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Antioxidant Characteristic of Pretreatment Hypericum Perforatum Oil Administration in a Rabbit Model of Palatal Mucosal Injury

Palatal Mukozal Hasar Oluşturulan Tavşanlarda Tedavi Öncesi Hypericum Perforatum Yağı Uygulamasının Antioksidan Özelliği

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Öz

Amaç: Bu çalışma, tavşanlarda tedavi öncesi (t) topikal Hypericum perforatum (HP) yağı uygulamasının antioksidan ve yumuşak doku yara iyileştirici özelliklerini belirlemek amacıyla yapıldı.
Gereç ve Yöntem: Otuz altı Yeni Zelanda albino tavşanı, rastgele olarak t-HP yağı (test, n = 18) ve t-zeytin yağı (kontrol, n = 18) gruplarına ayrıldı. Test ve kontrol gruplarındaki tavşanların her biri cerrahi öncesi topikal olarak HP veya zeytinyağı ile ön işleme tabi tutuldu. Alınan dişeti biyopsileri, re-epitelizasyon (RE) ve granülasyon dokusu olgunlaşması (GTM) açısından analiz edildi. Genel yara görünümü, eritem ve epitelyal birleşme skorlaması yapılarak klinik görünüm değerlendirildi. VEGF ve FGF-2 düzeyleri immünohistokimyasal olarak analiz edildi. Doku katalaz (CAT) ve malondialdehid (MDA) seviyeleri ELISA yöntemi ile belirlendi. Tüm değerlendirmeler cerrahi sonrası 3., 7. ve 14. günlerde gerçekleştirildi.
Bulgular: t-HP yağı uygulaması yapılan grupta, t-zeytinyağı uygulaması yapılan gruba göre daha yüksek epitelyal birleşme (7. günde) ve genel yara görünüm skorları (7. ve 14. günlerde) tespit edildi ($p < 0.05$). Diğer taraftan, RE skorları gruplar arasında farklı değildi ($p > 0.05$). Pozitif olarak boyanmış FGF-2 hücrelerinin sayısı, test grubunda kontrol grubundan daha yüksekti ($p < 0.05$), ancak gruplar arasında pozitif boyanmış VEGF damar sayısı açısından anlamlı bir fark yoktu ($p > 0.05$). t-HP yağı uygulaması, t-zeytinyağı uygulamasına göre doku CAT seviyelerini arttırırken, doku MDA seviyelerini düşürdü ($p < 0.05$).
Sonuç: Bu çalışmadan elde edilen sonuçlar, topikal HP yağının tedavi öncesi uygulanmasının antioksidan etkilere sahip olduğunu, taşıyıcısı olan zeytinyağı ile karşılaştırıldığında sekonder yara iyileşmesini hızlandırmak amacıyla kullanılabileceğini göstermiştir.
Anahtar Kelimeler: Katalaz, hypericum perforatum yağı, malondialdehid, zeytin yağı, yara iyileşmesi

Abstract

Objective: This study was aimed to determine the antioxidant and soft tissue wound healing properties of pretreatment (p) of topical Hypericum perforatum (HP) oil in rabbits.

Materials and Methods: Thirty-six New Zealand albino rabbits were randomly classified as p-HP oil (test, n = 18) and p-olive oil (control, n = 18) groups. Each groups of rabbits were topically pretreated with either HP or olive oil before the surgery. Gingival biopsies were excised and analyzed for re-epithelialization (RE), and granulation tissue maturation (GTM) and clinical appearance were scored for erythema, epithelial confluence and general wound appearance. Levels of VEGF and FGF-2 were assessed immunohistochemically. Tissue catalase (CAT) and malondialdehyde (MDA) levels were measured using ELISA. All analyses were performed on days 3, 7 and 14 after surgery.
Results: Higher epithelial confluence (on the 7th day) and general wound appearance scores (on the 7th and 14th days) were determined in p-HP oil than that of p-olive oil group ($p<0.05$). On the other hand RE scores did not differ between groups ($p>0.05$). The number of positively stained FGF-2 cells was higher in the test group than that of the control group ($p<0.05$), but there were no significant differences between the groups for the number of positively stained VEGF vessels. p-HP oil administration increased the tissue CAT levels and reduced the MDA levels compared to p-olive oil ($p<0.05$).
Conclusions: The results obtained from this study indicated that pretreatment with topical HP oil has antioxidant effects and may be used to accelerate the secondary wound healing compared to its base, olive oil.
Key Words: Catalase, hypericum perforatum oil, malondialdehyde, olive oil, wound healing

