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PROMOTION OF WOUND HEALING BY *HYPERICUM PERFORATUM* EXTRACT IN RABBIT

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Abstarct

The aim of this work was to evaluate the effect of hydroalcoholic extract of *Hypericum perforatum* (St John's Wort) (*SJW*) on wound healing in rabbit. Wound surface area and histopathological changes following topical application of various concentartions of *SJW* extract (2, 5 and 10% w/w) in eucerin base were tested on full thickness excision wound in rabbit. Such effect was compared with phenytoin cream (1%) as a standard healing agent. *SJW* extract significantly reduced the rate of wound healing compared to no-treatment, eucerin or phenytoin treated groups. 2% *SJW* extract cream showed better healing profile, and the rate of healing was significantly ($p<0.01$) shorter than other groups. *SJW* showed a considerable potential for wound healing possibly by promotion of fibroblast and myofibroblasts proliferation which results in higher rate of wound closure.

Keywords:

Saint John's Wort, Wound healing, Full thickness wound, Hydroalcoholic extract, Rabbit.

Introduction

Wound repair is a natural reaction to injury which results in restoration of tissue integrity. Repair process of wound will complete within 3 phases including: inflammation, proliferation and remodeling (1). There is a similarity between wound healing in human and certain mammalian species like rabbit. Therefore, in this work full thickness wound healing model of rabbit was studied. Numerous drugs with natural source such as: Gingko biloba, quince seed mucilage, yarrow and licorice(2,3,4) or chemical agents e.g. phenytoin, dexpanthenol, zinc oxide, ketanserin (5,6) have been employed to accelerate the rate of wound closure. *Hypericum perforatum* L. (Fam: Cluciaceae = Guttiferae) (St John's Wort) is a five petalled, yellow-flowered perennial weed common to the United States, Europe, and Asia. Small, dark red dots on the petals contain hypericin, one of many compounds found in this plant. Hypericum contains numerous biologically active constituents, including hypericin and its derivatives, hyperforin, flavonoids (hyperoside, rutin, quercetin, etc.), catechin, epicatechin, procyanidin B2, amino acid derivatives (melatonin, GABA), and essential oils (7). As a herbal remedy known since Greek and Roman times, St. John's Wort is used against ulcers, diabetes mellitus, the common cold, gastrointestinal disorders, jaundice, hepatic and biliary disorders in many countries (8).

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One of the major clinical effects of *SJW* is antidepressant effect (9). In many countries different dosage form of *SJW* are available as antidepressant, anxiolytic and sedative (10). However, more therapeutic effects have claimed for this plant. In folk medicine, it has been used to treat wounds, skin inflammation, asthma, convulsion, anemia and viral infection (11).

In a clinical study, it was demonstrated that oily extract of *Hypericum perforatum* promotes healing of surgical wounds from childbirth with caesarean section as a result of the increase in epithelial reconstruction (12). However, wound-healing activity of *Hypericum* species has been investigated less frequently when compared to antidepressant and other central nervous system activities. In this study, the wound healing property of hydroalcoholic extract of *SJW* was investigated on excisional wound of rabbit.

Materials and methods

Plant material: Dried aerial parts of *Hypericum perforatum (SJW)* was donated by Goldaru Pharmaceutical Co. Isfahan, Iran. The plant was taxonomically identified at department of botany, school of agriculture, Shahid Chamran University. The plant was powdered using a grinder; then 100g of this powder was placed in a beacker and 1000mL of ethanol 70% was added. The mixture was left at room temperature for 3 days. The extract was seperated and the remaining plant was extracted by more ethanol after 2 days. The extract was filtered by Wattman (No.10) filter paper and concentrated by a rotary evaporator (Hiedolph 230, Germany). Creams of *SJW* extract as 2%, 5% and 10% in eucerin base were prepared.

