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Title: Growth factor stimulation in traumatic ulcer healing with liquid rice hull smoke from Indonesia
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Kindly submit your revised manuscript within 21 from the date the decision was taken.

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KIK conceived and designed the study, conducted research, provided research materials, and collected and organized data. HMY analyzed and interpreted data. SYG wrote initial and final draft of article, and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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Deputy Editor-in-chief
Journal of Taibah University Medical Sciences

Reviewers comments:

Reviewer #2: This work presents on the presence of liquid rice hull smoke in the growth factor simulation for traumatic ulcer healing. This work is interesting however there are several comments which should be the authors before it could be accepted in this journal. There are a lot of work required;

1) English language needs to be improved thoroughly for this manuscript. Please seek English expert in this research area to proofread your manuscript.
2)Abstract - Write more precisely the objectives, methods, results and conclusion in abstract. .
3) The novelty and research gaps need to be addressed clearly in the introduction section. I do not see this in the introduction section.
4) Please provide supporting literature on previous studies conducted in this area in the introduction section.
5) Page 5 Lines 28-31, Statistical analysis section - Please provide the involved measurement and also state of the the group..... This sentence is not clear.
6) Please compare your current findings in Table 1 with results from literature.
7) Conclusions section - This content of this section is too limited. Please highlight the research methodology, key findings achieved, and future work.

Reviewer #3: 1- In abstract the abbreviation of liquid rice hull smoke should be standardized wherever they are (LR-HS or RH-LS)
2- the names of abbreviations of growth factors should be written in abstract.
3- In introduction; the 3rd paragraph in page 2-3 should given a references. From (The Study..... initiate tissue regeneration)
4- the name of TNF-a should be written in last paragraph of introduction.
5- In results: in Expression of PDGF the result of it in three day was mentioned as insignificant while in the table 1 it differed it was significant at three day? why / should be match with table.

6- the discussion is formulated well but it should be summarized more clearly ; the phrase in page 8 from M2 also facilitates..... till the to dissolve the extracellular matrix ... in page 9) It must be rewritten again without repeating the sentences).
7- the figures should be explained well and pointed with arrows inside the figure to pointed the study variables and tissues.

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Ms. Ref. No.: JTUMED-D-20-00804R2
Title: Growth factors stimulation in traumatic ulcer healing with liquid rice hull smoke
Journal of Taibah University Medical Sciences

Dear Ira,

I am pleased to inform you that your paper "Growth factors stimulation in traumatic ulcer healing with liquid rice hull smoke" has been accepted for publication in Journal of Taibah University Medical Sciences.

Thank you for submitting your work to Journal of Taibah University Medical Sciences.

Yours sincerely,

Salman Yousuf Guraya, FRCS, Masters MedEd (Dundee)
Deputy Editor-in-chief
Journal of Taibah University Medical Sciences

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Original Article

Running head: Growth factors stimulation by LR-HS

Growth factor stimulation for the healing of traumatic ulcers with liquid rice hull smoke

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Peer review under responsibility of Taibah University.

الملخص

أهداف البحث

تتطلب عملية الشفاء من قرحة مؤلمة عوامل النمو لإعادة بناء الأنسجة المفقودة بعد الانتهاء من عملية الالتهاب. وقد أظهر دخان قشرة بذرة الأرز السائل خصائص فريدة من نوعها مضادة للالتهابات. هذه الدراسة تحلل دور دخان قشرة بذرة الأرز السائل في تحفيز عوامل النمو في شفاء القرحة الرضية مثل عامل النمو الليفي، وعامل نمو البطانيي الوعائية، وعامل النمو المشتق من الصفائح الدموية وتعبير الكولاجين نوع-١.

طرق البحث

حصلنا على دخان قشرة بذرة الأرز السائل من الانحلال الحراري لهياكل الأرز. تم إنشاء القرحة المؤلمة في الزاوية الزبيبية السفلية للشفة لفئران ويستار، وعولجت بدخان قشرة بذرة الأرز السائل مرة واحدة في اليوم لمدة ثلاثة وخمسة وسبعة أيام. وعولجت مجموعة المراقبة بالماء المعقم. في وقت لاحق، تم التضحية بالفئران بعد العلاج وتم فحص أنسجة الزاوية الزبيبية السفلية للشفة فحصا باستخدام الصبغة الكيميائية المناعية لفحص تعبير عامل النمو الليفي وعامل نمو البطانيي الوعائية وعامل النمو المشتق من الصفائح الدموية والكولاجين نوع-١.

النتائج

أظهر علاج القرحة الرضية بدخان قشرة بذرة الأرز السائل زيادة في تعبير عامل النمو الليفي وعامل نمو البطانيي الوعائية وعامل النمو المشتق من الصفائح الدموية والكولاجين نوع-١. كما زاد تعبير عامل نمو البطانيي الوعائية تحت علاج دخان قشرة بذرة الأرز السائل مقارنة مع مجموعات العلاج لمدة سبعة أيام. وقد زاد تعبير عامل النمو الليفي والكولاجين نوع-١ تحت علاج دخان قشرة بذرة الأرز السائل مقارنة بمجموعات العلاج التي تستغرق خمسة أيام وسبعة أيام. زاد تعبير عامل النمو المشتق من الصفائح الدموية بعد العلاج مع دخان قشرة بذرة الأرز السائل لمدة ثلاثة خمسة وسبعة أيام.

الاستنتاجات

أثبتت هذه الدراسة أن دخان قشرة بذرة الأرز السائل يمكن أن يحفز على التعبير عن عوامل النمو أثناء شفاء القرحة الرضية باستخدام الصبغة الكيميائية المناعية. نقترح أن دخان قشرة بذرة الأرز السائل يمكن استخدامها كدواء عشبي لعلاج قرحة الفم.

