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# Effect of Metatera Extract on Wound Closure

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**Abstract:** Wounds represent a major health problem, in terms of morbidity and mortality, the primary goals of wound treatment are the rapid wound closure and a functional scar. There are medicinal plants used empirically for treatment of wounds such as metatera plant (Scrophulariaceae family). Our group demonstrated *in vitro* that metatera extract (ME) induces important events for wound closure as proliferation, migration and differentiation of fibroblast. The main objective of this work was to determinate the wound closure effect induced by metatera extract in an excisional *in vivo* model. Metatera extract included in a hydrogel (ME-H) was optimized for dairy topical application during 15 days. We analyzed the wound closure rate effect, the histopathology changes and the total collagen content in tissues treated with ME-H. The morphometric analysis showed that ME clearly improve wound closure. Moreover, histopathology changes induced by this extract suggest that ME induced different cellular events, such as fibroblast maturation and fibroblast and collagen fibers arrangement. Our results supported the ethnobotanic use of this plant for wound care in Mexico.

## INTRODUCTION

The primary function of the skin is to serve as a protective barrier against the environment. Loss of the integrity of the skin as a result of injury or illness may lead to major disability or even death. Every year in the United States more than 1.25 million people have burns and 6.5 million have chronic skin ulcers caused by pressure, venous stasis, or diabetes mellitus.

The primary goals of wound treatments are rapid wound closure and a functional and aesthetically satisfactory scar[1, 2].

Despite the considerable advances in the pharmaceutical industry, the availability of drugs capable of stimulating the process of wound healing is still limited. Only 1–3% of the drugs listed in Western pharmacopoeias are used on the skin or wounds; by comparison, at least one-third of herbal remedies are applied for this propose[3].

Moreover, medicinal plants are rich sources of new efficacious wound-healing substances[4]. Nowadays, Traditional Mexican Medicine is widely practiced and is viewed as an alternative to conventional medicine for wound healing in Mexican population.

In Hidalgo state (Mexico) a plant known as Metatera (Scrophulariaceae family) was used for wound healing. In our group we demonstrated *in vitro* that metatera extract induced different important events for wound healing such as proliferation, migration and differentiation of fibroblast[5].

However, no systematic studies have been carried out about the clinical evaluation of the wound healing activity of metatera plant. The main research goal of this study was to determinate the wound closure, the wound healing histological properties and the total content of collagen induced by the metatera extract-hydrogel (ME-H) which was prepared and optimized for a excisional *in vivo* model in rats.

## METHODS

**Animals:** Male Wistar rats of 220-240 g were maintained at 26°C under 12:12 hours light/dark cycle. Animals received food and water *ad libitum*. The ethic committee of the postgraduate section of ENMyH approved the experimental procedure of this study.

**Metatera formulation:** Metatera plant was collected from Hidalgo State, Mexico; and identified with the voucher specimen (IZTA-Flora Útil-UAM herbarium).

The aquoethanolic extract, was obtained by continuous reflux method described by Hidalgo[5]. The solvent was evaporated under reduced pressure in a rotary vaporator at 45°C. The extract yield was 81.97%. Then, the metatera extract (ME) was included into a hydrogel elaborated by Dermopharma enterprise at 20, 40 and 80 mg/ml (final concentrations) (ME-H).

***In vivo* excisional skin wound model:** Animals were anesthetized, the dorsal region was harvested and four full-thickness skin excision wounds of 1cm$^2$ were removed by surgery and covered with a commercial film (Tegaderm).

Then, experimental animals were divided into six random groups: control group without treatment (WOT); group treated with the hydrogel (vehicle); group treated with a commercial product for wound care (Kitoscell); and three experimental groups which were treated with the formulation at 20, 40 and 80 mg/ml of metatera extract-hydrogel (ME-H).

The healings were treated daily and animals were sacrificed at 15 days post-injure. A minimum of six rats were used for each experimental group.

**Morphometric analysis:** The healings were observed, photographed and measured immediately (day 0), and at 15 days post-surgery by Vernier caliper, calculating percentage of wound reduction of the original area.

**Histopathology analysis of wound tissue :** At 15 days post wounding, wound lesions with adjacent normal skin were removed and fixed in 4% buffered paraformaldehyde; the samples were then embedded in paraffin and sectioned at thickness of 5 µm.

The sections were stained with hematoxylin-eosin and Masson´s Trichome staining, and then samples were analyzed and photographed using the Olympus system CX31.

