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GREEN APPLICATION AND PROSPECTS OF WOUND HEALING APPLICATIONS ON ZEBRA FISH USING *Bougainvillea glabra* DERIVATIVES

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ABSTRACT

In traditional medicine, inflammatory disorders are treated with a variety of plants and herbs. Given the numerous side effects of modern medicine, indigenous drugs with fewer side effects should be sought out as a better alternative for treating inflammation with Bougainvillaea glabra Linn. It is now widely acknowledged that medicinal plants play an important role in traditional health care practices, providing insights into new areas of drug research and biodiversity conservation. Inflammation is a complex biological response by vascular tissues to harmful stimuli such as pathogens, damaged cells, and irritants. It is the organism's protective attempt to remove injurious stimuli while also initiating the tissue healing process, and it is thought to be the primary cause of rheumatoid arthritis. Drugs used to treat pain and inflammation have toxic side effects when taken on a long-term basis. As a result, efforts are being made to investigate promising plants, which could lead to the development of newer or safer drugs.

KEY WORDS: Herbal, Medicinal plant, Wound healing.

INTRODUCTION

Wound healing is divided into four highly integrated and overlapping phases: hemostasis, inflammation, proliferation, and tissue remodelling or resolution [1]. These phases and their biophysiological functions must occur in the correct order, at a specific time, and at an optimal intensity for a specific duration [2]. Many factors can interfere with one or more phases of wound healing, resulting in poor or impaired tissue repair. Wounds with impaired healing, such as delayed acute wounds and chronic wounds, have typically not progressed through the normal stages of healing[3]. Such wounds frequently develop pathologic inflammation as a result of delayed, incomplete, or uncoordinated healing[4]. Most chronic wounds are ulcers caused by ischemia, diabetes, venous stasis disease, or pressure[5]. Non-healing wounds affect approximately 3 to 6 million people in the United States, with people aged

65 and up accounting for 85% of these events. Non-healing wounds cause enormous health-care costs, estimated at more than $3 billion per year [2]. Laboratory investigations and clinical studies have provided a wealth of information on normal and impaired wound healing. More recently, extensive research has been conducted to better understand the critical factors that influence poorly healing wounds. While much remains to be discovered, these studies could lead to therapeutics that promote proper tissue repair and improve sluggish wound healing[6].

Wound healing is a dynamic process that consists of four continuous, overlapping, and precisely programmed stages. The events of each phase must be precise and regulated. Interruptions, anomalies, or delays in the process can result in delayed wound healing or a non-healing chronic wound. In adult humans, optimal wound healing consists of the following events:[7]

(1) Rapid haemostasis, [8]
(2) Proper inflammation,
(3) Mesenchymal cell differentiation.
Proliferation and migration to the wound site;
(4) Appropriate angiogenesis;[9]
(5) Prompt re-epithelialization (re-growth of epithelial tissue over the wound surface);
(6) Proper collagen synthesis, cross-linking, and alignment;
(7) Strengthening the healing tissue[10].

The first phase of haemostasis occurs immediately following wounding, with vascular constriction and fibrin clot formation. The clot and surrounding wound tissue release pro-inflammatory cytokines and growth factors, including TGF-β, PDGF, FGF, and EGF[11]. Once the bleeding is under control, inflammatory cells migrate into the wound (chemotaxis) and promote the inflammatory phase, which is characterised by the sequential infiltration of neutrophils, macrophages, and lymphocytes. [12]. Neutrophils play an important role in clearing invading microbes and cellular debris from the wound area, but they also produce substances like proteases and reactive oxygen species (ROS), which cause additional bystander damage[13].

Bougainvillaea glabra, which belongs to the *Nyctaginanceae* family, is a popular flowering ornamental and edible plant. *B. glabra*, also known as paper flower or lesser bougainvillaea, is a popular ornamental plant in many parts of the world due to its attractive and vibrant flowers, which come in a variety of colours such as pink, purple, red, and orange[14].

FIG 1: *Bougainvillea glabra*

B. glabra is used as a traditional medicine for different therapeutic conditions like insecticidal, anti-inflammatory[15], anti-diarrhoeal, anti-ulcer, anti-microbial (Edwin et al., 2007), and anti-diabetic [16] activities.

