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FULL PAPER

Wound healing potential of palm oil tocotrienols rich fraction

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Abstract

Diabetic patients often experience problems with their immune system activation and result in delayed wound healing. Slow and incomplete wound healing increases the risk of complications caused by infected wounds. Metformin has been used as a standard drug for diabetes treatment and it accelerates wound healing. However, intake of metformin may cause gastrointestinal symptoms including diarrhoea, nausea and abdominal discomfort. Therefore, a safe alternative to metforminis is required. While many research programs focus on *alpha*-tocopherol, in this paper the potency of tocotrienols in wound and diabetes management was investigated. Tocotrienol rich fraction (TRF) was tested for its ability to stabilize blood glucose, reduce lipid peroxidation, promote platelet-derived growth factor-BB and wound closure. In this study, the rodent model was used to investigate the effects of TRP in wound healing proficiency. The results showed that TRF was comparable to metformin in stabilizing blood glucose, promoting PDGF-BB in the blood during the initial wound healing stage and produced clean wound closure. Interestingly, the findings of this study showed TRF had higher potency than metformin in reducing lipid peroxidation that could delay wound healing. Hence, TRF could be a good alternative to metformin in wound and diabetes management.

1. Introduction

Wound healing is a series of well-orchestrated integrations and complex biological events. It requires four overlapping phases, which includes coagulation of white blood cells, inflammation, migration-proliferation of cells and tissue remodelling to complete the healing process (Tottoli *et al.*, 2020). The ideal wound healing is rapid and complete without infection and sepsis. In diabetic patients, the risk of incomplete or uncoordinated wound healing is high (Patel *et al.,* 2019). More recent evidence shows that diabetic patients are likely to experience disruption in haemostasis (Nurden *et al.,* 2008; Liu *et al.,* 2017), causing prolonged inflammation response (Schürmann *et al*., 2014), changes in growth factors and chemokines (Ochoa *et al.*, 2007), alteration in proliferation, granulation, angiogenesis (Altavilla *et al.*, 2001; Guo *et al.,* 2020; Okonkwo *et al.*, 2020) microcirculation (Lioupis, 2005) employment of macrophages, neutrophils and vasoconstriction (Goren *et al.*, 2009; Mirza *et al.*, 2009; Lin *et al.*, 2018) and oxidative stress (Johansen *et al*., 2005; Xu *et al*., 2020). These are among the factors delaying and impairing wound healing among diabetic patients and increase the potential of microbial infection with the chronic wound (Dong *et al.*, 2020; Xu *et al.*, 2020).

Metformin has been used as a standard drug for diabetes treatment. It was observed that treatment with metformin accelerated wound healing through modulation of wound repair mechanism (Inouye *et al.*, 2014; Yu *et al.*, 2016; Han *et al*., 2017; Qing *et al.*, 2019). However, intake of metformin may cause gastrointestinal symptoms including diarrhoea, nausea and abdominal discomfort, anorexia (Bailey and Turner, 1996). Not common but a high mortality rate of lactic

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acidosis or metformin-associated lactic acidosis (MALA) when an overdose of metformin is taken among patients with renal impairment (DeFronzo *et al.*, 2016; Blumenberg *et al.,* 2020). Metformin is also known to be a potential genotoxin (Amador *et al.*, 2012), embryotoxin (Li *et al.,* 2015), thus it should be avoided as a treatment for the pregnant women. Therefore, a safe alternative is required where Tocotrienols Tich Fraction (TRF) acts as a natural phytonutrient that can be extracted from crude palm oil. Tocotrienols have been gaining attention in the last two decades and are regarded as super vitamin E that promotes cardiovascular health, anti-cancer, immune modulation, neuro-protection, aid for cognitive function, skin protection and other clinical effects like anti-oxidant, anti-inflammatory properties (Meganathan and Fu, 2016).

