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Treatment of contact burn injury with hypericum perforatum: An experimental study

Temas tipi yanıklarda *Hypericum perforatum* (sarı kantaron) ile tedavi: Deneysel bir çalışma

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Abstract

Aim: Burns are one of the most difficult physical and psychological traumas that people face. Generally, protection and prevention strategies from burns are practiced. The young and the elderly are most likely to be affected tragically. The aim of the present study is to investigate the effect of *Hypericum perforatum* methanol extract-containing gel on the healing of burn wounds.

Materials and Methods: Forty male rats of the Sprague-Dawley strain were divided into four groups after a 4x4 area of their back was shaved, and an experimental burn was created with the direct contact of an aluminum metal stamp heated in boiling water for 15 seconds. There was no implementation on or treatment of the control group (Group 1). Burn wounds were irrigated with saline solution (Saline group, Group 2), and silver sulphadiazine 1% (Silverdin®) cream (Group 3), *Hypericum perforatum* methanol extract-containing gel (Group 4), and a placebo gel (Group 5) were applied topically 4 times a day after the contact burn. Histopathological analyses of the burned area were made at 4, 8, and 24 hours.

Results: The topical use of *Hypericum perforatum* methanol extract-containing gel in the experimental contact burns, histologically; resulted in the reduction of collagen discoloration, vascular damage and hair follicle and glandula sebaceous damage while preserving total number of hair follicles, number of vessels and epidermal thickness compared to Silver Sulphadiazine 1% (Silverdin®) cream treatment.

Conclusion: Positive effects of topical *Hypericum perforatum* gel were detected on experimental burns and its use might have beneficial effects on acute burn wounds.

Keywords: Hypericum perforatum, burns, injuries, wound healing.

Öz

Amaç: *Yanık insanların karşılaştığı en zor fiziksel ve psikolojik travmalardan biridir. Genellikle yanıktan koruma ve önleme stratejileri uygulanmaktadır. Yaşlılar ve gençler yanıktan en trajik olarak etkilenen popülasyondur. Bu çalışmada, metanol ekstraksiyonu yöntemiyle elde edilen Hypericum perforatum (sarı kantaron) jelinin uzun süreli olumlu etkilerinin araştırılması amaçlandı.*

Gereç ve Yöntem: *Kırk adet Sprague-Dawley cinsi erkek sıçanlar 100°C de 15 saniye kaynamış suda ısıtılmış metalle dağlanarak yakılıp 5 gruba ayrıldı. Kontrol grubuna hiçbir işlem ve tedavi uygulanmadı (Grup 1). Salin grubuna (Grup 2), yanık uygulaması yapılıp % 0, 9' luk NaCl solüsyonu ile irrigasyon yapıldı. Ticari bir ürün olan Silverdin® kremi (Grup 3), metanol ekstrasyonu ile elde eldilmiş Hypericum perforatum jeli (Grup 4) ve plasebo jel (Grup 5), aynı gün topikal olarak 4 kez uygulandı. Yanık alanı ve histopatolojik analizler 4., 8. ve 24. saatte biyopsi alınıp değerlendirildi.*

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Bulgular: *Metanol ekstrasyonu ile elde eldilmiş Hypericum perforatum jelinin deneysel yanık modeli sonrası topikal uygulaması; kollajen diskolorizasyonunu, vasküler hasar, kıl folikülü ve sebasöz bezlerde meydana gelen hasarı düşürürken ayrıca tüm damarların epidermal kalınlığının Silverdin®'e göre daha tedavi edici özellikte olduğu gözlemlendi.*

Sonuç: *Deneysel yanıklar üzerinde Hypericum perforatum jelinin olumlu etkilerinin olduğu ve kullanımının akut yanıklar üzerinde yararlı etkileri olabileceği görüldü.*

Anahtar Sözcükler: *Kantaron, yanıklar, yaralanmalar, yara iyileşmesi.*

Introduction

Burns are one of the most difficult physical and psychological traumas that people face. Generally, protection and prevention strategies for burns are well practiced; however, the young and the elderly are most likely to be affected tragically (1). *Hypericum perforatum* is an herb that belongs to the Hypericaceae family. It has antitumor (2), antiviral (3), antimicrobial (4), antibacterial (5), analgesic (6), and hepatoprotective (5) effects. *Hypericum perforatum* extract inhibits free radical output as well as the myeloperoxidase (7), cyclooxygenase-1, 5-lipoxygenase (8), inducible cyclooxygenase, and nitric oxide synthase stages (9). According to the literature, there are not enough clinical and experimental studies about *Hypericum perforatum* extract' s effects on patients with burns or on experimental burn models (10). Thus, if *Hypericum perforatum* extract, which is thought to be effective on burns and wound healing, has an effect on experimental contact type burns; it should be compared to other current burn treatments.

