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## Original Article / Orijinal Araştırma

# Comparison of the healing effects of silver sulfadiazine cream 1% and the extract of *Hypericum perforatum* on scald burn wound in a rat model

# Sıçan modelinde sıcak sıvı yanık yarası üzerine gümüş sülfadiazin %1 kremin ve *Hypericum perforatum* özütünün etkilerinin karşılaştırılması

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## Abstract

**Aim.** The aim of this study was to compare the healing effects of silver sulfadiazine (SS) cream 1% and the extract of *Hypericum perforatum* on scald burn wound in a rat model. **Methods.** The scald burn was developed on backs of 16 male and 16 female mature rats. The male and female rats were randomly divided into 4 groups (4 male and 4 female rats in per group): control, no drug administered; SS, SS cream 1% administered; *Hypericum perforatum* (HP), the extract of *Hypericum perforatum* administered; and SS plus HP groups, SS cream 1% and the extract of *Hypericum perforatum administered*. Tissue samples were obtained from all the study rats on days 1, 3, 10, and 17 and examined histologically by an experienced pathologist. **Results.** The healing effect of SS plus the extract of *Hypericum perforatum* was significantly better than the effects of other treatments on days 10 and 17. **Conclusions.** Extract of *Hypericum perforatum* provided a positive effect on scald burn wounds in the settings of rat model. It increased the amount of collagen and provided a meaningful anti-inflammatory effect in the wound area. With SS cream 1%, the extract of *Hypericum perforatum* had a synergistic effect on burn wound healing. Further studies are needed to determine the main mechanisms for the wound healing induced by the extract of *Hypericum perforatum*.

**Keywords:** *Hypericum perforatum*, extract, silver sulfadiazine, wound, burn

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## Özet

**Amaç.** Bu çalışmada rat modelinde %1 gümüş sülfadiazin kremin ve *Hypericum perforatum* özütünün sıcak sıvı yanık yarası üzerine iyileştirici etkilerinin karşılaştırılması amaçlandı. **Yöntem.** Onaltı erkek ve 16 dişi erişkin sıçanın sırtlarında sıcak sıvı yanığı oluşturuldu. Erkek ve dişi ratlar randomize olarak 4 gruba ayrıldı (her grupta 4 erkek ve 4 dişi olmak üzere): kontrol, herhangi bir ilaç uygulanmadı; gümüş sülfadiazine (GS), %1 GS krem uygulandı; *Hypericum perforatum* (HP), *Hypericum perforatum* özütü uygulandı ve GS artı HP, %1 GS krem ve *Hypericum perforatum* özütü uygulandı. Tüm çalışma gruplarındaki sıçanlardan 1, 3, 10 ve 17. günlerde doku örnekleri elde edilerek deneyimli bir patolog tarafından değerlendirildi. **Bulgular.** GS artı HP'nin birlikte uygulandığı grupta 10 ve 17. günlerde diğer tedavilere göre anlamlı olarak daha iyi iyileştirici etki saptandı. **Sonuçlar.** Oluşturulan sıçan modelinde, *Hypericum perforatum* özütü, sıcak sıvı yanık yaralarında olumlu etki sağladı. Yara bölgesinde kollojen miktarını arttırdı ve anlamlı antiinflamatuvar etki sağladı. Yanık yara iyileşmesinde %1'lik GS krem ile birlikte sinerjik etkisi oldu. *Hypericum perforatum* özütünün sağladığı yara iyileşmesindeki temel mekanizmaları anlamak için daha fazla çalışmaya ihtiyaç vardır.

**Anahtar sözcükler:** *Hypericum perforatum*, özüt, gümüş sülfadiazin, yara, yanık

## Introduction

Burn injury is one of the biggest trauma that a person would experience during own life. The management of burn injury is a troublesome process for both patient and physician. Dehydration and infection are the most common and important of issues that need to be overcome. Chronic diseases affected the metabolism as diabetes mellitus and chronic renal failure are particularly important on treatment process of children, elderly, and immune compromised patients.

