

The Effects of Virgin Coconut Oil on Fibroblasts and Myofibroblasts on Diabetic Wound Healing

WONG SK, RANGIAH T, BAKRI NSA, ISMAIL WNA, BOJENG EEF,
ABD RAHIMAN MA, SOLIMAN AM, GHAFAR N, DAS S, TEOH SL

Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.

ABSTRAK

Penyembuhan luka yang lambat adalah salah satu komplikasi utama diabetes melitus yang sering menyebabkan amputasi. Minyak kelapa dara (VCO) adalah minyak semulajadi yang kerap digunakan dalam rawatan luka. Ia mempamerkan aktiviti antioksidan dan antibakteria. Tujuan kajian ini adalah untuk menentukan kesan VCO terhadap fibroblas dalam proses penyembuhan luka diabetik. Luka kulit ketebalan penuh diambil daripada tikus diabetik yang tidak dapat rawatan, yang dirawat VCO dan perak sulfadiazine (SS). Tisu luka kemudiannya diproses untuk pewarnaan Verhoeff eosin dan imunohistologi untuk fibroblas dan miofibroblast. Analisis histologi menunjukkan peningkatan serat kolagen dengan epidermis yang utuh dalam kumpulan VCO berbanding dengan tisu luka tidak dirawat dan yang mendapat rawatan SS. Sel fibroblas dan miofibroblast diperhatikan lebih banyak dalam kumpulan tidak dirawat berbanding dengan kumpulan dirawat VCO dan SS. VCO mempercepatkan proses penyembuhan luka dengan ketara dalam tikus diabetik dengan meningkatkan proses epithelialisasi, penghasilan serat kolagen dan penguncupan luka. Hasil daripada penyelidikan ini mencadangkan VCO boleh digunakan untuk merawat luka diabetik.

Kata kunci: diabetes melitus, kulit, otot, regenerasi, vimentin

ABSTRACT

Delayed wound healing is one of the major complications of diabetes mellitus where it often leads to amputation. Virgin coconut oil (VCO) is a natural oil widely used to treat wounds and burns traditionally. It possesses potent antioxidant and antibacterial activities. This study aimed to determine the effect of VCO on the fibroblast population in diabetic wound healing. Full thickness cutaneous wound

Address for correspondence and reprint requests: Seong Lin Teoh. Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia. Tel: +603-9145 8635 Email: teohseonglin@yahoo.com.my

tissues were collected from non-treated, VCO-treated, and silver sulfadiazine (SS)-treated diabetic rats. The tissues were then subjected to Verhoeff eosin staining and immunohistology of fibroblast and myofibroblast. Histological analysis showed increased collagen deposition with intact epidermis in the VCO treated group compared to decreased collagen deposition with damaged epidermis in both non-treated and SS-treated groups. Interestingly, more fibroblasts and myofibroblasts were observed in the non-treated group compared to the VCO- and SS-treated groups. VCO significantly promoted wound healing process in diabetic rats via promoting re-epithelialization, and increasing collagen fibres deposition and wound contraction. The results suggested VCO can be used to treat diabetic wounds.

Keywords: cutaneous, diabetes mellitus, muscle, regeneration, vimentin

INTRODUCTION

Diabetes is a chronic disease that occurs when the pancreas does not produce sufficient insulin, or when the body cannot effectively use the insulin it produces (Teoh et al. 2009; WHO 2016). It is associated with reduced life expectancy and diminished quality of life due to its microvascular and macrovascular complications (ischemic heart disease, stroke and peripheral vascular disease) (Zar et al. 2012). Globally, around 422 million adults were diagnosed with diabetes in the year 2014, compared to 108 million in 1980 (WHO 2016). It was estimated that diabetes will affect 439 million adults by the year 2030 and further to increase to 592 million in adults by the year 2035 (Guariguata et al. 2014; Shaw et al. 2010).