Animals: Iranian rabbits of either sexes weighing 1.6-2.2kg were used during the study. Animals were purchased from Razi Institute, Karadje, Iran. Before and after surgery the animals were housed individually in aluminium cages. They were allowed to feed on a standard, commercial pellet diet (Shushtar, Khorakdam Co. Iran) supplemented with fresh vegetables and water *ad libitum*. The animals were maintained in a holding room illuminated with 12 h light/dark cycles. Room temperature was set at 23±2°C with humidity of 45% to 55%. A full thickness wound was made in the skin of the test animals according to the model of Cross et al. (13) and modified by Hemmati and Mohammadian (2). Hairs of lower back and left flank of the test animals were fully shaved and cleared, the desired area was locally sterilized and anaesthesized with the subcutaneous injection of lidocaine (2%). The animal was held in standard crouching position, and the mobile skin of flank was gently stretched and held by the fingers. A metal template measuring 20×20mm was placed on the stretched skin and an outline of the template was traced on the skin using a fine-tipped pen. The wound was made by excising the skin, within the border of the template to the level of loose subcutaneous tissue, using a size No.15 scalpel blade and a forceps. Wounds of animals in groups of 6 were treated topically with eucerin containing 1% phenytoin (Darupakhsh Co. Iran) as a standard healing agent, or eucerin containing *SJW* extract 0%, 2%, 5% and 10%. Wounds of control group were not treated with any healing agent.

The animals were subsequently returned to cages in the previously described holding room. Bedding in the cages were changed daily and cages were kept clean to avoid infection of wounds. Application of drugs was done using a sterile swap, twice daily. Test animals with infected wound were excluded from the study. Only one animal from non-treatment group had infection and was excluded, but substituted later. All ethical issues were

considered in surgery procedure and during the treatments.

In order to determine the rate of wound healing, animals from both test and control groups were held in the standard crouching position and outline of the wound was traced at 24 h interval on a transparent plastic sheet using a fine-tipped pen. Measurment errors were minimized by repeating each measurment three times and using an average of the measurment in all calculations.

The area of the wounds on the first day was considered as 100% and the wound areas on subsequent days were compared with the wound on the first day.

Healing percentages on different days of treatments were calculated as the difference between the initial wound surface area and that on the day of measurement.

In order to study the healing process histologically, a sample of skin was taken after 7 day of initiating wound and on the day that wound healing was completed macroscopically. Tissue sample was placed in fixative solution (10% neutral-buffered formalin) for at least 12–24 h, Tissue blocks were placed in formalin, dehydrated in a graded series of ethanol, embedded in paraffin, cut into 5 µm-thick serial sections. The sections then were stained with hematoxylin-eosin to identify inflammatory cells, granulation tissue and tissue structure.

Statistical analysis were performed using one way ANOVA followed by multiple comparison with Dunnet's test. The differences were considered significant when $p<0.05$.

Results

Comparison of animals treated with eucerin and non-treated control animals showed no differences and in both groups healing was completed within 21 days (Fig. 1).

In group treated with phenytoin, 15 days was required for complete healing (Fig. 2), while *SJW* extrcat creams of 2%, 5% and 10% reduced the rate of healing to 14, 15 and 16 days, repectively (Fig. 3).

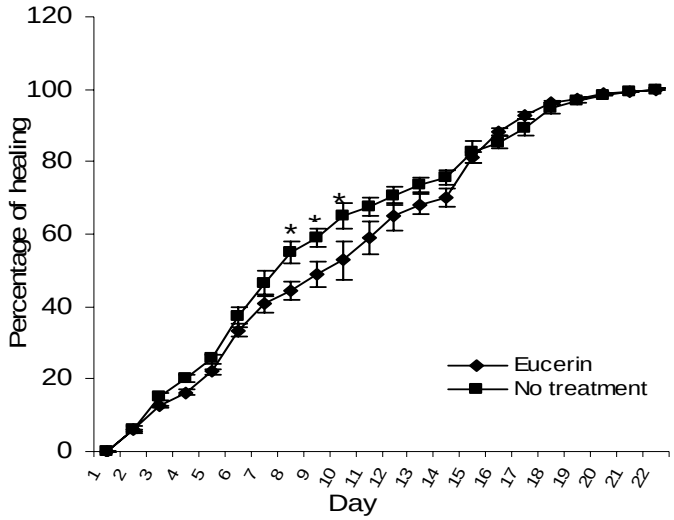

Fig. 1: Comparison of the wound healing in no-treatment and eucerin treated groups (n=6).Values significantly different from no-treatment are indicated as *($p<0.05$).

