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ORIGINAL ARTICLE

Wound Healing and Antibacterial Activities of 2-Pentadecanone in Streptozotocin-Induced Type 2 Diabetic Rats

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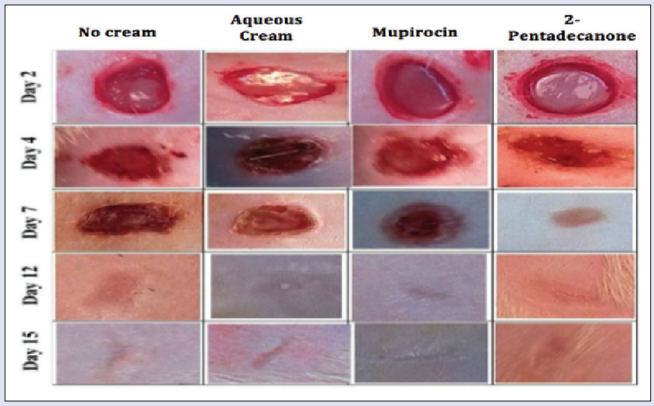
ABSTRACT

Background: Skin regeneration is critical in diabetic wound healing. Anti-inflammatory activity of 2-Pentadecanone (2-P), identified from *Marantodes pumilum* was reported in our previous studies. **Objective:** This study was aimed at evaluating 2-P wound healing and anti-bacterial activities. **Materials and Methods:** Fifteen streptozotocin-induced type 2 diabetic Sprague Dawley rats were divided into negative (N), positive (P), and 2-P treatment (2-P) groups. 2-P aqueous cream was applied daily to 5 mm excision wounds up to day 15. Wound closure rate was assessed by tracing the wound onto plastic sheets and were accessed using a stereoscope microscope. Antibacterial activity (minimal inhibition concentration and disc diffusion) was determined using the modified resazurin assay and Kirby-Bauer method. The skin samples were excised on days 2, 7, and 15 and were analyzed by Hematoxylin/Eosin staining. Semi-quantification analysis was done for re-epithelialization, polymorphonuclear leukocytes (PMNL), and fibroblasts proliferation. Hydroxyproline (collagen deposition) and glutathione (antioxidant) activities were evaluated. **Results:** The results showed that 2-P possess antibacterial activity against *Staphylococcus aureus*. It demonstrated significantly higher wound closure compared with negative and positive groups. It showed a higher effect on epithelial cell migration ($P < 0.05$) and collagen deposition ($P < 0.05$) compared to negative control group. However, 2-P effect on fibroblast proliferation ($P > 0.05$) and reduction in PMNL cells ($P > 0.05$) was not significantly different compared with negative control. **Conclusion:** It can be concluded that 2-P reveals antibacterial activity, wound closure, collagen deposition, and fibroblast proliferation. Thus, we suggest that 2-P has the potency to be used as an active ingredient in the formulation of a diabetic wound healing cream.

Key words: 2-Pentadecanone, diabetes mellitus, fibroblasts proliferation, polymorphonuclear leukocytes, re-epithelialization, *Staphylococcus aureus*, wound healing

SUMMARY

- 2-Pentadecanone was able to accelerate wound closure, possibly due to its ability to inhibit *Staphylococcus aureus*, increased epidermal migration, reduced inflammation, increased fibroblast proliferation and collagen deposition. This suggests 2-pentadecanone has the potential to be used as an active ingredient in the formulation of a diabetic wound healing cream.



Abbreviations used: DM: Diabetes mellitus; 2-P: 2-Pentadeconone; PMNL: Polymorphonuclear leukocytes; STZ: Streptozotocin; PDGF: Platelet-derived growth factor; TGF-β: Tissue growth factor beta; MMP: Matrix metalloproteinase; BFGF: Basic fibroblast growth factor; IL-1: Interleukin-1; ROS: Reactive oxygen species; MIC: Minimal Inhibitory concentration; DF: Disc Diffusion; H and E: H and E; KGF: Keratinocyte growth factor.

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INTRODUCTION

Diabetes mellitus (DM) or commonly known as diabetes, is a chronic lifelong metabolic disorder caused by impaired insulin secretion or impaired insulin action (or even both) leading to hyperglycemia.[1] According to the Geneva Global Report, DM is a serious health problem faced by 422 million worldwide.[2] In 2012, 3.7 million people died due to their diabetic state (40.5%) while others died due to complications caused by hyperglycemia (59.5%).[2] The Malaysian National Health Morbidity Survey III in 2006 reported that the national percentage of patients with lower limb amputations due to DM was 4.3%.

