

## The Effect of *Forsythia suspensa* Extract on the Wound Healing of Rats

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### ABSTRACT

We examined the wound healing effect and toxicity of *Forsythia suspensa* extract to verify the therapeutic usefulness on wound treatment. Additionally, we examined its antibacterial effect. The wound healing effect of *Forsythia suspensa* extract was compared with the tetrachlorodecaoxygen anion complex (TCDO), protein free haemodialysate (Haemyl), and physiological saline.

The wound areas on the 4th, 11th, 14th day of treatment with *Forsythia suspensa* extract, TCDO, and Haemyl were significantly smaller than with the saline treatment. The wounds on the 8th day of treatment with *Forsythia suspensa* extract were only significantly smaller than the saline treatment. On the 8th day of treatment with *Forsythia suspensa* extract, TCDO, and Haemyl accelerated the wound epithelization more than saline treatment. There was not a significant difference in liver enzyme activity (GOT, GPT) and blood cell count between the *Forsythia suspensa* extract and the saline treatment. In addition, the *Forsythia suspensa* extract did not inhibit bacterial proliferation.

These results suggest that *Forsythia suspensa* extract has great possibility of being developed into new wound healing drugs. Its pharmacological effects seem to have no relation with antibacterial effect.

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**Key Words:** *Forsythia suspensa* extract, Tetrachlorodecaoxygen anion complex(TCDO), Haemyl,  
Wound healing

### INTRODUCTION

A Wound is an injury of the continuity of living tissue by chemical and physical damage. Wound healing is a very complicated process. It includes a platelet aggregation, blood clotting, fibrin formation, inflammatory response to injury, and alteration in the ground substance. The capillar vessels proliferate, the wounds contract, and are reconstructed from the regeneration of

certain cell types, Therefore, we divide the wound healing processes into inflammatory-, substrate-, proliferative-, and maturation phase. In spite of many agents for wound healing, several wounds are very hard to heal.

In this study, we want to verify the wound healing effect of the *Forsythia suspensa* Nakai (*Forsythia*) (Lee, 1981; Ha 1995) and to compare its effect to the tetrachlorodecaoxygen anion complex (TCDO) (Hina *et al.*, 1984; Youngman *et al.*, 1985) and Haemyl (Broghammer *et al.*, 1965; Chung *et al.*, 1994). *Forsythia suspensa*

Nakai belongs to the Oleaceae and is distributed in southern regions of China and Korea. Its water extract tastes bitter, and contains forsythol and sterol compound, saponin, flavonoid, vitamin P, and oleanolic acid. It inhibits the proliferation of *E. Coli*, *Staphylococcus*, *Streptococcus*, *Diphtheria*, and *Salmonella* (Ha 1995). Though it is used as an antipyretic and wound healing agent in oriental medicine, its effect has not been clearly examined. The Haemyl, a protein-free haemodialysate, and tetrachlorodecaoxygen anion complex (TCDO) were developed in Germany, their wound healing effect have already been verified, and used intensively clinically. We examined the wound healing effects by assessment of wound areas and histological examination. Moreover, we examined the liver enzyme activity and hematological variance to determine its toxicity.

## MATERIALS AND METHODS

### Wound healing effect

1) **Animals:** Each group has 17 heads of Spargue-Dawley(S.D.) rat and were maintained at room temperature and allowed to access sterilized food (Samyang Co., Korea) and water ad libitum. Their body weights were describe in Table 1.

2) **Experimental wounds:** Rats were anesthetized by intraperitoneal injection of ketamine (Yuhanyanghaeng, Korea, 100 mg/kg). We shaved closely the backs of the rats with a fine-toothed electric clipper. After sterilization of the rats skin with mixed solution of boric acid and hydroperoxide, we made the wounds of 20 mm in diameter on the backs by stamping with plastic tube of 20 mm in diameters and extended down to the panniculus carnosus using sterile scissors (Fig. 1). The initial wound areas were described in Table 1.