In this study, TRF is proposed to have the potential in promoting wound healing among diabetic patients. Based on past research, TRF could improve body glucose utilization and insulin sensitivity (Fang *et al.,* 2010), effectively decrease blood glucose and glycated haemoglobin (Wan Nazaimoon and Khalid, 2002), improve dyslipidaemia while maintaining vessel wall integrity (Budin *et al.,* 2009), reverse neuropathic pain (Kuhad and Chopra, 2009), prevent cognitive deficits (Kuhad *et al.*, 2009) in diabetic animals, improve the glycaemic status and renal function (Siddiqui *et al.,* 2010) and prevent hyperglycaemia induced skeletal muscle atrophy associated with diabetes (Lee and Lim, 2018).

A few earlier studies showed the potential of TRF in diabetic wound healing through elevating antioxidants enzymes (Musalmah *et al*., 2005) improved glycaemia status and prevent DNA damage (Matough *et al.,* 2014), promote early regeneration of both epidermal and dermal components (Elsy *et al.,* 2017) and increase expression of genes (Xu *et al.,* 2017). However, it is still lack of investigation on the role of TRF in the modulation of the growth factors in wound healing.

In this paper, the potency of TRF in diabetic wound treatment is compared to the standard first-line drug-metformin using rat model. The antibacterial property in TRF was tested on infections in wounds and found that it increases the time of healing and reduce the quality of the wound and causes scarring. We also evaluated the potential of TRF in modulating wound contraction, regulation of PDGF-BB growth factor, managing oxidative stress by detecting its by-product malondialdehyde (MDA), as well as blood glucose control and bodyweight of the diabetic animal model.

2. Materials and methods

2.1 Anti-microbial test

The antibacterial property of the oil palm tocotrienols rich fraction was assessed based on the disk-diffusion method (Hudzicki, 2009; Chand, 2020). Several Gram-positive bacteria such as *Staphylococcus aureus*, *Listeria innocua* and Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica* serovar Typhi were included in the test. The bacterial stock cultures were obtained from the Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia Campus Bintulu. Bacteria cultures were first spread on an agar plates and labelled accordingly. Then, 6 mm paper discs (Whatman no.1) with different concentrations of TRF were aseptically transferred onto the agar plate and incubated at 37^{o}C for 24 hrs. A positive control (standard drug) and negative control (diluent- absolute ethanol) were included in the test. The diameter of the inhibition zone was measured to describe the antimicrobial potency of TRF.

2.2 Wound healing test on diabetics rats

The wound healing test was performed on diabetic rats after obtaining the approval of Institutional Animal Care and Use Committees, University Putra Malaysia (UPM/IACUC/AUP-R048/2019). Male Sprague Dawley (SD) rats of 300-400 g, supplied by the UPM Animal Resource Unit, Faculty Veterinary Medicine were used in this study. The rats were acclimatized for 14 days, kept in an open cage system with standard food (Altromin) and reverse osmosis (RO) water *ad libitum* and 12 hrs light cycles at temperature-regulated conditions at Comparative Medicine and Technology Unit (COMeT), UPM.

For diabetic induction, after 8 hrs of fasting, 65 mg kg^{-1} of streptozotoxin (STZ) (Sigma, St Louis, MO, USA) in 50 mM Sodium Citrate Buffer (Merck, Darmstadt, Germany), pH 4.5 was injected intraperitonial *(i.p.)* using a modified method (Furman, 2015). The rats had access to 10% sucrose (Merck, 8515) water on the day after induction and resumed RO water on the subsequent day. Feed was made available *ad libitum* all time during the experiment.

All SD rats proceeded for a blood glucose test after 8 hrs of fasting on the 4th day. Blood glucose level was measured using Glucometer (Accu-Chek Performa Blood Glucose Meter). Blood pricked at the tail end using a sterilized lancet. Eight rats with blood glucose levels more than > 8.0 mmol/L were selected and randomly assigned to 2 treatment groups.

