ABSTRACT

Objective: This study aims to investigate the effects of the *Heliotropium indicum* extract (HIE) on factor promoting wound healing in radical scavenging and inflammatory activity and growth factor promotion.

Methods: The radical scavenging capacity of HIE was evaluated by scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO) radicals. Furthermore, the anti-inflammatory activity of HIE was determined in a cellular model. RAW264.7 macrophage cells were treated with various concentrations of HIE before activating the treated cells with lipopolysaccharide (LPS). The nitrite concentration of activated macrophage was determined by the Griess reagent kit. The cell viability of RAW264.7 was evaluated by resazurin reduction assay as well as NIH3T3 fibroblast cells. In addition, production of the growth factors (transforming growth factor-β [TGF-β] and basic fibroblast growth factor [bFGF]) of fibroblast was determined by Elisa kit.

Results: HIE exhibited radical scavenging activity in the DPPH and NO radicals with half maximal inhibitory concentration (IC₅₀) at 0.22 mg/ml and 0.52 mg/ml, respectively. In a cellular study, HIE inhibited NO production in LPS-stimulated macrophage without cytotoxic effect to the cells with IC₅₀ at 87 µg/ml. Furthermore, HIE promoted fibroblast cell viability at 72 h of treatment and TGF-β and bFGF production at 24 h of treatment.

Conclusion: These results obtained in this study suggests that HIE promoted the factors which involved in wound healing processes, including anti-inflammatory effect with scavenged radical forming and inhibited activated-macrophage. Furthermore, HIE also stimulated growth factor production in fibroblast. These finding supported using traditional and folk medicine of *H. indicum* in wound treatment.

Keywords: *Heliotropium indicum*, Wound healing, Radical scavenging, Anti-inflammatory, Growth factors.
been used to cure various diseases in different traditional and folklore medicine system. In folk remedies, *H. indicum* has been used for treating fever, insect bite, diarrhea, skin rash, and herpes as well as wound and ulcer [11]. Furthermore, it has been reported that the application of the leaf paste of *H. indicum* to heal rheumatism and skin infections as well as fresh cuts and wounds [12]. Previous studies in the animal model reported the healing promotion of *H. indicum* extract [HIE (HE)] in the incision and infected wound [12] as well as diabetic wound [13].

This study aims to examine the HIE on the factor promoting wound healing including (a) *in vitro* radical scavenging activity, (b) anti-inflammatory activity in macrophage, (c) production of growth factors (TGF-β1 and bFGF) in fibroblast, and (d) viability of macrophage and fibroblast cells. These results provide scientific support of using *H. indicum* in folklore medicine and a new direction for the promotion of wound healing.

**METHODS**

**Plant material**

*H. indicum* Linn, procured from Khaen Dong District, Buri Ram Province, Northeastern Thailand. The leaves of the plant were cleaned with water then dried at 50°C for 72 h. The dried plant was ground and stored at room temperature in vacuum. The extract was prepared by maceration of the grounded plant in 70% ethanol and shook at 250 rpm with the shaker for 24 h. The macerated mixture was filtered for collected supernatant to evaporate at 50°C for 24 h. The crude extract of *H. indicum* leaves was stored at −20°C until used.

**2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of HIE**

The HIE was first dissolved in a 2% dimethyl sulfoxide (DMSO), approximately 75 µl of the dissolved HIE of variable concentrations (0.0156, 0.0312, 0.0625, 0.125, 0.25, 0.5, 1, and 2 mg/ml) was mixed with 150 µl of 0.2 mM DPPH (Sigma-Aldrich USA) solution (in methanol) and allowed to stand for 30 min without direct exposure to light. The absorbance was determined at 520 nm using a microplate reader. In addition, DMSO and L-ascorbic acid were used as negative and positive controls, respectively. The DPPH scavenging capacity of the experimental HIE is presented as a percentage of DPPH radical inhibition as below, where OD is the optical density:

\[
\text{%DPPH radical inhibition} = \left(\frac{\text{OD}_{\text{without extract}} - \text{OD}_{\text{with extract}}}{\text{OD}_{\text{without extract}}}\right) \times 100
\]

**NO radical scavenging of HIE**

In this research, sodium nitroprusside (SNP) (Sigma-Aldrich, USA) was utilized as the NO donor. Specifically, 10 mM of SNP in a pH 7.4 PBS solution was incubated with 1 ml dissolved HIE of variable concentrations (0.0156, 0.0312, 0.0625, 0.125, 0.25, 0.5, 1, and 2 mg/ml) at 25°C for 180 min. Approximately 100 µl of the resulting solution was withdrawn to react with a Griess Reagent kit (Promega, USA) whereby the solution was reacted with 20 µl sulfanilamide for 10 min and then 20 µl N-1-naphthylethylenediamine dihydrochloride for another 10 min. The reaction mixture absorbance was measured at 560 nm, and the NO concentrations were determined from nitrite (NO$_2^-$) in the standard curve of a standard nitrite solution. The reaction mixture absorbance was measured at 560 nm, and the NO concentrations were determined as the nitrite (NO$_2^-$) concentrations from the standard curve of a standard nitrite solution. About 0.2% DMSO and 100 µl of dexamethasone each with 1 µg/ml LPS were, respectively, used as the negative and positive controls.