MATERIALS AND METHODS

The plant flower were collected from the Botanical Garden Ooty. The collected plants were washed with running tap water in three times[17]. After washing the flower was dried under sun dry and shadow dry for 2 days. Third day, grind the flower at Mortar and Pestle for fine powder and seive it for getting very thin powder [18]

WOUND HEALING ACTIVITY

Adult zebrafish were wounded between the ages of 6 and 12 months. Tg(krt4:egfp)gz7, Tg(mpx:GFP)i114, Tg(lyz:EGFP)nz117, Tg(lyz:dsRED2)nz50, Tg(fli1a:EGFP)y1, Tg(kdrl:HSRAS:mCherry)s896, and Tg(hsp70l:dnfgfr1-EGFP)pd1 [19] [20] have been earlier described [21] [22]Adult fish were anaesthetized with 0.13% Tricaine (w/v) and placed on Whatman paper soaked in system water. A full-thickness wound was created on the left flank, directly anterior to the anal and dorsal fins[23]. The Erbium:YAG MCL29 Dermablate dermatology laser (Asclepion) was set to a frequency of 5 Hz, and two pulses with a strength of 500 mJ for smaller (20-30 mm) or 600 mJ for larger (30-40 mm) specimens were applied, resulting in a pulse strength of 7.1 or 8.5 J/cm2. Tg(hsp70l:dnfgfr1-EGFP) fish were transferred from 28°C to pre-warmed water at 40°C for one hour before returning to 28°C and being wounded four hours later. Heat shock treatments were administered every 24 hours[24], [25]. All zebrafish experiments were approved by the national animal care committee (LANUV Nordrhein-Westfalen; 8.87-50.10.31.08.130; 84-02.04.2012.A253) and the University of Cologne[26].

RESULT

BEFORE WOUND

We have established a rapid and reproducible technique for introducing wounds of approximately 2 mm in diameter onto the flank of adult zebrafish, using a clinical dermatology laser. [see figure a), b), c), d)]

a) b)

c) d)

AFTER WOUND

During mammalian wound healing, inflammation and re-epithelialization coincides with granulation tissue formation [figure A), C)], characterized by the invasion of fibroblasts, macrophages and blood vessels into the wound space underneath the neo-epidermis [figure C), D)] .

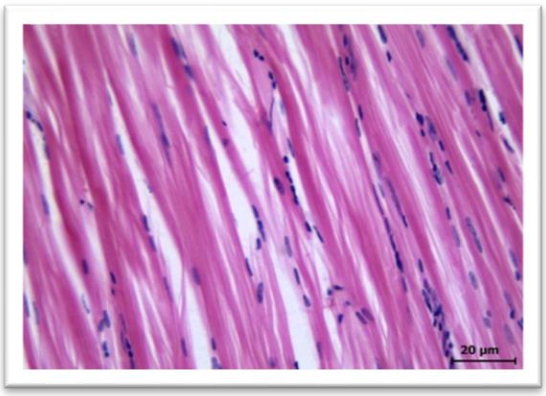
A) B)

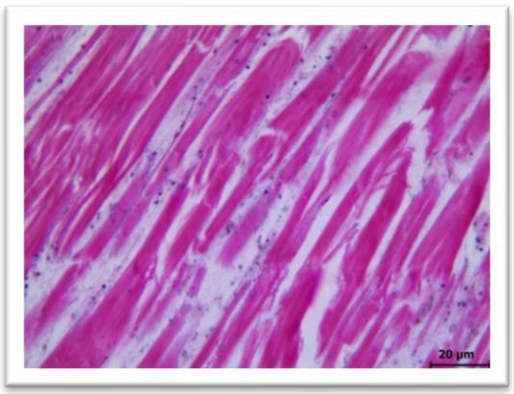
C) D)

WOUND HEALING DIFFERENTIATION

Histological and immunofluorescent analysis with a variety of markers demonstrates that the trunk skin of adult zebrafish is composed of overlapping scales, each of which is wrapped by a thin layer of dermal fibroblasts and a multi-layered epidermis. Epidermis and dermis are separated by a basement membrane, and dermis and underlying muscle by a layer of subcutaneous adipocytes [figure i, ii].

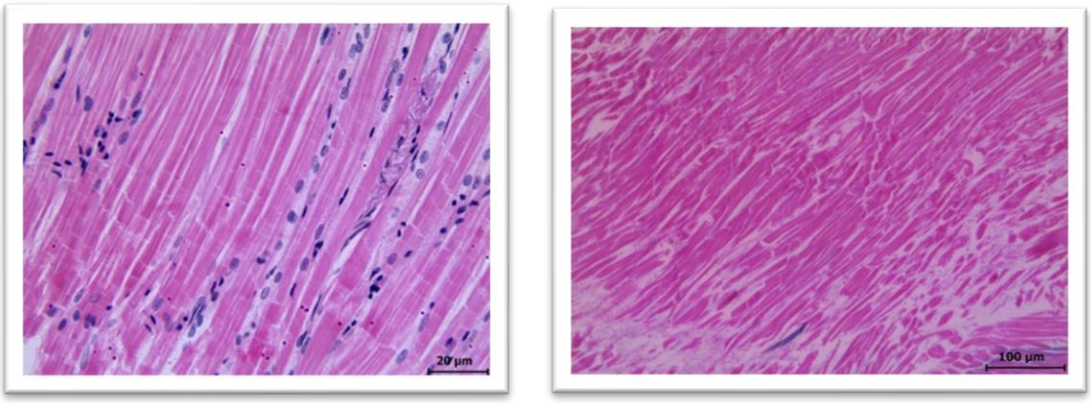
i. ii.