2.3 Wound creation

On the same day after the blood glucose check, the diabetic rats were anaesthetized by ketamine (50 mg/kg) and xylazine (5 mg/kg) i.p and proceeded for wound induction procedures, as per modified method (Moreira *et al.*, 2015). The dorsal fur was shaved and sterilized and two 6 mm full-thickness excisions were made using sterile 6 mm punch biopsies. The rats were separated into individual open cage systems after wound induction.

2.4 Tocotrienols rich fraction and metformin treatment via oral gavage

Metformin (Merck, Dramstadt, Germany) was fed to the diabetic rats in Treatment 1 as the positive control. Oil palm Tocotrienols Rich Fraction (TRF) supplied by SOP Green Energy Sdn. Bhd. was administrated to the diabetic rats in Treatment 2. The TRF used were of 50% concentration strength verified by the supplier and contained less than 25% α- tocopherol and more than 75% full spectrum of tocotrienols (consist of α, β, γ and δ - Tocotrienols). The HPLC analysis of the TRF is shown in Figure 1. Mass of 30 mg kg^{-1} metformin or 400 mg/kg of TRF were given once daily, from day 0 until day 10. The dosages were prepared using a sterilized syringe. Rats were restrained by holding the loose skin behind the ears and feeding using a curved metal cannula (Krinke, 2000) by oral gavage.

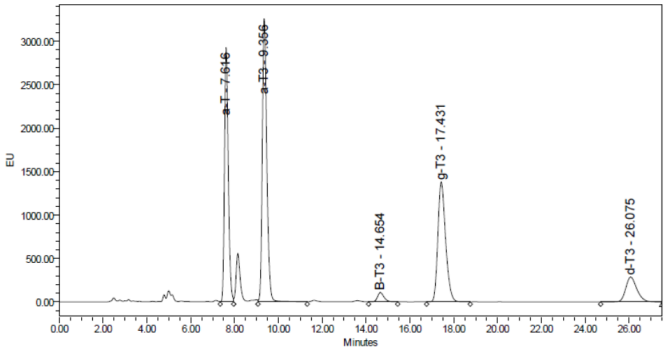


Figure 1. The HPLC chromatogram analysis of TRF supplied by SOP Green Energy

2.5 Monitoring bodyweight and blood glucose level

The bodyweight of rats was weighed before STZ induction at day-4 and after wound creation throughout the study period. The rat was put in a weighing bowl for bodyweight measurement using benchtop balance (AND, Fx1200 i). The blood glucose of the rat was measured on days 0, 2, 6 and 10, using test strips (Roche Accu-Chek Instant, 07819374).

2.6 Wound contraction

The wounded area was recorded on Day 0 immediately after wound creation and at day 2, day 6 and day 10. The areas of the wounds were measured after blood sampling. A transparent plastic sheet was pressed on the excisions sites to record the actual wound size. The area of the wound size was interpreted using the graph paper of 2×2 mm smallest scale.

The closure was expressed as a percentage of reduced wound area over the original wound area. The area of the epidermal tongue was used to assess the rate of wound contraction during healing time (Wall *et al.,* 2002). Wounds were considered closed if moist granulation tissue was no longer apparent and the wound appeared to be covered with epithelium (Greenhalgh *et al.*, 1990). Wound contraction was calculated based on the following formula:

Wound contraction (%) = (original wound area - current wound area)/(original wound area)×100

2.7 Blood sampling

Blood samplings were done on each rat from their tail vein using a sterile needle and sterile syringe (Terumo) on day 0 and day 2 with needle 25 G × 1" and using Cardiac puncture method on day 10 (Donovan and Brown, 2006). The experimental animals were anaesthetized *i.p.* by ketamine (50 mg/kg) and xylazine (5 mg/kg) while maintaining the temperature around 24° C to 27°C. If the vein was not visible, the tail was dipped into warm water (40°C). The tail was not rubbed from the base to the tip as it will result in leukocytosis. About 0.6- 1.0 mL per withdrawal of blood sample was collected according to IACUC guideline. Samplings were limited to Day 0, Day 2 and Day 10 throughout the 10 days observation to reduce stress to the experimental rats. This limitation inhibited the observation trend of platelet-derived growth factor-BB daily release pattern throughout the 10 days of observation, however, it was necessary because stress could affect the outcome of the study. Blood glucose levels were tested immediately and the balance of blood was kept in the centrifugal vial in a cooler box for the blood serum process on the same day.