Wound healing is defined as a complex process of replacing devitalized tissue layers.[3] In the normal wound healing process, the inflammatory phase promotes hemostasis which produces cardinal signs of inflammation such as erythema (redness of the wound area), edema (swelling of the wounded region), pain, and also the loss of

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function. An injury to the vascular tissue triggers the blood clotting mechanism and results in the coagulation of platelets. Platelet-derived growth factor (PDGF) and tissue growth factor beta (TGF-β) initiate chemotaxis and produces neutrophils.[4] These neutrophils debride the tissue by removing the foreign particles around the wounded area. On the other hand, macrophages also debride the wound by releasing inflammatory mediators such as basic fibroblast growth factor (BFGF) and interleukin-1 (IL-1).[4] IL-1 stimulates cell proliferation and angiogenesis through a balanced matrix metalloproteinase (MMP) activity. MMPs stimulate the development of a new extracellular matrix (ECM) via angiogenesis and tissue remodelling. MMP-1 digests collagen, weakening the membrane attached to the matrix. Hence, new epithelial cells are able to migrate across the collagen matrix to begin the proliferation of a new matrix.[5]

In diabetic patients, neuropathy and hyperglycemia significantly affect the ability of the wounds to heal.[6] The desensitization brought about by neuropathy makes a diabetic patient prone to injuries and infections prolonging the inflammatory stage of the chronic wound healing process.[7] The increase in neutrophil infiltration as a result of the prolonged inflammation leads to an increased production of reactive oxygen species. Moreover, some other factors cause the delay in wound healing such as decreased inflammatory reactions (tumor necrosis factor-alpha and nuclear factor-κB); impaired growth factors secretion; impaired angiogenic responses; macrophage activation; keratinocyte; and fibroblast migration.[8-10] 2-pentadecanone (2-P) was identified in our previous research from a purified extract of *Marantodes pumilum* (MP) using Gas chromatography/Mass Spectroscopy.

This purified extract was named Fraction A. Several studies have shown that this fraction has anti-inflammatory, ntinociceptive, and antipyretic activities.[11-13] It was found that Fraction A possesses major compounds; phthalic esters (17.8%), branched alkane hydrocarbons (11.72%), hydrocarbons (7.89%), Tricosane and Eisosane (5.25%), and Terpenes; (6.84%) summing up to (49.5%) of the total constituents (unpublished data from this research team). 2-P is a ketone and is has been identified in *Humulus lupulus*, *Cocos nucifera* and other oils.[14] Plants containing its analog 6,10,14-trimethyl-2-P such as *Peganum harmala* and *Eclipta alba* (L.) have been reported to improve wound healing activities.[15] *P. harmala* was reported to significantly increase fibroblast proliferation and accelerated wound closure.[16] Ethanoic extract of *E. alba* (L.) was found to increase skin tensile strength, improve wound contraction, increase hydroxyproline and antioxidant content.[15] This study aims to evaluate wound healing and anti-bacterial activities of 2-P a compound identified in identified in the leaf extrat of MP plant.