In burn treatment, appropriate antibiotherapy, regulation of fluid and electrolyte balance, and burn dressing under sterile conditions and adequate analgesia are basic principles. The factors as shape, depth, and percentage of burn wound also affect the hospitalization requirement, length of staying in hospital, and the treatment course. The burn treatment begins by the determination of burn depth and area; therefore, the assessment of extend of burn injury is very important for planning the treatment [1].

In the wound dressing of burns, silver sulfadiazine 1% is usually used [2]. In addition, various conventional methods are preferred to apply to the wound for burn wound care [3]. Traditionally, *Hypericum perforatum* and its extracts are in use for wound healing in our country, and in various parts of the world for healing of burn wounds.

In this study, we examined the healing effect of the extract extracted from *Hypericum perforatum* on the burn wound developed as scald burn. The aim of this study was to compare the healing effects of silver sulfadiazine cream 1% and the extract of *Hypericum perforatum* on scald burn wound in a rat model.



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## Material and Methods

### *Plant extract*

The aboveground portion of *Hypericum perforatum* plant have been picked from plateaus located in the eastern parts of the province of Antalya in the third week of June. The plants have been picked when flowering, and have been pulverized after dried in the shade.

Olive-oil extraction of the aboveground portions of *Hypericum perforatum* plant has been prepared. Fifty gram of dried plant was put into olive oil in 500 ml glass jar and has been waited in where under the sun light into olive oil, and the extract has been prepared by mixing every other days.

### *Animals*

The animals have been obtained from Cumhuriyet University Animal Laboratory after the approval of Animal Research Ethics Committee of our university. The 16 male and 16 female mature rats weight between 220 and 240 g were randomly divided into 4 groups (4 male and 4 female rats in per group): control, silver sulfadiazine (SS), *Hypericum perforatum* (HP), and silver sulfadiazine plus *Hypericum perforatum* (SS plus HP) groups. Tissue samples were obtained from all the study rats on days 1, 3, 10, and 17 and examined histologically by an experienced pathologist.

### *Experimental procedure*

In the study rats, xylazine HCl 3 mg/kg before anesthesia and ketamine HCl 90 mg/kg (sc) as anesthetic were used. Carprofen 4 mg/kg subcutaneously once a day was performed for analgesia. Scald burn wound was selected as burn model in the rats. Tap water was warmed to 90°C and burn was developed on the back of the rats previously shaved after exposed to hot water for 15 min. The developed burns were first or second degree. In the control group, rats were created and any dressing was not made. In the SS group, a dressing with silver sulfadiazine cream 1% was applied to the burn wound for 17 days. In the HP group, a dressing with the extract of *Hypericum perforatum* was applied to the burn wound for 17 days. In the SS plus HP group, a dressing with silver sulfadiazine cream 1% and extract of *Hypericum perforatum* was applied to the burn wound for 17 days. After the experiment, pentothal 200 mg/kg was given to rats intraperitoneally for euthanasia. Tissue samples were taken from back of the rats from the burn area as 2x2 cm on day 1, 3, 10, and 17 and they were put into sterile containers and were evaluated by an experienced pathologist (M.T.).

### *Histological assessment*

The samples were fixed in 10% buffered-formaldehyde for histological evaluation. The fixed samples were passed through alcohol and xylene series after washing with tap water for 12 hours. Then, blocks were prepared as embedding the samples into paraffin. As taking 4-5 µm sections from the prepared blocks, they were stained with hematoxylin-eosin. Stained-sections were evaluated for the findings listed in Table 1.



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### *Statistical analysis*

Kruskal-Wallis ANOVA and Mann Whitney-U test were used for statistical comparisons. A p value of less than 0.05 was accepted as significant.

