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## ANTIPROLIFERATIVE EFFECT OF LICORICE EXTRACT ON PROLIFERATION OF KELOID FIBROBLASTS

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### INTRODUCTION

Keloid formation is resulted from wound healing process and influenced by various factors such as age, gender, heredity, and ethnicity. Wound healing, as a normal biological process in the human body, is accomplished through different phases: hemostasis, inflammation, proliferation, and remodeling. For a wound to heal effectively, all four phases must occur in the right sequence. Different factors may interfere with one or more phases of this process. Scars are areas of fibrous tissue that replace normal skin (or other tissue) after injury. A scar results from the biological process of wound repair in the skin and other tissues of the body and constitutes a natural part of the healing process. Scarring is considered abnormal when the amount of fibrosis is excessive or suboptimal, as in hypertrophic, atrophic, or keloidal scars; when it affects normal function; and when it is symptomatic. (Nurul Syazana et al, 2001)

The present study was to determine the antiproliferative effect of Licorice (*glycyrrhiza glabra*) extract on keloid fibroblasts and the inhibitory activity of Licorice extract on TGF- $\beta$  and IL-6 production that played an important role in keloid pathogenesis.

### MATERIALS AND METHODS

#### Plant material and preparation of plant extracts

Licorice roots were purchased from the local Thai market and after that they were cleaned, dried in the hot air oven, and grounded to be a coarse powder. The powder was extracted with ethanol. The ethanolic extract was filtered and concentrated under reduced pressure. After that the ethanolic extract was dissolved in water and partitioned into four fractions by various solvents as follow: hexane, dichloromethane, n-butanol, and ethyl acetate. The ethanolic extract was tested on the proliferation of keloid fibroblast and the production of TGF- $\beta$ . Then the ethanolic extract and various fractions were studied on IL-6 secretion.

#### Antiproliferative effect of ethanolic Licorice extract on keloid fibroblasts

Keloid fibroblasts were seeded in a 96-well plate at a density of  $7.5 \times 10^4$  cells/ml in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum and incubated in a humidified atmosphere of 95% air and 5% CO<sub>2</sub> for 3 days at 37°C. After confluence, cells were incubated in fresh Dulbecco's modified Eagle's medium containing lipopolysaccharide and interferon- $\gamma$  under the same conditions for 12 h. Licorice extract at a concentration of  $1 \times 10^{-4}$  g/ml was added to cells and then incubated for 48 h. Cell proliferation was determined by MTT assay.

#### Determination of inhibitory activity of ethanolic Licorice extract on TGF- $\beta$

Keloid fibroblasts were grown in a 96-well plate at a concentration of  $1 \times 10^4$  cells/ml in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum and incubated in a humidified atmosphere of 5% CO<sub>2</sub> for 3 days at 37°C until confluence. Cells were then incubated in fresh Dulbecco's modified Eagle's medium containing interferon- $\gamma$  under the same conditions for 48 h. A  $1 \times 10^{-4}$  g/ml Licorice extract was added to cells and incubated for 24 h. Cells were then washed and supernatant were obtained for analysis using an ELISA kit.

#### Determination of inhibitory activity of ethanolic Licorice extract and various fractions on IL-6

Murine macrophage cell line RAW264.7 (American Type Culture Collection) were cultured in a 96-well plate at a concentration of  $1 \times 10^5$  cells/ml in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum and incubated in a humidified atmosphere of 5% CO<sub>2</sub> for 3 days at 37°C until confluence. The medium was subsequently removed and each well was washed with DMEM without fetal calf serum. The cells were pre-incubated with ethanol, hexane, dichloromethane, butanol and ethyl acetate extracts and stimulated by lipopolysaccharide (100 ng/ml). Then they were incubated for 12, 24, 48 h at 37°C in an atmosphere of 5% CO<sub>2</sub>. After incubation, the supernatants were removed and kept in -70°C until cytokines analysis. The IL-6 levels were quantified by ELISA kit according to the manufacture's protocols.

**RESULTS AND DISCUSSION**

**Antiproliferative effect of ethanolic Licorice extract on keloid fibroblasts**

**Table 1.** Antiproliferative effect of Licorice extract on keloid fibroblasts at the concentration of  $1 \times 10^{-4}$  g/ml

Sample	concentration (g/mL)	%Inhibition
Licorice extract	$1 \times 10^{-4}$	45.4

Table 1 shows the antiproliferative effect of Licorice extract on keloid fibroblasts compared with the control cell without sample test. After incubation for 48 h ,sample was determined by MTT assay. Licorice extract inhibited fibroblast proliferation for 45.4 % at the concentrations of  $1 \times 10^{-4}$ g/mL.

**Determination of inhibitory activity of ethanolic Licorice extract on TGF-β**

**Table 2.** Inhibitory activity on TGF-β production of Licorice extract, at the concentration of  $1 \times 10^{-4}$  g/ml.

Sample test	concentration (g/mL)	TGF-beta (pg/mL)	% inhibition
Negative control	-	54	-
Licorice extract	$1 \times 10^{-4}$	52.6	2.6

Table 2 exhibits the study of Licorice extract on the inhibition of TGF-β secretion. Sample was tested in comparison to negative control without sample test. After incubation for 24 h, sample was determined by using an ELISA kit . The inhibitory activity of this extract on TGF-β production is found to be 2.6% at the same concentration

**Determination of inhibitory activity of ethanolic Licorice extract and various fractions on IL-6**

**Table 3** Inhibitory activity on IL-6 production of ethanolic extract and various fractions, at the concentration of  $1 \times 10^{-4}$  g/ml.

Sample test	concentration (g/mL)	% inhibition
Ethanolic extract	$1 \times 10^{-4}$	62.9
Hexane extract	$1 \times 10^{-4}$	-71.4
Butanol extract	$1 \times 10^{-4}$	-62.6
Dichloromethane extract	$1 \times 10^{-4}$	21.8
Ethly acetate extract	$1 \times 10^{-4}$	86.2

The inhibitory activity of Licorice extract on IL-6 production, as presented in Table. 3, is found to be 62.9 % at the concentration of  $1 \times 10^{-4}$  g/mL. From this result, the ethanolic extract is likely to have antiproliferative effect on keloid fibroblast by reducing the secretion of IL-6. To determine the active fraction and confirm the activity, the ethanolic extract was successively partitioned by hexane, dichloromethane, ethyl acetate and n-butanol. The result showed that the ethyl acetate extract is found to be most inhibitory activity for 89.2 % whereas dichloromethane extract has low activity and the hexane and butanol extracts is found to be no activity.

## CONCLUSION

In this in vivo study, the ethanolic Licorice extract possessed the antiproliferative effect on keloid fibroblasts by reducing the level of IL-6. This inhibition activity of the extract was found to be present in ethyl acetate fraction of this plant. Detailed mechanisms and cytotoxic profile are needed to be further investigated. Our data suggest that Licorice is potent herb for further research on the prevention of keloid scars.

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