SCIENTIFIC CULTURE

SCIENTIFIC CULTURE, Vol. 12, No.5.1, (2026), pp. 427-438
Open Access. Online & Print

www.sci-cult.com

DOI: 10.5281/zenodo.12511035

WOUND SPRAY WITH *GARCINIA MANGOSTANA* PERICARP EXTRACT IMPROVES WOUND HEALING IN A DIABETIC RAT MODELS

Kelvin Soo[2,3], Hanan Kumar Gopalan[1], Tong Woei Yenn[1,2,6*], Leong Chean Ring[2], Tan Wen-Nee[4], Mushtak T.S. Al-Ouqaili[5], Utami Maisari[7]

[1]*Universiti Kuala Lumpur, Institute of Medical Science Technology, A1, 1, Jalan TKS 1, Taman Kajang Sentral, 43000 Kajang, Selangor, Malaysia.*
[2]*Universiti Kuala Lumpur, Malaysian Institute of Chemical and Bioengineering Technology, Lot 1988 Kawasan Perindustrian Bandar Vendor, Taboh Naning, Alor Gajah, Melaka, Malaysia.*
[3]*Furley Bioextracts Sdn Bhd, 7, Villaraya, Villaraya Industrial Park 3, 23rd Mile, Jalan, Jalan Sungai Lalang, 43500 Semenyih, Selangor, Malaysia.*
[4]*Chemistry Section, School of Distance Education, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia.*
[5]*Department of Microbiology, College of Medicine, University of Anbar, Ramadi, Iraq.*
[6]*Department of Biotechnology and Bioengineering, School of Biosciences and Technology, Gagotias University, U.P, Greater Noida, 203201, India.*
[7]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Islam Indonesia, Yogyakarta, 55584, Indonesia. *wytong@unikl.edu.my.*

ABSTRACT

Microbial infection is a frequent complication of diabetic foot ulcers, with up to 82% of ulcers being infected at initial stage of diabetes. *Garcinia mangostana* is widely grown as fruit tree in south east Asia, and the its wound healing effect is well reported. However, the efficacy of the ready-to-use form product has not been investigated. Therefore, in this study, we aimed to evaluate the wound healing efficacy of Spray 8, a wound spray using *G. mangostana* pericarp extract as active ingredient. The animals were subjected to induction of diabetes mellitus using streptozotocin. The wound healing efficacy was evaluated via histological, microbiological and molecular approaches. In the group treated with Spray 8, the time required for wound closure was reduced to 21 days. Based on histological examination, treatment with Spray 8 significantly increased epithelialisation and the granulation tissue deposition. Besides, the spray also promotes angiogenesis during the proliferation and remodelling phases. Spray 8 also reduced the inflammatory cell infiltration. A 99.9% reduction in bacterial burden was observed on Day 14. In conclusion, wound spray with *G. mangostana* extract significantly improved the wound healing in diabetic rat models.

Keywords: *Diabetic wound, Garcinia mangostana, Antimicrobial activity, Mangosteen pericarp, Wound healing, Wound spray.*

Funding Source

This study received no specific grant from any funding agency.

Ethical Approval

All animal protocols in this study were reviewed and approved by the Animal and Research Ethics Committee, Universiti Kuala Lumpur (UniKL/REC/2021/01).

1.0 Introduction

Diabetes mellitus is a chronic condition that affects millions of people. It is anticipated that the incidence will increase by 7.7% by 2030, with 439 million patients worldwide (Chen et al., 2025). Pednekar et al. (2015) found that amputation risk in diabetic patients with foot ulcers was 150 times higher., which is always associated with inadequate wound management. The diabetic ulcer also considerably increases the health care expenditures of patients and the nation. Therefore, appropriate wound management with an

antimicrobial dressing is required to improve the patients' quality of life (Chen et al., 2025).

To accelerate the healing of the chronic wounds, antiseptic spray products are applied topically, particularly for home health care (Biter et al., 2014; Verbeken et al., 2020). Silver ions are commonly used as active ingredient in the wound spray products. However, there have been concerns raised about the emergence of bacteria resistance to heavy metal ions (Tong et al., 2017). Additionally, prolonged use of heavy metal preparations might cause the skin to accumulate heavy metal particles. Hence, an alternative is needed to replace the usage of heavy metal ions. Today, natural products have been added in wound care products to promote the healing of wound. The phytochemical constituents of the plants are able to reduce the microbial load and promote blood clotting (Thangapazham et al., 2016). *Garcinia mangostana* is a fruit tree with a pyramidal crown that grows slowly. The large calyx at the stem end caps the round-shaped fruits, which have 4 to 8 triangular remnants of the stigma in the shape of a rosette at the apex (Misra et al., 2009). This plant is widely grown as a component in mixed crop plant in south east Asian countries (Semangoen et al., 2025). The major phytochemicals are xanthone derivatives. The major constituents are alpha and gamma mangostin (Misra et al., 2009). To date, more than 60 xanthone derivatives were isolated from the pericarp of G. *mangostana* and their pharmacological activities were also widely reported (Misra et al., 2009).

In previous studies, we reported the potential use of *G. mangostana* extract to improve the healing of diabetic wounds (Ring et al., 2019). The extract exhibited broad spectrum antimicrobial activities on 3 Gram positive bacteria, 3 Gram negative bacteria, and all test fungi (Ring et al., 2019). Furthermore, nitric oxide production from lipopolysaccharide-stimulated RAW 264.7 cells was significantly reduced with the presence of *G. mangostana* extract. Inflammation is a significant pharmacological target for inducible nitric oxide. As a result, the inhibition of nitric oxide production by extract may have potential therapeutic value in the context of inflammation (Chen et al., 2008). The wound healing effect of *G. mangostana* extract was reported in many studies (Charernsriwilaiwat et al., 2013; Gondokesumo et al., 2020; Qian et al., 2021; Tatiya-Aphiradee at al., 2019). However, the efficacy of the ready-to-use form product has not been investigated. Besides, there is no evidence of efficacy via transcriptomic approach, and the wound healing mechanism is unknown. Therefore, in this study, we aimed to evaluate the wound healing efficacy of Spray 8, a wound spray using *G. mangostana* pericarp extract as its main active ingredient on diabetic rat models. The wound healing efficacy of Spray 8 was evaluated via histological and molecular approaches. Besides, the effect of topical application of Spray 8 on the microbial load of diabetic ulcers was also assessed.

2.0 Materials and Methods

2.1 Test substance

The test substance Spray 8 was provided by Furley Bioextracts Sdn Bhd, Semenyih, Malaysia. The spray was stored at room temperature (25±2°C) during the study.