**Hydroxyproline (Hyp) detection:** Collagen concentrations were determined by measuring hydroxiproline content in fresh skin samples after digestion with acid[6, 7].

## STATISTICAL ANALYSIS

Statistical significance was analyzed using two tailed Student's t test. Data that tested significantly different using analysis of variance ($p < 0.05$). All analysis was performed using software NCSS (Statistica 10.0).

## RESULTS

**Metatera extract improve wound closure rate:** The *in vivo* excisional model used give us clean healings reducing the infection risk and let us observe the cellular, histological and morphometric changes that occurs during wound healing process [8]. Four full 1cm$^2$ thickness were done on the dorsal shaved of male Wistar rats, and monitored for 15 days post wounding. For this work the ME was included in a hydrogel for topical application to maintain the microambient[9]. Macroscopically, we observed wound reduction in all groups, although, we found that almost all the healings treated with 80 mg/ml of ME-H were closed (>90%, data not showed), without scab and scarless, in comparison with the other groups **(Figure 1).** These results correlated with the percentage of wound reduction; while in controls groups the percentage of wound reduction was only 96-97 %, using 40 and 80 mg/ml ME-H, the reduction was 99.8-99.9 % **(Figure 1).**

These results demonstrated that ME-H improved wound closure at 40 and 80 mg/ml per day in comparison with the + Control, vehicle and with the WOT groups. Our results are similar to other studies with plants of the same family; Lau *et al.* [10, 11] in a diabetic model of rats observed that healings treated with the extract of *R. rehmenniae* (Scrophulariaceae family) showed wound reduction between 8 to 18 days, in comparison with control group. Murthy *et al*. [12] in an *in vivo* model observed that the percent rate of wound contraction in rats, treated orally with *B. monniera* extract (25mg/kg), was from 32.2% on day 4 to 85.4% on day 12 and 92.1% to 100% from day 14 to day 20, respectively, the effect was better than in control groups.

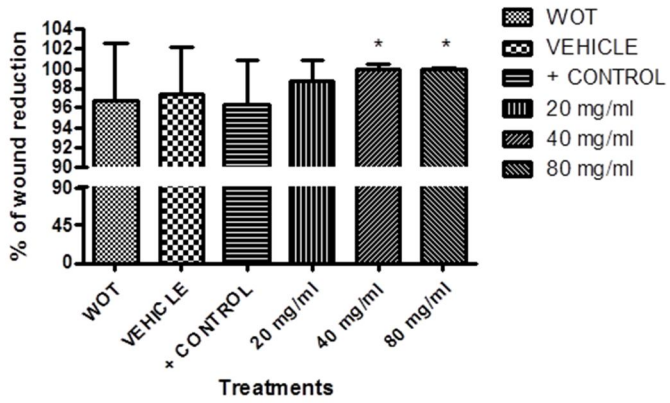


**Figure 1:** Wound closure rate. Percentage of wound reduction at 15 days post-wound healing in wounds without treatment (WOT), wounds treated with hydrogel (vehicle), wounds treated with KitosCell (+control), and wound treated with 20, 40 and 80 mg /ml of ME-H respectively, * $p<0.05$.

**Histopathology changes induced by metatera extract:** Due ME-H improve the wound closure, we analyzed the histopathology changes induced by this plant at 15 days; at this time, all wounds showed the presence of fibroblast and reduction of inflammatory cells. In WOT, vehicle, + control, and 20 mg/ml ME-H, the epidermis was not clearly formed, while in 40 and 80 mg/ml ME-H the epidermis was obvious **(Figure 2. upper panel)**. Harish *et. al.*, [13] evaluated lupeol isolated from the *Paniculatus celastrus* extract, they found that lupeol at 8 mg/ml has a wound healing activity, it stimulated reepithelization, with increase in granulation tissue and collagen. Moreover, in our work, groups treated with ME-H at 40 and 80 mg/ml showed large and orientated fibroblast into the tissue **(Figure 2. Upper panel).**