Abstract

Objective

The healing process of a traumatic ulcer requires growth factors to rebuild the lost tissue after the inflammatory process has been completed. Liquid rice hull smoke (LR-HS) has shown unique anti-inflammatory

properties. This study analyses the role of LR-HS in growth factor stimulation for the healing of traumatic ulcers, such as fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and collagen type 1 (COL-1) expression.

Methods

We obtained LR-HS from the pyrolysis of rice hulls. Traumatic ulcers were created in the labial fornix incisive inferior of Wistar rats and treated with LR-HS once a day for 3, 5, and 7 days. The control group was treated with sterile water. Each animal was sacrificed after treatment, and its labial fornix incisive inferior tissues were biopsied and immunohistochemically stained to examine FGF, VEGF, PDGF, and COL-1 expression.

Result

The treatment of traumatic ulcers with LR-HS showed an increase in FGF, VEGF, PDGF, and COL-1 expression. VEGF expression increased under LR-HS treatment compared with the control 7-day treatment groups ($p < 0.000$). FGF and COL-1 expression increased under LR-HS treatment compared with the control 5- and 7-day treatment groups ($p < 0.000$). PDGF expression increased after treatment with LR-HS for 3, 5, and 7 days ($p < 0.000$).

Conclusion

This study has demonstrated that LR-HS can induce the expression of growth factors during the healing of a traumatic ulcer using immunohistochemical staining. We suggest that LR-HS can be used as a herbal medicine for oral ulcer therapy.

الكلمات المفتاحية: الكولاجين نوع-١; عامل النمو الليفي; دخان قشرة بذرة الأرز السائل; عامل النمو المشتق من الصفائح الدموية; قرحة مؤلمة

Keywords: COL-1; FGF; Liquid rice hull smoke; PDGF; Traumatic ulcer

Introduction

Liquid smoke is produced by biomass decomposition through pyrolysis.[1] The use of this liquid for traditional treatments in Indonesia is still controversial because liquid smoke is highly acidic[2] and contains the polyaromatic hydrocarbon benzopyrene.[3]

In Indonesia, liquid smoke can be obtained from biomass such as coconut shells[2] and rice hulls.[4] These are available in large quantities in Indonesia as waste products of the coconut and rice industries. The resultant liquid smoke is a natural product that is able to stimulate healing in some pathological conditions. Liquid rice hull smoke (LR-HS) has a low toxicity,[4] is able to decrease blood glucose levels in diabetics,[5] and has anti-inflammatory properties.[6] Other forms of liquid smoke have also been proven to stimulate the healing of oral mucosal wounds, such as traumatic ulcers. The liquid smoke from coconut shells has the ability to stimulate anti-inflammatory properties in traumatic ulcers by inhibiting the production of pro-inflammatory cytokine by macrophages through the inhibition of nuclear factor kappa b (NF-kB), which causes delays in healing.[7] This inhibition of macrophages stimulates fibroblast proliferation and collagen synthesis.[8] The application of liquid smoke to a traumatic ulcer not only stimulates healing,[9] it also provides an analgesic effect.[10]

The study of stimulating oral mucosal healing is not only focused on the inhibition and prevention of long-term inflammation; oral mucosa can delay healing through saliva and microorganisms that support infection.[11] Anti-inflammatories and antiseptic drugs, such as mouthwash, are always given to a patient with an oral mucosal wound, such as a traumatic ulcer. However, anti-inflammatories alone are not able to promote the healing of oral wounds, as they are not able to stimulate growth factors during the healing process.[12] In some cases, a traumatic ulcer is unable to heal completely with topical anti-inflammatories, and growth factor stimulation ~~application~~[12] is necessary. Growth factors are the proteins released by the immune cells to initiate the healing of an oral wound.[13] Fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and collagen type 1 (COL-1) are the first growth factors released by macrophages, fibroblasts, and endothelial cells to initiate tissue regeneration.[14]

LR-HS contains phenolic compounds that have anti-inflammatory effects[15-17] given that they down-regulate tumour necrosis factor α (TNF-α) expression[17] and have thus been proven capable of accelerating the inflammatory process.[18] Since TNF-α is a cytokine that induces the inhibitor of nuclear factor-κB (IκB) kinase (IKK) activity, the degradation of I-kB, and activates NF-kB, the down-regulation of TNF-α results in NF-kB inhibition.[15,16] This prompts a switch from classically-activated macrophages (M1) to alternatively-activated macrophages (M2). Therefore, M2 polarisation becomes more dominant.[19] M2 secretes growth factors, including FGF, VEGF, PDGF, and COL-1, which contribute to healing via fibroblast proliferation and collagen production.[20-24] The application of LR-SH as a traumatic ulcer treatment has never been studied before, and its potential has not yet been proven. Based on the description above, LR-HS has the auspicious potential to stimulate FGF, VEGF, PDGF, and COL-1 because it has been proven to down-regulate NF-kB. Hence, an *in vivo* study of LR-HS is necessary.

Materials and Methods

Liquid rice hull smoke (LR-HS)

Rice hulls (*Oryza sativa* L) were obtained at Tumpang village's rice processing centre. The LR-HS used in this study was produced via the pyrolysis process, as described in Arundina et al. (2020).[4]

Animals

This research was conducted on 30 male Wistar rats aged 2 months and weighing around 120 g ~~to~~ 160 g at the Laboratory of Animal Testing, Department of Biochemistry, Faculty of Medicine, Airlangga University. The Wistar rats were housed in communal cages, with two rats per cage. The environment was maintained at room temperature (27 °C) and artificially lit for a duration of 12 hours ~~:~~ light and 12 hours' dark. The rats had free access to a standard diet and water.