2.2 Test animals and ethics

In this research, 36 male Sprague Dawley rats devoid of anomalies were utilised. Animal and Research Ethics Committee (AREC), Universiti Kuala Lumpur (UniKL/REC/2021-01) reviewed and approved all animal protocols in this study. All procedures were conducted in accordance with the protocol approved by AREC and the code of practice for the care and use of animals for scientific purposes. This investigation was also reported in accordance with the ARRIVE guidelines' principles. Efforts were made to minimize suffering in test animals, and only the minimum number of test animals required to generate reliable data was used in this study. At the start of this research, the rats were 12 weeks old and weighed between 200 and 250 grammes. Twelve rats were assigned to each of the three groups: (I) the 7-day treatment group, (II) the 14-day treatment group, and (III) the 21-day treatment group. Four rats were kept in an open stainless-steel cage (59.5 × 38 × 20 cm^3) with a solid bottom and pine sawdust as bedding. They were maintained on a 12-hour light–dark cycle. The animal cages were disinfected daily with 70% ethanol. Throughout the duration of the study, the rats were fed a standard rat diet (Specialty Feed, Australia) and provided with ad libitum access to tap water in polycarbonate bottles.

2.3 Induction of diabetes mellitus

The animals were subjected to induction of diabetes mellitus via injection of streptozotocin (Sigma) (Shi et al., 2018). The rats were fasted overnight before receiving an intraperitoneal injection of 65 mg/kg streptozotocin dissolved in 0.1 M sodium citrate buffer (pH 4.5). Blood sample was withdrawn through a tail vein puncture, and the blood glucose was measured by using an Accu-Check Glucometer 48 hours following induction. The threshold value of fasting blood glucose was >11 mmol/L. Thus, a constant reading of >11 mmol/L were considered as diabetic. Fasting blood glucose was monitored constantly to ensure their diabetic state. The diabetic state was then

maintained for about 4 weeks to obtain a chronic type 2 diabetes mellitus.

2.4 Intraperitoneal glucose tolerance test (IPGTT)

The diabetic state of the rats was confirmed via an intraperitoneal glucose tolerance test (IPGTT) before testing (Feng et al., 2017). Briefly, each rat was administered glucose (100 mg/kg) via intraperitoneal injection, and a blood sample was collected via tail puncture at 0, 30, 60, 90, and 120 minutes. The blood glucose was determined using an Accu-Check Glucometer. All of the rats subjected to this test showed a glucose reading of >11 mmol/L, and thus the diabetic state of the rats was confirmed. A week after successfully inducing diabetes, the animals were used to create the wound healing models.

2.5 Draize skin irritation test

The animals' dorsal surfaces were shaven with an electric clipper twenty-four hours prior to the application of the test substance. This was performed with caution to prevent skin injury that could alter the permeability. As an antiseptic, methylated spirit was administered to the shaved area to prevent bacterial infection. The test substance was then topically applied to the free surface of the skin. The application site in terms of erythema or oedema was examined at 24, 48, and 72 h to observe changes or dermal reactions (Kamel et al., 2019).

2.6 Wound models

All animals received intraperitoneal injections of ketamine (5 mg/kg) and xylazine (2 mg/kg) to induce anaesthesia. Under anaesthesia, the animals' fur was removed by using an electric clipper to shave the dorsal back of the rodents. Prior to creating the wound, methylated spirit was administered to the shaved portion as an antiseptic. Two circular wounds, 8 mm in diameter, were made using a sterile surgical biopsy punch (Robbins Instruments) on the dorsal surface of the animals. The wound was treated with Spray 8 twice daily for 21 days, while sterile saline water (RinsCap) twice daily was applied for control group. During the study, researchers were aware of group allocation, but they were blinded to group assignment for sample analysis. The wound contractions were measured using a digital calliper (Mitutoyo Japan) on the wounded margin, and the time of epithelialization was measured as the number of days needed for the eschar to peel off completely without leaving any trace of a raw wound. Throughout the trial, the rats' body weights were checked every day.

2.7 Histological analysis

Some of the rats were carbon dioxide euthanized at each interval (7, 14, and 21 days), and wound samples were removed and dissected (Thangapazham et al., 2016). The samples were sectioned at 5 µm on a microtome after being fixed in 10% formalin. For histological investigation, the paraffin-embedded sections were stained with hematoxylin and eosin. Epithelial gaps in the photographs were evaluated, and the total area of the granulation tissues was observed under light microscope.

2.8 Bacterial load assessment

The superficial swab sampling method was used to collect specimens from the surface of the wound areas. Sterile cotton swabs were used to collect the samples and the swabs were suspended in sterile phosphate-buffered saline (pH 7). Serial dilutions of the suspensions were plated on blood agar (Oxoid) to determine the bacterial load of the wound (Thangapazham et al., 2016).

2.9 Tissue samples preparation

At 7, 14, and 21 days post-wounding, full-thickness dorsal biopsies were harvested from the rats. The wounds were excised and bisected flush at the wound margin. The wound tissues were divided in half, and the tissues were snap-frozen in liquid nitrogen before being stored at 70 °C until analysis**.**

2.10 Total RNA extraction and quantitative real time polymerase chain reaction (RT-PCR)

Each specimen was ground in liquid nitrogen using a mortar and pestle in order to isolate the RNA. The TRIzol® Reagent (Thermo Fisher) was used to extract total RNA in accordance with the manufacturer's instructions. cDNA was synthesised from total RNA using random decamers and reverse transcriptase (Invitrogen) according to the manufacturer's instructions (Feng et al., 2017; Khalid et al., 2019; Oh et al., 2010). cDNA synthesised from 50 ng of total RNA was used for real-time PCR. The quantitative real-time RT-PCR analysis utilised gene-specific primers for the target genes and beta-actin as the housekeeping gene. Real-time PCR was conducted using the Step One Real-Time PCR System (Life Technologies) and SYBR Green Real-Time PCR Master Mix (Life Technologies).

2.11 Statistical analysis

The statistical analysis was conducted using version 24 of Statistical Programme for the Social Sciences (SPSS) (IBM, United States). Percentages, means, and standard deviations were calculated for variables when applicable. To evaluate the effectiveness of the test groups, a one-way ANOVA

was performed, and $p \leq 0.05$ was deemed statistically significant.

3.0 Results and Discussion

3.1 Draize skin irritation test

The test was performed to assess the dermal irritation of the test substance (Kamel et al., 2019). In the present study, no sign of redness and itching was observed when Spray 8 was applied to the shaved back of each test animal. Oedema refers to swelling brought on by an excess of fluid trapped in the body tissues, whereas erythema refers to skin redness brought on by increased blood flow in superficial capillaries (Oh et al., 2010). The skin irritation indexes for erythema and oedema were both 0. This suggested that Spray 8 did not cause erythema or oedema on the skin. The irritation index has indicated that the extract has a non-irritant nature based on information from the dermal irritation scoring system of the Draize and Environmental Protection Authority. The results from this study were similar to many other research articles that have studied the pericarp extract of *G. mangostana* (Saptarini & Hadisoebroto, 2017; Lee et al., 2010; Zuo et al., 2018).