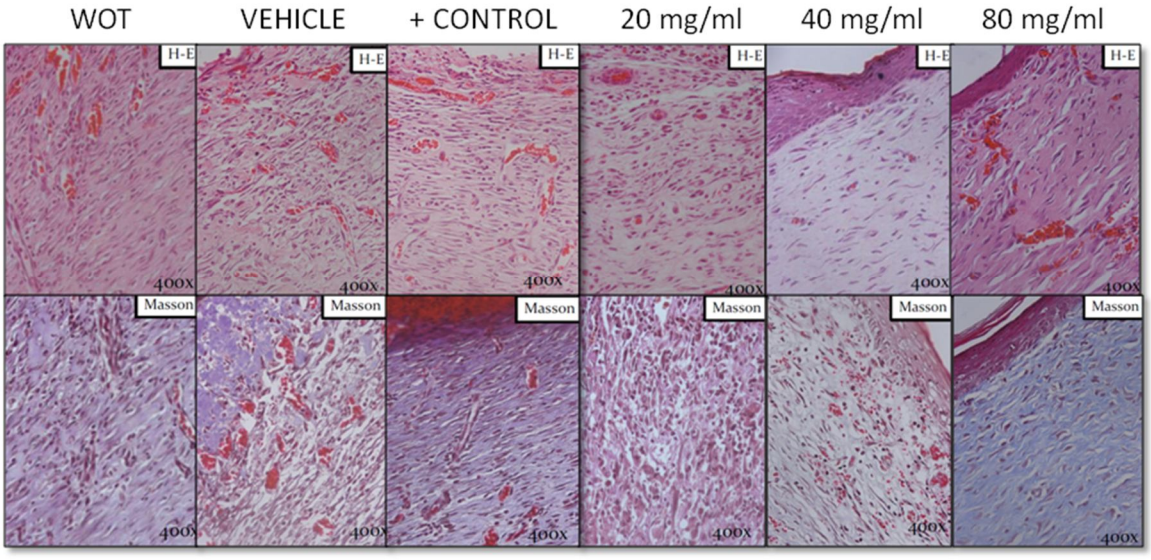


**Figure 2:** Histopathology analysis. Representative photomicrographs of H&E (upper panel) and Masson's trichome (lower panel) staining at 15 days post-wound healing in wounds without treatment (WOT), treated with hydrogel (vehicle), treated with KitosCell (+control), and treated with 20, 40 and 80 mg /ml of ME-H.

**Metatera extract modulate collagen arrangement:** Furthermore, collagen is a major protein of the extracellular matrix and is the component that contributes to wound strength. Interestingly, analysis of collagen content showed that healings treated with 80 mg/ml ME-H displayed mature collagen fibers, which had a better arrangement in comparison with the other groups. Although the + Control group also showed oriented collagen fibers, this collagen was immature. In the other groups collagen fibers was disorganized and immature **(Figure 2. lower panel).** These findings suggest that the 80 mg/ml ME-H induce deposition, maturation and better arrangement of collagen fibers. The decreased collagen content in the other groups might be due to prolonged inflammatory phase, where the degradation of collagen is more important than the synthesis *per se* [14]. Healings treated with *Acalypha indica* extract exhibited dense and parallel arrangement of thick collagen fibers on day 14 in comparison with control groups in which undifferentiated keratinocytes and irregular packing of collagen fibers were found [15] . However, assessment of collagen content in wound tissues from control and the ME-H treated groups did not show an augment in total collagen in comparison with controls groups **(Figure 3)**. These results suggest that ME-H improve wound closure regulating different cell and molecular pathways in early phases of the process.

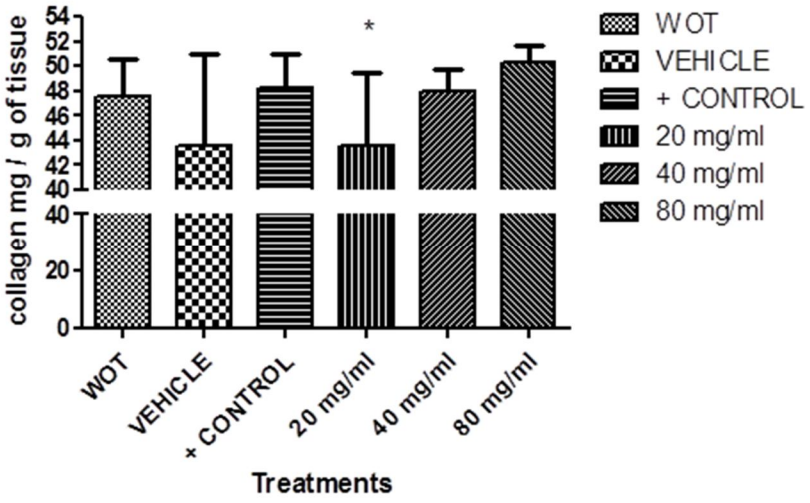


**Figure 3:** Total content of collagen mg/ g of tissue determinate by hydroproline measure. at 15 days post-wound healing in wounds without treatment (WOT), wounds treated with hydrogel (vehicle), wounds treated with KitosCell (+control), and wound treated with 20, 40 and 80 mg /ml of ME-H respectively * $p<0.05$.

