RESEARCH ARTICLE

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Effects of Propolis and *Hypericum perforatum* Cream on Burn Wound Healing

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This study aimed to evaluate the effects of Propolis (Pr) and *Hypericum perforatum* (HP) cream on a number of biochemical and histopathological parameters in burn wounds in a rat model. Forty two female 3-month-old Wistar Albino rats were assorted into three equal main groups. Each of main groups was assorted into two subgroups for postoperative 7 and 14 days follow-ups. Bilaterally 10 mm in diameter second degree thermal burn wounds were created on the back skin of all rats under general anaesthesia. The burned skin areas in the first, second and third groups were coated with placebo cream (control), 50% Pr cream, and 50% HP cream twice a day, respectively (n= 14 for each group). The rats that completed postoperative follow-up periods of 7 and 14 days were euthanized and skin samples were examined biochemically and histologically. Results showed that Pr had more antioxidant property than the other groups. Pr and HP increased the burn wound healing effect with its encouraging property for epithelisation and granulation tissue and collagen (P<0.05). In conclusion, local application of 50% Pr cream and 50% HP cream are significantly efficacious in healing of burn wounds.

Key Words: *Propolis, Hypericum perforatum, wound healing, burn, rat*

Propolis ve *Hypericum perforatum* Krem'in Yanık Yara İyileşmesi Üzerine Etkileri

Bu çalışmada, Propolis (Pr) ve *Hypericum perforatum* (HP) kreminin, bir rat modelinde yanık yaralarında bir dizi biyokimyasal ve histopatolojik parametreler üzerindeki etkilerini değerlendirmeyi amaçladık. Kırk iki dişi 3 aylık Wistar Albino rat üç eşit ana gruba ayrıldı. Bu ana grupların her biri, 7 ve 14 günlük postoperatif takipler için iki alt gruba ayrıldı. Genel anestezi altında tüm ratların sırt derisi üzerinde iki adet 10 mm çapında ikinci derece termal yanık oluşturuldu. Birinci, ikinci ve üçüncü gruplardaki yanmış deri bölgeleri sırasıyla plasebo krem (kontrol), %50 Pr krem ve % 50 HP krem ile kaplandı (her grup için n= 14). Postoperatif 7 ve 14 günlük takip sürelerini tamamlayan ratlar ötenazi edildi ve deri örnekleri biyokimyasal ve histolojik olarak incelendi. Sonuçlar Pr'nin diğer gruplara göre daha fazla antioksidan özelliği olduğunu gösterdi. Pr ve HP, epitelizasyon, granülasyon dokusu ve kollajen için teşvik edici özelliği ile yanık yara iyileşme etkisini arttırdı (P<0.05). Sonuç olarak, %50 Pr ve %50 HP kremilerinin topikal uygulamaları yanık yaralarının iyileşmesinde etkilidir.

Anahtar Kelimeler*: Propolis, Hypericum perforatum, yara iyileşmesi, yanık, rat*

Introduction

Burned tissues and cells are destroyed by thermal injury and ischemic processes. The indirect destruction is derived in part from disorders in circulation of blood with ischemia, infarctions, stasis, and thrombosis (1, 2).

Propolis (Pr) is a product of the honey bees. Pr is a natural drug commonly used since ancient times. It contains phenolic acids, amino acids, cinnamic acid, flavanoids, terpens and caffeic acid. Topical application of Pr has been found to be efficacious in producing a granulating bed and controlling infection (3-5).

It is reported that *Hypericum perforatum* (HP) have sedative, antidepressant, antispasmodic, and antiseptic effects as well as wound-healing effects (6, 7). The active contents of HP are hypericin and hyperforin. Hypericin is the most the effective component of HP, which is responsible from the wound healing (8-11).

This study aimed to evaluate the effects of Propolis and *Hypericum perforatum* cream on a number of clinical, biochemical and histopathological parameters in burn wounds in a rat model.

Material and Methods

Chemicals: Placebo cream, Pr extract cream and HP oil cream were used in this study.

Pr used in this study was obtained from beekeepers in Elazığ, Turkey. Extract of Pr was prepared and used during this study as defined by Isla et al. (12). Frozen Pr was crushed in a mortar. Then, the resulting powder of Pr was extracted with ethanol with continuous stirring for 24 hour at room temperature. The suspension was let to precipitate for 3 days. The supernatant was concentrated in a evaporator and the sediment was blended with equal quantity of placebo cream.

HP oil (Aksu Vital, 20 mL) was mixed within equal amount of placebo cream, and HP cream was prepared.