MATERIALS AND METHODS

Antimicrobial activity: Minimal inhibitory concentration

All reference organism *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *S. aureus* (ATCC 25923) and Ampicillin antibiotic (Sigma Aldrich, USA) were from the Microbiology Laboratory, UCSI University. The minimum inhibitory concentration was determined using the modified Resazurin assay.[17] A concentration of 2 mg/ml of Resazurin dye was used in this experiment. The assay required bacteria inoculum of concentration 5 × 105 CFU/ml (*E. coli* (ATCC 25922) - 0.004 nm, *P. aeruginosa* (ATCC 27853) - 0.020 nm, and *S. aureus* (ATCC 25923) - 0.005 nm in each well. Each well had a final volume of 200 µl (20 µl of bacteria inoculum of 5 × 106 CFU/ml). The drug concentrations ranged from 50 to 1000 µl/ml of 2-P 20 mg (Sigma Aldrich, USA) was prepared in 600 µl of 6% hexane and was topped up with 9.4 ml Milli Q water. Ampicillin antibiotic (Sigma Aldrich, USA) was prepared by mixing 10 mg in 10 ml milli-Q water. All of the drugs used were filtered with a 0.22 µm syringe filter. Plates were prepared under aseptic conditions. A sterile 96 well plate was labeled [Figure 1]. A volume of 100 µL of test drug was pipetted into the first row of the plate. To all other wells, 50 µL of nutrient broth was added. Serial dilutions were performed using a multichannel pipette. Tips were discarded after use such that each well had 50 µL of the test drug in serially descending concentrations. To each well 10 µL of resazurin indicator solution was added. Using a pipette 30 µL of × 3.3 strength isosensitized broth was added to each well to ensure that the final volume was single strength of the nutrient broth. Finally, 10 µL of bacterial suspension (5 × 106 cfu/mL) was added to each well to achieve a concentration of 5 × 105 cfu/mL. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each plate had a set of controls: A column with a broad-spectrum antibiotic as positive control (Ampicillin antibiotic, Sigma Aldrich, USA) in serial dilution, a column with all solutions with the exception of the test drug, and a column with all solutions with the exception of the bacterial solution adding 10 µL of nutrient broth instead. The plates were prepared in triplicate and placed in an incubator set at 37°C for 18–24 h. The color change was then assessed visually. Any color changes from purple to pink or colorless were recorded as positive. The lowest concentration at which color change occurred was taken as the minimal inhibitory concentration (MIC) value. The average of three values was calculated and that was the MIC for the test drug and bacterial strain.

Antimicrobial activity: Disc diffusion method

Disc diffusion (DF) was done based on the Kirby-Bauer method as described in the protocol taken from the American Society for Microbiology.[18]

Animal study and drug formulation

A total of 15 Sprague Dawley rats (160–250 g; n = 3, two rats for histological evaluation and on rat for biochemical analysis) were kept in the animal holding room with a controlled temperature of (23°C ± 2°C) for 12 h light/12 h dark cycle and fed with food pellets and water *ad libitum*. The rats were left to acclimatize for 2 weeks before commencing the experiment. At the beginning of the experiment, the rats were divided into two groups – normal and diabetic. 2-P (0.521 g) was mixed with 5.1618 g aqueous-based cream (Pharmaniaga, Malaysia) making a 10% w/w formulation. Bactroban™ (Mupirocin) (Glaxo Smith Kline,

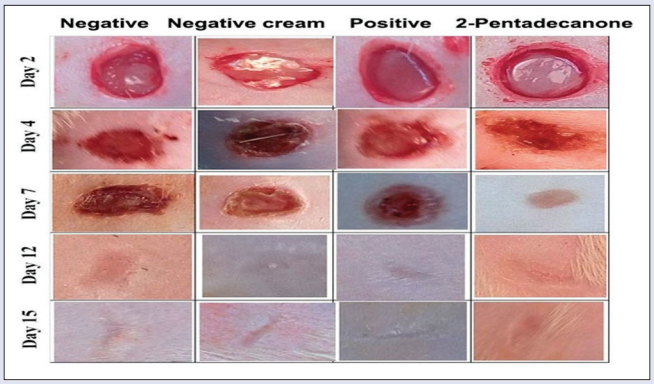

Figure 1: Macroscopic pictures of wound closure of 2-pentadeconone (0.5%w/w) compared with negative (saline 0.9%), negative cream (aqueous cream), positive (mupirocin) on the normal (left), and diabetic (right) rats

Malaysia) was used as positive control. Ethics were obtained from Ethics board UCSI University.

Streptozotocin type 2 diabetes induction

Animals were injected with a single intraperitoneal dose of streptozotocin (60 mg/kg) dissolved in sterile, cold citrate buffer (0.1M sodium citrate, pH 4.5). Blood samples were taken intravenously for glucose analysis (Accu-chek Performa using the glucose oxidase principle) on three occasions (before diabetic induction, 30 days after the induction and just before the dissection). Animals with blood glucose levels higher than 11 mmol/L were considered as type 2 diabetic models.[17] To confirm diabetes, in addition to blood glucose the other diabetic markers; glycated hemoglobin (HbA1c), urea, creatinine, alkaline phosphatase, aspartate transaminase, alanine aminotransferase, triglycerides, high-density lipoproteins, and low-density lipoproteins were also evaluated.

