IN-VITRO ANALYSIS OF HEALING PROPERTIES OF FLAXSEED (LINUM USITATISSIMUM) EXTRACT ON HUMAN GINGIVAL FIBROBLAST (HGF) AND HUMAN OSTEOBLAST-LIKE (SAOS2) CELLS

BY

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INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

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ABSTRACT

The process of tooth extraction causes injury to the soft and hard tissues. The restoration of the damage tissues and the healing process takes few weeks in normal cases but could also extended to months in cases with complication. The bioactivities of flaxseed (*linum usitatissimum*) extract; also known as Biji Rami in Malay, such as antimicrobial, antioxidant and anti-inflammatory effects including the extract's ability to enhance healing of skin wounds *in-vivo* have been reported. However, the effect of the extract on the socket healing process (post tooth extraction) has not been demonstrated and described. Thus, the current research might be the first *in-vitro* study investigating the effect of flaxseed extract on the socket healing process using two different cell lines; human gingival fibroblast (HGF) and human osteoblast-like (SaOS2) cell lines, representing the soft tissue and bone, respectively, both of which are involved in the process to mimic socket healing. Additionally, the effect of flaxseed extract on the expression of wound healing-related genes was also analysed on these cell lines. Flaxseed was extracted using soxhlet extraction method with both absolute ethanol (ABS EtOH) and 60% ethanol in water (60% EtOH) as an extraction medium. The active components of flaxseed extract from both extractions were assessed using gas chromatography mass spectrum (GC-MS) which demonstrated many active compounds such as lignan, oleic acid and linoleic acid. The activity of both cell lines against flaxseed extract in both ABS EtOH and 60% EtOH were analysed using colorimetric MTT (3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay in three different time lines (24,48, and 72 hours) which revealed the HGF and SaOS2 cell proliferation activity in both extracts increasing by time. Wound healing activity was performed *via* scratch assay on both cell lines and the results were recorded in a time line manner (18,24,48,72, 96 and 120 hours) to closely resembled the wound healing ability of the extract. Interestingly, the results illustrated cell regeneration in the wound area occurs as early as 18 hours' post flaxseed extract treatment which lasted for 5 days. Lastly, some wound healing related genes; *EGFR*, *BMP2*, and *FGF2* were measured using real time q-PCR to confirm the wound healing activity of flaxseed extract on HGF and SaOS2 cell lines. The cells treated with flaxseed extract revealed a significant increase in the expression of the mentioned wound healing related genes. ABS EtOH flaxseed extract managed to increase the expression of these genes in both cell lines in significantly higher levels ($p < 0.05$) than the negative control, all the results were compared to a negative control to prove the activity of the extract. This study indicated that both absolute ethanol and ethanol/water flaxseed extract have wound healing effects on the cells that are involved in the process of socket healing, due to the major active components present in the extract such as lignan, linoleic acid and oleic acid; all of which aid in the process of wound healing, due to their high anti-inflammatory and anti-oxidant effects. This study also confirms that flaxseed extract elevates the expression of wound healing related genes hence it enhances the wound healing process. Therefore, flaxseed extract can be considered as a potential wound healing agent for oral wounds. Future *in vivo* analysis of oral wound in animal model will further justify the positive effects of flaxseed extract as a natural-based wound healing agent.