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Animals: Forty two, 2-month old, weighing 210-240 g, female Wistar rats were used in the study. This study was approved by The Experimental Animals Local Ethical Committee of the Firat University, Turkey (Protocol No: 2014/127). Animals were anaesthetised with an intramuscular injection of 80 mg/kg ketamine hydrochlorure (100 mg/mL, Ketasol, Richter Pharma AG) and 5 mg/kg xylazine hydrochlorure (23.32 mg/mL, Rompun, Bayer).

Thermal Injury: The skins of the backs of the animals were shaved and burn wounds of 10 mm diameter were obtained. Skin burn models were constituted as defined by previous studies (13, 14). Animals were exposed to second-degree skin burns with 10 mm diameter brass probe. The probe was put in boiling water until thermal balance was reached. This probe was then put on the skin of back of the animals for 15 s without applying pressure.

Treatment Protocol: The rats were divided into 3 groups. Immediately after burn, placebo cream (Group control), 50% Pr, and 50% HP cream were performed locally to the burned wounds in the first, second, and third groups respectively (n= 14 for each group). Each main group was separated into 2 subgroups for followed up on the 7^{th} and 14^{th} days. The topical implementations were repeated twice a day. Clinically, the burned wounds were observed in all groups every day. Seven and 14 days later, the burned skin specimens were taken from animals for biochemical and histological examinations.

Biochemical Analysis: In the skin homogenate, the malondialdehyde (MDA) levels were measured in accordance with the method of Placer et al. (15). The skin catalase (CAT) activity was measured according to the method of Aebi (16). Concentration of protein was measured in accordance with to the method of Lowry et al. (17). The glutathione (GSH) level was measured according to the method of Sedlak and Lindsay (18). The glutathione peroxidase (GSH-Px) activity in skin tissue was evaluated in accordance with the method of Matkovics et al. (19). The superoxide dismutase (SOD) activity in skin tissue was measured in accordance with the method of Sun et al. (20).

Histological Examination: The skin samples were fixed in 10% neutral buffered formalin, and were cut into 5 µm sections. The sections were stained with haematoxylin and eosin, and examined by light microscopy. The samples were evaluated with regard to epithelisation, collagen deposition and granulation tissue, inflammatory cell infiltration, and angiogenesis. Sections were evaluated semiquantitatively as no (-), mild (+), medium (++) and severe (+++).

Statistical Analysis: SPSS (22.0 version) was used in statistical analyses. After Normality test, One-Way test ANOVA were used to detect differences among the groups. Significant differences were further compared with Tukey HSD test. Within each group, the Independent Samples t–test was used to calculate statistical differences between the 7^{th} and 14^{th} days. Values are presented as means±SEM. P<0.05 was considered as significant.

Results

Mortality was not observed in the animals during this study. Clinically, it was observed that a less redness, swelling and heat in the Pr and HP groups than in the control group.

The mean tissue levels of GSH, GSH-Px, MDA, CAT and SOD in all groups are shown in Table 1. On day 7^{th}, the local usage of Pr and HP reduced the MDA level (P<0.001). In the Pr group, levels of GSH, CAT, and GSH-Px were increased on the 7^{th} day (P<0.001). On the 14^{th} day, a significant decrease was found in the MDA level in the Pr group (P<0.001), besides an increase in the CAT, GSH, GSH-Px and SOD levels (P<0.001) (Table 1).

Histological evaluations of the wounds on 7th and 14th days are shown in Table 2. It was found that the levels of epithelisation, collagen and granulation tissue deposition on the 7th and 14th days were lesser in the control group than in the Pr and HP groups (P<0.05). In addition, it was found that the severe oedema seen in the granulation tissue of the control group decreased in the Pr and HP groups (Figure 1).

Table 1. The mean tissue levels of GSH, GSH-Px, MDA, CAT and SOD (values shown as mean±SEM)