## CONCLUSIONS

Metatera extract included in a hydrogel for topical application, clearly improve wound closure, these two concentrations induced different cellular events, such as the maturation and arrangement of fibroblast, the re-epithelialization enhanced, the modulation of collagen fibers arrangement. The result of the present study supported the ethnobotanic use of this plant for healing of wound in Mexico. Further experiments currently under study let us know the metabolites responsible of the healing effect, and the cellular and molecular pathways induced by this extract.

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**REFERENCES**

1. A. Singer and R. Clark. Cutaneous Wound Healing. *N. Engl. J Med,* 1999, 341(10):738-746.
2. R. Clark, K. Ghosh and M. Tonnesen. Tissue Engineering for Cutaneous Wounds. *J Invest Dermatol,* 2007, *127*(October 2006):1018–1029.
3. B. Kumar, M. Vijayakumar, R. Govindarajan and P. Pushpangadan, Ethnopharmacological approaches to wound healing-exploring medicinal plants of India. *J. Ethnopharmacol.,* 2007, 114:103–13.
4. G. Blazso, M. Gábor, F. Schönlau and P. Rohdewald. Pycnogenol accelerates wound healing and reduces scar formation. *Phytother Res.* 2004, 18: 579-581.
5. A. Hidalgo. Determinación del efecto cicatrizante del extracto acuoetanólico de la planta Bacopa procumbens en la línea celular 3T3 de fibroblastos de ratón. Tesis de Maestría en Biomedicina Molecular. ENMH IPN. 2010.
6. D. Prockop and S. Underfriend. Aspecific method for the analysis of hidroxyproline in tissues and urine. *Anal. Biochem,* 1960,1: 228-239.
7. P. Muriel and R. Deheza. Fibrosis and glycogen stores depletion induced by prolonged biliary obstruction in the rat are ameliorated by metadoxine. *Liver Int.,* 2003, 23: 262-268.
8. X. Liu, P. Lee, C. Ho, V. Lui , Y. Chen, C. Che and K. Wong. Silver nanoparticles mediate differential responses in keratinocytes and fibroblasts during skin wound healing. *ChemMedChem*., 2010, *5*(3):468–75.
9. P. Martini, C. Mazzatenta and G. Saponati. Efficacy and tolerability of fitostimoline in two different forms (soaked gauzes and cream) and citrizan gel in the topical treatment of second-degree superficial cutaneous burns. *Dermatol. Res. Pract,* 2011, 2011: 978291.
10. T. Lau, Y. Chan, C. Lau, C. Chan, C. Lau, K. Fung and Y. Ho. Investigation of the Effects of Chinese Medicine on Fibroblast Viability : Implications in Wound Healing. *Phytother Res.,* 2007, 21(10):938–947
11. T. Lau, F. Lam, K. Lau, Y. Chan, K. Lee, D. Sahota and C. Lau. Pharmacological investigation on the wound healing effects of Radix Rehmanniae in an animal model of diabetic foot ulcer. *J. Ethnopharmacol,* 2009, 123(1):155-62.
12. S. Murthy, M. Gautam, S. Goel, V. Purohit, H. Sharma and R. Goel. Evaluation of in vivo wound healing activity of Bacopa monniera on different wound model in rats. *Biomed Res. Int.,* 2013, 2013:972028.
13. B. Harish, V. Krishna, H. Santosh Kumar, B. Khadeer Ahamed, R. Sharath and H. Kumara Swamy. Wound healing activity and docking of glycogen-synthase-kinase-3-beta-protein with isolated triterpenoid lupeol in rats. *Phytomedicine,* 2008, *15*(9):763–7.

14. S. Sasidharan, S. Logeswaran and L. Latha. Wound Healing Activity of Elaeis guineensis Leaf Extract Ointment. *Int. J. Mol. Sci,* 2012, 13:336–47.

15. M. Ganeshkumar, T. Ponrasu, R. Krithika, K. Iyappan, V. Gayathri and L. Suguna. Topical application of Acalypha indica accelerates rat cutaneous wound healing by up-regulating the expression of Type I and III collagen. *J. Ethnopharmacol.,* 2012, 142 (1):14–22.

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