3.2 Intraperitoneal glucose tolerance test (IPGTT)

Following 3 weeks of monitoring from the day of induction, the random blood glucose reading for the rats was 15mmol or above. IPGTT was performed to verify that the rats were in a diabetic condition. The glucose solution was injected intraperitoneally into the animal models, and the glucose levels were measured after 2 hours. The average blood glucose level was 13.4 ± 1.4 mmol/L. These results indicate that the diabetic animal models were successfully developed (Feng et al., 2017). Thus, all of the animal models were selected for the wound portion of the study.

3.3 Wound size

The macroscopic evaluation of the wound revealed that the time taken for complete wound closure was significantly shortened in the wound treated with Spray 8 ($p \leq 0.05$). Furthermore, by Day 7, we had also observed significantly greater epithelial coverage in the wounds tested with Spray 8 and the wound was fully covered with wound crust on Day 14. Spray 8 improved wound healing compared with that of the control treatment, with a statistical significance ($p \leq 0.05$) at days 7, 14, and 21. The mean time for closure of the Spray 8 treated wounds was 21 days. In contrast, complete wound closure was not observed in the control group, even after 21 days of treatment. Moreover, the treatment with Spray 8 had significantly accelerated the wound closure at Day 7 and 14, with 28.47±3.9% and 62.32±4.8% closure.

3.4 Histological analysis

The anti-inflammatory activity of the *G. mangostana* pericarp extract was reported by Chomnawang et al., 2007. Figure 1 shows the progress of the wound healing induced by the spray. Histological observation showed significant differences between Spray 8 treated wounds and the control wounds on Day 7 in terms of new tissue organisation, cellular infiltration, and the collagen matrix deposition. Heavy cellular infiltration was observed in the control, with poor collagen formation. The heavy cellular infiltration indicated the inflammatory response of the test animals. However, tissue reconstruction and granulation were observed in the wounds tested with Spray 8 as immature granulation tissues were observed along with some collagen formation. Therefore, the presence of granulation in the tissues signified the beginning of the tissue regrowth stage.

On Day 14, treatment with Spray 8 resulted in significant granulation tissue formation while, no significant improvement in wound healing was observed in the control group. Furthermore, poorly formed tissues were found in the control group. Treatment with Spray 8 significantly increased epithelialisation and granulation tissue deposition. Spray 8 significantly improved tissue regeneration, as evidenced by a well-differentiated epidermis and a significantly denser dermis. Blood supply's significance in wound healing has been reported (Bagodi et al., 2025; Wang et al., 2016). Histological observation revealed that Spray 8 had significantly increased neovascularisation on Day 14, compared with that of the control. The formation of new blood vessels was observed, and visible collagen fibre formation was also observed in the Spray 8 treated wounds. This indicated good perfusion to the wounded area.

The biological effects of *G. mangostana* extract on wound healing are diverse. It was observed that the wound contracting ability was significantly better compared to control. Figure 2 showed that almost a complete healing occurred in the treated animals, which was achieved on Day 21. In contrast, ulceration and oedema were still observed on the wound sites in the control group, which signified poor inhibition of the microbial growth on the wound. Moreover, 21 days following treatment, a complete re-epithelialisation of the wounds treated with Spray 8 had occurred, that exhibited denser granulation tissue and more extensive hair follicle development. It has been reported that a plant-based wound healing remedy should accelerate at least two different processes of wound healing. In this regard, Spray 8 with *G. mangostana* extract has met these criteria as this spray increased wound contraction and neovascularisation, thus reduced

the epithelisation time. The effect of Spray 8 on the contraction and epithelisation of the wounds suggests a possible effect in promoting the migration and proliferation of the epithelial cells.

3.5 Bacterial load assessment

Bacterial load in the wound plays an important role in wound recovery. The high bioburden of a chronic wound would delay the tissue remodelling process. Figure 3 shows the bacterial burden of the wound healing induced by Spray 8. The wounds treated with Spray 8 exhibited a significantly lower bacterial burden, with a statistical significance on Day 7, 14, and 21 ($p \leq 0.05$). A 99.9% reduction in bacterial load was observed on Day 14 via daily topical application of Spray 8. The antimicrobial efficiency of *G. mangostana* pericarp extract was also reported (Liakos et al., 2014; Ragasa et al., 2010). Bacterial colonisation of the wound slowed down the wound healing, and reducing the wound's bacterial load could accelerate the healing process.

3.6 Quantitative real time polymerase chain reaction (RT-PCR)

An ideal drug for diabetic wound dressing should promote rapid contraction of wound that leads to quick healing and reduce the wound epithelisation time (Xu et al., 2025). Three overlapping phases comprised the wound healing process: inflammation, proliferation, and remodelling. Following skin injury, the platelets promoted clot formation and other release factors that attracted immune cells to the wound site (Dhivya et al., 2015). This marks the beginning of the inflammatory phase, which usually lasted for 14 days or more. This was a dynamic process whereby numerous immune cells took part by secreting cytokines and growth factors that assisted in clearing the microbes at the wound site and promoted healing. Various cytokines, including TNF-α and IL-1 were produced by neutrophils at the wound site and used to recruit the fibroblasts and epithelial cells (Witko-Sarsat et al., 2000). While macrophages that were important for the phagocytic process released growth factors like PDGF, TGF-β, β-FGF as well as cytokines like TNF-α, interleukin 1 (IL-1), and IL-6 (Figure 4). In thisstudy, the expression of IL-1, IL-6, and TNF-α were higher in the control group compared to the wounds treated with Spray 8, throughout the study ($p \leq 0.05$). These results were in agreement with the observations by microscopic examination of the wound tissue as well as the bacterial load at the wound site. The bacterial load was high, and wound closure was not observed in the control group, even after 21 days of treatment. Higher expression of cytokines at the wound site might reflect the continuous infiltration of the immune cells, including macrophages, into the wound site due to the relatively high microbial load of the placebo group (Cianci et al., 2010). In chronic wounds, the proliferative and remodelling stages did not occur readily. The wound was unable to heal because it persisted in the inflammatory phase, which does not promote tissue regeneration (Wolcott et al., 2009). The application of Spray 8 targets and corrects the cellular causes of protracted inflammation in chronic wounds, and may be an effective means of restoring the wound to a state of healing.