Traumatic ulcer inducement

A ketamine/xylazine cocktail was used to anaesthetise the Wistar rats. A long incision, of about 10 mm, was made using a round stainless steel blade at the labial fornix incisive inferior to induce traumatic ulcers.[9] Ulcer inducement was considered to have been successful if verifien ~~after~~ 24 hours, a yellowish-white ulcer with an erythematous halo occurred.[8]

After confirmation of the painful ulcers, Wistar rats were randomly assigned to the control group (15 rats) or the experimental group (15 animals). Ulcers were treated topically using the intraoral dropping method. In the control group, the traumatic ulcers were treated using sterile water; in the experimental group, LR-HS was used, in a dose of 20 µL/20 g once daily for 3, 5, and 7 days.[2]

Growth factor expression on traumatic ulcers

After 3, 5, and 7 days of treatment, the rats were discharged and their fornix incisive inferior tissues were biopsied. To identify the expression of FGF, VEGF, PDFG, and COL-1, immunohistochemical (IHC) staining was performed using FGF *(FGF-2 mouse monoclonal, Santacruz biotechnology)*, VEGF *(anti-VEGFA rabbit polyclonal, Abcam, USA)*, PDGF *(PDGF-A antibody mouse monoclonal, Santacruz biotechnology)*, and COL-1 *(COL-1A mouse monoclonal, Santacruz biotechnology)* antibodies, respectively. A light microscope *(Nikon H600L microscope; Nikon, Japan)* with a magnification of 400 ~~x~~ with five fields of view and a single blind operator was used to take all measurements.

Statistical analysis

The data were presented as mean ± standard deviation (mean ± SD) for each measurement in both the treatment group and the control group. Afterwards, the data in the treatment and control groups were analysed using an independent *t*-test with the significance set at $p < 0.01$. Statistical analysis was done using SPSS 22.00 for Windows.

Results

The expression of FGF on traumatic ulcers

FGF expression on traumatic ulcers increased after treatment with LR-HS for 3–7 days (Figure 1). Only treatment durations of 5 and 7 days were sufficient to achieve an FGF expression that was significantly higher than in the control group ($p = 0.000$ and $p = 0.002$, respectively); a 3-day treatment was insignificant (Table 1).

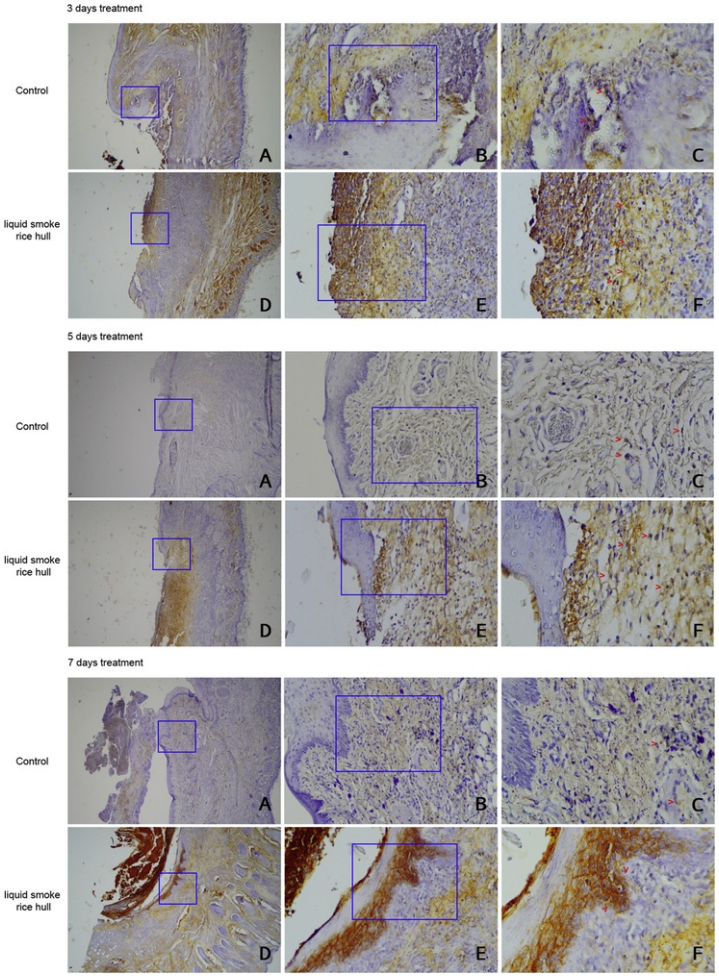

Figure 1 FGF expression in traumatic ulcer tissue. (A and B) magnification 40×; (B and E) magnification 100×; (C and F) magnification 400×.

alt-text: Figure 1

Table 1 The expression of growth factors in oral traumatic ulcers.

alt-text: Table 1

Days of treatment	Marker	Group		*p* value
		LS-RH	Control	
3	FGF	6.80 ± 1.35	6.60 ± 2.30	0.872^{ns}
	VEGF	7.80 ± 2.59	7.20 ± 2.77	0.733^{ns}
	COL-1	6.20 ± 2.28	5.80 ± 2.28	0.789^{ns}
	PDGF	8.40 ± 0.89	3.60 ± 1.82	0.002*

5	FGF	14.00 ± 1.46	6.80 ± 1.79	0.000*
	VEGF	12.20 ± 2.59	7.80 ± 2.04	0.019[ns]
	COL-1	14.00 ± 1.41	7.20 ± 2.17	0.001*
	PDGF	10.60 ± 1.82	6.20 ± 1.30	0.003*
7	FGF	16.20 ± 3.03	8.20 ± 1.92	0.002*
	VEGF	18.20 ± 3.42	9.00 ± 2.00	0.002*
	COL-1	17.80 ± 1.92	8.60 ± 2.07	0.000*
	PDGF	16.00 ± 3.54	7.40 ± 1.82	0.003*

Fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), collagen type-1 (COL-1), and platelet-derived growth factor (PDGF).