3) **Treatment of wounds:** We treated the wounds as described in Table 1. We used TCDO (Oxorine, Hanwha Pharmacy, Korea),

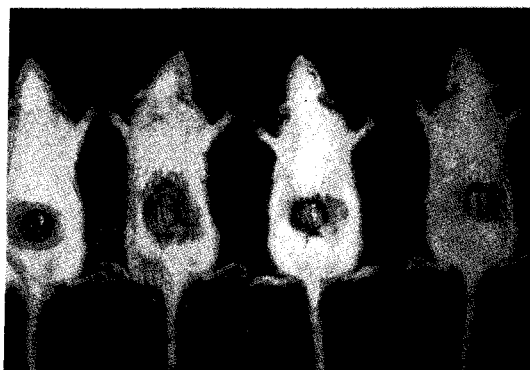


Fig. 1. Photograph of experimental wound model.

Table 1. Summary of important information of the animal study

| Group     | No. of animal | Body weight(g) before experiment | Initial wound area(mm <sup>2</sup> ) | Daily treatment   |
|-----------|---------------|----------------------------------|--------------------------------------|---|
| Control   | 16            | 153.5±45                         | 353±48                               | twice inoculation of physiological saline                         |
| TCDO      | 16            | 150±42                           | 342±38                               | twice inoculation of 1 ml TCDO                                    |
| Haemyl    | 16            | 149±44                           | 320±28                               | One i.p. injection of 1 ml Haemyl and twice inoculation of saline |
| Forsythia | 16            | 149±51                           | 346±51                               | drinking of extract ad libitum and twice inoculation of saline    |

(Mean±S.D.)

haemodialysate (Haemyl, Hanlim Pharmacy, Korea), and Forsythia suspensa extract. We made the extract of Forsythia suspensa by boiling 100 g dried Forsythia suspensa seed in 600 ml water for 2 hours. This original extract was diluted with water (1:10, V/V) and given to rats instead of drinking water.

4) **Assessment of wound surface:** In the experiment to test wound healing effect, results were represented by the ratio of healed wound area against initial wound area.

$$\text{Wound area(\%)} = \frac{100 \times \text{TL} \times \text{TS} \times 3.14}{\text{IL} \times \text{IS} \times 3.14}$$

TL: the longest half diameter of the treated wound

TS: the shortest half diameter of the treated wound

IL: the longest half diameter of the initial wound

IS: the shortest diameter of the initial wound

5) **Histological test:** On the 4th, and 8th day of treatment, the rats with the smallest wounds in each group were sacrificed and were taken skin samples. The skin samples were stained by the hematoxyline-eosine staining method. The granulation and epithelization of the wounds were observed by microscope (Olympus, Japan).

6) **Antibacterial test:** The inhibitory zone in diameter (mm) of bacterial proliferation was calculated by the agar diffusion test to determine antibacterial effect. After inoculations of bacterias (200  $\mu$ l) on the agar plate, 200  $\mu$ l of Forsythia suspensa extract or Povidone iodine as a kind of antiseptic agent (Hyundai Pharmacy, Korea) were transferred to the paper disc (8 mm in diameter, Toyo, Japan) on the agar plate, and incubated for 24 hours at 37°C. Agar plates were prepared from dissolving of heat extract 5g, peptone 15g, NaCl 5g, K<sub>2</sub>HPO<sub>4</sub> 5g, agar 15g in 1 L water. We used Staphylococcus aureus R-209, Bacillus subtilis IAM 1069, E. coli AB 1157, and Salmonella typhimurium SL 1102. We triplicated this experiment.

7) **Toxicological Tests:** The 12 male rats (S.D.) for each group were kept for two months at room temperature allowing to drink the diluted Forsythia extract with water (1:1, V/V), or drink water ad libitum. Their body weights at the beginning of the experiment were 147  $\pm$

44 g for the control group, and 149  $\pm$  45 g for the experimental group (Mean  $\pm$  S.D.). We determined the activity of GOT and GPT in serum by using an enzymatic kit (Asan, AM101-K, Korea) and the numbers of blood cells by using an automatic test instrument (Coulter T-450) to see the liver toxicity and the influence of blood formation.

8) **Statistical analysis:** All data were analyzed statistically by t-test and Duncan's multiple-range test using SAS software.

