraham *et al.*, 2000; Schreml *et al.*, 2010).

* Malaysian Palm Oil Board (MPOB)
6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.
E-mail: zaizuhana@mpob.gov.my

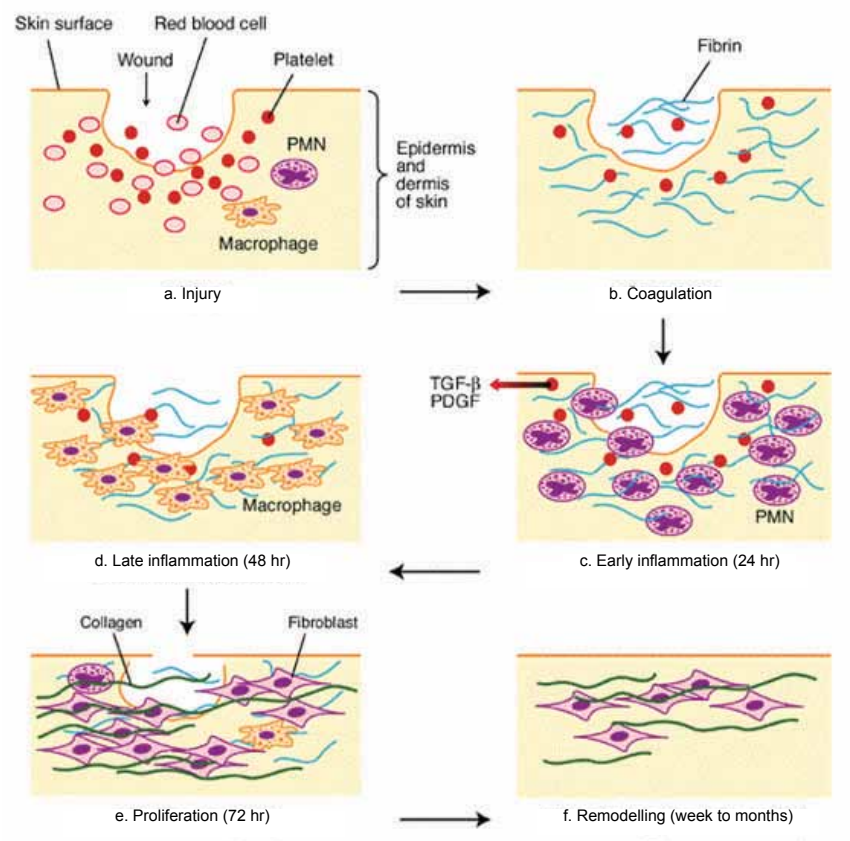
Inflammatory phase

Whether it is injury to the skin or any other type of tissue injuries, the consequence is an immediate onset of what we called acute inflammation (Broughton *et al*., 2006), which is a local protective reaction to various stresses (Pastore *et al.*, 2011) that is the most important phase in wound healing. It is one of the earliest events that determine the fate and quality of wound repair. It is controlled by various inflammatory signals, whether to initiate, maintain or resolve the degree of inflammation (Gál *et al.*, 2009; Roy *et al.*, 2011).

Proliferative phase

One of the primary inflammatory responses to skin lesions is to support subsequent regeneration of new functional tissue (Falanga, 2000; Atiyeh and Hayek, 2005). In the proliferative phase, cellular activity predominates. There are three main actions involved: (i) Establishment of a permeable barrier (*e.g.* re-epithelialisation), (ii) Production of sufficient blood supply (*e.g.* angiogenesis), and (iii) Strengthening of the injured skin tissue (*e.g*. granulation) (Granick *et al*., 2006; Fonder *et al*., 2008; Korting *et al.*, 2010).

Basically re-epithelialisation consists of rebuilding an epidermis from the adjacent cells after the skin is injured. It involves several steps which include the movement of epidermal cells (keratinocytes) into the wound area. The propagation of these cells is essentially useful for growth and progression as well as migration of epithelial formation. Then, the new epithelium differentiates into a stratified epidermis, finally forming a basement membrane



Source: Beanes *et al.,* (2003).

Figure 1. Phases of wound healing.

zone that joins both epidermis and the underlying dermis (Pierce *et al*., 1992; Stallmeyer *et al*., 1999; Wiechula, 2003; Li *et al*., 2007).

Angiogenesis is another main stage in wound repair (Stojadinovic *et al*., 2008; Öztürk and Ermertcan, 2011). Angiogenesis is defined as the formation of new blood vessels from the existing ones that are adjacent to or neighbouring the injured site. In normal adult tissues, cutaneous blood vessels are inactive. However, when injury occurs, microvascular endothelial cells start the angiogenic process that involves activation of these cells into growing or penetrating into the wound, proliferation, forming a tubule structure and repairing the injured membrane. Eventually, a newly formed vasculature is produced as the tissue begins to remodel (Sporn *et al*., 1987; Herlyn and Malkowicz, 1991; Gailit *et al*., 1996; Nussler and Billiar, 2000; Shivhare *et al*., 2010). The main functions of these newly formed blood vessels are to provide nutrition as well as sufficient oxygen to the growing tissue. Cytokines are also secreted out by the endothelial cells during the process, which further facilitates the healing episodes.