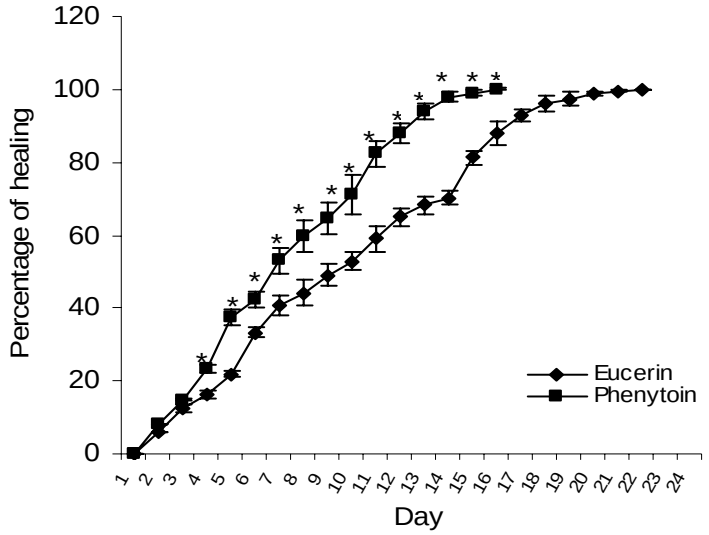

Fig. 2: Comparison of the wound healing in eucerin and phenytoin treated groups (n=6). Values significantly different from eucerin are indicated as *($p<0.05$).

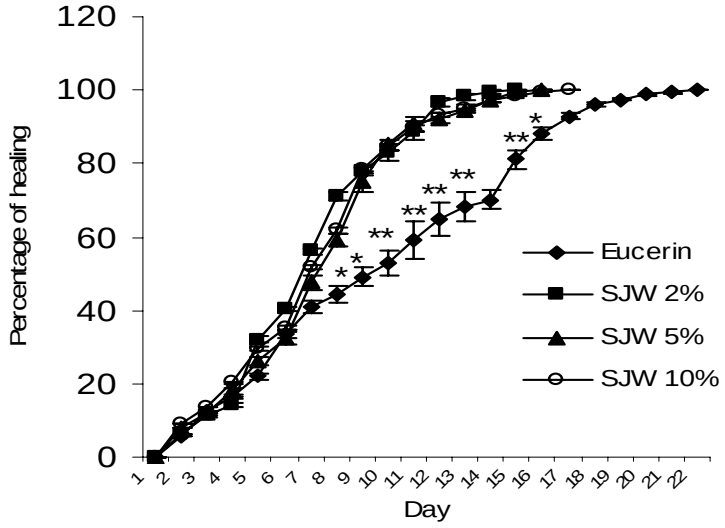

Fig. 3: Comparison of the wound healing in eucerin and *SJW* (2, 5, and 10%) treated groups (n=6). Significant differences of eucerin group with *SJW* groups are indicated as *($p<0.05$) and **($p<0.001$).

SJW extract cream of 2% produced best healing rate and better than phenytoin. Significant differences ($p<0.05$) between eucerin group and 2% *SJW* exctract cream was observed at second day of treatment until the end of treatment course (Fig. 4). Histological examination of wounded or repaired skin tissue showed earlier formation of granulation tissue and proliferation of fibroblast in *SJW* treated tissues compred to control or untreated tissues. On day 14 of 2% *SJW* treatment epiderm layer is smililar or even thicker than untreated tissue after 21 days (Figs. 5-8).

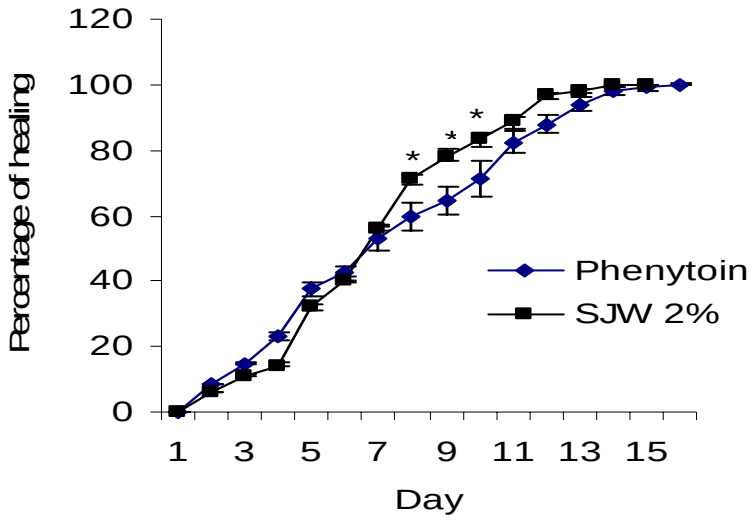

Fig. 4: Comparison of the wound healing in phenytoin and *SJW* 2% treated groups (n=6). Values significantly different from phenytoin are indicated as *($p<0.05$).