	Day	Groups		
		Control	Pr	HP
GSH (nmol/g tissue)	7	3.21 ± 0.07[a]	4.63 ± 0.09[b]	3.33 ± 0.09[a]
	14	4.09 ± 0.06[a]	5.00 ± 0.06[b]	3.95 ± 0.10[a]
P<		0.01	0.05	NS
GSH-Px (U/g protein)	7	30.87 ± 0.63[a]	39.30 ± 0.55[b]	29.85 ± 0.34[a]
	14	34.27 ± 0.61[a]	42.05 ± 0.60[b]	34.67 ± 0.64[a]
P<		0.05	0.05	NS
MDA (nmol/g tissue)	7	13.36 ± 0.35[a]	8.59 ± 0.21[b]	11.63 ± 0.19[c]
	14	9.58 ± 0.26[a]	6.08 ± 0.13[b]	10.07 ± 0.31[a]
P<		0.001	0.001	NS
CAT (katal/g protein)	7	2.22 ± 0.06[a]	3.32 ± 0.06[b]	2.23 ± 0.06[a]
	14	2.58 ± 0.03[a]	4.07 ± 0.07[b]	2.62 ± 0.04[a]
P<		0.01	0.01	NS
SOD (U/g protein)	7	12.19 ± 0.16[a]	14.21 ± 0.22[b]	13.20 ± 0.26[c]
	14	14.73 ± 0.29[a]	16.53 ± 0.20[b]	15.57 ± 0.18[a]
P<		0.01	0.01	0.05

[a,b,c]: Values in the same row with different superscripts are significantly different (P<0.001).
NS: Non-significant.

Table 2. Histological evaluation of the wounds on 7th and 14th days (values shown as mean±SEM)

	Day	Groups		
		Control	Pr	HP
Epithelisation	7	0.16 ± 0.16^{aA}	0.83 ± 0.16^{bB}	0.66 ± 0.21^{bB}
	14	2.16 ± 0.16^{bA}	2.83 ± 0.16^{aB}	2.66 ± 0.16^{aB}
Granulation tissue and collagen	7	0.83 ± 0.16^{aA}	1.83 ± 0.16^{bB}	1.66 ± 0.16^{bB}
	14	2.16 ± 0.16^{bA}	2.83 ± 0.16^{aB}	2.83 ± 0.16^{aB}
Angiogenesis	7	0.33 ± 0.21^{a}	0.33 ± 0.16^{a}	0.33 ± 0.16^{a}
	14	1.16 ± 0.16^{b}	1.66 ± 0.22^{b}	1.50 ± 0.22^{b}
Inflammatory cell	7	2.66 ± 0.21^{a}	2.66 ± 0.21^{a}	2.50 ± 0.22^{a}
	14	0.66 ± 0.21^{b}	0.50 ± 0.22^{b}	0.66 ± 0.22^{b}

[a,b]: For epithelisation, granulation tissue and collagen, angiogenesis, and inflammatory cell, the different letters indicate significant differences between the 7th and 14th days (P<0.05).

[A,B]: Values in the same row with different superscripts are significantly different (P<0.05).