## RESULTS

### Wound area

Figures 2 and 3 show the wound area percentage to initial wound on the 4th, 8th, 11th, and 14th day of treatment. Table 2 describes the results of the Duncan's multiple-range test. These results show significantly more effective wound healing by treatment of TCDO, Haemyl, and Forsythia than physiological saline in the control group. The wound areas were not significantly different by treatment of TCDO, Haemyl, or Forsythia extract on 4th, 11th, and

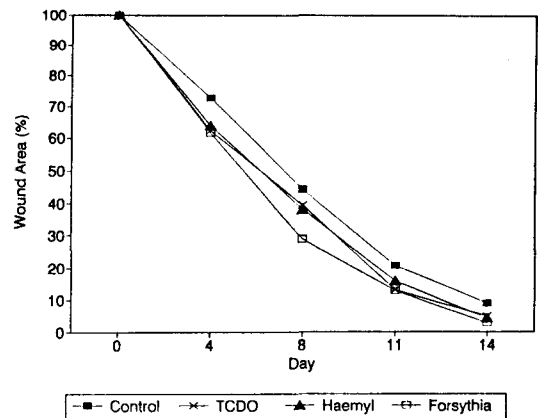


Fig. 2. Wound healing effect by treatment with Haemyl, TCDO, Forsythia suspensa extract, and saline for control group. The wound area (%) for each day was calculated with the initial wound area as 100%.

14th day of treatment. But the wound area of Forsythia treatment on the 8th day of treatment was significantly smaller than the other groups.

### Histological Test

Figures 4 and 5 show the histological observation of the wound on the 4th, 8th day of treatment. Figure 3, photographs of skin samples on 4th day of treatment, shows the intensive accumulation of inflammatory cells on the wound surface and intensive proliferation of

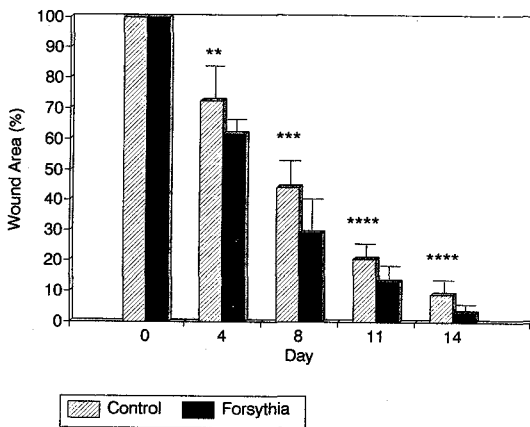


Fig. 3. The differences of wound areas between the Forsythia suspensa extract and control group. The wound areas of the extract treatment group were very significantly smaller than control group. (Mean S.D., \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ , \*\*\*\*:  $P < 0.0001$ )

capillary in every group. But the skin preparation on the 8th day of treatment with TCDO, Haemyl, and Forsythia extract show normal skin construction with thick epidermis and clear basal membrane with clearly divided dermises and epidermis. But the saline treatment group show many inflammatory cell and very thin epidermis.

### Antibacterial test

Fig. 6 shows the results of the agar diffusion test to determine the antibacterial activity of Forsythia suspensa extract. About a 10 mm inhibitory zone of bacterial proliferation by inoculation of Forsythia suspensa extract on the disc were much less than 20 mm as an acceptable size for antibacterial effect. But inoculation of povidone iodine inhibited bacterial proliferation much more than 20 mm. These results show that Forsythia suspensa extract has no antibacterial effect.

### Toxicological test

Liver enzyme activities Fig. 7 shows the results of the activity test for liver enzyme after 2 months of oral administrations of Forsythia suspensa extract which was diluted with drinking water (1:1, V/V) or normal drinking water. The values of GOT, GPT were minimally increased by administration of Forsythia suspensa extract, but these were not significantly different.