In order to regenerate tissues at wound site, wound cells proliferate and migrate during the proliferation stage. The 3 phases of proliferation include fibroplasia, granulation, and epithelialisation (Nisbet et al., 2010). Granulation tissue is deposited, and keratinocytes migrate over it to close the laceration (Patra et al., 2015). Granulation tissues replace the damaged vasculature during angiogenesis. Epidermal cells, fibroblasts, vascular endothelial cells, and macrophages contributed to angiogenesis by the production of β-FGF, TGF-β, and VEGF. In the present study, a significant difference in the expression level of VEGF and MMMP-3 was observed between the control and the wounds treated with Spray 8 ($p \leq 0.05$). Despite this, no significant differences were observed in the EGF and IGF3 expression. Hypoxia regulated the proliferative effects of VEGF, stimulating VEGF-induced angiogenesis via adenosine. (Du et al., 2015). Thus, *G. mangostana* extract in Spray-8 could possible promote angiogenesis as well as granulation tissue. However, Spray-8 might not have a prominent effect on epithelialization since the expression of EGF and IGF-3 was not upregulated in the wound site. The process of remodelling constitutes an integral component of the resolution phase of the healing process. The egress of inflammatory cells is accompanied by a reduction in the population of cells that secrete growth factors. We also looked into the expression marker for the growth factors such as GM-CSF and TGF-beta. A significant difference in these growth factors was found between the placebo group and the one treated with Spray-8 on Day 7 and 14 post-treatment. This indicated that Spray-8 had a prominent effect during the inflammation and proliferation phases and, to a lesser extent, during the remodelling phase.

4.0 Conclusions

In conclusion, Spray 8 with *G. mangostana* extract significantly improved the wound healing in the diabetic wound model. It promoted wound healing by (I) reducing the bioburden of the wound, (II)

promoting the formation of granulation tissues and blood vessels on the wound, and (III) reducing the inflammatory cell infiltration.

Acknowledgement
We are thankful to Universiti Kuala Lumpur.

References

1. Biter, L.U., Beck, G.M., Mannaerts, G.H., Stok, M.M., Van der Ham, A.C., Grotenhuis, B.A., 2014. The use of negative-pressure wound therapy in pilonidal sinus disease: a randomized controlled trial comparing negative-pressure wound therapy versus standard open wound care after surgical excision. Dis. Colon. Rectum. 57(12), 1406-1411.
2. Charernsriwilaiwat, N., Rojanarata, T., Ngawhirunpat, T., Sukma, M., Opanasopit, P. 2013. Electrospun chitosan-based nanofiber mats loaded with *Garcinia mangostana* extracts. Int. J. Pharm. 452(1-2), 333-343.
3. Chen, L.G., Yang, L.L., Wang, C.C., 2008. Anti-inflammatory activity of mangostins from *Garcinia mangostana*. Food Chem. Toxicol. 46(2), 688-693.
4. Chomnawang, M.T., Surassmo, S., Nukoolkarn, V.S., Gritsanapan, W., 2007. Effect of *Garcinia mangostana* on inflammation caused by *Propionibacterium acnes*. Fitoterapia. 78(6), 401-408.
5. Cianci, R., Pagliari, D., Pietroni, V., Landolfi, R., Pandolfi, F., 2010. Tissue infiltrating lymphocytes: the role of cytokines in their growth and differentiation. J. Bio. Regul. Homeost. Agents. 24(3), 239-242.
6. Dhivya, S., Padma, V.V., Santhini, E., 2015. Wound dressings–a review. BioMed. 5(4), 128-131.
7. Du, X., Ou, X., Song, T., Zhang, W., Cong, F., Zhang, S., Xiong, Y., 2015. Adenosine A2B receptor stimulates angiogenesis by inducing VEGF and eNOS in human microvascular endothelial cells. Exp. Biol. Med. 240(11), 1472-1479.
8. Feng, X.T., Tang, S.Y., Jiang, Y.X., Zhao, W., 2017. Anti-diabetic effects of Zhuoduqing formula, a Chinese herbal decoction, on a rat model of type 2 diabetes. Afr. J. Tradit. Complement. Altern. Med. 14(3), 42-50 (2017).
9. Gondokesumo, M.E., Sumitro, S.B., Handono, K., Pardjianto, B., Widowati, W., Utomo, D. H., 2020. A computational study to predict wound healing agents from the peel of the mangosteen *Garcinia mangostana* L. extract. Int. J. Bioautomation. 24(3), 265-276.
10. Kamel, R., El-batanony, R., Salama, A., 2019. Pioglitazone-loaded three-dimensional composite polymeric scaffolds: A proof of concept study in wounded diabetic rats. Int. J. Pharm. 570, 118667.
11. Khalid, R.S., Khan, I., Zaidi, M.B., Naeem, N., Haneef, K., Qazi, R.E.M., Salim, A., 2019. IL-7 overexpression enhances therapeutic potential of rat bone marrow mesenchymal stem cells for diabetic wounds. Wound Repair Regen. 27(3), 235-248.
12. Lee, L.T., Tsai, Y.F., Hu, N.Y., Wang, C.W., Huang, K.K., Hsiao, J.K., Munekazu, I., 2010. Anti-arthritis effect of mangostins from *G. Mangostana*. Biomed. Prev. Nutr. 3(3), 227-232.
13. Liakos, I., Rizzello, L., Scurr, D.J., Pompa, P.P., Bayer, I.S., Athanassiou, A., 2014. All-natural composite wound dressing films of essential oils encapsulated in sodium alginate with antimicrobial properties. Int. J. Pharm. 463(2), 137-145.
14. Bogadi, S., Uddin, M. E., Karri, V. V. S. R., Begum, R., Abullais, S. S., Reza, M. S., & Haque, M. S. (2025). Hypoxia inducible factor HIF-1α stabilization as a transformative approach in overcoming diabetic wound healing challenges. *Journal of Drug Delivery Science and Technology*, 107296.
15. Misra, H., Dwivedi, B.K., Mehta, D., Mehta, B.K., Jain, D.C., 2009. Development and validation of high performance thin-layer chromatographic method for determination of [alpha]-mangostin in fruit pericarp of mangosteen Plant (*Garcinia mangostana* L.) using ultraviolet-visible detection. Rec. Nat. Prod. 3(4), 178-182.
16. Semangoen, T., Chotigawin, R., Sangnim, T., Chailerd, N., Yodkeeree, S., Pahasup-Anan, T., & Huanbutta, K. (2025). Antimicrobial efficacy of mangosteen (Garcinia mangostana) Peel extracts in airborne microbial control within livestock farming environments. *Microbial Pathogenesis*, *204*, 107618.
17. Nisbet, H.O., Nisbet, C., Yarim, M., Guler, A., Ozak, A., 2010. Effects of three types of honey on cutaneous wound healing. Wounds. 22(11), 275-283.
18. Chen, X., Zhang, L., & Chen, W. (2025). Global, regional, and national burdens of type 1 and type 2 diabetes mellitus in adolescents from 1990 to 2021, with forecasts to 2030: a systematic analysis of the global burden of disease study 2021. *BMC medicine*, *23*(1), 48.
19. Oh, S.H., Bae, B.K., Kim, T.G., Kwon, Y.S., Lee, J.H., Lee, K.H., 2010. Effective treatment of facial redness caused by atopic dermatitis using intense pulsed light systems. Dermatol. Surg. 36(4), 475-482.
20. Patra, C., Boccaccini, A.R., Engel, F.B., 2015. Vascularisation for cardiac tissue engineering: the extracellular matrix. Thromb. Haemost. 113(03), 532-547.
21. Pednekar, S.N., Pol, S.S., Kamble, S.S., Deshpande, S.K., 2015. Drug resistant anaerobic infections: Are they complicating diabetic foot ulcer? Int. J. Healthcare Biomed. Res. 3(03), 142-148.
22. Qian, D., Florenly, F., Liena, L., Purba, D.R., 2021. Effectiveness of ethanol extract mangosteen peel (*Garcinia*