MATERIALS AND METHODS

Materials

Carboxy methyl cellulose (Na-CMC, average Mw~90.000) was purchased from Sigma (USA). Ethanol was obtained from TEKEL (Turkey). High Performance Liquid Chromatography (HPLC) grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). The ultrapure water used in the analysis was obtained from an in-house ultrapure water system (Sartorius Arium 611, Sartorius-Stedim, Goettingen, Germany). Pseudohypericin, hypericin, and hyperforin were purchased from Merck (Darmstadt, Germany).

Preparation of solvent extracts

Hypericum perforatum was collected from nature and the dried material was grinded homogeneously with a mechanical grinder. About 6 g of ground *Hypericum perforatum* were extracted with 300 mL of methanol (Merck, ≥99 purity) at 11°C for 30 minutes and then at 23°C for 30 minutes in an ultrasonically bath. After the sonication procedure, the samples were centrifuged at 7 g for 10 minutes. Supernatant was taken, 300 mL methanol was added, and then the extraction procedure was repeated four times to collect the supernatants. The collected supernatants were combined to obtain methanol extract. The methanol was removed by a rotary evaporator and lyophilized.

HPLC sample preparation

Hypericum perforatum extract was made from 50 mg of grinded material which is sonicated with 5 mL methanol for four times. All of the clear extracts were combined and diluted in 30 mL of methanol. Samples were filtered through 0.45 µm PTFE filters (Sartorius AG, Gottingen, Germany) prior to injections to remove non-dissolved particles. The final concentration of the *Hypericum perforatum* per 1 g of the gel was 5%.

HPLC analysis

HPLC analyses were performed on Shimadzu SCL-10VP. A Hichrom column (C18, 250×4.6 mm, 5 µm), Hichrom guard column (C18, 10×4.6 mm, 5 µm), and DAD detector were used. The mobile phase consisted of water with 0,3 % acetic acid (A), acetonitrile (B), and methanol (C). The gradient programs were as follows: (0–10 min, 100 % A; 10–30 min, 85 % A, 15 %; 30-40 min, 70 % A, 20 % B, 10 % C; 30-40 min, 10 % A, 75% B, 15 % C; and 40-55 min, 5 % A, 80 % B, 15% C.) The detection of wavelength, flow rate, and column temperature were set to 270 and 590 nm, 1 mL/min, and 30°C, respectively. The retention times for pseudohypericin, hypericin, and hyperforin were determined to be 44, 91 min, 49, 03 min and 51, 12 min, respectively.

Preparation of placebo gel

Placebo gel formulation was prepared by dispersing 7,5 % w/w Na-CMC in distilled water and adding 2 % ethanol to form a homogeneous dispersion under continuous stirring until a homogeneous gel was formed.

Preparation of gel containing Hypericum perforatum methanol extract

Gel formulation was prepared by dispersing 7,5 % w/w Na-CMC in distilled water to form a homogeneous dispersion. Five-percent *Hypericum perforatum* methanol extract was dissolved in 2 % ethanol and the solution was added gently to the Na-CMC dispersion under continuous stirring. The mixture was stirred gently until a homogeneous gel was formed (11).

Experimental procedure

The experiments were performed in accordance with the regulations specified by Ege University's, Animal Ethical Committee and conformed to national guidelines (Guide for the Care and Use of Laboratory Animals-Institute of Laboratory Animal Resources Commission on Life Sciences National Research Council [USA]) on the care and use of laboratory animals. During the experiment, they were kept in 24±2°C at room temperature, in appropriate lighting conditions; (12 hours in a sunny/dark cycle), and in cages that were cleaned once every day. Food and water were given ad libitum, and shavings were used as mounting. Twelve hours before the study, the animals were given only water (12).