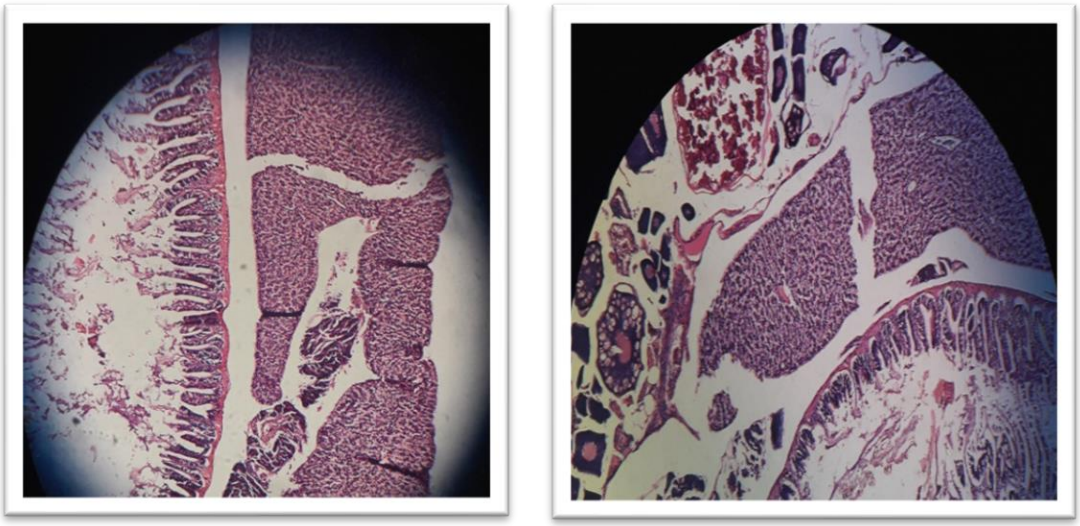
Histologically, a granulation-like tissue in zebrafish wounds is first visible at 2 days post wounding (dpw), reaches maximal size at 4 dpw, and has started to regress again at 6 dpw [figure iii, iv]. Analysis at later stages after wounding demonstrates the progressive regression of the granulation tissue so that at 10 dpw very few cells can be seen beneath the neo-epidermis. New scales are forming, and the collagen distribution resembles that of an unwounded region.

iii. iv.



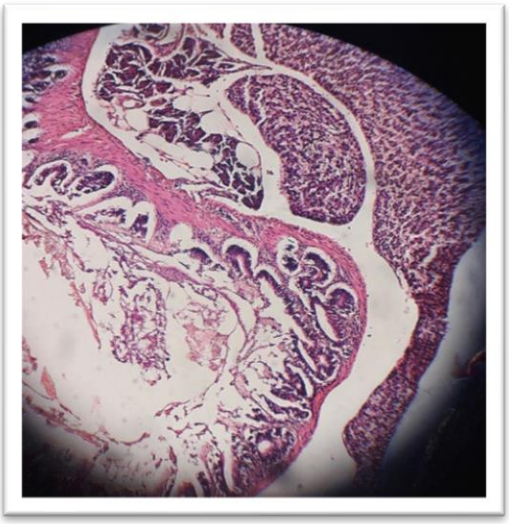
Sections reveal that introduced wounds have initially lost all epidermal and dermal cells, including the scales, and the subcutaneous adipocytes, while underlying muscle tissue is undamaged [figure a), b)]. At 7 hpw, a thin neo-epidermis covers most of the wounded surface and by 24 hpw the wound is completely re-epithelialized, with a neo-epidermis of multiple cell layers.