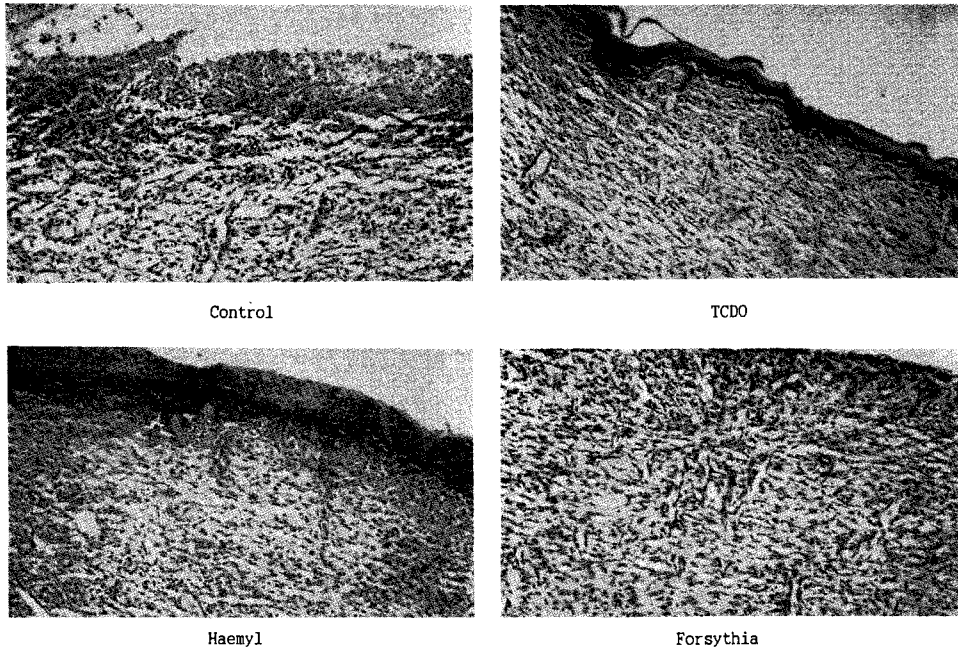
### Hematological test

Fig. 8 shows the results of the hematological test after 2 months of oral administration of di-

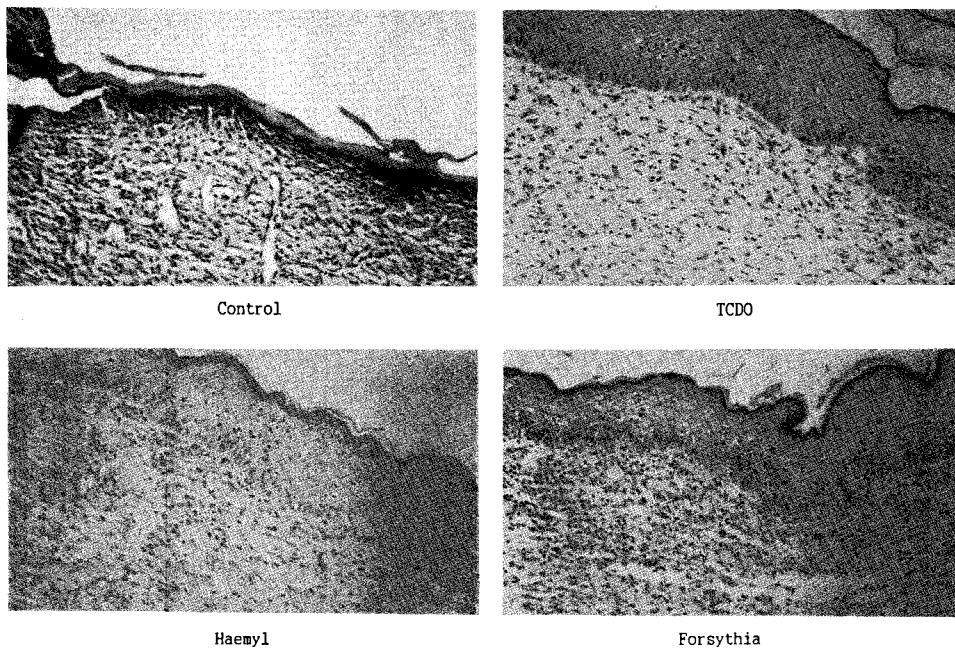
Table 2. The results of Duncan's multiple-range test. The different subscripts means statistic difference with  $P < 0.05$