The differences in the expression of each growth factor between liquid rice hull smoke (LS-RH) treatment and the control group using an independent *t*-test.

[ns] not significant.

* significant at $p < 0.01$.

The expression of VEGF on traumatic ulcers

VEGF expression on traumatic ulcers increased after treatment with LR-HS from 3–7 days (Figure 2). Only a treatment duration of 7 days was sufficient to achieve a VEGF expression that was significantly higher than in the control group ($p = 0.002$); 3- and 5-day treatments were insignificant (Table 1).

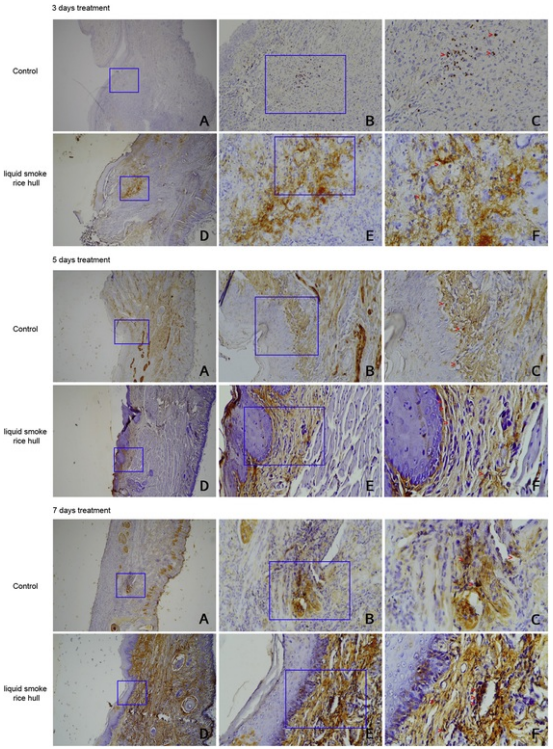

Figure 2 VEGF expression in traumatic ulcer tissue. (A and B) magnification 40×; (B and E) magnification 100×; (C and F) magnification 400×.

alt-text: Figure 2

The expression of COL-1 on traumatic ulcers

COL-1 expression on traumatic ulcers increased after treatment with LR-HS for 3–7 days (Figure 3). Only treatment durations of 5 and 7 days were sufficient to achieve a COL-1 expression that was significantly higher than in the control group ($p = 0.001$ and $p = 0.000$, respectively); a 3-day treatment was insignificant (Table 1).

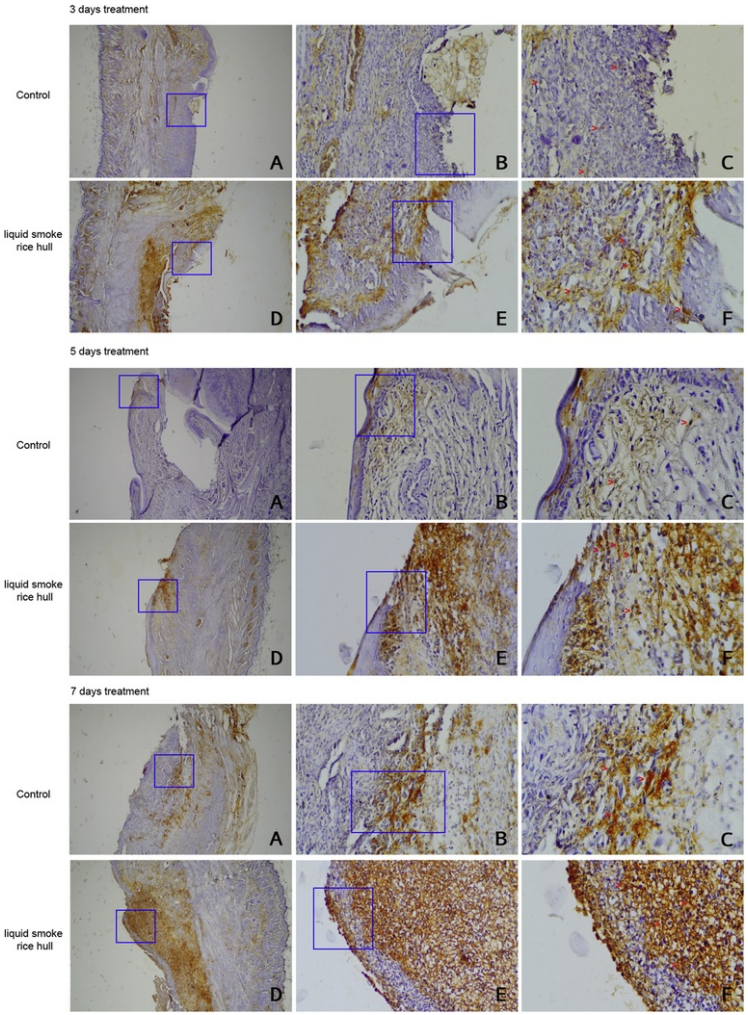

Figure 3 COL-1 expression in traumatic ulcer tissue. (A and B) magnification 40×; (B and E) magnification 100×; (C and F) magnification 400×.

alt-text: Figure 3

The expression of PDGF on traumatic ulcers

PDGF expression on traumatic ulcers increased significantly after treatment with LR-HS for 3–7 days (Figure 4 and Table 1). Treatment durations of 3, 5, and 7 days were sufficient to achieve a PDGF expression that was

significantly higher than in the control group ($p = 0.002$, $p = 0.003$, and $p = 0.003$, respectively) (Table 1).