Experimental protocol

Twenty-five minutes before the study, intraperitoneal 200 mg/kg paracetamol (Perfalgan, Bristol-Myers Squibb) were given to each 40 Sprague-Dawley strain male rats weighing between 250 and 350 g for analgesia; a 0, 8 -1, 3 mL/kg intraperitoneal dose of ketamine was given to provide general anesthesia. A preoperative dose of intramuscular cefazolin sodium (0.1 mg/ kg) was administered for infection prophylaxis. The aluminum metal stamp (4x4 cm, square shaped, total weight 85 g) heated in boiling water (100°C) for 15 seconds was applied on the rats dorsum, which had previously been shaved (12, 13). Thus, a standard burned area model was provided. The rats were randomly divided into five groups. There was no implementation or treatment on the control group (Group 1, n=8). On the second group, there was no implementation or treatment after the contact burn and irrigation with saline solution (0.9%) (Group 2, Saline Group, n=8). On the third group, after the contact burn and irrigation with saline solution (0.9 %), silver sulphadiazine 1 % (Silverdin®) cream, which is prescribed as a commercial product for burn treatment, was applied topically four times a day (every 6 hours) (Group 3, n=8). On the fourth group, *Hypericum perforatum* methanol-extract containing gel was applied topically four times a day (every 6 hours) (Group 4, n=8). And on the fifth group, a placebo gel was applied topically four times a day (every 6 hours) (Group 5, n=8). The rats were housed individually after surgery to prevent auto-cannibalization. Also, in every 500 mL of their water, codeine phosphate and 30 mg of paracetamol were added. The rats were sacrificed after measurements. The animals were killed by cervical dislocation.

The most prevalent topical treatment for partial thickness burns is silver sulfadiazine 1 % (SSD) (14,15). More recent studies have shown that the healing of partial thickness burns is delayed with the use of SSD (16,17), indicating the need for a better burn dressing. İn this study, the effect of *Hypericum perforatum* on wound healing is compared on the same standard burned model with the almost negligible effect of SSD on wound healing.

Analysis of test subjects

Body weight measurement

To understand the loss of water in test subjects, an electronic balance (electronic weighing system, Naugra Export, Ambala) that measures dry/wet weights at 0 and 24 hours was used.

Burn areas analysis

Photos at a 10 cm distance from the test subject's burn wounds were taken planimetrically at 4, 8, and 24 hours. These photos were analyzed with the Pro-Image Express program (Media Cybernetics, Inc., USA) (Figure-1).

Histopathological analysis

An incisional biopsy was taken from the rats' back skin at 4, 8 and 24 hours with a 1x1 cm rectangle shape (Figure-2). Paraffin blocks were prepared after taking biopsies and the material was fixed with 4% formaldehyde. After 5µm thickness sections were taken on a Leica RM 2145 model microtome, they were stained with H&E and examined randomly under light microscopy with X10, X20, and X40 enlargements to analyze the recovery of the wounds on the skin in term of edema, neutrophil (PMNL) infiltration, collagen discoloration, the number of veins, and glandula sebacea damage, with modified Verhofstad Scoring histopathologically evaluated by two blinded histologists (18) (Table-1). Biopsy materials were taken from all animals belonging to every group at 4, 8, and 24 hours. Five histological sections were taken from each animal's skin, and in every preparation the epidermis thickness from 20 different areas was used in the Image-Pro Express program (Media Cybernetics, Inc., USA),and the average results were recorded (Figure-1).

Table-1. Histopathological Evaluation; Modified Verhofstad Scoring Table.

Score	Edema	PMN infiltration	Collagen discoloration	Vein drainage	Hair follicle damage	Glandula sebacea
0	None	Normal	None	None	None	None
1	Light	Light	Light	Light	Light	Light
2	Pronounced	Pronounced	Pronounced	Pronounced	Pronounced	Pronounced
3	Dense	Dense	Dense	Dense	Dense	Dense

PMN: Neutrophil.