Wound healing process is known to be impaired in diabetic patients (Hussan et al. 2014). Impaired wound healing associated with diabetes are caused by multiple complex

pathophysiological mechanisms, such as decreased growth factors and cytokines production, vascular abnormalities, impaired inflammatory cells and epidermal barrier function, reduced keratinocyte and fibroblast migration and proliferation, suppressed cell mediated immunity, and imbalance between the accumulation and remodelling of extracellular matrix components (Hussan et al. 2018). This leads to the wound to remain open and unhealed for months, increasing the risk of infection and eventually lead to amputation. Oxidative stress developed in diabetes, may contribute to the impaired wound healing, e.g. continuous secretion of pro-inflammatory cytokine and matrix metalloproteases, impaired fibroblast and keratinocyte function (Dunnill et al. 2017). Modulating oxidative stress using antioxidants may be a useful therapeutic approach to treat diabetic wounds.

Currently, the use of natural products has gained momentum

during the past decades. Numerous natural product treatment were shown effective in promoting wound healing in diabetic rats, i.e. *Aloe vera*, *Curcuma longa*, *Momordica charantia*, *Piper betel* and tocopherol (Hussan et al. 2014; Hussan et al. 2018; Keat et al. 2010; Teoh et al. 2012; Teoh et al. 2009). The virgin coconut oil (VCO) is rich in antioxidants and were known to exert cardioprotective and vasculoprotective effects (Kamisah et al. 2016; Subermaniam et al. 2015).

To date, there is paucity of published data on the effect of VCO in diabetic wound healing. There is a need to assess the effect of VCO in diabetic wound healing especially on fibroblast populations so as an evidence of reason for applying VCO on diabetic wound in order to improve the healing process and outcome.

MATERIALS AND METHODS

Animals

In total, 36 male Sprague Dawley rats weighing 200-250g were obtained from the Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia (UKM). The animals were divided into three groups: a non-treated control group (n=12), a VCO-treated group administered topically (n=12) and a treated group with standard silver sulfadiazine (SS) administered topically (n=12). SS is commonly used to manage wound infection in full thickness injuries (Tarameshloo et al. 2012). Experimental rats were fed with commercial rat feed and water *ad libitum*. All experimental procedures

were conducted under ethical approval by UKM Animal Ethics Committee.

Induction of Diabetes

Diabetes was induced in the experimental rats by a single intravenous dose of streptozotocin (55 mg/kg of body weight) following overnight fasting (Hussan et al. 2014). The fasting blood glucose level was recorded using glucose meter (Accu-Check Advantage, Roche Diagnostics, Germany) three days after streptozotocin administration. The rats with fasting blood glucose levels above 8 mmol/L was recruited for the wound healing study (Hussan et al. 2014).

Wound Creation

We followed the previous protocol for wound creation in the experimental rats (Teoh et al. 2009). Wounds were created on day 10 following diabetes induction. Briefly, a total of four full-thickness skin excision wounds were created using a 6mm diameter punch biopsy needle (Integra LifeSciences, France) on the dorsal aspect of the anaesthetized experimental rats.

Treatment of Wounds and Wound Tissue Collection

In the treated groups, each rat was treated topically by 2ml of VCO and SS daily, using a micropipette 24 hours after wounding until the end of experiment. VCO was purchased from a local dealer. The oil was extracted mechanically without heating or chemical used. The wound was left

open during the entire experimental period. A total of 6 animals from each group were sacrificed at day 7 and 14 after wounding, and the wounded skin tissues were collected for histological analysis.

Histological Analysis

The skin tissues collected were fixed in 10% formalin, followed by dehydration through a series of alcohol solution (50-100%), cleared in xylene and embedded in paraffin wax (Hussan et al. 2014). Serial sections of 5m thickness were obtained using a microtome (Leica, German), and stained with Verhoeff eosin.