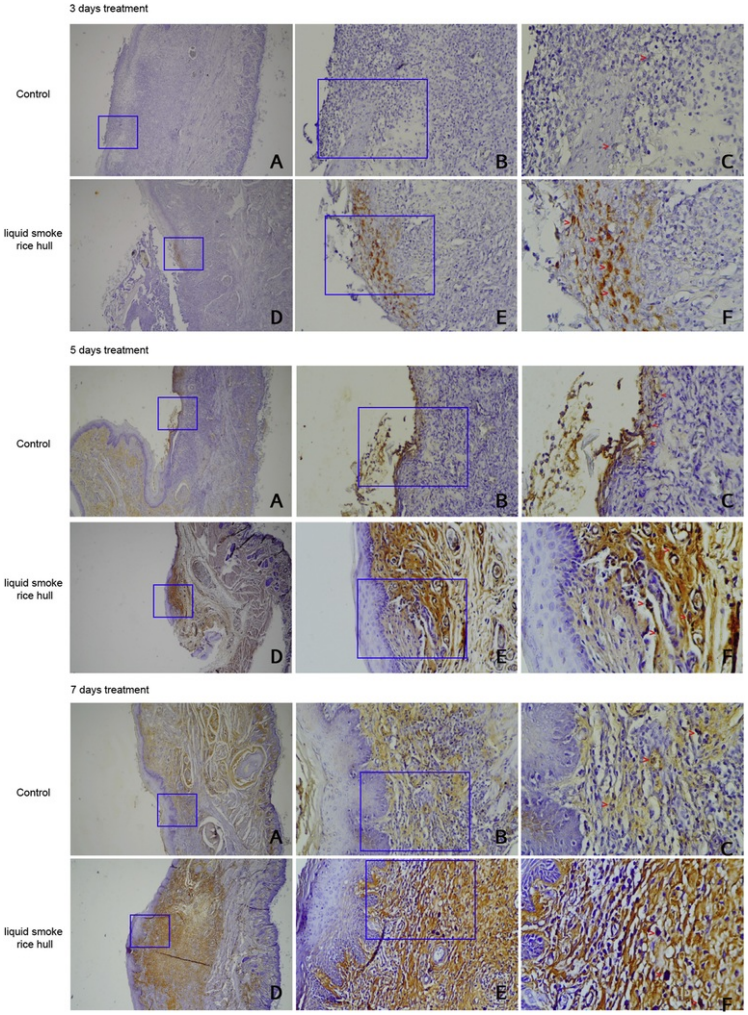

Figure 4 PDGF expression in traumatic ulcer tissue. (A and B) magnification 40×; (B and E) magnification 100×; (C and F) magnification 400×.

alt-text: Figure 4

Discussion

Wound healing is a normal biological process that includes four interdependent and overlapping phases.[25] However, today, there are many agents that can be used to accelerate wound healing, but these are derived from chemicals that have the potential to create adverse side effects and often come at a monetary cost ~~high cost~~.[26] Therefore, new substances capable of accelerating the wound healing process are still needed. Wound healing requires a complex process in which keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets have their own roles to play. These cells undergo several steps to restore the epithelium, including migration and proliferation. They regulate cellular response in the wound healing phase with the aid of growth factors, such as PDGF, FGF, VEGF, and COL-1.[27,28]

In this context, several *in vivo* studies with animal models have shown that natural ingredients with anti-inflammatory and antioxidant properties produce good results in accelerating wound healing. In this study, LR-HS

treatment increased FGF, VEGF, PDGF, and COL-1 expression compared to in the control group. The mechanism that might be involved uses the anti-inflammatory and antioxidant properties of phenolic compounds, such as phenol, guaiacol, and 4-ethyl-2-methoxy phenol, which are present in LR-HS.[6] In a ~~the~~ previous study, liquid smoke has been shown to have anti-inflammatory effects and the ability to inhibit NF-kB activation in macrophages.[7] Given the antioxidant ability, reactive oxygen species (ROS) production was inhibited, resulting in a decrease in IkB kinase (IKK) complex activity, thus preventing I-κB degradation and inhibiting NF-kB activation.[15,16] NF-kB is the main pathway for regulating the equilibrium of cellular redox state and inflammatory responses.[29] During oxidative stress situations, I-kB kinase is activated, and NF-kB is released. NF-kB causes pro-inflammatory mediator transcription at the nuclear site, such as interleukin 1β (IL-1β), interleukin 6 (IL-60, and tumour necrosis factor α (TNF-α). Excessive production of pro-inflammatory cytokine can cause delayed wound healing.[30] Excessive production of ROS at wound sites produces toxic effects, causing inflammation and the degradation of repair mechanisms.[31]

The phenolic compounds in LR-HS can interfere with the NF-kB and IKK signalling pathways by inhibiting ROS and cytokine production, such as TNF-α.[32] TNF-α is a pro-inflammatory cytokine, and in chronic wounds, its levels have been shown to be upregulated locally and systemically, causing delays in wound healing.[33] Therefore, agents that can block the secretion of TNF-α or the interaction between TNF-α and its receptors can help heal chronic wounds. In this study, it was noted that treatment with LR-HS significantly downregulated TNF-α. Uncontrolled migration of neutrophils at a wound bed is known to trigger excessive production of ROS and proteases that encourage systemic inflammation and cause increased tissue harm. These cells also release TNF-α and other pro-inflammatory cytokines, which are, in turn, responsible for chronic inflammatory responses.[31]