**Table 1. Histological findings used for the evaluation of treatment modalities of the healing of burn wounds.**

| Pathological lesion | Score |
|---|---|
| Necrosis in epidermis | 1 |
| Necrosis in epidermis and upper part of dermis | 2 |
| Necrosis in the whole of epidermis and dermis | 3 |
| Thrombosis in subcutaneous capillaries | 2 |
| Mild subcutaneous hyperemia | 1 |
| Severe subcutaneous hyperemia | 2 |
| Mild subcutaneous edema | 1 |
| Severe subcutaneous edema | 2 |
| Mild increase in collagen tissue | 1 |
| Severe increase in collagen tissue | 2 |
| Mild polymorphonuclear leukocyte infiltration | 1 |
| Severe polymorphonuclear leukocyte infiltration | 2 |
| Mild mononuclear leukocyte infiltration | 1 |
| Severe mononuclear leukocyte infiltration | 2 |

## Results

We found that mononuclear cell (MNC) infiltration increased and subcutaneous edema decreased after the dressing with both silver sulfadiazine cream 1% and the extract of *Hypericum perforatum* compared to the control group after first day of burn wound care ($p>0.05$) (Table 2).

When necrosis was evaluated in tissue taken on the third day, the necrosis decreased significantly in control group compared with other groups ($p<0.05$).When subcutaneous hyperemia, edema, and subcutaneous collagen increase were evaluated, these parameters decreased in the combined treatment groups ($p<0.05$).When the other parameters have been compared, those are not significant statistically ($p>0.05$) (Table 2).

On tenth day, all the evaluated parameters dealing with the injury have decreased significantly in the all group administered study drug compared to the control group. Whereas, there are no significant difference in the comparing drugs with each other ($p<0.05$). With regard to the subcutaneous capillary thrombosis, the study groups were found as similar ($p>0.05$). When subcutaneous hyperemia was evaluated, the hyperemia decreased after using the extract of *Hypericum perforatum* alone or combined with silver sulfadiazine cream 1% ($p<0.05$). When subcutaneous edema was evaluated, it has significantly decreased with study drugs ($p<0.05$), but we did not found an additional decrease with their combined use ($p>0.05$). When subcutaneous collagen increase was evaluated, it was significantly higher in all the groups used the extract of *Hypericum perforatum* but the effect of combined treatment on the collagen increase was significantly



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higher than the extract of *Hypericum perforatum* alone ($p<0.05$). When subcutaneous polymorphonuclear leukocyte infiltration was evaluated, it increased in the groups administered the extract of *Hypericum perforatum* ($p<0.05$) (Table 2).