Immunohistological Analysis

Sections were deparaffinized, rehydrated, and washed in phosphate-buffered saline (pH 7.4). Fibroblasts and myofibroblasts were examined by immunohistochemical stains. Fibroblasts were identified by mouse monoclonal anti-vimentin antibody (Abcam, Cat# ab8978, 1:500) and myofibroblasts were identified with mouse monoclonal anti-smooth muscle actin antibody (Abcam, Cat# ab7817, 1:500). The primary antibodies were incubated with a biotinylated anti-mouse secondary antibody (Animal Research Kit™, Dako) in phosphate buffered solution with 1% bovine serum albumin for 30 minutes, followed by blocking solution for a minimum of 5 minutes. Three separate sections of each wound were examined by light microscopy. The total number of fibroblasts and myofibroblasts were

counted in 5 randomly picked 40x magnification fields in 3 tissue sections of each group.

Statistical Analysis

All data were presented as mean \pm standard error of mean (SEM). Statistical analysis was performed using a two-way ANOVA followed by Tukey's post hoc test. A value of $p < 0.05$ was considered to be significant. All statistical analyses were performed using SPSS software (version 22; SPSS Inc., Chicago, USA).

RESULTS

Light Microscopy Analysis on Wound Tissues

Day 7 (Figure 1) showed intact epidermis in VCO treated compared to the damaged epidermis in non-treated and silver sulfadiazine treated groups. On day 14 (Figure 2), all groups showed complete epithelialization. However, only VCO treated group showed significant dermal epidermal interdigitation. VCO group also showed high collagen fibres arranged in a very well organised manner along with fibroblasts stimulating the normal skin.

Fibroblasts Population in Diabetic Wounds

Vimentin was widely expressed in fibroblasts. VCO and silver sulfadiazine treatment caused significant reduction in the fibroblast population in wound tissues of day 7 and 14 compared

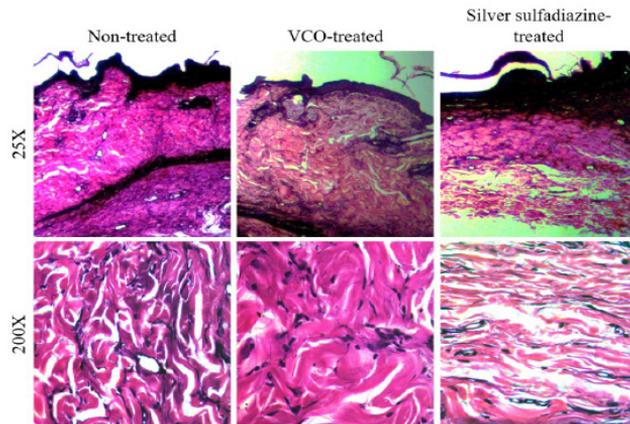


Figure 1: Skin tissue at day 7 following wound creation (Verhoeff eosin staining).

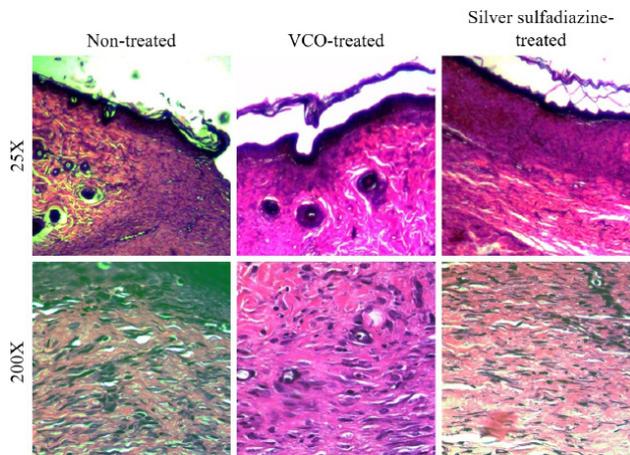


Figure 2: Skin tissue at day 14 following wound creation (Verhoeff eosin staining).