Excision wound model and treatment

Before inducing excision wounds on the animals, they were anesthetized with Ilium-Xylazil 100 (Troy laboratories), Ketamil (Troy laboratories), and distilled water (1:1:5).[19,20] The fur on the dorsal surface was shaved using a sterile surgical blade, and the back was sterilized using 70% alcohol swabs. The excision wounds were inflicted using a 5 mm biopsy punch. Both Normal and Diabetic rats had 6 wounds each, divided with the following labels:

i. Negative with no treatment (N) ($n = 2$; two wounds side-by-side)
ii. Positive control (Mupirocin) (P) ($n = 2$)
iii. 2-Pentadeconone (2-P) ($n = 2$).

Treated wounds had formulated drugs applied with a sterile cotton bud daily for 15 days, after cleaning the wound area with saline. The normal group was added to check there was any difference in the effect of the drug on normal and diabetic group. Animals were sacrificed with diethyl ether asphyxiation on days 2, 4, 7, 12, and 15. Histology was analyzed on days 2, 7, and 15.

Evaluation of wound healing rate

The wounds were assessed every day by observing the wound area under a stereoscope microscope (Toup Tek Co.Ltd,China) coupled with ToupView 3.7© software to measure the area of the wounds. The wound closures of positive, negative, and treated groups were measured using equation 2 (20);

Equation 2: Wound closure % = wound area (day Y–day X)/(day X) ×100%

Histology and staining

The excised skin tissue was fixed in 10% neutral-buffered formalin for 24 h at room temperature followed by tissue processing Embedded tissue was sectioned into 5 µm slices by a manual microtome (Sakura) which were then stained using H and E. All histopathological analyses were reviewed by the same pathologist.

Hydroxyproline and glutathione assays

Day 7, tissue samples were hydrolyzed in the evaluation of antioxidant levels. While on full wound closure on tissue samples from day 12 was used in the evaluation of hydroxyproline activity. Skin tissue at least 3–4 mm thickness with full-length coverage of the wound was excised for biochemical testing where; 10 mg was hydrolyzed in 100 µL concentrated NaOH (6N) at 120°C. Hydroxyproline assay kit (Sigma) was used to analyze the collagen content following kit instruction. The recycling system-glutathione assay (Abnova) was used to evaluate either the reduced or total glutathione present on this skin samples following the kit instructions.

Histological analysis

A quantitative and semi-quantitative method was used to evaluate the re-epithelialization, polymorphonuclear leukocytes (PMNL), and fibroblasts proliferation at ×40 using the Axio Vert. A1 inverted fluorescence microscope with quantitative scoring described by.[21] Two trained independent observers in a randomized blinded fashion analyzed the histology slides. Zeiss 2.1 black edition software was used for the histological analysis.

Statistical analysis

The data were represented as mean ± standard deviation. Data analysis was carried out using GraphPad Prism 7.0 software. Data of percentage wound closure were analyzed using two-way ANOVA and Bonferroni's multiple range test was applied for *post hoc* analysis. The semi-quantitative statistical analysis of the epithelialization and fibroblast scoring was performed using one-way ANOVA followed by nonparametric Kruskal–Wallis test followed by Dunn's multiple comparison tests. $P < 0.05$ was considered significantly different.

RESULTS

Antibacterial activity

The antibacterial activity of 2-P was evaluated against *E. coli* (Gram negative), *P. aeruginosa* (Gram negative) and *S. aureus* (Gram positive) and it was found that 2-P exhibited inhibition of *S. aureus* in the DF and minimal inhibition concentration experiments. The result of DF and minimal inhibition concentration tests was summarized in Table 1.

Wound closure percentage

Full wound closure was observed on day 12 in treated and untreated groups. There was no other significant difference in wound healing from days 4 to 15 in NT. Scarring was less in 2-P treatment compared to the untreated groups on day 15. In the diabetic group, 2-P showed significantly higher wound closure on day 4 ($P < 0.001$) [Figure 1]. Positive control mupurin in the diabetic group did not reduce scarring on day 15 [Figure 2 and Table 2].