mangostana L.) in accelerating post-tooth extraction healing in Wistar rats. Bp. Int. Res. Exact Sci. J. 4(1), 22-30.
23. Ragasa, C.Y., Crisostomo, C.J.J., Garcia, K.D.C., Shen, C.C., 2010. Antimicrobial xanthones from *Garcinia mangostana* L. Philipp Sci. 47, 63-75.
24. Ring, L., Yenn, T., Suzana, W., Tan, W., Siti, Z.B.A., Nurul, A.M.J., Muhammad, S.A.R., 2019. Antimicrobial wound dressing film utilizing nano-cellulose and gelatin as drug delivery system for *Garcinia mangostana* L extract. Malays. J. Microbiol. 15(5), 373-378.
25. Saptarini, N.M., Hadisoebroto, G., 2017. The activity of cellulase enzymes of *Ganoderma applanatum* and *Ganoderma tropicum*. J. Appl. Pharm. Sci. 7(04), 134-137.
26. Shi, R., Lian, W., Han, S., Cao, C., Jin, Y., Yuan, Y., Li, M., 2018. Nanosphere-mediated co-delivery of VEGF-A and PDGF-B genes for accelerating diabetic foot ulcers healing in rats. Gene Ther. 25(6), 425-438.
27. Tatiya-Aphiradee, N., Chatuphonprasert, W., Jarukamjorn, K., 2019. Anti-inflammatory effect of *Garcinia mangostana* Linn. pericarp extract in methicillin-resistant *Staphylococcus aureus*-induced superficial skin infection in mice. Biomed. Pharmacother. 111, 705-713.
28. Thangapazham, R.L., Sharad, S., Maheshwari, R.K., 2016. Phytochemicals in wound healing. Adv. Wound Care. 5(5), 230-241.
29. Tong, W.Y., Abdullah, A.Y.K., Rozman, N.A.S., Wahid, M.I.A., Hossain, M.S., Ring, L.C., Tan, W.N., 2017. Antimicrobial wound dressing film utilizing cellulose nanocrystal as drug delivery system for curcumin. Cellulose. 25(1), 631-638.
30. Verbeken, G., Verween, G., De Vos, D., Pascual, B., De Corte, P., Richters, C., Jennes S., 2020. Glycerol treatment as recovery procedure for cryopreserved human skin allografts positive for bacteria and fungi. Cell Tissue Bank. 13(1), 1-7.
31. Wang, Y., Han, G., Guo, B., Huang J., 2016. Hyaluronan oligosaccharides promote diabetic wound healing by increasing angiogenesis. Pharm. Rep. 68(6), 1126-1132.
32. Witko-Sarsat, V., Rieu, P., Descamps-Latscha, B., Lesavre, P., Halbwachs-Mecarelli, L., 2000. Neutrophils: molecules, functions and pathophysiological aspects. Lab Investig. 80(5), 617-653.
33. Wolcott, R.D., Kennedy, J.P., Dowd, S.E., 2009. Regular debridement is the main tool for maintaining a healthy wound bed in most chronic wounds. J. Wound Care. 18(2), 54-56.
34. Xu, R., Luo, G., Xia, H., He, W., Zhao, J., Liu, B., Yao, Z., 2015. Novel bilayer wound dressing composed of silicone rubber with particular micropores enhanced wound re-epithelialization and contraction. Biomaterials. 40, 1-11.
35. Zuo, J., Yin, Q., Wang, L., Zhang, W., Fan, Y., Zhou, Y.Y., Wang, G.D., 2018. Mangosteen ethanol extract alleviated the severity of collagen-induced arthritis in rats and produced synergistic effects with methotrexate. Pharm Biol. 56(1), 455-464.

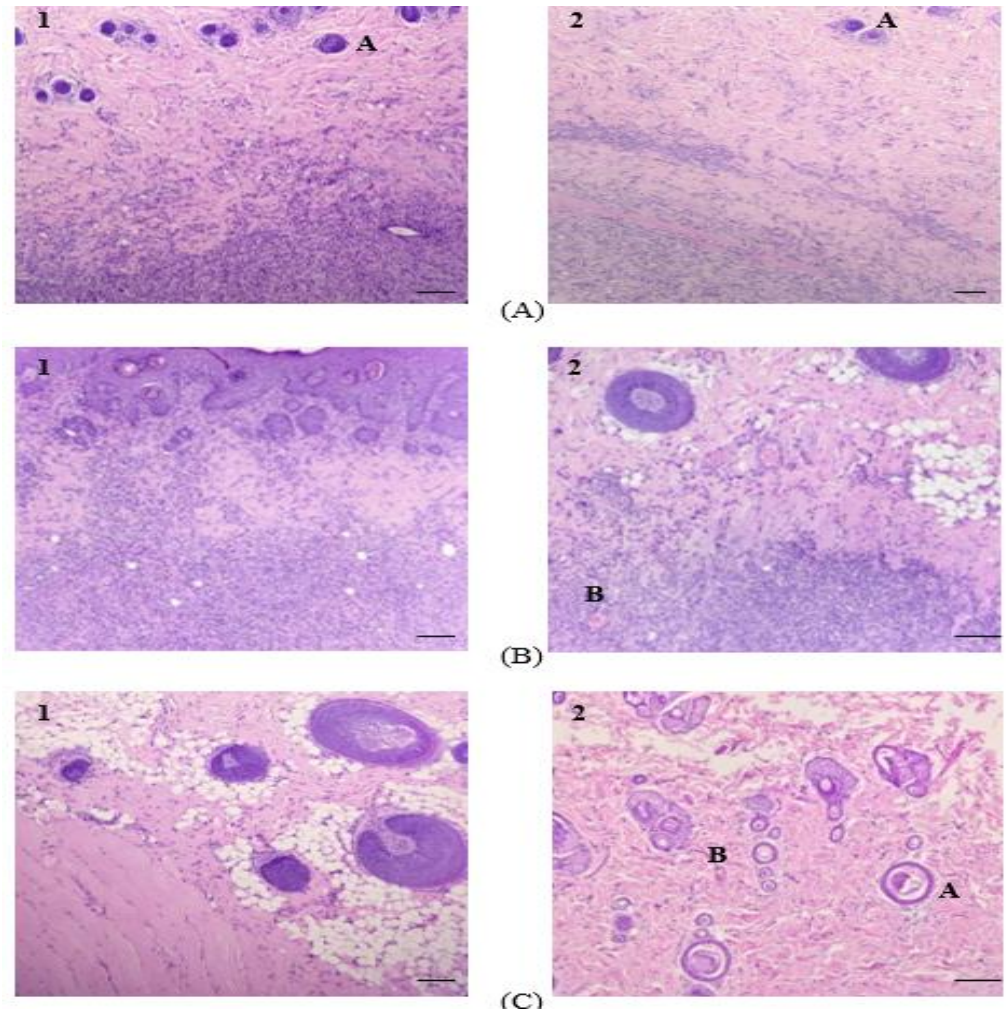


Figure 1: Histological examination of the wound tissues stained with haematoxylin and eosin on (A) Day 7, (B) Day 14 and (C) Day 21. Bars: 10 µm. The column 1 corresponds to the tissues from control group, and column 2 to those treated with Spray 8. Label A refers to the hair follicles, and B refers to blood vessels.