**NO production in the lipopolysaccharide (LPS)-stimulated macrophage**

The RAW264.7 macrophage cells (2×10$^5$ cell/ml) were pretreated with the dissolved HIE of variable concentrations (6.25, 12.5, 25, 50, and 100 µg/ml) in a 96-well plate and incubated at 37°C for 24 h. The pre-treated cells were stimulated with 1 µg/ml of LPS and incubated for another 24 h. The NO concentrations were determined from nitrite (NO$_2^-$) in the stimulated-cell supernatant using a Griess Reagent Kit whereby 100 µl of the supernatant was reacted with 20 µl sulfanilamide for 10 min and with 20 µl N-1-naphthylethylenediamine dihydrochloride for another 10 min. The reaction mixture absorbance was measured at 560 nm, and the NO concentrations were determined as the nitrite (NO$_2^-$) concentrations from the standard curve of a standard nitrite solution. About 0.2% DMSO and 100 µM of dexamethasone each with 1 µg/ml LPS were, respectively, used as the negative and positive controls.

**Determination of cell viability of RAW264.7 macrophage cells**

The viability of the residual macrophage cells after the NO assay, given HIE concentrations of 6.25, 12.5, 25, 50, and 100 µg/ml, was examined by resazurin (Sigma-Aldrich, USA) reduction assay, whereby the residual cells were incubated for 2 h at 37°C in 100 µM fresh DMEM containing 50 µg/ml resazurin. The reaction mixture absorbance was determined at 560 against 600 nm. The cell viability of the RAW264.7 macrophage cells was presented as percentage cell viability using the following formula:

\[
\text{%Cell viability} = \left(\frac{\text{OD}_{560} - \text{OD}_{600} \text{with extract}}{\text{OD}_{560} - \text{OD}_{600} \text{without extract}}\right) \times 100
\]

**Determination of cell viability of NIH3T3 fibroblast cells**

The NIH3T3 fibroblast cells (4×10$^5$ cell/ml) were seeded in a 96-well plate and incubated at 37°C for 24 h. The cells were treated with the variable of HIE concentrations at 6.25, 12.5, 25, 50, and 100 µg/ml, was examined by resazurin (Sigma-Aldrich, USA) reduction assay, whereby the cells were incubated for 4 h at 37°C in 100 µl fresh DMEM containing 50 µg/ml resazurin. The reaction mixture absorbance was determined at 560 against 600 nm. The cell viability of the NIH3T3 fibroblast cells was presented as percentage cell viability using the above formula.

**TGF-β and bFGF production in fibroblast cells**

The NIH3T3 fibroblast cells (4×10$^5$ cell/ml) were seeded into 24-well plate at 37°C for 24 h. The cells were treated with the variable of HIE concentration at 2.5, 25, and 50 µg/ml for another 24 h. The supernatant was collected to determine TGF-β and bFGF levels, respectively, using TGF-β Mouse Elisa kit (Abcam, USA) and bFGF Mouse Elisa kit (Sigma-Aldrich, USA) according to the manufacturer’s protocol.

**Statistical analysis**

In this study, data from at least three independent experiments carried out in triplicate. The statistical data were expressed as mean with a standard error of the mean. The negative control group was compared against the experimental group using one-way ANOVA with Tukey’s honestly significant difference (HSD) post hoc test, with the 5% (*p<0.05) and 1% (**p<0.01) significance level.
RESULTS

DPPH scavenging capacity of HIE

In this study, the DPPH scavenging capacity of HIE is evaluated by the percentage of DPPH free radical inhibition and half maximal inhibitory concentration (IC_{50}). IC_{50} is the IC at which the DPPH radicals are scavenged by 50%. The results revealed that with 0.0156–2 mg/ml (0.0156, 0.0312, 0.0625, 0.125, 0.25, 0.5, 1, and 2 mg/ml) of HIE concentrations, HIE significantly reduces DPPH radicals, achieving the inhibition performance in the range of 29.17±5.62–90.78±1.17% (Fig 1); and an IC_{50} of 0.22 mg/ml.

Table 1: DPPH and NO half maximal inhibitory concentrations of HIE and L-ascorbic acid

| Testing compound | IC_{50} of free radical scavenging (mg/ml) |
|------------------|------------------------------------------|
|                  | DPPH | NO |
| HIE              | 0.22 | 0.52 |
| L-ascorbic acid  | 0.030| 0.034|

DPPH: 2,2-diphenyl-1-picrylhydrazyl. NO: Nitric oxide. HIE: Heliotropium indicum extract. IC_{50}: Half maximal inhibitory concentration.