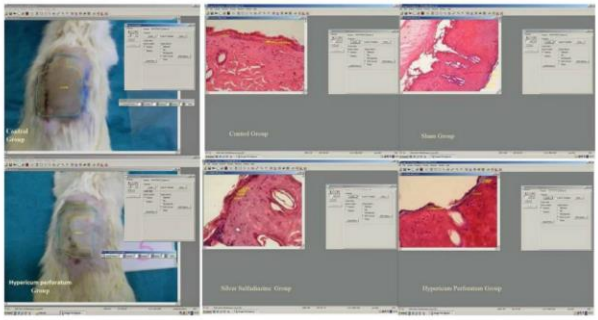

Figure-1. The evaluation of burnt areas of control and Hypericum perforatum groups at 4 hours with Image-Pro Express program. The evaluation of epidermis thickness in all groups at 24 hours with Image-Pro-Express program.

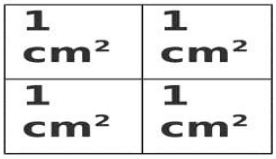

Figure-2. This schematic drawing shows the standard biopsy sites on the dorsum on each rats.

Statistical assessment

The statistical analysis of the data was performed using the Statistical Package for Social Sciences (SPSS) 14.0 (SPSS, Inc., Chicago, IL, USA) program. The data were analyzed using the Kruskal-Wallis, Friedman, Wilcoxon, and Mann-Whitney *U*-tests, and p values <0.05 were regarded as statistically significant.

RESULTS

Plant extract

The methanol extract of the plant was characterized by HPLC (Figure-3) analysis. According to the analysis, appreciable amounts of hypericin, hyperforin, and pseudohypericin were detected in the *Hypericum perforatum* extract using a DAD* detector. The HPLC analyses of the *Hypericum perforatum* extract is shown in the Table-2.

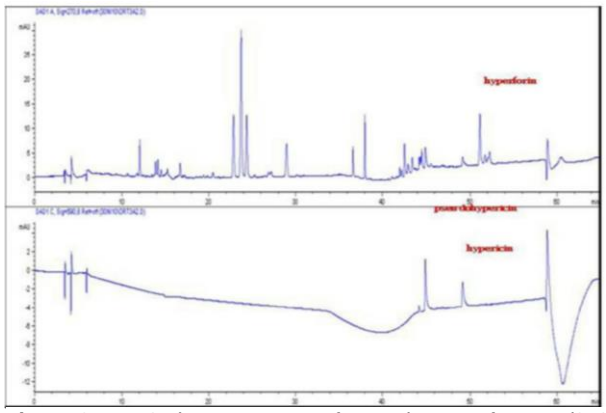

Figure-3. HPLC chromatograms of *Hypericum perforatum* (270 and 590 nm).

Results of body weight assessment

According to these results at Table-3 there was not a statistically significant difference between the groups.

Burn area analysis results

The burn area analysis results are given in Table-4. According to the burn area comparison for the 4-8-24 hour data, in the group 4 the burned area was statistically significantly lower than the area in experimental burn created groups ($p<0.05$).

Table-2. Results of HPLC Analyses of the *Hypericum perforatum* Extract.

Sample	RT** (min)	Mean area±SD	RSD%*** Area
Pseudohypericin	44.917	58.60±2.55	4.34
Hypericin	49.034	38.05±1.77	4.65
Hyperforin	51.122	97.10±0.70	0.73

DAD: Diode Array Detection (This detector is used in HPLC analysis and here it is used to obtain the molecules in the extract). **RT: Retention time. ***RSD: Relative standard deviations

Table-3. The Data of Mean±SD Body Weight in all Groups at 0 and 24 Hours.

Groups	0 hour	24 hours
Group 1 (Control group)	218.1±25.8	186.4±37.0
Group 2 (Saline group)	194±34	184.2±30.7
Group (Silverdin® cream group)	250.8±27.3	238.8±31.0
Group 4 (*Hypericum perforatum* gel group)	184±11.9	169.1±12.4
Group 5 (Placebo group)	214.7±14	199.1±12.4

Table-4. The Data of Average of Burn Areas (Cm2) in All Experimental Burn Groups at 0, 8 and 24 Hours.