2.8 Blood serum preparation; platelet-derived growth factor-BB and malondialdehyde testing

Blood serums were collected from centrifugation at 1,000×*g* for 15 mins at 4°C after allowing blood to stand at below 25°C for 2 hrs.

The blood serums were stored at -80°C in the freezer until the testing date. The serum was used for plasma-derived growth factor-BB, Platelet-derived growth factor -BB (E-EL-R0537-Elabscience) and Malondialdehyde, MDA (E-EL-0060-Elabscience) content testing using Elisa kit– based on manufacturer's procedure (Elabscience, Texas, USA).

Table 1. The Inhibitory zone of tocotrienols rich fraction (TRF) in the antimicrobial assay.

Bacteria	*Listeria innocua*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Salmonella enterica* serovar Typhi	Remarks
0.5% Ethanol	-	7 mm	7 mm	7 mm	-	Negative Control
30 mg/L	-	7 mm	7 mm	7 mm	-	Concentration 1
48 mg/L	-	7 mm	7 mm	7 mm	-	Concentration 2
105 mg/L	-	7 mm	7 mm	7 mm	-	Concentration 3
Standard Drug	29 mm (Meropenem 10 µg)	38 mm (Ciprofloxacin 1 µg)	35 mm (Meropenem 10 µg)	40 mm (Ampicillin 10 µg)	30 mm (Meropenem 10 µg)	Positive Control

2.9 Statistical analysis

Statistical analyses were conducted using SAS one way ANOVA followed by Tukey's test. *P* values below 0.05 were considered significant.

3. Results

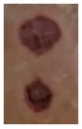
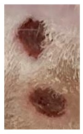
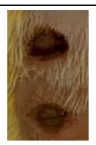
3.1 Antimicrobial assay

From the antimicrobial test result using the disc diffusion method (Table 1), it shows that oil palm tocotrienols rich fraction (TRF) at concentration 30 mg/L to 105 mg/L had no antimicrobial activity against *S. aureus*, *L. innocua*, *E. coli*, *P. aeruginosa* and *S. enterica* ser, Typhi.

3.2 Wound contraction

Wound closure was monitored for 10 days (Table 2). Rats fed with either metformin or tocotrienols rich fraction performed equally, with no significant difference in the percentage of wound contraction. The rate of wound contraction progressed steadily at Day 2 with 39.2±1.4% for metformin treatment and 33.3±0% for TRF treatment, at Day 6 at 56.5±6.3% for metformin treatment and 59.3±6.4% for TRF treatment and Day 10 at 100±0% for metformin treatment and TRF. No worsening of wound condition or abnormal observation at wound area was observed in both treatment groups. The wound with 100% contracted at Day 10 was observed and the wound was covered with a layer of epithelium without moist granulation.

Table 2. Wound appearance and wound contraction percentage on Day 0, 2, 6 and 10

Treatment/ Wound	Wound Size, mm^2			
	Day 0	Day 2	Day 6	Day 10
T01- Metformin				
	0.0±0.0%	19.6±18.3%	50.4±19.8%	89.8±13.6%
T02- TRF				
	0.0±0.0%	18.3±14.7%	47.1±18.9%	97.0±5.5%

Due to the scab formation, the wound recovery rate on Day 2 and Day 6 varied slightly as shown in Table 2. However, the wound recovery rates on Day 10 were compared after the removal of the scab. The wound closure for rats treated with TRF appeared to be more neat and complete.