خلاصة البحث

تتضمن عملية قلع الأسنان إصابة الأنسجة الرخوة والصلبة. تستغرق استعادة هذا الضرر وعملية الشفاء أسابيع قليلة في الحالات العادية إلى شهور في الحالات القصوى. تم الإبلاغ عن الأنشطة الحيوية لمستخلص بذور الكتان المعروف ببيجي رامي بالغة الماليزية مثل التأثيرات المضادة للميكروبات ومضادات الأكسدة والمضادة للالتهابات بالإضافة إلى قدرة المستخلص على تعزيز التئام الجروح الجلدية في الجسم الحي. مع ذلك، لم يتم الإبلاغ عن تأثير الاستخراج على عملية التئام السنخ (ما بعد قلع الأسنان). قد تكون هذه أول دراسة في المختبر تركز على وتحقق من تأثير مستخلص بذور الكتان على عملية التئام التجويف باستخدام سطرين مختلفين من الخلايا الليفية اللثوية البشرية وخلايا شبيهة للعظم البشري، واختيار هذين الخطين الخلويين نظرًا لحقيقة أنها تمثل الأنسجة الرخو والعظام وكلاهما يشارك في عملية محاكاة التئام التجويف. تم استخلاص بذور الكتان باستخدام طريقة (سوكلت) استخراج التي تحتوي على كلاً من الإيثانول بنسبة ٦٠٪ من الإيثانول في الماء كوسيط استخلاص والإيثانول المطلق، وتم تقييم المكونات النشطة لمستخلص بذور الكتان باستخدام الطيف الكتلي اللوني للغاز الذي أظهر العديد من المركبات النشطة مثل الليغنان وحمض الأوليك وحمض اللينوليك. تم قياس نشاط كلا الخطين الخلويين ضد مستخلص بذور الكتان في كل من الإيثانول بنسبة ٦٠٪ في الماء والإيثانول المطلق باستخدام مقايسة اللونية (٣- (٤،٥-ديميثيازول-٢-يل) -٢،٥-ثنائي فينيل رباعي بروميد البروميد) MTT في ثلاثة خطوط زمنية مختلفة (٢٤ و٤٨ و٧٢ ساعة) والتي شجبت نشاط تكاثر كلا الخلايا ولكلا المستخلصين مع مرور الوقت. بالنسبة لفحص نشاط التئام الجروح، تم إجراء اختبار خدش على سطري الخلايا وتم تسجيل النتائج بطريقة الخط الزمني (٩٦،٧٢،٤٨،٢٤،١٨ و١٢٠ ساعة) للمساعدة عن كثب في قدرة المستخلص على التئام الجروح، والنتائج يوضح تجديد الخلايا في منطقة الجرح في وقت مبكر من ١٨ ساعة بعد العلاج بخلاصة بذور الكتان التي تستمر لمدة ٥ أيام. أخيرًا، تم قياس بعض الجينات المتعلقة بالتئام الجروح (*EGFR*، *BMP2*، *FGF2*) باستخدام تقنية ال RT-qPCR ولتأكيد نشاط التئام الجروح لمستخلص بذور الكتان على خطوط الخلايا تظهر الخلايا المعالجة بمستخلص بذور الكتان تعبيرًا متزايدًا بشكل ملحوظ عن الجينات المذكورة ذات الصلة بالتئام الجروح. تمكن مستخلص بذور الكتان بالإيثانول المطلق من زيادة التعبير عن الجينات المذكورة في كلا الخلايا بمستويات هامة (p <٠,٠٥) اعلى من نسبة التحكم السلبي. تم مقارنة جميع النتائج مع عنصر تحكم سلبي لأثبات فعالية المستخلص. تؤكد هذه الدراسة أيضًا أن مستخلص بذور الكتان يرفع من التعبير عن الجينات ذات الصلة بالتئام الجروح، وبالتالي فهو يعزز عملية التئام الجروح. تشير هذه الدراسة إلى أن كلاً من الإيثانول والإيثانول / المائي لمستخلص بذور الكتان لهما تأثيرات على التئام الجروح على الخلايا التي تشارك في عملية التئام السنخ، لذلك يعتبر مستخلص بذور الكتان عاملًا محتملاً في التئام الجروح في الفم.

APPROVAL PAGE

The thesis of Sama Naziyah Shaban has been approved by the following

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Sama Naziyah Shaban

Signature ..... Date .....

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I declare that the copyright holders of this thesis are jointly owned by the student and IIUM.

Affirmed by Sama Naziyah Shaban

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