Groups	4 hours (cm^2)	8 hours (cm^2)	24 hours (cm^2)
Group 2 (Saline group)	12.8	12.3	11.8
Group 3 (Silverdin® cream group)	12.4	14.3	8.1*
Group 4 (*Hypericum perforatum* gel group)	6.8*	5.9*	4.7*
Group 5 (Placebo group)	12.5	11.8	9.4

**Statistically significant.*

Histopathological analysis results

All groups were scored histopathologically according to a modified Verhofstad scoring system. The histopathological images are given in Figures 4-5-6.

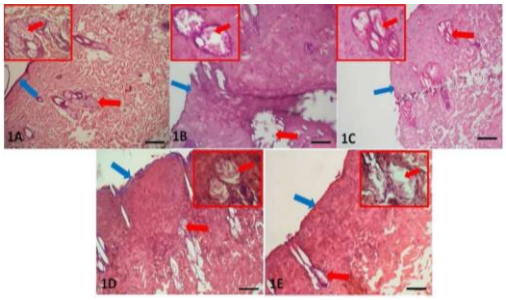

Figure-4. The histopathological view of experimental groups for 4 hours. H&E staining. Blue arrow indicates the epidermis and red arrow indicates the glandula sebacea. 1A. Group 1 (Control group) X10 magnification. 1B. Group 2 (Saline group) X20 magnification. 1C. Group 3 (Silverdin® application) X20 magnification. 1D. Group 4 (Hypericum Perforatum methanol extract containing gel application) X10 magnification. 1E. Group 5 (Placebo gel application) X20 magnification (Scale Bar X10=500 µm, X20=250 µm, X40=125 µm). 398x213 mm (72 × 72 DPI).

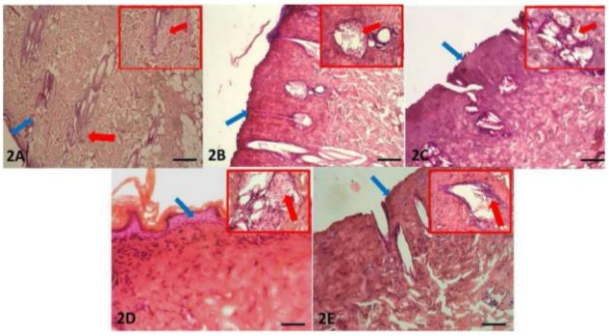

Figure-5. The histopatholological view of experimental groups for 4 hours. H&E staining. Blue arrow indicates the epidermis and red arrow indicates the glandula sebacea. 2A. Group 1 (Control group) X10 magnification. 2B. Group 2 (Saline group) X10 magnification. 2C. Group 3 (Silverdin® application) X20 magnification. 2D. Group 4 (Hypericum Perforatum methanol extract containing gel application) X20 magnification. 2E. Group 5 (Placebo gel application) X20 magnification (Scale BarX10= 500 µm, X20= 250 µm, X40=125 µm). 300×160mm (150 × 150 DPI).

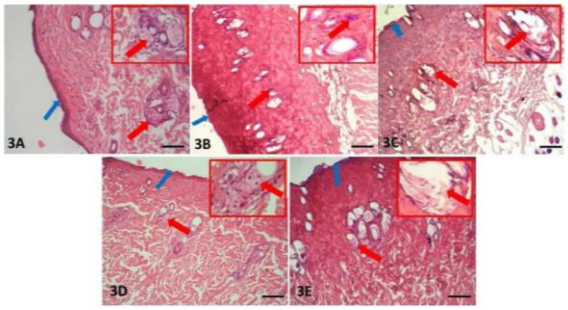

Figure-6. The histopatholological view of experimental groups for 4 hours. H&E staining. Blue arrow indicates the epidermis and red arrow indicates the glandula sebacea. 3A. Group 1 (Control group) X10 magnification. 3B. Group 2 (Saline group) X20 magnification. 3C. Group 3 (Silverdin® application) X20 magnification. 3D. Group 4 (Hypericum Perforatum methanol extract containing gel application) X20 magnification. 3E. Group 5 (Placebo gel application) X10 magnification (Scale Bar X10=500 µm, X20= 250 µm, X40=125 µm). 300×160mm (150 × 150 DPI).

Histopathological PMN infiltration analysis

In all groups, there was an increase of PMN infiltration from the 4th hour to the 8th hour. The highest results were on the 8th hour, and there was a decrease, the 24th hour neared. This change was clear, especially for the third and fourth groups, and statistically it was lower than the other groups (p< 0, 05).