3.3 Platelet-derived growth factor-BB (PDGF-BB) Content

The blood serum of the rats in the experiment was extracted on Day 0, Day 2 and Day 10. From the result shown in Table 3, the PDGF-BB increased on Day 2 after wound incision. Assessment based on Day 2 and Day 10 showed no significant differences between treatments for PDGF-BB content.

Table 3. Platelet derived growth factor-BB in experimental rats' blood serum on Day 0 and Day 10

Treatment	Day 0, pg/mL	Day 2, pg/mL	Day 10, pg/mL
T0- Metformin	2223±113[a]	2590±298[a]	2430±266[a]
T1- TRF	2095±129[a]	2590±348[a]	2508±372[a]

Values are presented as mean±SD. Values with the same superscript within the same column are not significantly different according to Tukey Test (p = 0.05).

3.4 Malondialdehyde (MDA) content

Elisa assay showed both Metformin and TRF treated group showed no significant difference in their malondialdehyde (MDA) content in blood serum on Day 0 (Table 4). However, on Day 10, the MDA concentration in TRF treated group showed MDA concentration dropped, significantly lower than the metformin group ($P < 0.05$). The MDA started at 237 ng/mL at Day 0 and reduced to 198 ng/mL at Day 10 for TRF treated group. While Metformin treated group, MDA tested 228 mg/mL before treatment roughly maintained at Day 10.

Table 4. Malondialdehyde (MDA) in experimental rats' blood serum on Day 0 and Day 10

Treatment	Day 0, ng/mL	Day 10, ng/mL
T0- Metformin	228±25[a]	222±37[a]
T1- TRF	237±20[a]	198±17[b]

Values are presented as mean±SD. Values with the same superscript within the same column are not significantly different according to Tukey Test (p = 0.05).

3.5 Blood glucose and bodyweight

The blood glucose and bodyweight of the experimental rats were monitored for 10 days after wound incision (Figure 2 and Figure 3). There is no significant difference between both treatments groups in their blood glucose level (Figure 2). Both experiencing reduction in blood glucose 6 days after metformin or TRF treatment. On Day 10, both TRF and metformin treated group had reached 25-26 mmol/L of blood glucose.

Bodyweights of both treatment groups decreased after STZ injection, then the decrease in weight ceased and was maintained from Day 1 onwards. The overall bodyweight among rats treated with TRF was slightly lower than that in metformin-treated rats.

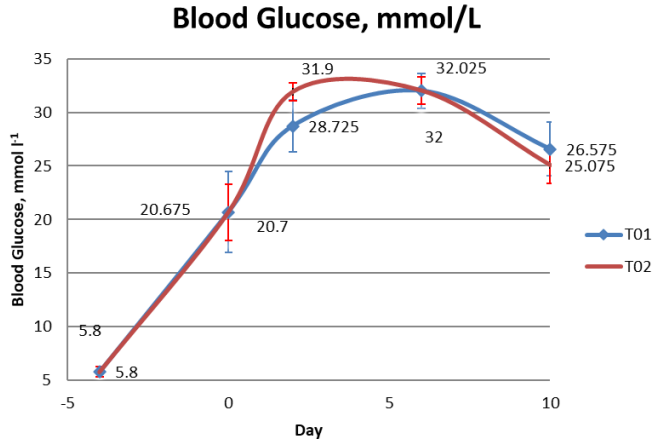


Figure 2. Mean blood glucose (mmol/L) of Metformin treated (T01) and TRF Treated group (T02) at initial, Day 0 (4 days after STZ induction), Day 2, Day 6 and Day 10.