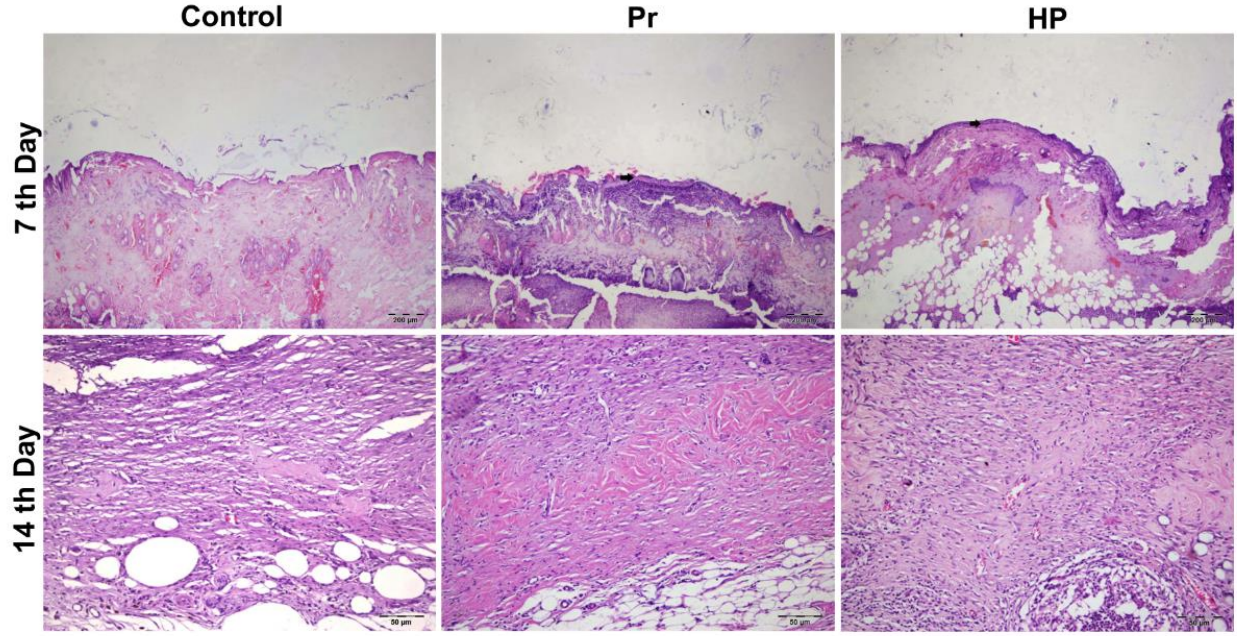

Figure 1. The microscopic images of the burned skin wounds on days 7 and 14. Seventh day: Group control: insufficient epithelisation; Group propolis (Pr): more improved epithelisation (arrow); Group *Hypericum perforatum* (HP): more improved epithelisation (arrow) (H&E). Fourteenth day: Group control: appearance of oedematous immature granulation tissue; Group propolis (Pr): appearance of less oedematous mature granulation tissue; Group *Hypericum perforatum* (HP): appearance of less oedematous mature granulation tissue (H&E).

Discussion

Alternative treatment methods have been commonly used in treatment of diseases since ancient times. For many years the effects of herbal medication on wound healing have been reported. Herbal products seem to have effect with less or no toxicity comparing to the synthetic drugs (21-23). In this study, stimulatory effects of Pr and HP on burn wound healing were studied in clinical, biochemical, and histological terms.

Experimental animals such as rats have been used in wound healing studies (13, 24, 25). In this study, the burn wound model used is easy and reproducible.

In accordance with previous studies (2, 14), the back skin of the rat seems to be an appropriate model for the study of burn wound healing and we used this model due to its simplicity.

Appropriate wound healing requires equilibrium between antioxidants and oxidative stress. Increase of reactive oxygen species inhibits wound healing, and also in a reduction in some free radical scavengers to cause tissue damage. Antioxidants increase the healing of wounds by decreasing the damage that is caused by free oxygen radicals (26-28).

Numerous antioxidants have been used in the treatment of wound both experimentally and clinically. Among them; Vitamins A, C, E, β-carotene, glutathione, SOD, dimethyl sulfoxide, melatonin, selenium, uric acid, ceruloplasmin, coumarine, ubiquinol, glycolic acid, and caffeic acid phenethyl ester could be mentioned (4, 14).

Pr is a natural medication that has been used since ancient times. In the last decades, Pr has attracted investigators' attention owing to several pharmacological and biological properties (3). It was notified that the antimicrobial (29) and anti-inflammatory activity (5) of Pr is owing to caffeic acid phenethyl ester. Some researchers (12, 28), reported that antioxidative effect of Pr.

The time-dependent reductions in MDA level and statistical increments in all antioxidants on day 14 were determined in both control and Pr groups when compared with the values on day 7 (Table 1). This situation may be explained with the natural increase in wound healing by the time and subsequent improvements in oxidant/antioxidant balance. In addition, results of this study clearly demonstrated that Pr plays a role positively in healing of skin burn wounds by its anti-inflammatory and antioxidant effects.

It is reported that the HP extract has anti-inflammatory and antioxidant effects (6, 7, 11, 30). In this study, MDA and SOD levels were significantly different in HP group compared to the control group on day 7 ($P<0.001$). A statistical increase was observed in only SOD activity between days 7 and 14 in HP group ($P<0.05$) (Table 1). However, it is believed that HP does not have strong antioxidant properties compared with Pr.

The accumulation of collagen demonstrates wound healing (31). Pr was used as an antiseptic and cicatrizant in burn wound treatment (4). It was reported that Pr extract have important anti-inflammatory properties and has reduced oedema in inflammation via this feature (5).

It was reported that HP has stimulating effects of fibroblast migration, collagen accumulation, and revascularisation (9, 10, 32).

In this study, on the 7th and 14th days, granulation tissue, collagen accumulation and epithelisation in animals in the Pr and HP groups were higher compared with the control group ($P<0.05$), as shown by the previous studies (9, 32, 33). In accordance with by the previous studies (3, 4), it was found that the serious oedema observed in the control group decreased in the Pr and HP groups.

It is known that HP increased wound healing (33). Samadi et al. (34) showed that HP aided in healing caesarean wounds.

The results acquired in this study indicated that the antioxidant property of Pr was more than that of HP. Pr and HP were found to accelerate wound healing probably by increasing the formation of collagen deposition, epithelial and granulation tissue. It is also thought that antioxidant property of Pr contribute to the wound healing.

Consequently, results of this study remarked that Pr and HP skin creams could be applied in treatment of burn wounds and it may be an alternative to the traditional methods.

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