Inhibited NF-kB and TNF-α resulted in more dominant M2 polarisation through a switch from M1 to M2 polarisation.[19] M2 secretes growth factors, including TGF-β, FGF, VEGF, PDGF, and COL-1, which contribute to healing, fibroblast proliferation, and collagen production.[20–24] Platelets, macrophages, endothelial cells, fibroblasts, and keratinocytes are sources of PDGF. M2 macrophages produce PDGF, resulting in the inducement of α-SMA expression in fibroblasts.[24] M2 also facilitates tumour development by producing proteases, such as metalloproteinase-9 (MMP-9), to degrade the extracellular matrix and releasing growth factors (such as VEGF, FGF, and PDGF) for a proliferation of endothelial cells and micro vessel formation.[34]

M2 macrophages play a role in the formation of new vessels, and increased macrophage numbers correlate with a high micro-vessel density during this phase. This study has shown that increased macrophages promote VEGF, as there is an increase in VEGF of ~~on~~ Days 5 and 7 after LR-HS treatment. VEGF plays a role in inducing permeable blood vessels and vascular permeability in the injured area, suggesting that VEGF contributes to vascular permeability ~~of vascularity~~ during the early stages of the traumatic ulcer healing process. ~~from the healing process of traumatic ulcers in the early stages~~.[35,36] VEGF plays a role in triggering cell proliferation and differentiation during the process of angiogenesis and increasing endothelial cell proliferation, differentiation, and migration.[37,38] VEGF increases leukocyte rolling, which is very important for inflammatory cell mobility from the bloodstream to the tissues––a feature of the inflammatory response.[39] In the proliferation phase, several types of cells work together to repair tissue, leading to total wound healing. Keratinocytes migrate to the wound area to perform repairs during the epithelialisation process. The new vessels function by supplying oxygenated blood and nutrients, which are needed to support the activity of the cells that are involved in this proliferation phase.[36]

PDGF is a potent mitogen and chemo-attractant for mesenchymal cells, which play a role in the regulation of cell growth, cell division, angiogenesis, the stimulating of macrophages and neutrophil chemotaxis, myofibroblast and fibroblast proliferation, and chemotaxis, as well as smooth muscle cells' secretion of other growth factors from macrophages.[28,40]

FGF is well-known for its efficacy in healing skin wounds; it can enhance fibroblast activation and proliferation by stimulating collagen accumulation and endothelial cell division. FGF thus promotes angiogenesis, which plays a significant role in cell repair.[27,41] FGF also induces COL-1 synthesis; hence, it plays a pivotal role in wound healing.[41]

The increase in COL-1 may be due to increased FGF and fibroblast proliferation,[2,41] as this study has shown that RHLS increased FGF, thus increasing the proliferation of fibroblasts. Fibroblasts are the most commonly found cell in connective tissues throughout the body and are the principal extracellular matrix (ECM) source. Fibroblasts produce ECM ground substance (glycosaminoglycans, such as glycoproteins and hyaluronan), adhesive proteins (fibronectin and laminin), and structural proteins (elastin fibrous collagen).[42] The rate of collagen synthesis is considerably higher on the second day after an operation and continues at a high level until at least the seventh post-operative day.[43] Collagen plays a key role in skin structure maintenance and is important for firm, healthy skin. There are around 28 different collagens that occur in vertebrates, with COL-1 being the most abundant. COL-1 is known for its rope-like structure and its promotion of wound healing by increasing tensile strength in large, open dermal wounds.[44–47]

Conclusion

Based on analysis using immunohistochemical staining, LR-HS can induce the expression of the growth factors FGF, VEGF, PDGF, and COL-1 during traumatic ulcer healing. A 7-day treatment duration was sufficient for FGF, VEGF, PDGF, and COL-1 expression. This finding strengthens the assertion that LR-HS can be used as herbal medicine in oral ulcer therapy.

Recommendation

It is recommended that further studies be conducted to isolate each component of LR-HS and assess their pharmacodynamic and pharmacokinetic properties to strengthen their role in the treatment of traumatic ulcers.

Source of funding

This work is supported by the Ministry of Higher Education, Republic of Indonesia 2020 in the schema Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT), under the grant number 607/UN3.14/PT/2020.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical approval

This study was performed in strict accordance with the Guide for the Care and Use of Laboratory Animals, National Health Research and Development Ethics Standard and Guidelines Council (2017), Minister of Health, Republic of Indonesia. The protocol was approved by the Ethical Clearance of Health Experiment Committee, Faculty of Dental Medicine, Airlangga University, Surabaya under registered-number 132/HRECC.FODM/IV/2019 (approval date was April 4, 2019).

Authors' contributions

IA designed the study, acquired funding, and revised the draft article. ID conducted research, acquired funding, and revised the draft article. MDCS organised, analysed, and interpreted the data; acquired funding; and wrote the initial draft of the article. EM conducted research and co-wrote the initial draft of the article. NMA conducted research and co-wrote the initial draft of the article. All authors have critically reviewed and approved the final draft of the article and are responsible for the manuscript's content and similarity index.

References

1. W.E. Triastuti, P.A. Budhi, E. Agustiani, R.A. Hidayat, R. Retnoningsih and A.A. Nisa', Caracterization of liquid smoke bamboo waste with pyrolysis method, *IPTEK J Proc Ser* **3**, 2019, 114–117.

2. M.D.C. Surboyo, I. Arundina, R.P. Rahayu, D. Mansur and T. Bramantoro, Potential of distilled liquid smoke derived from coconut (Cocos nucifera L) shell for traumatic ulcer healing in diabetic rats, *Eur J Dent* **13** (2), 2019 May 5, 271–279.