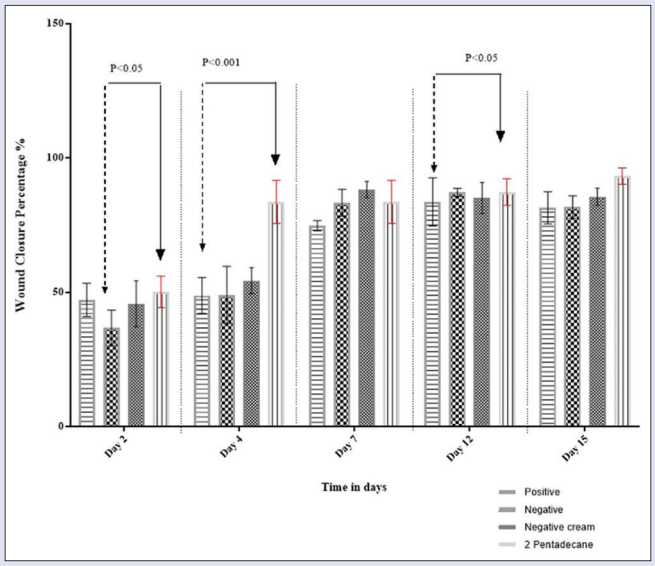

Figure 2: Effect of 2-pentadeconone (0.5% w/w) type 2 diabetes wound closure percentage. Data were expressed as mean ± standard deviation and were analyzed by two-way analysis of variance followed by Dunnett's test

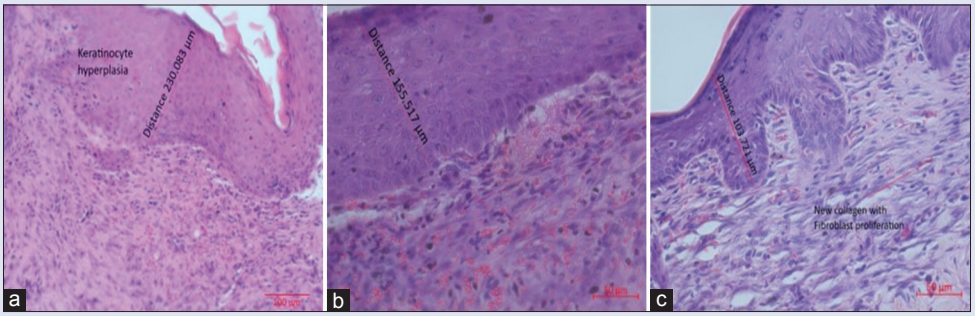

Figure 3: Histopathology sections on day 7 (×40) (a) Negative group: Hyperplasic epidermis observed (230 µm), mild inflammation, granulation tissue formed but not matured (b) mupirocin group: hyperplasic epidermis (155 µm), mild inflammation observed. (c) 2-pentadeconone group: Reduced hyperplasia (104 µm), increased fibroblast proliferation and connective tissue accumulation

Table 1: *In vitro* antibacterial activity of test compounds *Staphylococcus aureus*

Tested microorganisms	2-P		Ampicillin	
	MIC	DF	MIC	DF
Staphylococcus aureus	>200 µg/ml	11 mm	<12.5 µg/ml	31 mm

MIC: Minimal inhibition concentration; 100–1000 µg/ml used compared to 12.5–200 µg/ml (ampicillin); DF: Disc diffusion; zone of inhibition by 2-P at 1000 µg/disc (mm) compared with ampicillin at 1000 µg/disc (mm). 2-P: 2-pentadecanone

Table 2: Hydroxyproline and glutathione activity of skin tissue on control and diabetic groups treated with 2-pentadecanone

	Glutathione (mM)		Hydroxyproline (nM)	
	Normal	Diabetes	Normal	Diabetes
Untreated skin	0.914±0.010^{D}	1.050±0.002^{D}	13.31±0.688	13.02±0.298^{A}
Negative control	1.011±0.001	1.233±0.013	11.89±0.271	18.24±1.662
Positive control	1.038±0.011^{D}	1.462±0.024^{D}	13.25±0.120	21.18±1.585
2-pentadeconone	1.229±1.003^{D}	1.228±0.001	14.55±0.019	13.19±0.112^{A}

Data expressed as mean±SD (n=3) and analyzed by one-way ANOVA followed by Tukey's multiple range test for each parameter separately. ^{A}P<0.05, ^{B}P<0.01, ^{C}P<0.001, ^{D}P<0.0001 compared to the negative day 12 (hydroxyproline assay) and negative day 7 (glutathione assay). SD: Standard deviation

Hydroxyproline and glutathione activity

Wounds treated with 2-P in the hyperglycemic control no significant difference compared with the negative group. Treatment with 2-P significantly increased the hydroxyproline activity.