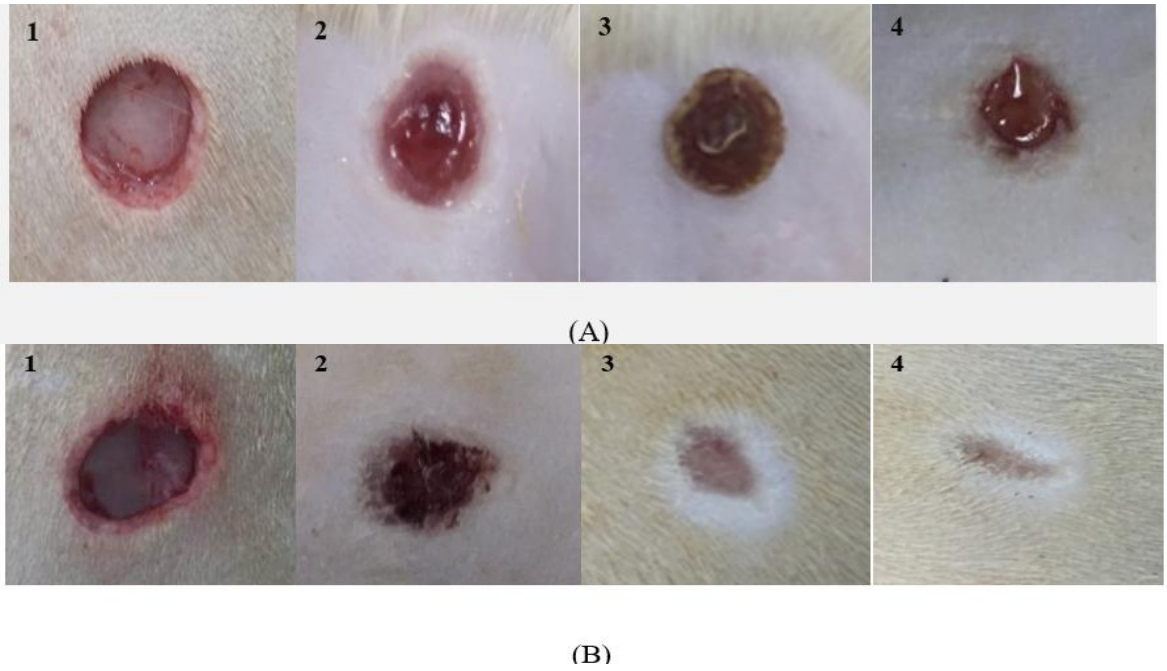


Figure 2: Macroscopic observations of the wounds treated with (A) control (n=12) (B) Spray 8 (n=12). Wound 1 were observed on Day 1, wound 2 on Day 7, wound 3 on Day 14 and wound 4 on Day 21.

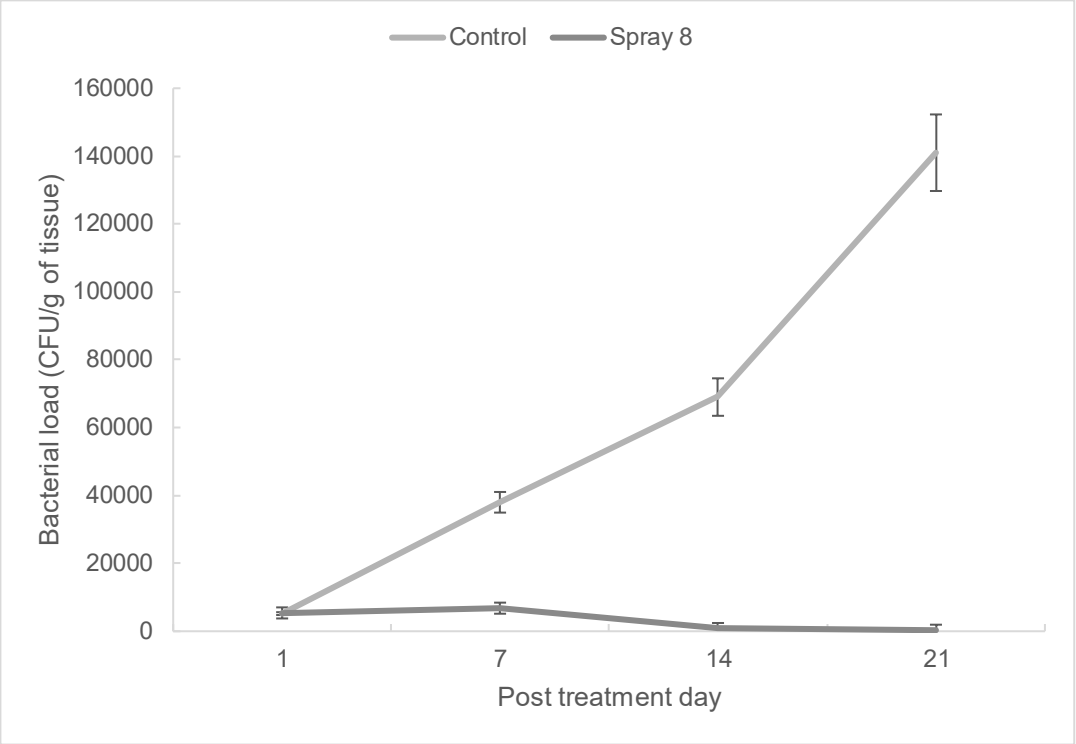
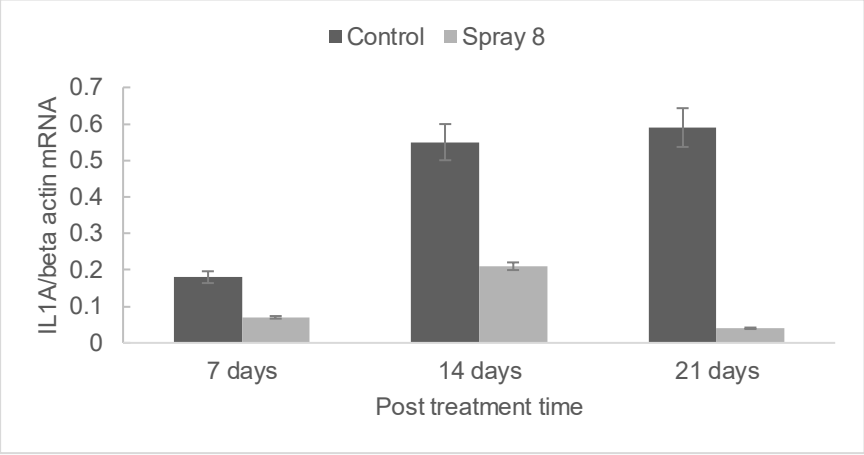
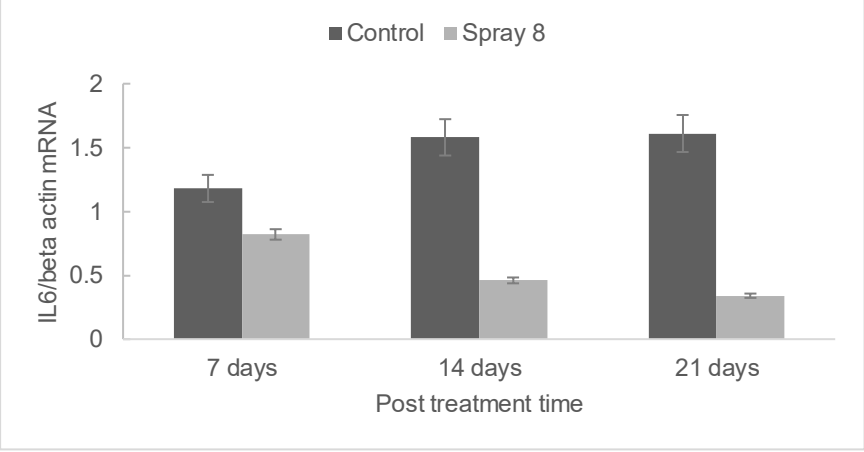