3. C.T. Nithin, C.G. Joshy, N.S. Chatterjee, S.K. Panda, R. Yathavamoorthi, T.R. Ananthanarayanan, et al., Liquid smoking - a safe and convenient alternative for traditional fish smoked products, *Food Contr* **113** (January), 2020, 107186.

4. I. Arundina, T. Tantiana, I. Diyatri, M.D.C. Surboyo and R. Adityasari, Acute toxicity of liquid smoke of rice hull (Oryza sativa) on mice (Mus musculus), *J Int Dent Med Res* **13** (1), 2020, 91–96.

5. J.Y. Yang, E. Moon, S.H. Nam and M. Friedman, Antidiabetic effects of rice hull smoke extract on glucose- regulating mechanism in type 2 diabetic mice, *J Agric Food Chem* **60** (30), 2012, 7442–7449.

6. S. Phil Kim, J. Young Yang, M. Young Kang, J. Cheol Park, S. Hyun Nam and M. Friedman, Composition of liquid rice hull smoke and anti-inflammatory effects in mice, *J Agric Food Chem* **59** (9), 2011 Apr, 4570–4581.

7. M.D.C. Surboyo, F.Y. Mahdani, D.S. Ernawati, A. Sarasati and F. Rezkita, The macrophage responses during diabetic oral ulcer healing by liquid coconut shell smoke: an immunohistochemical analysis, *Eur J Dent* **14** (3), 2020 Jul 24, 410–414.

8. M.D.C. Surboyo, I. Arundina and R.P. Rahayu, Increase of collagen in diabetes-related traumatic ulcers after the application of liquid smoke coconut shell, *Dent J (Majalah Kedokt Gigi)* **71** (32), 2017, 71–75.

9. M.D.C. Surboyo, D.S. Ernawati, I. Arundina and R.P. Rahayu, Oral ulcer healing after treatment with distilled liquid smoke of coconut shell on diabetic rats, *J Krishna Inst Med Sci Univ* **8** (2), 2019, 70–79.

10. M.D.C. Surboyo, T. Tantiana and I. Arundina, Analgesic effect of coconut shell (Cocos nucifera L) liquid smoke on mice, *Dent J (Majalah Kedokt Gigi)* **45** (3), 2012, 156–160.

11. C. Politis, J. Schoenaers, R. Jacobs and J.O. Agbaje, Wound healing problems in the mouth, *Front Physiol* **7**, 2016 Nov 2, 1–13.

12. R. Tripathi and K. Tripathi, Management of non healing oral ulcer in diabetic patient using topical application of epidermal growth factor: a case report, *Scholars Acad J Biosci* **3** (8), 2015, 640–643.

13. B. Behm, P. Babilas, M. Landthaler and S. Schreml, Cytokines, chemokines and growth factors in wound healing, *J Eur Acad Dermatol Venereol* **26** (7), 2012 Jul, 812–820.

14. F. Zarei and M. Soleimaninejad, Role of growth factors and biomaterials in wound healing, *Artif Cells Nanomed Biotechnol* **46** (sup1), 2018 Oct 31, 906–911.

15. M.S. Hayden and S. Ghosh, Regulation of NF-kB by TNF family cytokines, *Semin Immunol* **26** (3), 2014, 253–266.

16. K. Lingappan, NF-κB in oxidative stress, *Curr Opin Toxicol* **7**, 2018, 81–86.

17. M. Friedman, Rice brans, rice bran oils, and rice hulls: composition, food and industrial uses, and bioactivities in humans, animals, and cells, *J Agric Food Chem* **61** (45), 2013 Nov 13, 10626–10641.

18. I. Arundina, I. Diyatri, T. Kusumaningsih, M.D.C. Surboyo, E. Monica and N.M. Afanda, The role of rice hull liquid smoke in the traumatic ulcer healing, *Eur J Dent* **1–7**, 2020.

19. Y. Zhou, T. Zhang, X. Wang, X. Wei, Y. Chen, L. Guo, et al., Curcumin modulates macrophage polarization through the inhibition of the toll-like receptor 4 expression and its signaling pathways, *Cell Physiol Biochem* **2015** (3), 2015, 631–641.

20. D.T. Ploeger, N.A. Hosper, M. Schipper, J.A. Koerts, S. De Rond and R.A. Bank, Cell plasticity in wound healing: paracrine factors of M1/M2 polarized macrophages influence the phenotypical state of dermal fibroblasts, *Cell Commun Signal* **11** (1), 2013, 1–11.

21. M. Akiyama, H. Yasuoka, K. Yoshimoto and T. Takeuchi, CC-chemokine ligand 18 is a useful biomarker associated with disease activity in IgG4-related disease, *Ann Rheum Dis* **77** (9), 2018, 1386–1387.

22. D.A. Chistiakov, V.A. Myasoedova, V.V. Revin, A.N. Orekhov and Y.V. Bobryshev, The impact of interferon-regulatory factors to macrophage differentiation and polarization into M1 and M2, *Immunobiology* **223** (1), 2018, 101–111.

23. Y. Bi, J. Chen, F. Hu, J. Liu, M. Li and L. Zhao, M2 macrophages as a potential target for antiatherosclerosis treatment, *Neural Plast* **2019**, 2019 Feb 21, 1–21.

24. J.E. Glim, F.B. Niessen, V. Everts, M. van Egmond and R.H.J. Beelen, Platelet derived growth factor-CC secreted by M2 macrophages induces alpha-smooth muscle actin expression by dermal and gingival fibroblasts, *Immunobiology* **218** (6), 2013, 924–929.