Histology analysis

Epithelialization evaluation diabetic group

In the diabetic control, the negative group showed defined wound margin on day 2 and day 4, the wound had fully re-epithelized, however, it was observed that on day 7 the epidermis had thickened (hyperplasia) [Figure 3]. The wound keratinocytes had fully differentiated by day 12. In the positive group, re-epithelialization was <50% on days 2 and 4. By day 7, the positive group showed extreme hyperplasia with detachment of epidermis from dermis. 2-P had no clear wound margin on day 2 but showed >50% migration on day 4; this confirmed the significantly accelerated wound closure on day 4. Statistical analysis comparison of epithelialization amongst the diabetic group, 2-P, and mupirocin [Figure 4]. The ANOVA nonparametric analysis found a significant difference in the medians (P < 0.05); however, there was no significant difference in the multiple comparisons test with Dunn's test.

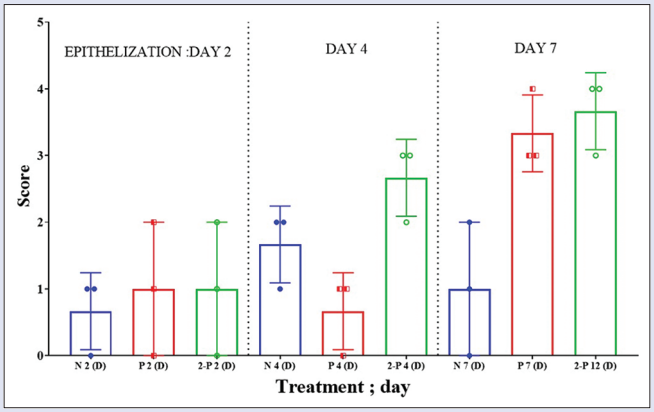

Figure 4: Epithelialization histopathological analysis with no significant difference compared with the Negative group

Polymorphonuclear leukocytes evaluation diabetic group

Negative control in the diabetic group showed high PMNL cells on the wound margin on days 2 (wound margin) and 4 (granulation tissue). Mupirocin had observed PMNL cells on the wounds cut edges and subcutaneous layer on days 2 and 4 but not as much as the negative group. 2-P had moderate amounts of PMNL cells on days 2 and 7 compared with the negative group [Figure 5]. The nonparametric ANOVA statistical comparison of PMNL cells in the diabetic group found significance with Kruskal–Wallis P value summary of P < 0.001; this shows the difference in the groups median values. However, there was no significant difference after a multiple comparison tests with Dunn's test. The PMNL cells analysis is represented in Figure 6.

Fibroblasts evaluation diabetic group

Fibroblast proliferation in the diabetic group was low on day 7 in the negative group and as shown in Figure 4(A). Positive control had mild amounts of fibroblast on days 2 and 4 but high amounts on day 7 compared with the negative group. 2-P showed high amounts of fibroblasts on days 7 and 12 [Figure 7 and Figure 8]. Nonparametric analysis found significant difference in the medians between the groups (P < 0.001); however, there was no significant difference in the multiple comparisons group with Dunn's test.

DISCUSSION

S. aureus has been reported to be the most frequently isolated bacteria in diabetic foot infections and has been found to negatively influence the wound healing sequence.[22] It has been shown to reduce growth factor

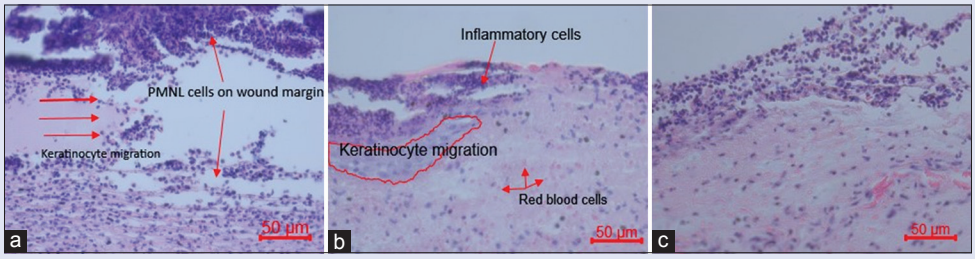

Figure 5: Histopathology sections on day 2 (×40) (a) Negative group: High number of polymorphonuclear leukocytes cells and clear signs of epidermal migration (b) Mupirocin: Reduced polymorphonuclear leukocytes cells and signs of keratinocyte migration (c) 2-pentadeconone group: Reduced Inflammatory cells and delayed keratinocyte migration