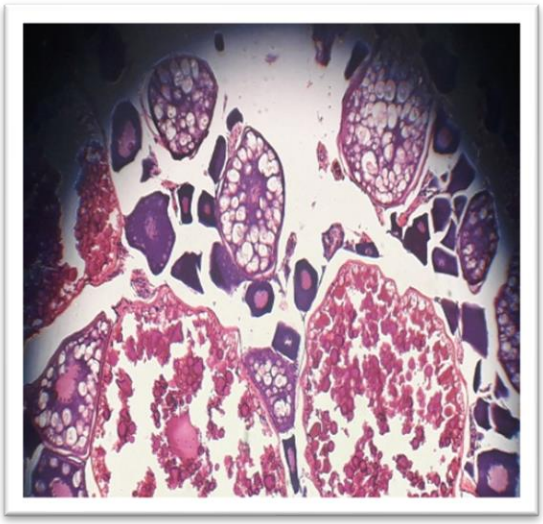
a) b)



A vital dye penetration assay, where methylene blue is absorbed by damaged tissue but not undamaged or regenerated epidermis, reveals rapid re-establishment of the barrier by 12 hours post-wounding (hpw). [figure c), d)]

c) d)





DISSCUSSTION

Over the wound healing process, skin cells such as fibroblasts migrate to the wound site and proliferate to restore skin integrity and generate new granulation tissue.

Thus, proper fibroblast proliferation and migration are required for the formation of granulation tissue, which serves as the foundation of the new dermis. In this study, the effects of *B. glabra* extracts on fibroblast migration were assessed using the scratch assay, a useful method for simulating cell migration during wound healing *in vivo*[27]. The effect of extracts on fibroblast migration was calculated as a percentage of wound closure. In comparison to the control group, the treated group had a wound closure rate of approximately 100%. *B. glabra* extracts significantly increased the natural migration rate of fibroblasts into the wounded area at all concentrations tested, as well as wound closure rates when compared to the control group[28].

CONCLUSION

Wound healing is a complex biological process that includes hemostasis, inflammation, proliferation, and remodelling. This process involves a large number of cell types, such as neutrophils, macrophages, lymphocytes, keratinocytes, fibroblasts, and endothelial cells. Multiple factors can impair wound healing by affecting one or more stages of the process, which are classified as local or systemic factors. These factors have complementary effects. Single or multiple factors may play a role in any of the individual phases, influencing the overall outcome of the healing process.

REFERENCES

[1] R. Richardson, K. Slanchev, C. Kraus, P. Knyphausen, S. Eming, and M. Hammerschmidt, "Adult zebrafish as a model system for cutaneous wound-healing research," *Journal of Investigative Dermatology*, vol. 133, no. 6, pp. 1655–1665, 2013, doi: 10.1038/jid.2013.16.

[2] M. J. Hardman and G. S. Ashcroft, "Estrogen, not intrinsic aging, is the major regulator of delayed human wound healing in the elderly," *Genome Biol*, vol. 9, no. 5, May 2008, doi: 10.1186/gb-2008-9-5-r80.

[3] N. B. Menke, K. R. Ward, T. M. Witten, D. G. Bonchev, and R. F. Diegelmann, "Impaired wound healing," *Clin Dermatol*, vol. 25, no. 1, pp. 19–25, Jan. 2007, doi: 10.1016/j.clindermatol.2006.12.005.

[4] K. I. Shingel, M. P. Faure, L. Azoulay, C. Roberge, and R. J. Deckelbaum, "Solid emulsion gel as a vehicle for delivery of polyunsaturated fatty acids: Implications for tissue repair, dermal angiogenesis and wound healing," *J Tissue Eng Regen Med*, vol. 2, no. 7, pp. 383–393, 2008, doi: 10.1002/term.101.

[5] "j.1600-0757.2007.00211.x".

[6] Y. Wu, J. F. Wang, P. G. Scott, and E. E. Tredget, "Bone marrow-derived stem cells in wound healing: A review," *Wound Repair and Regeneration*, vol. 15, no. SUPPL. 1. Sep. 2007. doi: 10.1111/j.1524-475X.2007.00221.x.

[7] M. A. M. Loots, E. N. Lamme, J. Zeegelaar, J. R. Mekkes, J. D. Bos, and E. Middelkoop, "Differences in Cellular Infiltrate and Extracellular Matrix of Chronic Diabetic and Venous Ulcers Versus Acute Wounds," 1998.

[8] M. E. Swift, A. L. Burns, K. L. Gray, and L. A. Dipietro, "Age-Related Alterations in the In¯ammatory Response to Dermal Injury."

[9] J. A. Greco *et al.*, "The effect of weight loss surgery and body mass index on wound complications after abdominal contouring operations," *Ann Plast Surg*, vol. 61, no. 3, pp. 235–242, Sep. 2008, doi: 10.1097/SAP.0b013e318166d351.