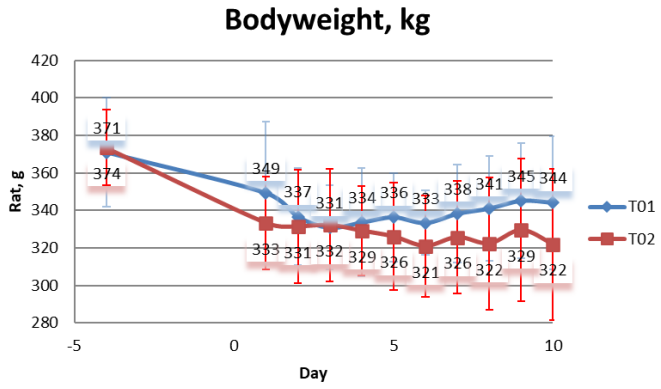


Figure 3. Mean bodyweight of Metformin treated (T01) and TRF Treated group (T02) at initial, Day 1 till Day 10

4. Discussion

The oil palm tocotrienols rich fraction (TRF) did not demonstrate any antimicrobial inhibitory property against *S. aureus, L. innocua, E. coli, P. aeroginosa and S. enterica* ser. Typhi at concentration 30 mg/L to 105 mg/L h. From the research by Al-Salih *et al.* (2013) that vitamin E in concentration more than 50 IU/mL could inhibit *S. aureus* and *Staphylococcus epidermidis* and *P. aeruginosa* was sensitive to 200 IU/mL of vitamin E while *E. coli* was sensitive to 400 IU/mL (Al-Salih *et al*., 2013) However, based on Cheah and Gan (2000) research findings, there was no antimicrobial effect asserted by vitamin E alpha-tocopherol which is in agreement with our findings. In Al-Salih *et al*. (2013), the source of vitamin E used was not mentioned, thus it could result in a different outcome. Vitamin E comprises of two subfamilies that include tocopherols and tocotrienols and these compounds differ in structure and dietary source (Lee and Han, 2018), hence their potency in preventing microbial growth could be varied.

Although TRF did not display any antibacterial activity, the wound contraction progressed steadily from Day 2, 6 and 10 and showed the potential of TRF on diabetic wound healing, with no sign of infection and sepsis. During the observation period, we observed that the scab formed started on the end of Day 1 (Table 2) turned dried at day 6 and the scab started to get smaller when granulation started until a pink layer of epithelial cell appeared at the wound at Day 10. The non-delayed wound contraction rate for TRF treated wounds agreed with Musalmah *et al.* (2002) when compared to the wound contraction and complete epithelialization an animal model, normal and diabetic SP male rats. The TRF treated rats reached >90 % wound closure on Day 10. In the same testing, untreated diabetic rats had merely <20% of recovery (Musalmah *et al.*, 2002). In addition, Xu *et al.* (2017) also reported that epoxidated tocotrienols tested accelerated the wound contraction (Xu *et al.,* 2017). The effectiveness of TRF with wound recovery without delays is also evidenced in the diabetic and non-diabetic animals models (Abu Dayyih *et al.,* 2020).

Okizaki *et al.* (2015) used using STZ induced diabetic and non-diabetic animal model on wound healing and showed that the wound contraction was delayed in STZ animals during the early phase of the healing period with reduction of the recruitment of macrophages into the wound granulation tissue, that contributed to the delay in wound healing and angiogenesis compared to normal animal model (Okizaki *et al.,* 2015). Yu et al. (2016) found that the wound contraction rate on STZ induced diabetic animal treated with metformin reached 90% at Day 10 with 6 mm circular wound. Their study suggested that metformin improve BM-EPC functions in STZ-induced diabetic mice, which was possibly dependent on the AMPK/ *eNOS* pathway (Yu *et al.,* 2016). Our study suggested that TRF is comparable to Metformin in wound healing

performance based on the results in Table 1, Table 2 and Table 3. The STZ induced diabetic rats fed with metformin or TRF in our study showed non-delay of wound healing.