**Table 2. Histological findings of study groups on days 1, 3, 10, and 17.**

| Groups | Days | Necrosis | Subcutaneous capillary thrombosis | Subcutaneous hyperemia | Subcutaneous edema | Subcutaneous collagen increase | Subcutaneous PMNL infiltration | Subcutaneous MNC infiltration |
|---|---|---|---|---|---|---|---|---|
| C vs. SS | 1 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | ***0.002*** | 0.141 |
| C vs. HP | 1 | 0.591 | 0.317 | 1.000 | 1.000 | 1.000 | ***0.023*** | 0.141 |
| C vs. SS plus HP | 1 | 1.000 | 0.317 | 1.000 | ***0.007*** | 1.000 | ***0.013*** | ***0.023*** |
| SS vs. HP | 1 | 0.591 | 0.317 | 1.000 | 1.000 | 1.000 | ***0.023*** | 1.000 |
| SS vs. SS plus HP | 1 | 1.000 | 0.317 | 1.000 | ***0.007*** | 1.000 | ***0.044*** | 0.298 |
| HP vs. SS plus HP | 1 | 0.591 | 1.000 | 1.000 | ***0.007*** | 1.000 | 0.049 | 0.298 |
| C vs. SS | 3 | ***0.007*** | 0.606 | 1.000 | ***0.023*** | 1.000 | 0.141 | 0.254 |
| C vs. HP | 3 | ***0.006*** | 1.000 | 1.000 | ***0.002*** | 0.060 | ***0.000*** | 0.060 |
| C vs. SS plus HP | 3 | ***0.019*** | 0.221 | ***0.013*** | ***0.000*** | 1.000 | 0.080 | 0.480 |
| SS vs. HP | 3 | 0.141 | 0.606 | 1.000 | 0.254 | 0.060 | ***0.007*** | 0.317 |
| SS vs. SS plus HP | 3 | 0.562 | 0.480 | ***0.013*** | ***0.046*** | 1.000 | 0.724 | 0.617 |
| HP vs. SS plus HP | 3 | 0.625 | 0.221 | ***0.013*** | 0.285 | ***0.046*** | ***0.011*** | 0.170 |
| C vs. SS | 10 | ***0.001*** | 0.742 | 0.103 | ***0.037*** | 0.529 | 0.089 | 0.139 |
| C vs. HP | 10 | ***0.000*** | 0.143 | ***0.029*** | ***0.003*** | ***0.036*** | ***0.003*** | 0.117 |
| C vs. SS plus HP | 10 | ***0.000*** | 0.221 | ***0.004*** | 0.082 | ***0.001*** | ***0.001*** | ***0.006*** |
| SS vs. HP | 10 | 1.000 | 0.298 | 0.202 | 0.248 | ***0.035*** | 0.133 | 1.000 |
| SS vs. SS plus HP | 10 | 0.355 | 0.409 | ***0.011*** | 0.626 | ***0.000*** | 0.063 | 0.181 |
| HP vs. SS plus HP | 10 | 0.285 | 0.838 | 0.082 | 0.117 | ***0.025*** | 0.617 | 0.157 |
| C vs. SS | 17 | ***0.030*** | 0.530 | 0.591 | 0.060 | 0.060 | 0.254 | ***0.002*** |
| C vs. HP | 17 | ***0.010*** | 0.141 | 0.122 | 0.060 | ***0.035*** | 0.060 | ***0.001*** |
| C vs. SS plus HP | 17 | ***0.006*** | 0.880 | 0.082 | ***0.046*** | ***0.000*** | 0.200 | 0.170 |
| SS vs. HP | 17 | 0.298 | 0.317 | 0.298 | 1.000 | ***0.035*** | 0.317 | ***0.044*** |
| SS vs. SS plus HP | 17 | 0.204 | 0.617 | 0.221 | 1.000 | ***0.000*** | 0.922 | ***0.023*** |
| HP vs. SS plus HP | 17 | 0.838 | 0.170 | 0.880 | 1.000 | ***0.025*** | 0.350 | ***0.003*** |

C: control group; SS: SS group; HP: HP group; SS plus HP: SS plus HP group. PMNL, polymorphonuclear leukocyte.

On day 17, overall, with regard to the healing, the combination of study drugs was better than study drugs alone. When subcutaneous collagen increase was evaluated, it was significantly higher in the all groups used the extract of *Hypericum perforatum* but the effect of combined treatment on the collagen increase was significantly higher than that of the extract of *Hypericum perforatum* used alone ($p<0.05$). When the other parameters was evaluated, they have not been statistically significant among the study groups ($p>0.05$).



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## Discussion

Various plants have been used for wound care since ancient times. Plant and plant extracts have continued to use for the management of many diseases though their importance is decreased nowadays at the age of modern medicine. Nowadays, 60% of the world population and 80% of developing communities prefer such treatments [4]. Because of subgroups of *Hypericum* species is more especially in European and Asian countries, it is among commonly used plants for several diseases including migraine, peptic ulcer, wound healing, and antiparasitic, antispasmodic, or antiseptic drugs [5, 6].

All stages of burn wound care are a troubled process for both the patient and the doctor. After its difficulties in the early period, contracture development and scar formation in joint regions are main problems that were encountered. Age of patient, immunocompromised state, diabetes mellitus, chronic renal failure, cancer, chemotherapy, wideness of burn area are some factors that affect the outcome negatively. The wound healing effect of *Hypericum* species has been investigated in many experiments, but there has not been any study on their healing effect of scald burn wounds. For this purpose, we investigated the healing effect of the extract of *Hypericum perforatum* on the scald burn wounds.