[10] E. Evans, J. G. Moggs, J. R. Hwang, J.-M. Egly, and R. D. Wood, "Mechanism of open complex and dual incision formation by human nucleotide excision repair factors," 1997.

[11] A. M. Vincent, J. W. Russell, P. Low, and E. L. Feldman, "Oxidative stress in the pathogenesis of diabetic neuropathy," *Endocrine Reviews*, vol. 25, no. 4. pp. 612–628, Aug. 2004. doi: 10.1210/er.2003-0019.

[12] S. E. Wozniak, L. L. Gee, M. S. Wachtel, and E. E. Frezza, "Adipose tissue: The new endocrine organ? a review article," *Digestive Diseases and Sciences*, vol. 54, no. 9. pp. 1847–1856, Sep. 2009. doi: 10.1007/s10620-008-0585-3.

[13] C. Quattrini, M. Jeziorska, A. J. M. Boulton, and R. A. Malik, "Reduced Vascular Endothelial Growth Factor Expression and Intra-Epidermal Nerve Fiber Loss in Human Diabetic Neuropathy," 2008, doi: 10.2337/dc07.

[14] K. A. Radek, E. J. Kovacs, R. L. Gallo, and L. A. Dipietro, "Acute ethanol exposure disrupts VEGF receptor cell signaling in endothelial cells," *Am J Physiol Heart Circ Physiol*, vol. 295, pp. 174–184, 2008, doi: 10.1152/ajpheart.00699.2007.-Physio.

[15] A. A. Tandara and T. A. Mustoe, "Oxygen in Wound Healing - More than a Nutrient," *World Journal of Surgery*, vol. 28, no. 3. pp. 294–300, Mar. 2004. doi: 10.1007/s00268-003-7400-2.

[16] D. Hofman and B. A. Hons, "J O U R N A L O F WO U N D C A R E VO L 1 6 , N O 5 , M AY 2 0 0 7 2 2 7 Use of topical corticosteroids on chronic leg ulcers."

[17] "Managing patients with metastatic colorectal cancer on bevacizumab."

[18] R. Glaser and J. K. Kiecolt-Glaser, "Stress-induced immune dysfunction: implications for health," 2005. [Online]. Available: www.nature.com/reviews/immunol

[19] K. A. Radek, E. J. Kovacs, and L. A. DiPietro, "Matrix proteolytic activity during wound healing: Modulation by acute ethanol exposure," *Alcohol Clin Exp Res*, vol. 31, no. 6, pp. 1045–1052, Jun. 2007, doi: 10.1111/j.1530-0277.2007.00386.x.

[20] S. Guo and L. A. DiPietro, "Critical review in oral biology & medicine: Factors affecting wound healing," *J Dent Res*, vol. 89, no. 3, pp. 219–229, Mar. 2010, doi: 10.1177/0022034509359125.

[21] D. Hofman and B. A. Hons, "J O U R N A L O F WO U N D C A R E VO L 1 6 , N O 5 , M AY 2 0 0 7 2 2 7 Use of topical corticosteroids on chronic leg ulcers."

[22] "00129334-200702000-00009".

[23] B. C. Tong and A. Barbul, "Cellular and Physiological Effects of Arginine," 2004.

[24] G. D. Krischak, O. Surgeon, ; P Augat, ; L Claes, ; L Kinzl, and ; A Beck, "The effects of non-steroidal anti-infl ammatory drug application on incisional wound healing in rats."

[25] V. D. F. de Mello *et al.*, "Effect of weight loss on cytokine messenger RNA expression in peripheral blood mononuclear cells of obese subjects with the metabolic syndrome," *Metabolism*, vol. 57, no. 2, pp. 192–199, Feb. 2008, doi: 10.1016/j.metabol.2007.08.024.

[26] L. T. Sørensen *et al.*, "Acute Effects of Nicotine and Smoking on Blood Flow, Tissue Oxygen, and Aerobe Metabolism of the Skin and Subcutis," *Journal of Surgical Research*, vol. 152, no. 2, pp. 224–230, Apr. 2009, doi: 10.1016/j.jss.2008.02.066.

[27] "Why combine a foam dressing with ibuprofen for wound pain and moist wound healing?" [Online]. Available: http://www.worldwidewounds.

[28] N. Morimoto, S. Takemoto, T. Kawazoe, and S. Suzuki, "Nicotine at a Low Concentration Promotes Wound Healing," *Journal of Surgical Research*, vol. 145, no. 2, pp. 199–204, Apr. 2008, doi: 10.1016/j.jss.2007.05.031.