Wound contraction is a major contributor to the healing of full-thickness open wounds compared with incisional wounds. Wounds with square edges contract more rapidly than circular wounds (Ramasastry, 2005). According to Ramasastry (2005), wound contraction started 4 to 5 days after wounding. The process involves the centripetal movement of the wound edge toward the centre of the wound. Maximal wound contraction continues for 12 to 15 days, as long as the wound remains open. Wound contraction progresses at an average of 0.6 to 0.75 mm/day. The tissues that have the greatest laxity demonstrate the greatest degree of wound contraction. The starting of wound contraction need platelet-derived growth factor-BB (PDGF) then only proceeds into remodelling (Ramasastry, 2005). Table 3 shows that the experimental rats fed with either metformin or TRF produced a sufficient amount of PDGF at the initial of wound healing (Day 0 to Day 2) prevented the wound to turn into a chronic non-healing wound. This agreed with the findings of Xu *et al.* (2017) on tocopherols and tocotrienols that induces VEGF and PDGF expression, which played important roles in cell proliferation and migration in wound healing.

PDGF-BB is the first of few growth factors delivered to the wound site by degranulated platelets as initial signals for activation of neutrophils, macrophages and fibroblasts. The response on hemostasis and inflammatory on blood serum was tested on Day 2 and noted there was a peak in PDGF-BB. The raise of PDGF -BB after injury agreed with PDGF roles in inflammatory (Steed, 1997) and initial wound recovery mechanism (Bennett *et al*., 2003). Based on Doxey *et al.* (1995), platelet-derived growth factor levels in wounded diabetic rats showed no response of PDGF until Day 20, while non-diabetic rats would have a hike in the expression of platelet-derived growth factor (PDGF) at Day 5. It is suggested that the diabetic state inhibits cellular PDGF expression in diabetes wounds (Doxey *et al.,* 1995). Since rats in both experimental groups in this study showed an increase in PDGF levels on Day 2, therefore it indicates the potential use of both metformin and TRF in wound management in diabetic animals. Towards the end of wound closure, the PDGF-BB in blood serum would gradually drop due to the diversification of growth factors and cytokines that take place for rebuilding with new granulation and extracellular matrix as well as developing a new network of blood vessels (Stadelmann *et al.*, 1998).

Malondialdehyde (MDA) is the product of lipid peroxidation that occurs during oxidation stress; it is generated by reactive oxygen species (ROS) (Marnett, 1999). Due to the diabetes systemic condition, there are higher MDA in the wound or ROS activities involved compared to non-diabetic wounds (Baynes, 1991; Rasik and Shukla, 2000). ROS acts as signalling molecules and defence systems while it is detrimental to cells and tissue, it stimulates the initiation of inflammatory reactions (Johansen *et al.,* 2005; Paswan *et al.,* 2020).

In the development of complications in diabetes, it is suggested that the oxidative stress may be amplified by a continuing cycle of metabolic stress, tissue damage and cell death, leading to increased free radical production and compromised free radical inhibitory and scavenger systems, which further exacerbate the oxidative stress (Baynes, 1991).

TRF treatment group showed a significant reduction of MDA compared to metformin-treated on Day 10 (Table 4). Musalmah *et al.* (2002) and Musalmah *et al* (2005) reported that diabetic rats would experience significantly higher plasma MDA levels compared to the normal rate, but the MDA of the diabetic rat could reduce significantly with *α*-tocopherol (Musalmah *et al.*, 2002; Musalmah *et al.*, 2005). Our study showed that tocotrienol (TRF) could also reduce the plasma MDA, indicates its potent antioxidants and significantly reduced the lipid peroxidation levels in the wounds as measured by the reduction in MDA levels. *Alpha*-tocopherol and *alpha*-tocotrienol are both vitamin E constituents having the same aromatic chromanol "head" but differing in their hydrocarbon "tail": tocopherol with a saturated and tocotrienol with an unsaturated isoprenoid chain (Sen *et al*., 2006). This small difference in molecular structure allows tocotrienols to cover a larger surface area of the cell membrane more quickly, hence making them more effective as antioxidants. Serbinova *et al.* (1991) had compared the antioxidant potent of both tocotrienols and tocopherols and reported that alpha-tocotrienol is 40-60 times better than alpha-tocopherol in (Fe^{2+} + ascorbate)- and (Fe^{2+} + NADPH)-induced lipid peroxidation in rat liver microsomal membranes and 6.5 times more effective in protecting cytochrome P-450 against oxidative damage. The unsaturated isoprenoid side chain of the tocotrienols, which had been suggested to provide higher mobility, allowing more efficient and uniform distribution into the bilayer cell membranes (Serbinova *et al.,* 1991). This makes tocotrienol a potential topical application for wound recovery treatment with potent anti-oxidant properties. While alpha-tocopherol research still occurs at a much greater level than tocotrienol research, the benefit of tocotrienols has to gain more attention, driven by a growing body of science.