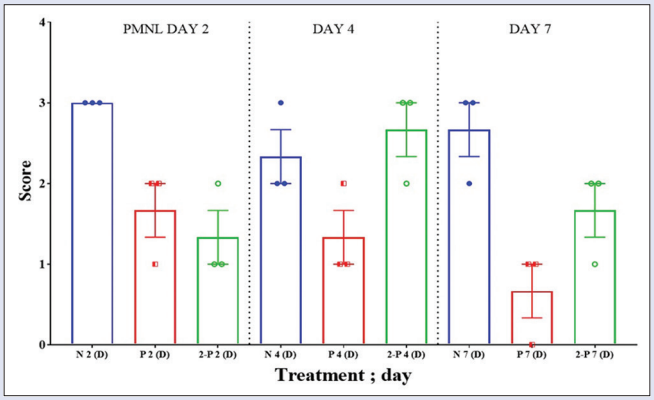

Figure 6: Polymorphonuclear leukocytes histopathological analysis with no significant difference compared with the negative group

activity, reduce cellular proliferation, elevate inflammation markers and increase proteases.[23] The inhibitory effect of 2-P on *S. aureus* which was observed in this study may explain the reason why there was a reduction PMNL in 2-P-treated group. It may have also contributed to the wound closure activity of 2-P. The wound healing process is a complex and highly-integrated cascade of events that repairs and restores the integrity of the skin in response to an injury.[21] Re-establishing the epithelial barrier and new epithelial layer formation to cover the wound is very crucial step in wound healing. The process of epidermal migration is initiated by the release of fibroblast which causes the generation of other molecules such as collagen, cytokines, PDGF, TGF-β, keratinocyte growth factor (KGF), BFGF, and insulin-like growth factor-1. Fibroblasts also assemble collagen molecules into fibers, which are cross-linked and organized into bundles.[4] The increasing content of wound collagen correlates with increasing tensile strength. The molecules released initiates chemotaxis and produces neutrophils which stimulate proliferation and angiogenesis through a balanced MMP activity. MMPs stimulate the development of a new ECM via angiogenesis and tissue remodeling. Mmp-1 digests collagen, weakening the membrane attached to the matrix. Hence, new epithelial cells are able to migrate across the collagen matrix to begin the proliferation of a new matrix.[5] 2-P treatment on day 4 showed significant ($P < 0.05$) wound closure and also improved the tissue remodeling effect on the wound. The significant epithelial migration observed in 2-P-treated group especially on day 4 and subsequently the wound closure might be because of the stimulation of the release fibroblast which could cause the generation of other molecules such as collagen, cytokines, PDGF, TGF-β, KGF, basic fibroblast growth, insulin-like growth factor-1 which are necessary to enhance wound healing. However, the detailed mechanism of action needs more research investigation.

PMNL are the most abundant white blood cells in the blood circulation system, consisting of neutrophils and macrophages that are mostly found at the injury site under the scabbing.[24] PMNL cells are dominant in the scabbing area in the first 2 days of wound healing, their number then decreases as the debris and bacteria count diminish, however higher PMNL accumulation has been reported in diabetic wounds.[17,18] Studies by Guo and Dipietro have shown increased PMNL activity delays wound healing. It has been reported that in chronic wounds, PMNLs could be present continuously because of constantly trauma such as pressure, infection, leukocyte-trapping or ischemic-reperfusion injury. Hence, the recruitment of PMNL cells is being constantly activated. In this study, 2-P decreased the PMNL infiltration in diabetic wound healing, thereby reducing the inflammation and enhancing the wound healing. This result is in consonance with our previous reports which purified mp extract showed anti-inflammatory effect.[12,13,25] There have been studies that have suggested that the crude and purified extract has anti-inflammatory activities such as the reduction of pro-inflammatory mediators and reduction in eosinophil infiltration. This study could suggest that 2-P plays a major role in mp anti-inflammatory activity.

Fibroblasts are cells that secrete procollagen and elastic fibers.[26] Procollagen is cleaved by enzymes into collagen that crosslinks. The cross-linked collagen strengthens the dermis by providing tensile strength and resistance to mechanical forces.[26] Elastic fibers resist deformational forces and restore the skin to its resting shape.[26] Days 12 onward, is characterized by the increased collagen content which brings about an increase in elasticity/tensile strength.[17] 2-P treatment induced the proliferation of fibroblasts even though it was not significantly different from the negative. The fibroblast proliferation could have been the major influence in the increase wound closure as the collagen deposition was also increased.