25. Y.H. Yen, C.M. Pu, C.W. Liu, Y.C. Chen, Y.C. Chen, C.J. Liang, et al., Curcumin accelerates cutaneous wound healing via multiple biological actions: the involvement of TNF-α, MMP-9, α-SMA, and collagen, *Int Wound J* **15** (4), 2018, 605–617.

26. C. Anlas, T. Bakirel, F. Ustun-Alkan, B. Celik, M. Yuzbasioglu Baran, O. Ustuner, et al., In vitro evaluation of the therapeutic potential of Anatolian kermes oak (Quercus coccifera L.)as an alternative wound healing agent, *Ind Crop Prod* **137** (December 2018), 2019, 24–32.

27. Y.H. Song, Y.T. Zhu, J. Ding, F.Y. Zhou, J.X. Xue, J.H. Jung, et al., Distribution of fibroblast growth factors and their roles in skin fibroblast cell migration, *Mol Med Rep* **14** (4), 2016, 3336–3342.

28. S. Yamakawa and K. Hayashida, Advances in surgical applications of growth factors for wound healing, *Burn Trauma* **7**, 2019, 1–13.

29. J.D. Wardyn, A.H. Ponsford and C.M. Sanderson, Dissecting molecular cross-talk between Nrf2 and NF-κB response pathways, *Biochem Soc Trans* **43**, 2015, 621–626.

30. M. del Carmen Villegas-Aguilar, Fernández-Ochoa Á, M. de la Luz Cádiz-Gurrea, S. Pimentel-Moral, J. Lozano-Sánchez, D. Arráez-Román, et al., Pleiotropic biological effects of dietary phenolic compounds and their metabolites on energy metabolism, inflammation and aging, *Molecules* **25**, 2020, 1–27.

31. H.S. Yaseen, M. Asif, M. Saadullah, Asghar S. Mahrukh, M.U. Shams, et al., Methanolic extract of Ephedra ciliata promotes wound healing and arrests inflammatory cascade in vivo through downregulation of TNF-α, *Inflammopharmacology* **28**, 2020 May 8, 1691-1704.

32. P. Limtrakul, S. Yodkeeree, P. Pitchakarn and W. Punfa, Suppression of inflammatory responses by black rice extract in RAW 264.7 macrophage cells via downregulation of NF-kB and AP-1 signaling pathways, *Asian Pac J Cancer Prev APJCP* **16** (10), 2015, 4277–4283.

33. K.W. Finnson, S. McLean, G.M. Di Guglielmo and A. Philip, Dynamics of transforming growth factor beta signaling in wound healing and scarring, *Adv Wound Care* **2** (5), 2013, 195–214.

34. N. Jetten, S. Verbruggen, M.J. Gijbels, M.J. Post, M.P.J. De Winther and M.M.P.C. Donners, Anti-inflammatory M2, but not pro-inflammatory M1 macrophages promote angiogenesis in vivo, *Angiogenesis* **17** (1), 2014, 109–118.

35. M. Rodrigues, N. Kosaric, C.A. Bonham and G.C. Gurtner, Wound healing: a cellular perspective, *Physiol Rev* **99** (1), 2019, 665–706.

36. K.E. Johnson and T.A. Wilgus, Vascular endothelial growth factor and angiogenesis in the regulation of cutaneous wound repair, *Adv Wound Care* **3** (10), 2014, 647–661.

37. G.K. Kolluru, S.C. Bir and C.G. Kevil, Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing, *Int J Vasc Med* **2012**, 2012, 1–30.

38. D.S. Ernawati and A. Puspa, Expression of vascular endothelial growth factor and matrix metalloproteinase-9 in Apis mellifera Lawang propolis extract gel-treated traumatic ulcers in diabetic rats, *Vet World* **11** (3), 2018, 304–309.

39. M. Detmar, L.F. Brown, M.P. Schön, B.M. Elicker, P. Velasco, L. Richard, et al., Increased microvascular density and enhanced leukocyte rolling and adhesion in the skin of VEGF transgenic mice, *J Invest Dermatol* **111** (1), 1998, 1–6.

40. S. Gökşen, B. Balabanlı and Ş. Coşkun-Cevher, Application of platelet derived growth factor-BB and diabetic wound healing: the relationship with oxidative events, *Free Radic Res* **51** (5), 2017, 498–505.

41. R. De Araújo, M. Lôbo, K. Trindade, D.F. Silva and N. Pereira, Fibroblast growth factors: a controlling mechanism of skin aging, *Skin Pharmacol Physiol* **32** (5), 2019, 275–282.

42. R.T. Kendall and C.A. Feghali-Bostwick, Fibroblasts in fibrosis: novel roles and mediators, *Front Pharmacol* **5** (MAY), 2014, 1–14.

43. S. Zhou, J. Salisbury, V.R. Preedy and P.W. Emery, Increased collagen synthesis rate during wound healing in muscle, *PloS One* **8** (3), 2013, 8–11.

44. S. Ricard-Blum, The collagen family, *Cold Spring Harb Perspect Biol* **3** (1), 2011, 1–19.

45. R.I. Schwarz, Collagen I and the fibroblast: high protein expression requires a new paradigm of post-transcriptional, feedback regulation, *Biochem Biophys Rep* **3**, 2015, 38–44.

46. M. Xue and C.J. Jackson, Extracellular matrix reorganization during wound healing and its impact on abnormal scarring, *Adv Wound Care* **4** (3), 2015, 119–136.

47. S. Chattopadhyay and R.T. Raines, Collagen-based biomaterials for wound healing, *Biopolymers* **23** (1), 2014, 1–7.

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