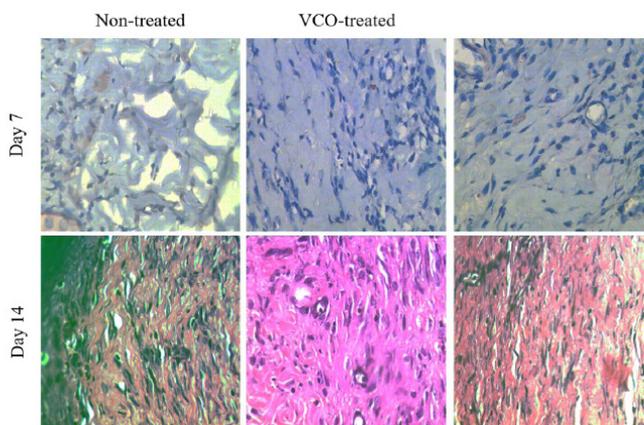


Figure 3: Skin tissue stained with anti-vimentin at day 7 and 14 wounds. Magnification X200. VCO and silver sulfadiazine treatments showed reduced fibroblast population in wound tissues of day 7 and 14 compared to non-treated group.

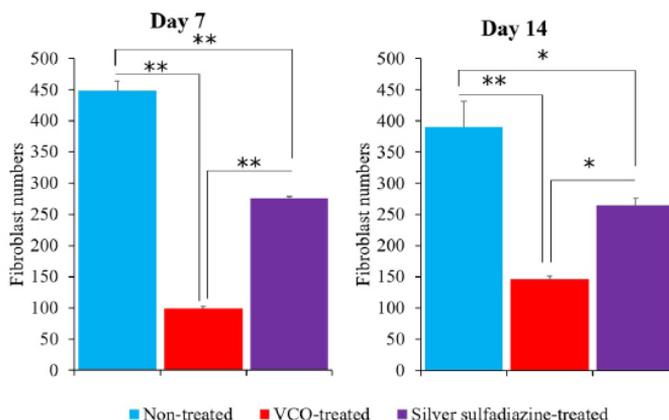


Figure 4: Effect of VCO and silver sulfadiazine in fibroblasts of day 7 and 14 wound tissues. *p<0.05, **p<0.001, one-way ANOVA followed by post-hoc Tukey test.

to non-treated group (Figure 3 & 4). Fibroblasts were present more abundantly at day 14 compared to day 7. The highest number of fibroblasts was found in the non-treated group whereas the least number of fibroblasts was noted in VCO treated group in day 7. It showed significant difference ($p < 0.001$) between the fibroblast numbers of treated wounds compared to the non-treated wounds. The fibroblast numbers for day 14 showed similar trends.

Myofibroblasts Population in Diabetic Wounds

Anti-smooth muscle actin antibody was used as myofibroblast marker and myofibroblasts cytoplasmic staining. Myofibroblasts formed a parallel band in VCO and silver sulfadiazine-treated wound but appeared patchy in non-treated wounds (Figure 5). Interestingly, in terms of cell count in day 7 (Figure 6), non-treated group had the highest number of myofibroblasts

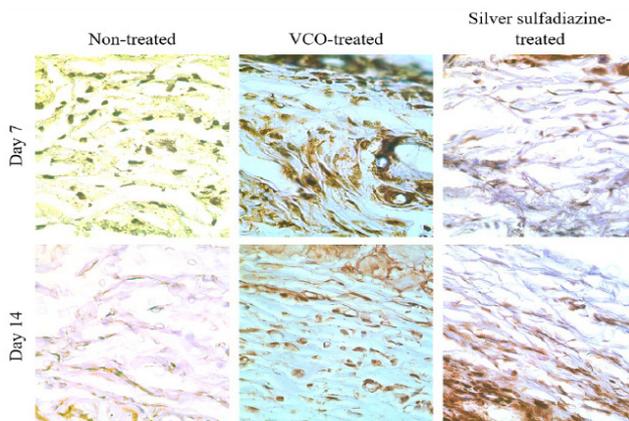


Figure 5: Skin tissue stained with anti-smooth muscle actin at day 7 and 14 wounds. Magnification x200. Myofibroblasts formed a parallel band in VCO and silver sulfadiazine-treated wound but appeared patchy in non-treated wounds.