Hydroxylation of proline and lysine in the formation of collagen fibers has been reported as an indicator for collagen synthesis.[27] Chua *et al.*[28] reported the stimulation of collagen after using mp on *in vitro* dermal fibroblast cells; however, they did not report the bioactive ingredient responsible for the effect. The synthesis of collagen is crucial to building the integrity of the wound, which is impaired in diabetic wounds.[29] This study suggests that the collagen increasing ability of the drug on the dermal fibroblasts could be due to the activity of 2-P. The increase of the fibroblast proliferation observed in 2-P compared to positive control maybe because of the ability of 2-P to initiate a chemotactic event that produces neutrophils which stimulates proliferation and angiogenesis through a balanced MMP activity. The study done by rasik and Shukla,[9] reported the down-regulation of reduced-glutathione activity in the skin of diabetic wounds. Studies conducted by Norhaiza *et al.*[30] where they reported that mp crude extract possessed antioxidant activity. Compounds with antioxidant activity have been shown to upregulate glutathione activity.[31,32] 2-P was not able to significantly increase the

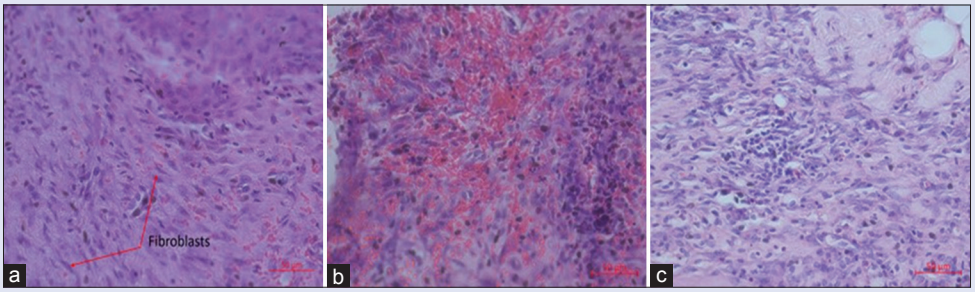

Figure 7: Fibroblast proliferation histopathology analysis (×40) (a) Negative group: Reduced fibroblast proliferation (b) mupirocin group: Mild proliferation of fibroblasts (c) 2-pentadeconone group: Increased fibroblast proliferation

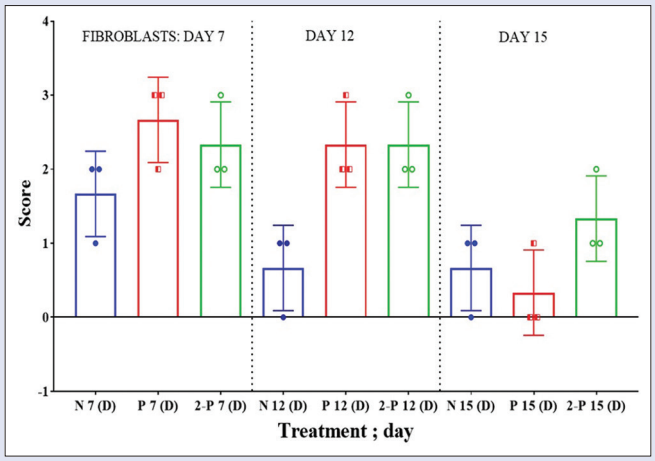

Figure 8: Fibroblast proliferation histopathological analysis with no significant difference compared with the negative group

glutathione activity compared with the negative group. However, based on the structure, it has the ability to donate electrons that have the ability to scavenge free radicals.

Standard Protocol on Approvals, Registrations, Patient Consents, and Animal Protection:

Animal ethics approval was obtained from Ethics board UCSI University.

CONCLUSION

In this study, 2-P was able to accelerate wound closure, possibly due to its ability to inhibit *S. aureus*, increased epidermal migration, reduced inflammation, increased fibroblast proliferation and collagen deposition. This suggests 2-P has the potential to be used as an active ingredient in the formulation of a diabetic wound healing cream.

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Conflicts of interest

There are no conflicts of interest.

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