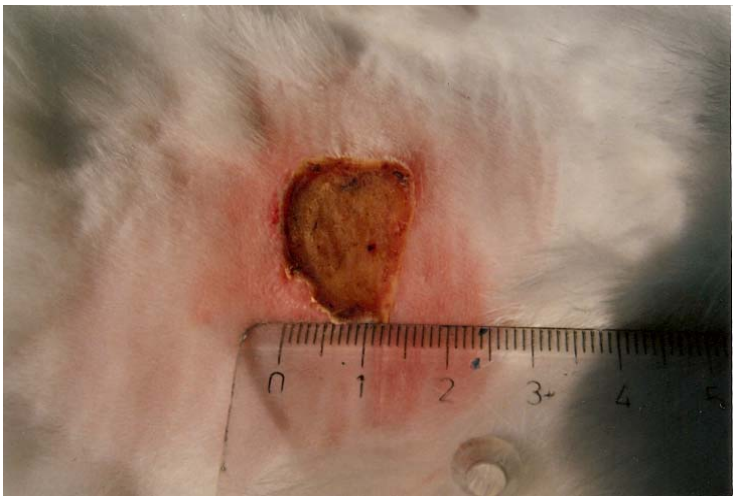

Fig. 5: Macroscopic depiction of wound 7 days after treatment with *SJW* 2%. Wound contracture and redness of area are seen. Scab still is attached to the surface of wound.

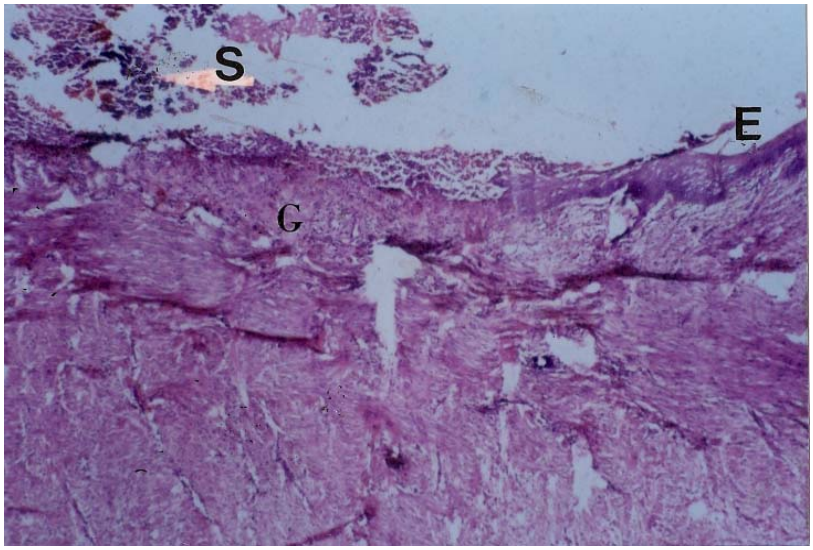

Fig. 6: Photomicrograph of wound 7 days after treatment with *SJW* 2% wound. Scab (S) and granulation tissue (G) is seen in the wound area. Incomplete epiderm (E) is seen in the upper right of photograph (H&E x40).