Silver sulfadiazine is the most preferred and used topical antibiotic in almost all over the world. The agent, preferred because of resistance has not developed, is board-spectrum effective to gram-positives, many of gram-negatives and some fungal agents. Its application and use is easy and does not cause additional pain on the wound. It may cause to transient leukopenia in continuous use for a long time. In addition, silver sulfadiazine can cause to melanin increase in skin. In our study, we preferred silver sulfadiazine as a study drug because of these properties.

In the current study, subcutaneous edema decreased in the drug used groups after first day and necrosis decreased on the third day compared to the control group; however, there are no differences with the use of the extract of *Hypericum perforatum* compared to the use of silver sulfadiazine alone. It implies that the extract of *Hypericum perforatum* is not superior to silver sulfadiazine with regard to the healing effect after the tissue damage of scald burn. In the terms of inflammation, it has been determined that subcutaneous edema and hyperemia significantly decreased in the groups used the extract of *Hypericum perforatum* to silver sulfadiazine, in addition to the increase of polymorphonuclear leukocytes that was higher in groups used silver sulfadiazine. The findings support that the anti-inflammatory effect and immune response are more potent with the use of the extract of *Hypericum perforatum* on third day of wound healing. As expected, the parameters such as necrosis, capillary thrombosis related tissue damage was less in the groups used the study drugs on 10th day of wound healing. The reason for this can be that the healing happened better in the groups used the study drugs. Tissue healing factors, particularly amount of collagen, was significantly higher in all the groups used the study drugs including the extract of *Hypericum perforatum*. Interestingly, collagen increase was the greatest in the groups combined the extract of *Hypericum perforatum* and silver sulfadiazine. These findings support that combined use of these drugs have synergistic effect on tissue healing and collagen increase. There are no significant difference for mononuclear cell (MNC) infiltration among the study groups. The assessment of subcutaneous edema and



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hyperemia with the significant decreases of polymorphonuclear leukocyte count in the groups used the extract of *Hypericum perforatum* has implied that anti-inflammatory effect of the extract of *Hypericum perforatum* has continued on 10th day. When we look at the 17th day, it has been seen that tissue damage parameters decreased, and the amount of collagen and MNC infiltration increased. Again, these findings suggest that collagen increase and wound healing was the best in the use of combined drugs. Similarly, Fenner et al. [7] have suggested that *Hypericum* species have antifungal effects, and Medina et al. [3] have suggested that hyperforin showed the anti-inflammatory effect by inhibiting cyclooxygenase-1 and 5-lipoxygenase and hyperforin found as related with pro-inflammatory response of leukocytes. In the study performed by Mukherjee et al. [8], it has been determined that chloroform, acetone and methanol extract of *Hypericum hookerianum* leaves and stems have antibacterial effect to *Pseudomonas Cepacia, Bacillus Subtilis, Bacillus Megaterium, Bacillus Coagulans, Staphylococcus Aureus* and *Escherichia Coli*. The most inhibitor effect has been determined at the 400 g/mL dosage of methanol extract [8]. In the study performed in 2010 by Süntar et al. [9], it has been also showed that fibroblast and collagen increases were more in the extract of *Hypericum perforatum*. When the activity of chicken embryo fibroblast was evaluated in the study performed by Öztürk et al. [10], it has been showed that *Hypericum perforatum* plant extract increased collagen synthesis of fibroblast and accelerated the forming of polygonal collagen. Because of the effects seen on wound healing was likewise on the burn wound, we suggest that the plant would be tried in the burn treatment. It has been observed that the extract of *Hypericum perforatum* has the positive effect on burn wound by increasing the amount of collagen and showing the anti-inflammatory effect, but it has synergistic effect on wound healing when combined with silver sulfadiazine.

In conclusion, the findings of current study support that the healing effects of the extract of *Hypericum perforatum* on scald burn wound used alone or in combination with silver sulfadiazine cream 1% in a rat model. Further studies are necessary to determine the main mechanisms for this extract induced wound healing.

## Conflict of Interest

The authors declare that no scientific and/or financial conflicts of interest exist with other people or institutions.

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