| Group     | Wound area(%) |                      |                      |                      |                        |
|-----------|---------------|----------------------|----------------------|----------------------|------------------------|
|           | 0 day         | 4th day              | 8th day              | 11th day             | 14th day               |
| Control   | 100           | 73 ± 11 <sup>a</sup> | 44 ± 10 <sup>a</sup> | 21 ± 4 <sup>a</sup>  | 8.9 ± 4.4 <sup>a</sup> |
| TCDO      | 100           | 62 ± 6 <sup>c</sup>  | 39 ± 10 <sup>a</sup> | 13 ± 4 <sup>c</sup>  | 4.9 ± 3.7 <sup>b</sup> |
| Haemyl    | 100           | 64 ± 9 <sup>c</sup>  | 38 ± 8 <sup>a</sup>  | 16 ± 4 <sup>bc</sup> | 4.3 ± 3.0 <sup>b</sup> |
| Forsythia | 100           | 62 ± 5 <sup>d</sup>  | 29 ± 11 <sup>b</sup> | 13 ± 5 <sup>c</sup>  | 2.8 ± 2.5 <sup>b</sup> |

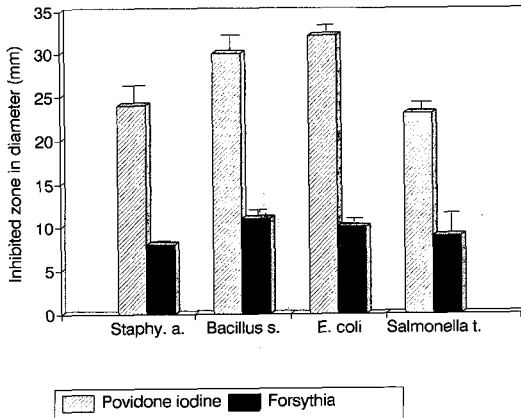
(Mean ± S.D.)



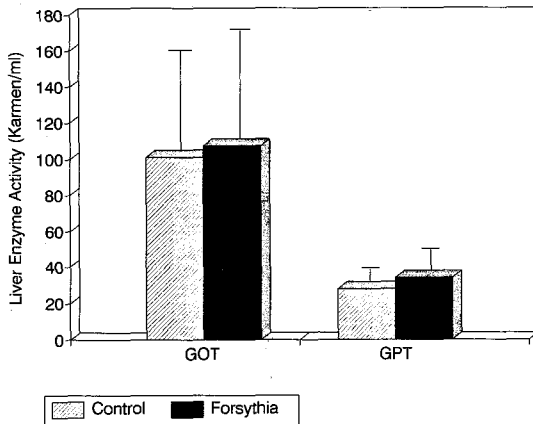
**Fig. 4.** Histological finding of wounds on the 4th post operative day (H & E,  $\times 100$ ). There are many inflammatory cells and capillary vessels in the wound.



**Fig. 5.** Histological finding of wounds on the 8th post operative day (H & E, X100). The TCDO, Haemyl, and Forsythia suspensa extract treated wounds showed regular skin structure with a clear division between epidermis and dermis, but the control group showed intensive inflammatory reaction on the wound surface.

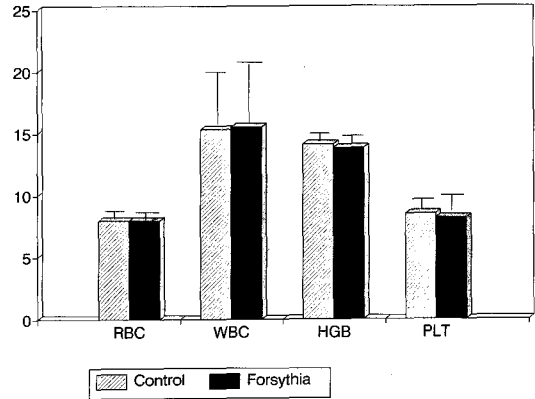


**Fig. 6.** The sizes of inhibitory zones of bacterial proliferation on the agar plates. The Forsythia suspensa extract inoculated disc showed about 10 mm in diameter inhibitory zone of bacterial proliferation which is much less than an acceptable size of 20 mm for antibacterial effect of povidone iodine.