Granulation in normal healing usually starts to restructure 3 to 4 days after injury. This is characterised by the formation of new and growing skin tissue that consists of 60% of the blood vessels from the process of angiogenesis. Furthermore, the build-up of fibroblast cells to form temporary extra-cellular matrices, which are rich in fibronectin, act as a platform for the cells to migrate into the wound area and become the substitute for the wounded skin (Granick *et al*., 2006; Singer and Dagum, 2008).

Remodelling phase

The tissue remodelling phase can last up to two years. It continues to develop the healing process from the formation of a fibrin clot in the early inflammatory stages until the tissue is replaced by the granulation tissue that is rich in Type III collagen and blood vessel formation. Subsequently, the granulation tissue will also be replaced by a collagenous scar, predominantly of Type 1 collagen (Witte and Barbul, 1997). The synthesis of this collagen continues until it reaches maturity. The phase may proceed over many months.

ANTIOXIDANT THERAPY IN SKIN WOUND HEALING

Oxidative stress has been reported to reduce the enzymatic antioxidant activities and diminution of non-enzymatic antioxidants in acute or chronic wounds (Schultz *et al*., 2000; Visse and Nagase, 2003; Lawrence and Diegelmann, 2014). It has been observed that oxidative stress is more pronounced in chronic rather than acute wounds (Shetty *et al*., 2008). Administration of wounds with antioxidants would be a rational way to counter oxidative damage of tissues and to enhance healing (Medina and Radomski, 2006).

A study done by Atiyeh and Hayek in 2005 shows that Raxofelast (hydrophilic vitamin E analog) reduces oxidative stress

by decreasing lipid peroxidation as well as edema in diabetic wounds. It also manages to stimulate reepithelisation, neovascularisation, and propagation of fibroblasts, synthesis and development of the extra-cellular matrix needed in wound healing (Atiyeh and Hayek, 2005). This article also suggests that antioxidants may protect the diabetic wound cells from oxidative stress produced by high glucose levels (Marx *et al*., 1999). According to Roy *et al*. (2011), under high glucose levels, fibroblasts show significant reduction in their ability to contract and resistance in producing the growth factor (important in inducing proliferation). High glucose levels also induce apoptosis in keratinocytes (Marx *et al*., 1999). Glutathione (an antioxidant) is also shown to restore the capability of fibroblasts to contract and furthermore protects keratinocytes from apoptosis. Other types of antioxidants such as ascorbic acid, selenite, vitamin E, carotenoids and Q10 are found to reverse the resistance of fibroblast in secreting growth factors arising from high glucose levels (Hehenberger and Hansson, 1997). A combination of vitamin E, sodium pyruvate and fatty acids is shown to enhance wound repair after laser surgery. Unsaturated fatty acids act as a replacement source for damaged membrane fatty acids while sodium pyruvate and vitamin E act as antioxidants (Gailit *et al*., 1996).

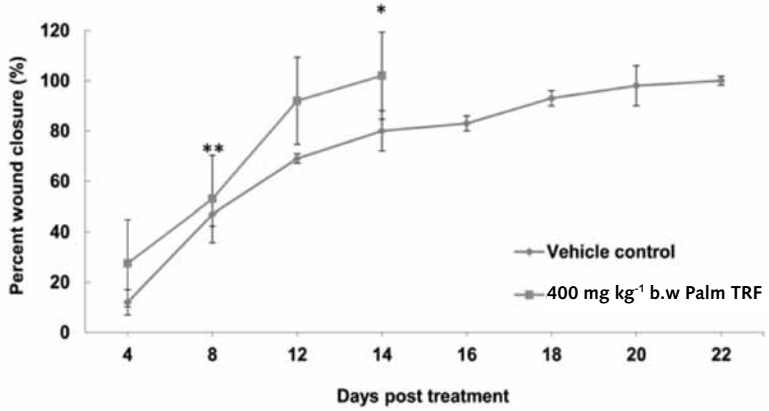
PALM TOCOTRIENOL FOR SKIN WOUND HEALING

It has always been the quest of researchers and clinicians to search for safe, efficient and zero downside of a healing agent. Thus, the emergence of natural products as therapeutic agents for skin wound healing has been seen as the way forward. It is a well-known fact that vitamin E is a potent antioxidant, providing the ability to reduce the occurrence and severity of any pathological event in the skin (Johnston *et al*., 2011).