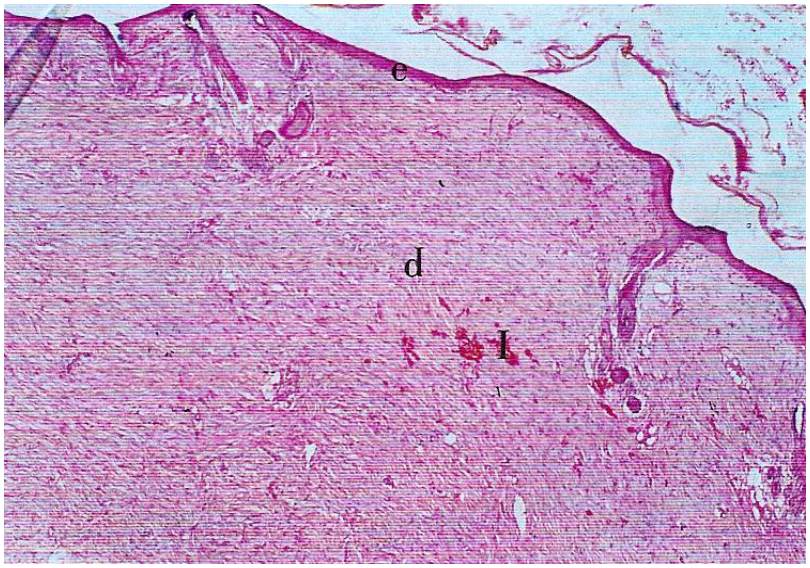

Fig. 7: Photomicrograph of wound 14 days after treatment with *SJW* 2%. Epiderm (e) and derm (d) layers are completed. Slight inflammation is evident (H&E x40).

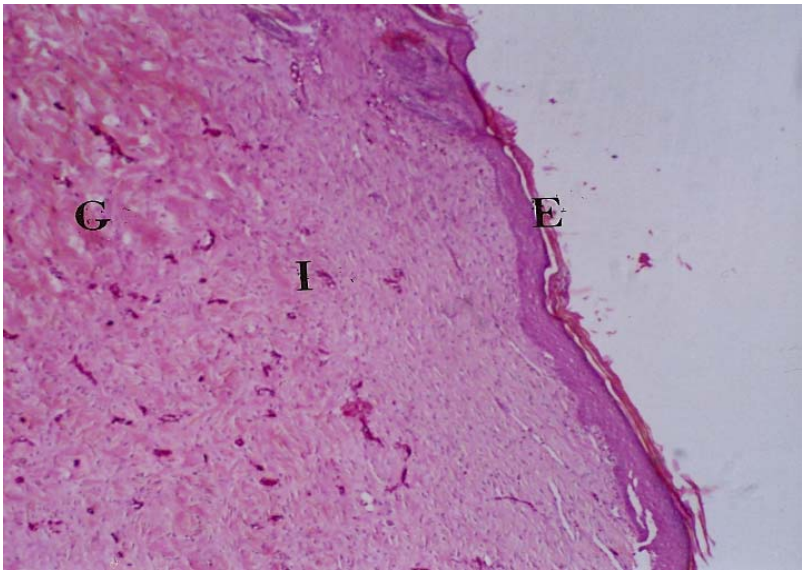

Fig. 8: Photomicrograph of untreated skin tissue 21 after wounding. Epiderm (E) is relatively complete but some inflammation (I) close to granulation tissue (G) is seen (H&E x40).

Discussion

Wound healing is a complex phenomenon involves various phases e.g. coagulation, inflammation, collagenation, wound contraction and epithelization (14). While the phases between coagulation to collagenation are intimately inter-linked, the phases of wound contraction and epithelization are independent to each other and run concurrently(15). In the present study, excision wound model for contraction and granulation tissue was employed. Numerous studies have been done regarding the pharmacological properties of *SJW* (16). However, wound healing effect of this plant has not been tested by standard methods. Our results defenitely indicat that extract of *SJW* is able to promote and accelerate the rate of wound healing. This conclusion comes from the faster contraction of wounds treated with *SJW* extract compared to control or untreated wound. *SJW* extract was more potent than phenytoin which is commercially available for clinical use.

The course of healing by 2% *SJW* extract was 14 days which is 1 day shorter than phenytoin which is considerable advantage for this extract. The percentage of healing with SJW extract was significantly higher than eucerin from the second day of tratement and continued until the completion of healing. The histopathological studies also confirm the the quantitative results.

The exact mechanism of *SJW* extract in wound healing can not be proposed from the present findings. However, significant differences of *SJW* extract treated groups suggesting that *SJW* extract may be effective in one or more phases of wound healing. Perhaps it is able to promote the granulation tissue, myofiroblast proliferation and contraction for the faster closure of wound. Active ingredients of SJW such as hyperforin, flavonoids (hyperoside, rutin, quercetin, etc.), catechin, epicatechin, procyanidin B_2, and essential oils (17) may contribute to the

healing effects of *SJW* extract. Presence of oligomers such as cathechin tannins in *SJW* extract (18) may cause coagulation of surface proteins and prevention of wound infection and assist the wound for faster closure and healing (19). Active ingredients of SJW may also stimulate the cytokines and growth factors involved in wound healing. However, more studies are required to elucidate the exact mechanism of *SJW* extract in wound healing.

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