**Fig. 7.** The liver enzyme activity (GOT, GPT) from rat serum after intake of Forsythia suspensa extract for 2 months. The enzyme activities were increased minimally by treatment with the extract, but these were not significantly different with the control group.

luted Forsythia suspensa extract with drinking water (1:1, V/V) or normal drinking water. The number of erythrocyte (RBC), leukocyte (WBC),



**Fig. 8.** Haemogram of rats after intake of Forsythia suspensa extract for 2 months. The number of blood cells between the two groups were not significantly different. (RBC: mill./ $\mu$ l, WBC: thsd./ $\mu$ l, PLT: 0.1mill./ $\mu$ l, HGB: g/dl)

platelet (PLT), and hemoglobin concentration (HGB) were similar between two groups.

## DISCUSSION

Wound healing can be disturbed by a contamination of wound surface or a decrease of oxygen tension in tissue from inhibited blood circulation (Kühne *et al.*, 1985). Currently, many kinds of drugs are used clinically to accelerate wound healing. One of them is TCDO and Haemyl. The wound healing mechanisms of TCDO are increasing of the oxygen tension in the wound and the antibacterial effect (Kühne *et al.*, 1985; Zenker *et al.*, 1986). Haemyl, a protein free haemodialysate, increases the permeation of metabolically important substances, such as glucose and phosphate, in perinecrotic zones and promotes cell respiration of various organs (Kern *et al.*, 1965; Riede *et al.*, 1974; Wickingen 1960). The wound healing effect of these substances has been established clinically and useful for chronic wounds (Heinz *et al.*, 1985). Though the water extract of Forsythia suspensa Nakai was known to accelerate the wound

healing in oriental medicine, it has not been proven. The wound area (Fig. 2, Table 2) and histological test (Fig. 5) showed Forsythia suspensa extract healed the experimental wounds of rats equivalently with TCDO and Haemyl treatment on the fourth, eleventh, fourteenth day of treatment. Moreover the healing effects of Forsythia extract on the 8th day was significantly more effective than TCDO and Haemyl. This means the therapeutic effect of Forsythia extract is at least equivalent to the TCDO and Haemyl. All three agents were significantly more effective in healing the experimental wounds of rats than the control group. The liver enzyme activity (Fig. 7) and haemogram (Fig. 8) of the the Forsythia suspensa extract treated rats did not significantly differ from the control group in spite of the intaking of high concentrated Forsythia suspensa extract (1:1) and a long duration (2 months) of treatment. The antibacterial effects of the Forsythia suspensa extract, which was suggested by a reference (2), was not shown in the agar diffusion test in this study (Fig. 6). For the present, we can't explain the mechanism of wound healing effects of the Forsythia suspensa extract, but its therapeutic effect was verified by this study. Therefore, we think it is possible to develop a new wound healing drug by using the Forsythia suspensa extract.

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=국문초록=

## 백서의 창상치유에 미치는 연교액기스의 효과

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연교액기스가 창상치료제로서의 사용가능성을 검증하기 위하여 연교의 창상치유 효과와 독성에 대하여 조사하였으며 아울러 연교액기스가 항생작용이 있는지도 조사하였다. 연교의 창상치유 효과를 Tetrachlorodecaoxygen anion complex(TCDO) 및 단백질을 제거한 혈액투석액(Haemyl)과 생리식염수의 창상치료 효과와 비교하였다.

4일, 11일, 14일째의 창상면적은 연교액기스, TCDO, Haemyl의 치료에 의하여 대조구인 생리식염수의 치료구에 비해 유의하게 적었다. 그러나 8일째의 창상면적은 다만 연교액기스의 치료구만 유의하게 적었다. 조직학적 검사에서도 연교액기스, TCDO, Haemyl의 치료구가 생리식염수의 치료구에 비해 표피화가 촉진되었다. 간 효소활성도(GOT, GPT), 혈구의 수에 있어서 연교액기스의 투여가 대조구에 비하여 어떤 유의적 차이가 보이지 않았으며 그외에도 연교액기스는 병원균의 증식을 억제하지 않았다.

이런 결과들은 연교액기스가 장차 창상 치료제로서의 개발 가능성이 시사하였으며 그의 약리작용은 항생작용과 무관한 것으로 추정된다.