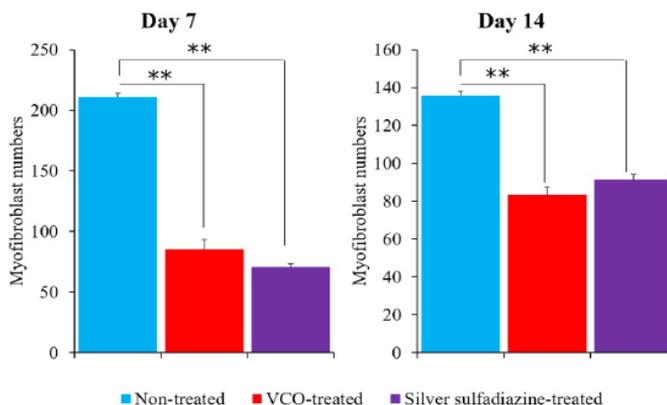


Figure 6: Effect of VCO and silver sulfadiazine in myofibroblasts of day 7 and 14 wound tissues. ** $p < 0.001$, one-way ANOVA followed by post-hoc Tukey test.

whereas silver sulfadiazine group had the lowest number of myofibroblasts. There was significant difference between myofibroblast numbers of treated wounds compared to the non-treated wounds. However, there was no significant difference between myofibroblast number of VCO and SS-treated wounds. Similarly, for day 14, non-treated group had the highest myofibroblast number while VCO and silver sulfadiazine-treated group had low count for myofibroblasts.

DISCUSSION

The healing process of a wounded skin consists of several stages; hemostasis, inflammation, proliferation or granulation, and remodelling or maturation (Latiff et al. 2010). These stages require complex collaborative efforts from many different cells to achieve optimum healing of the skin wound (Hussan et al. 2018; Latiff et al. 2010). In diabetes, the normal wound healing process is impaired, mainly resulted from the oxidative

stress by free radicals (Elsy et al. 2017). VCO is widely known as an antioxidant agent, due to the phenolic compounds present (Marina et al. 2009). In addition, VCO treatment increased antioxidant enzymes levels and prevented lipid peroxidation, in both *in vitro* and *in vivo* studies (Nevin & Rajamohan 2006). So, to improve the healing process of diabetic wound, the oxidative stress can be eliminated by using VCO to suppress the reactive oxygen species production. VCO was shown to promote wound healing in young rats when applied topically (Nevin & Rajamohan 2010). We have previously shown VCO to be able to promote wound healing in diabetic rats by promoting re-epithelialization and collagen synthesis (Soliman et al. 2018). The current study focussed on the effect of VCO on fibroblasts and myofibroblasts in diabetic wounds.

Previous studies showed diabetes affects wound healing by delaying the re-epithelialization process (Hussan et al. 2014; Teoh et al. 2009). The present study showed that VCO enhanced the

re-epithelialization of the wounded skin. On day 7, light microscopic analysis showed intact epidermis in VCO treated wound tissue, compared to the damaged epidermis for non-treated and SS-treated groups. Complete re-epithelialization was noticed in all groups on day 14, but only VCO treated group was found to have significant dermal interdigitation, to hold epidermis and dermis together and to provide strength to endure shearing forces or any insult to the skin. So, VCO treated group is proven to heal much faster than the silver sulfadiazine treated group and the non-treated group, which aids significantly in delayed wound healing in diabetes patient. Numerous natural products promoted re-epithelialization in diabetic rats. Similar to VCO, administration of *M. charantia* and *P. betle* extracts, and tocopherol cream had accelerated re-epithelialization and wound contraction rate in diabetic rats (Hussan et al. 2014; Hussan et al. 2018; Keat et al. 2010; Soliman et al. 2018; Teoh et al. 2012; Teoh et al. 2009; Zailan et al. 2010).