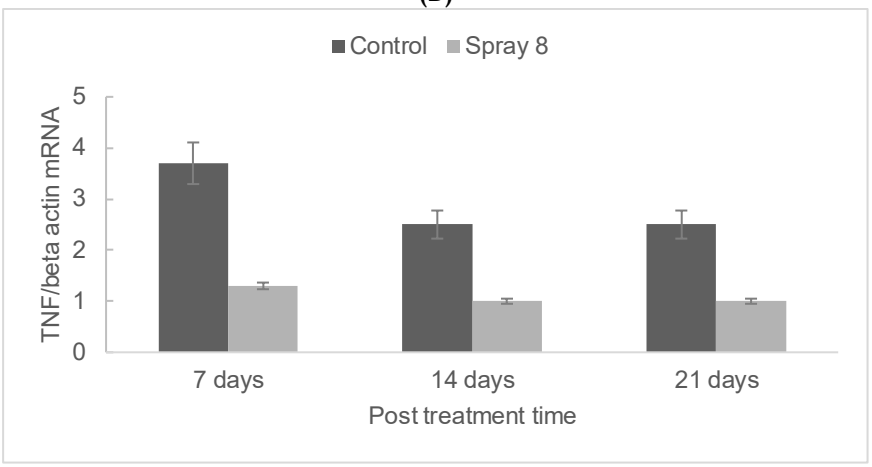
Figure 3: A 99.9% reduction in bacterial burden was observed on Day 14 via daily topical application of Spray 8 (n=12).



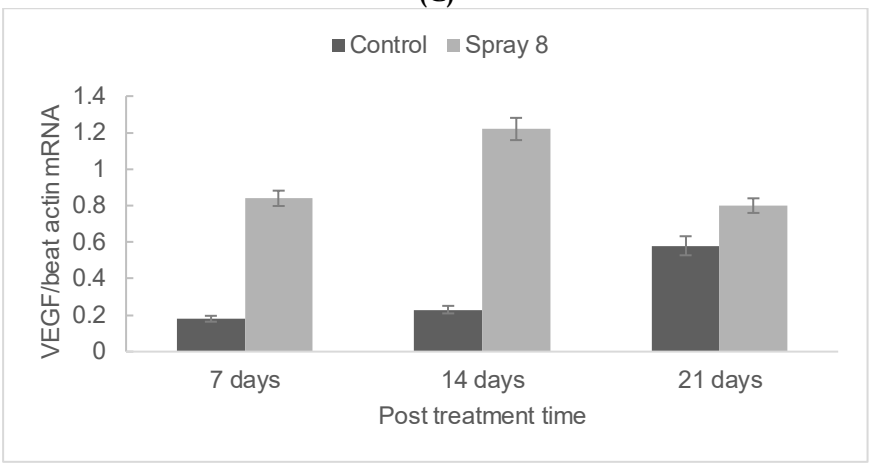
(A)



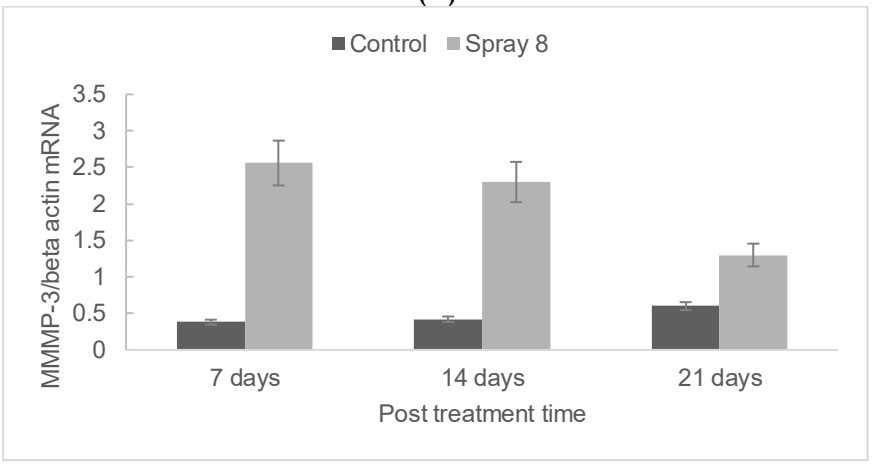
(B)


Control
Spray 8
TNF/beta actin mRNA
5
4
3
2
1
0
7 days
14 days
21 days
Post treatment time