Edema analysis

Edema quantity decreased in all groups over time. This decrease was statistically significant only for the fourth and fifth groups between the 4th and 24th hours (p< 0, 05). Group 4 showed a statistically significant decrease at the 24th hour in comparison with the other groups (p< 0, 05).

Collagen discoloration analysis

In all groups, there was the highest value of collagen discoloration after the burn at the fourth hour, and it decreased closer to the 8th and 24th hours. This change was statistically significant in all groups except Group 1 (p< 0, 05). There is a clear difference between Group 4 and the other groups regarding decreasing collagen discoloration at the 24th hour. This difference was found to be statistically significant (p< 0, 05).

Vein damage analysis

In all groups, the highest value of vein damage was at the 4th hour, and it decreased closer to the 8th and 24th hours. In regards to time, this difference was found to be statistically significant for groups 2, 4, and 5 (p< 0, 05). However, there was a statistically significant difference between Group 4 and the other groups (2, 3, and 5) at the 8th hour (p< 0, 05).

Hair follicle damage analysis

In all groups, the highest value of hair follicle damage was at the 4th hour after the burn, and it decreased closer to the 8th and 24th hours. There was a statistically significant difference between Group 4 and the other groups (2, 3, and 5) at all hours (p< 0, 05).

Glandula sebacea damage analysis

Damage increased closer to the 4th, 8th, and 24th hours in Group 2, but this is not statistically significant. In groups 3, 4, and 5, it decreased closer to the 4th, 8th, and 24th hours. This decrease is statistically significant in Group 4 compared with other groups (p< 0, 05).

Epidermis thickness

The data for mean epidermis thickness are given in Table-5. There was a statistically significant difference between Group 1 and groups 2, 3, and 5 at the 4th, 8th, and 24th hours (p> 0, 05). However, the

difference between Group 1 and Group 4 was not found to be statistically significant. In Group 4, there was a statistically significant increase compared with the other experimental burn groups (p< 0, 05).

Table-5. The Data of Mean Epidermis Thickness (µm) İn all Groups at 0, 8 and 24 Hours.

Groups	4 hours (µm)	8 hours (µm)	24 hours (µm)
Group 1 (Control group)	35.7	35.6	35.6
Group 2 (Saline group)	8.7	10.2	12.4
Group (Silverdin® cream group)	11.1	13.6	14.9
Group 4 (*Hypericum perforatum* gel group)	27.5*	30.8*	34.7*
Group 5 (Placebo group)	12.9	16.6	19.8

**Statistically significant.*

DISCUSSION

According to the American Burn Association, there are 500,000 people who are treated for burns annually, and 45,000 people out of that 500,000 need to be treated at the hospital in US. Most patients are treated in emergency services and are then discharged; however, 3,500 people die because of burns annually (19). Pain control and local care of the burn area after resuscitation and stabilization in treatment is highly important. Meyerholz et al. (20) defined trusted histopathological parameters and morphological changes to examine the severity of burns on an acute thermal rat model. They found that the severity and depth of a burn with hair follicle damage, collagen discoloration, vein damage, epidermal changes, and subepidermal vesicle genesis are correlated with burn contact time when all these morphological parameters are evaluated in terms of determining the severity of burn. Different kinds of topical agents are used frequently for minor burns. The most common is 1 % silver sulfadiazine because its implementation is easy, and its toxicity is minor (11). In our study, silver sulphadiazine 1 % (Silverdin®) cream a smaller effect than expected in acute time periods. As a result, wound area, reductions of 37.6 % and 15.83 % was observed, respectively, in the experimental and control groups (21).

CONCLUSION

According to the histopathological results, a comparison between treatment with gel containing *Hypericum perforatum* methanol extract and treatment with silver sulphadiazine % 1 (Silverdin®) cream showed that burn wounds is healing better with *Hypericum perforatum*. The present data also suggest that the effect of *Hypericum perforatum* methanol extract on burn healing might be related to its antioxidant effect and its effect of stimulating the proliferation of fibroblasts. Our study was performed in an animal model, and these findings would be useful for further clinical experiments.

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