Fibroblasts are critical in supporting wound healing process, by continuously secreting extracellular matrix content such as collagen, angiogenic factors, cytokines and immunomodulatory substances (Khamaisi et al. 2016). Fibroblasts in diabetes patients demonstrated a reduced migration and were functionally less effective compared to healthy individuals (Khamaisi et al. 2016). However, in the present study, we showed fibroblast population was the highest in the non-treated group, compared to VCO

and SS-treated groups, in day 7 and day 14 after wounding. Despite most abundant fibroblasts were present in the non-treated diabetic wounds, however, due to abnormal fibroblasts function evidenced by the least collagen fibres deposition in the non-treated group, wound healing process was delayed.

Myofibroblasts possess a very essential role in wound healing process. It portrays important contractile apparatus similar to that of smooth muscle, and plays a central role in wound contraction (Bochaton-Piallat et al. 2016). For myofibroblasts, they were found most abundantly in non-treated group in day 7 and day 14, the least in SS-treated group in day 7, and the lowest in VCO-treated group in day 14. In this study, myofibroblasts were noted to appear in form of parallel band in VCO and silver sulfadiazine-treated wounds but appear patchy in non-treated wounds. This disorganization of myofibroblasts in non-treated wounds may affect the wound contraction and the whole wound healing process. In addition, diabetic fibroblast has lower contractibility compared to normal fibroblast (Wang et al. 2019). Taken together with the impaired epithelialization in the control group, the wound contraction rate were reduced in diabetic wounds, which was reflected by the impaired re-epithelialization process and myofibroblasts contraction.

CONCLUSION

VCO treatment showed significant role in improving wound healing process in

diabetic rats. VCO also promoted the dermal and epidermal interdigitation and provide strength to the epithelial tissue.

ACKNOWLEDGEMENT

The authors thank Mr. Shaiful Ridzwan Bin Sapri for the technical assistance. This study was supported by the Faculty of Medicine, Universiti Kebangsaan Malaysia (FF-2017-174 and FF-2017-176).