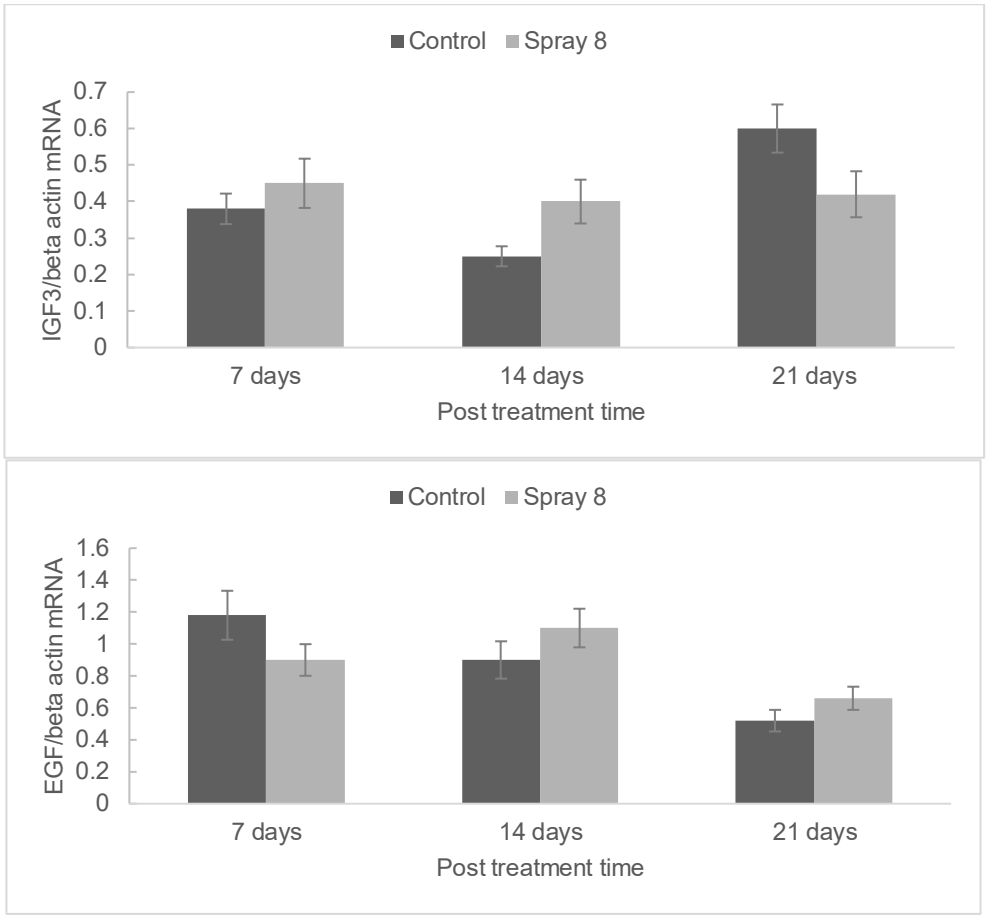
(C)


Control
Spray 8
VEGF/beat actin mRNA
1.4
1.2
1
0.8
0.6
0.4
0.2
0
7 days
14 days
21 days
Post treatment time

(D)


Control
Spray 8
MMMP-3/beta actin mRNA
3.5
3
2.5
2
1.5
1
0.5
0
7 days
14 days
21 days
Post treatment time

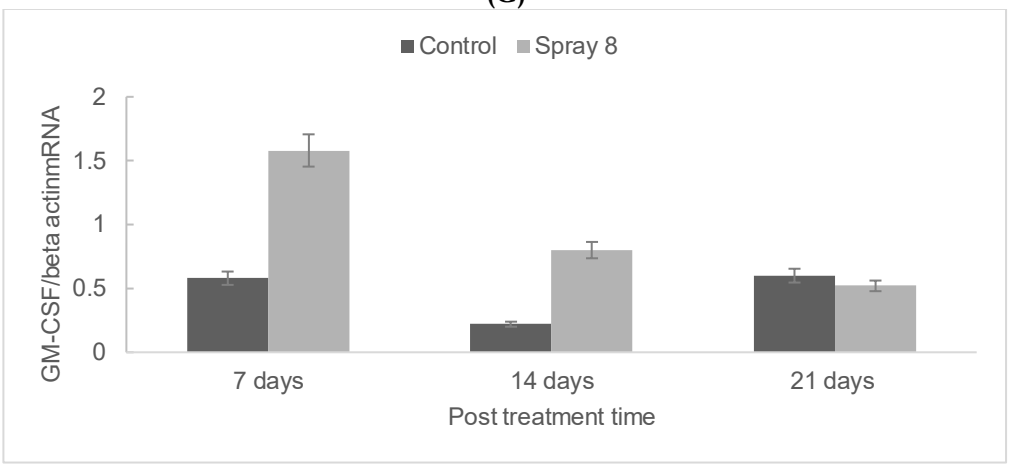
(E)


Control
Spray 8
IGF3/beta actin mRNA
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
7 days
14 days
21 days
Post treatment time

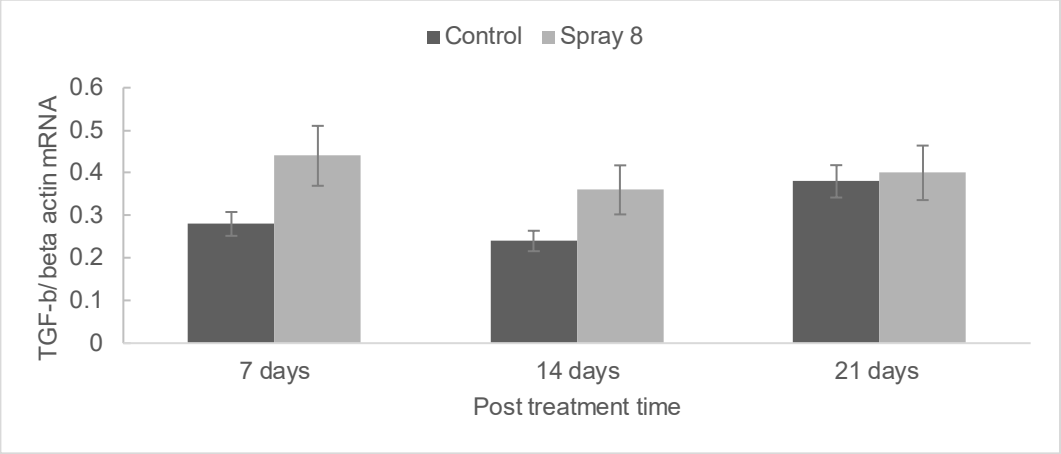
Control
Spray 8
EGF/beta actin mRNA
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
7 days
14 days
21 days
Post treatment time

(F)

(G)


Control
Spray 8
GM-CSF/beta actinmRNA
2
1.5
1
0.5
0
7 days
14 days
21 days
Post treatment time

(H)



(I)

Figure 4: Effect of Spray 8 topical application on the expression of (A) IL1A (B)IL6 (C) TNF (D) VEGF (E) MMMP-3 (F) EGF (G)IGF3 (H) GM-CSF and (I) TGF-b on the diabetic animal models.