Lipid peroxidation generates a high level of free radicals. The free radicals impair the normal wound healing process by being injurious to a keratinocyte, endothelial cells, capillary permeability and collagen metabolism. Hence, oxidative stress induces cellular dysfunction and retards angiogenesis and the healing process. The inhibition of lipid peroxidation can restore impaired vascular endothelial growth factor expression and stimulates wound healing (Altavilla *et al.,* 2001). Therefore, the ability of TRF in bringing down lipid peroxidation and regulating oxidative stress would increase the potent use of TRF in wound healing.

Other than wound healing, the potential of TRF in diabetes management is also of interest to this study. The blood glucose level of all rats increased from an initial 5.8 mmol/L to 21 mmol/L after 4 days of STZ induction. Administration of STZ destroyed pancreatic *b*-cells, leading to the inhibited insulin secretion, thereby increased blood glucose levels.

The blood glucose on day 2 after administration of metformin or TRF had significantly lowered the blood glucose and the metformin group had lower blood glucose than the TRF supplementary group. Metformin is the first-line therapy for type 2 diabetes mellitus to lower both basal and postprandial plasma glucose (PPG). Metformin works by inhibiting the production of hepatic glucose, reducing intestinal glucose absorption and improving glucose uptake and utilization (Gong *et al.*, 2012).

On day 6 and day 10, the blood glucose level of both metformin and TRF treated remained slow-down, 26.5 mmol l^{-1} for the metformin-treated group and 25.0 mmol L^{-1} for TRF treated group. This indicates TRF is able to stable down the blood glucose as good as metformin. This finding provides additional support to the work of Wan Nazaimoon and Khalid (2002), that claimed TRF can stabilize the hike of the blood glucose level and prevent the further rise of glycated haemoglobin content significantly in STZ- induced diabetic rats compared to the negative control. They also postulated that TRF affects protecting the total damage of *b*-cells by STZ or glucotoxicity (Wan Nazaimoon and Khalid, 2002). Another study using STZ induced diabetes rats, with TRF oral supplement after the STZ induction, reported TRF lowers the blood glucose and improved dyslipidemia on the blood vessel wall (Budin *et al.*, 2009).

Monitoring bodyweight is also an important component in diabetes management. For diabetic patients, insufficient insulin prevents their bodies from getting glucose from the blood into the body's cells. The loss of ability to obtain glucose as an energy source switched of energy source to the catabolism of protein and fats, which lead to a reduction in bodyweight. A previous study by Zolali *et al.* (2020), where metformin-treated (50 and 100 mg/kg) STZ induced diabetic rats showed dropped in bodyweight (Zolali *et al.*, 2020). The bodyweight of the experimental rats in this study reduced sharply after the STZ induction to approximately 6 to 10 %. After being supplemented with either metformin or TRF, the bodyweight stabilized but was not able to gain its previous weight.

4. Conclusion

Tocotrienols Rich Fraction (TRF) showed potential in managing diabetes and assist in wound healing. The oxidative stress experienced among the diabetic rats can be reduced with the administration of TRF where a significant reduction of MDA, the product from lipid peroxidation was noted. TRF also timely promoted the growth factor, PDGF-BB which is important for clean wound closure. Based on these findings, TRF may be considered a promising supplement in diabetic management.

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