REFERENCES

- Bochaton-Piallat, M.L., Gabbiani, G., Hinz, B. 2016. The myofibroblast in wound healing and fibrosis: answered and unanswered questions. *F1000Res* 5: 752.
- Dunnill, C., Patton, T., Brennan, J., Barrett, J., Dryden, M., Cooke, J., Leaper, D., Georgopoulos, N.T. 2017. Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. *Int Wound J* 14(1): 89-96.
- Elsy, B., Khan, A.A., Maheshwari, V. 2017. Effect of vitamin E isoforms on the primary intention skin wound healing of diabetic rats. *Our Dermatology Online* 8(4): 369-75.
- Guariguata, L., Whiting, D.R., Hambleton, I., Beagley, J., Linnenkamp, U., Shaw, J.E. 2014. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract* 103(2): 137-49.
- Hussan, F., Teoh, S.L., Muhamad, N., Mazlan, M., Latiff, A.A. 2014. *Momordica charantia* ointment accelerates diabetic wound healing and enhances transforming growth factor- β expression. *J Wound Care* 23(8): 400-407.
- Hussan, F., Yahaya, M.F., Teoh, S.L., Das, S. 2018. Herbs for effective treatment of diabetes mellitus: medicinal chemistry and future therapeutic options. *Mini Rev Med Chem* 18(8): 697-710.
- Kamisah, Y., Ang, S.M., Othman, F., Nurul-Iman, B.S., Qodriyah, H.M. 2016. Renoprotective effect of virgin coconut oil in heated palm oil diet-induced hypertensive rats. *Appl Physiol Nutr Metab* 41(10): 1033-8.
- Keat, E.C., Razak, S.S., Fadil, N.M., Yusof, F.M., Chan, L.H., Chyi, F.K., Teoh, S.L., Das, S., Latiff, A.A., Mazlan, M. 2010. The effect of *Piper betel* extract on the wound healing process in experimentally induced diabetic rats. *Clin Ter* 161(2): 117-20.
- Khamaisi, M., Katagiri, S., Keenan, H., Park, K., Maeda, Y., Li, Q., Qi, W., Thomou, T., Eschuk, D., Tellechea, A., Veves, A., Huang, C., Orgill, D.P., Wagers, A., King, G.L. 2016. PKC δ inhibition normalizes the wound-healing capacity of diabetic human fibroblasts. *J Clin Invest* 126(3): 837-53.
- Latiff, A.A., Teoh, S.L., Das, S. 2010. Wound healing in diabetes mellitus: traditional treatment modalities. *Clin Ter* 161(4): 359-64.
- Marina, A.M., Man, Y.B., Nazimah, S.A., Amin, I. 2009. Antioxidant capacity and phenolic acids of virgin coconut oil. *Int J Food Sci Nutr* 60(Suppl 2): 114-23.
- Nevin, K.G., Rajamohan, T. 2006. Virgin coconut oil supplemented diet increases the antioxidant status in rats. *Food Chem* 99(2): 260-6.
- Nevin, K.G., Rajamohan, T. 2010. Effect of topical application of virgin coconut oil on skin components and antioxidant status during dermal wound healing in young rats. *Skin Pharmacol Physiol* 23(6): 290-7.
- Shaw, J.E., Sicree, R.A., Zimmet, P.Z. 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 87(1): 4-14.
- Soliman, A.M., Teoh, S.L., Ghafar, N.A., Das, S. 2018. Virgin coconut oil and diabetic wound healing: histopathological and biochemical analysis. *European Journal of Anatomy* 22(2): 135-44.
- Subermaniam, K., Mohd Saad, Q.H., Kamisah, Y., Othman, F. 2015. Effects of virgin coconut oil on the histomorphometric parameters in the aortae and hearts of rats fed with repeatedly heated palm oil. *International Journal of Bioscience, Biochemistry & Bioinformatics* 5(2): 120-31.
- Tarameshloo, M., Norouzian, M., Zarein-Dolab, S., Dadpay, M., Mohsenifar, J., Gazor, R. 2012. *Aloe vera* gel and thyroid hormone cream may improve wound healing in Wistar rats. *Anat Cell Biol* 45(3): 170-7.
- Teoh, S.L., Latiff, A.A., Abd Hamid, N.A., Wan Ngah, W.Z., Mazlan, M. 2012. Evaluation of topical tocopherol cream on cutaneous wound healing in streptozotocin-induced diabetic rats. *Evid Based Complement Alternat Med* 2012: 1-6.
- Teoh, S.L., Latiff, A.A., Das, S. 2009. The effect of topical extract of *Momordica charantia* (bitter melon) on wound healing in nondiabetic rats and in rats with diabetes induced by streptozotocin. *Clin Exp Dermatol* 34(7): 815-22.
- Wang, X.T., McKeever, C.C., Vonu, P., Patterson, C., Liu, P.Y. 2019. Dynamic histological events and molecular changes in excisional wound healing of diabetic DB/DB mice. *J Surg Res* 238: 186-97.
- WHO. 2016. *Global report on diabetes*. France:

WHO.

- Zailan, N., Abdul Rashid, A.H., Das, S., Abdul Mokti, N.A., Hassan Basri, J., Teoh, S.L., Wan Ngah, W.Z., Mohd Yusof, Y.A. 2010. Comparison of *Chlorella vulgaris* dressing and sodium alginate dressing: an experimental study in rats. *Clin Ter* **161**(6): 515-21.
- Zar, C.T., Teoh, S.L., Das, S., Zaiton, Z., Farihah, H.S. 2012. Use *Piper sarmentosum* as an effective antidiabetic supplement in South East Asia: a review. *Clin Ter* **163**(6): 505-10.

Received: 20 Mar 2019

Accepted: 5 Aug 2019