Effect of HIE on NO production and cell viability in LPS-stimulated macrophage

In this study, the RAW264.7 macrophage cells were treated with variable concentrations of HIE (6.25, 12.5, 25, 50, and 100 µg/ml) for 24 h before activating with 1 µg/ml LPS. Furthermore, DMSO and 100 µM of dexamethasone each with LPS were, respectively, used as the negative and positive controls. In Fig 2a, the experimental results showed that the nitrite (NO_{2}⁻) concentration in the LPS-stimulated macrophage was decreased in HIE-treated cells, achieving the inhibition performance in the range of 16.64±1.70–54.87±1.86% (Fig 2a); and an IC_{50} of 87 µg/ml. The NO inhibition is more obvious in the treatment with 12.5–100 µg/ml HIE concentrations. The dexamethasone-treated cells (positive control) achieved NO inhibition performance by 64.51±6.63% (Fig 2a).

In Fig 2b, the viability of the RAW264.7 macrophage cells was assessed by a resazurin reduction assay and the effective HIE concentrations determined. DMSO and dexamethasone each with 1 µg/ml LPS were, respectively, used as the negative and positive controls. In general, the cell viability and NO production are positively correlated. Given the non-cytotoxicity of HIE, the HIE-treated cells could achieve high NO inhibition performance with no effect to cell viability.

Effect of HIE on fibroblast cell viability

The cell viability of the NIH3T3 fibroblasts cells was examined by resazurin reduction assay. DMSO was used as negative control. In Fig 3, the fibroblast cells were treated with variable concentration of HIE 6.25–100 µg/ml. The results indicate that the cell viability of HIE-treated cell for 24, 48, and 72 h trend to increase. However, the cell viability of 24 and 48 h of treatment was not significantly different from the control (DMSO treated-
cells). For 72 h of HIE-treated cells, the cell viability is more pronounced in treatment with 50 µg/ml HIE concentration-treated cell (Fig. 3).

Effect of HIE on TGF-β1 and bFGF production in fibroblast cells
The growth factors, TGF-β1, and bFGF are determined by ELISA kit. The results exhibited that HIE with a concentration of 12.5, 25, and 50 µg/ml significantly increase the production of TGF-β1 (Fig. 4a) and bFGF (Fig. 4b).

DISCUSSION
Wound healing is the coordination and dynamic process that involves in many cell types, including macrophages and fibroblasts as well as their cytokines and growth factors. Macrophages promote and resolute inflammation, remove apoptotic or debris cells, and support cell proliferation, which makes equable of wound healing process [14]. However, macrophages have the potential to disturb different phases of repair, and persistent macrophage activation can lead to maladaptive, chronic inflammation, and dysfunction wound healing [15]. Macrophages continue to produce NO and ROS in prolonging inflammation that can cause to wound surrounding cell injury and chronic wound [16]. The previous study demonstrated that the chronic wound both infected and noninfected wounds improved by antioxidant, where it scavenges the excess free radicals to reduce the damage caused. In addition, the previous study revealed the correlation of radical scavenging properties of Sphaeranthus amaranthoides and wound healing improvement in mice [17]. This current study showed that HIE effectively scavenged free radical, DPPH, and NO. Based on these results, radical scavenging activity of HIE belongs to the enhancement of wound healing.

Moreover, the results also revealed the anti-inflammatory property of HIE through inhibition of NO production in activated macrophages. In general, although NO is a physiological signaling molecule, inordinate inducible NO becomes oxidative molecule which can interact with O$_3$ to produce cytotoxic oxidant peroxynitrite (ONOO$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^
function and production can help wound repair, especially the chronic wounds which presented growth factor deficiency.

In an animal model study, HIE promoted the healing of excision (normal and infected), incision, and dead space wound models [12], and excision wound in streptozotocin-induced diabetic rats [13]. The findings from our research in the cellular study provide a better understanding of the function of HIE in wound healing improvement. Given antioxidant, anti-inflammatory properties, and growth factors promotion of HIE, the extract could thus be applied to encourage chronic wound healing and increase the possibility of therapeutic application of HIE in wound healing. However, further phytochemical studies of HIE are required to separate the active compounds responsible for these pharmacological activities.

CONCLUSION

This experimental research has investigated the scavenging ability of HIE of variable concentration (0.0156–2 mg/ml) on DPPH and NO free radicals; anti-inflammatory property through inhibition of NO production in LPS-stimulated macrophage cells; and cell viability of LPS-stimulated macrophage and fibroblast cells, given the HIE concentration of 12.5–50 µg/ml. The experimental results revealed that the DPPH and NO scavenging performance and the HIE concentration are positively correlated, with the corresponding IC50 of 0.22 and 0.52 mg/ml. In addition, inhibition of NO production in LPS-stimulated RAW264.7 macrophage cells is also positively correlated to the HIE concentrations with no effect to cell viability. The experimental results also revealed that the cell viability of fibroblast trend to increase in 72 h of HIE treatment. Moreover, the results also presented the promotion of TGF-β1 and bFGF growth factors production in HIE-treated fibroblast cells. The findings demonstrate the benefit of HIE in wound healing promotion.

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