The tocotrienol-rich fraction (TRF) is a natural, non-toxic antioxidant compound derived from palm oil. It is a 'cocktail' of tocotrienol fractions and alpha-tocopherol. These two known vitamin E forms (tocotrienols and tocopherol) are closely related chemically, but have varying degrees of biological activities. They can be further separated into four homologues, α-, β-, γ- and δ (Liu *et al*., 2012). It is well documented that when compared with tocopherol, tocotrienol is a much more powerful antioxidant.

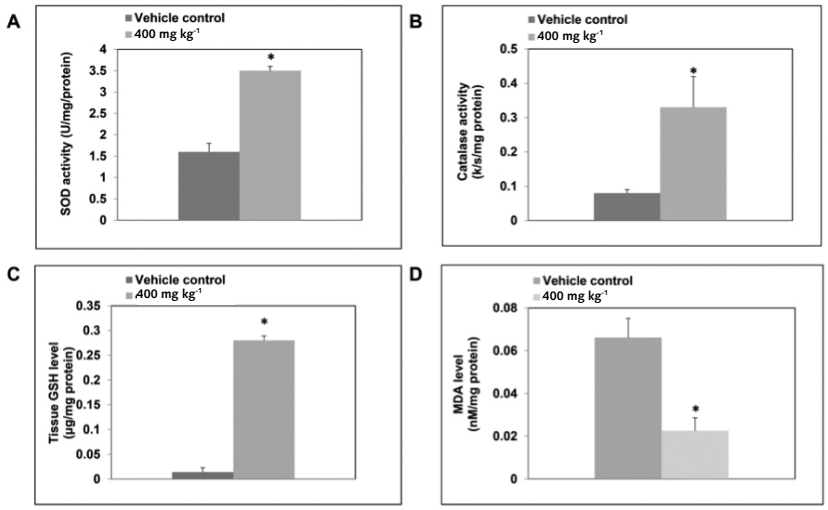
In relation to wound healing, palm tocotrienol is also known to directly benefit tissue repair and regeneration, and indirectly benefit the immune function. A study done by Musalmah and co-workers in 2005 showed that 200 mg kg^{-1} body weight of palm TRF provided as a supplement to streptozotocin-induced diabetic rats enhanced cutaneous healing and increased free radical-scavenging enzyme activities more than the same amount of alpha-tocopherol. Furthermore, in an excision wound experiment that we conducted (unpublished data), it was found that 400 mg kg^{-1} body weight of palm TRF accelerated wound closure in normal rats compared with the vehicle control (refined bleached and deodorised (RBD)-stripped vitamin E) group. Wound closure was nearly 100% after 16 days of administration of palm TRF whereas the vehicle control took another eight days (*Figure 2*).

We also examined the antioxidant status of the wound and our findings revealed that superoxidase dismutase (SOD) activity in the wound tissues was increased in rats treated with palm TRF compared with rats with the vehicle control (*Figure 3a*). Catalase (*Figure 3b*) and glutathione (GSH) levels in the tissues were significantly increased in rats treated with palm TRF compared with the ones with the vehicle control (*Figure 3c*). However, malondialdehyde (MDA) level was significantly decreased in the palm TRF-treated group compared with the control (*Figure 3d*).



Note: *p<0.001 *vs.* vehicle control; **p<0.05 versus *vs.* control

Figure 2. Effect of palm TRF on an excision wound model.



Note: *p<0.001 *vs.* vehicle control. Values are mean + SD of six replications.

Figure 3. Effects of palm TRF on antioxidant parameters: (a) SOD activity; (b) Catalase activity in newly formed tissue; (c) Decreased GSH level in newly formed tissue; (d) Lipid peroxidation-MDA level in wound tissue.

Oxidative stress, a consequence of overproduction of reactive oxygen species (ROS), definitely causes cytotoxicity to the cells and thus slows down the healing process (Manoj and Murugan, 2012). Reduced ROS has become the objective in wound repair especially for chronic lesions (Dissemond *et al*., 2002). Hence, antioxidants such as SOD, catalase and glutathione are significant as they speed up the process by abolishing the free radicals. They are known to extinguish the superoxide radical and thus prevent the damage of cells caused by free radicals (Korting *et al*., 2010). According to Shivhare *et al*. (2010), a significant change in the antioxidant profile with elevated levels of malondialdehyde contributed to delayed wound healing in immunocompromised rats. Here, we have shown that rats fed with palm TRF at a dose of 400 mg kg^{-1} body weight had increased activity of SOD, catalase as well as GSH; however, this dose decreased the MDA level compared with the vehicle control.

CONCLUSION

Palm tocotrienol is a good antioxidant. Palm TRF accelerates skin wound healing and can be potentially used as a therapeutic agent for wound repair. It provides protection against the burst of free radicals in the wound site as well as enhances the acceleration of the wound healing process. Palm TRF may also provide benefits in regulating abnormal or impaired skin wound healing.

ACKNOWLEDGEMENT

The authors wish to thank the Director-General, MPOB for permission to publish this article.

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