1. Introduction

Hypericum perforatum (HP) also popularly known as St. John's Wort (SJW) is one of the most intensively studied medicinal plant and has growing interest in recent years. Naphthodianthrones, acylatedphloroglucinol derivatives, flavonoids and biflavonoids are the chemical constituents of the HP [1]. The plant has wide range of therapeutic effects. Beside its antidepressant effect, HP possesses antioxidant, antifungal, antiinflammatory, antimycobacterial and antiviral characteristics [2, 3]. In traditional medicine, HP has been used both orally and topically to treat wounds, skin inflammation, cuts, burns, gastritis, hemorrhoids, peptic ulcers and bacterial infections [4]. The home made olive oil maseration of HP is widely used externally for its antiinflammatory and mainly for accelerating wound healing effects [5-7].

Wound healing involves complex interactions between inflammatory mediators and cells which results in tissue reconstitution. This process includes hemostasis, inflammation, cell proliferation, and ends with the tissue remodeling [8]. During this well organized process, a wide range of growth factors, chemokines/cytokines, enzymes, adhesion molecules are released in the extracellular matrix [9]. Additionally, reactive oxygen species, lipid peroxidation products, antioxidant systems and the balance between them play a significant role in regulating normal wound healing. Although reactive oxygen species are essential to overcome the microorganisms, excessive and uncontrolled oxidative stress causes elongation of inflammation processes which results delayed wound healing [10].

Chemotherapeutics including antibiotic and antiseptic agents are widely utilized in the field of oral diseases and periodontology. Because of the noteworthy side effects in the chemical compounds of these drugs [11], herbal therapies including plant extracts are growing interest in the recent years [12]. Tanideh et al. [13] studied the therapeutic effect of topical and systemic forms of HP on oral mucositis treatment. The researchers demonstrated that daily application of both HP treatment groups reduced inflammation and expedidated the healing of oral mucositis in hamsters. Recently, our research group investigated the wound healing effect of olive oil formulation of HP in rabbit palatal mucosa and compared with its base, olive oil. Although clinical and histomorphometric results showed HP oil was superior than that of its base, immunohistological and biochemical results revealed that administration of HP oil twice a day did not provide a significant benefit to secondary wound healing in rabbits [14].

Prophylactic administration or pretreatment with nutritional elements such as vitamin B-complex, vitamin C and dietary calcium [15, 16] or mediators [17] are utilized to accelarate the wound healing process. For instance, the effect of prophylactic platelet-activating factor (PAF) antagonist application was studied on both gastric [17] and oral mucosal ulsers [18]. In comparison to therapeutic administration, the wound healing process was accelerated with the prophylactic administration through increased in COX2 enzyme expression and reduction in mucosal apoptosis, TNF-α and NOS-2 activity.

In literature, it has been reported that the potential mechanism of Hypericum perforatum oil for topical wound healing was inhibition of TNFα-induced NF-κB activation [1]. Moreover, Lawrence et al. [19] indicated that inhibitors of NF-kB revealed anti-inflammatory effect when applied before the inflammatory stimulus, conversely the application caused prolongation of inflammatory responses when used therapeutically (16). In our recent study, HP oil treatment did not reveal additional wound healing effects in the oral mucosa of rabbits [14]. Therefore, in the aforementioned literature, we hypothesed that mucosal wound healing could be enhanced by pretreatment administration of HP oil. Thus, in the present study we decided to further study the antiinflammatory and antioxidant effects of HP oil–pretreatment on mucosal wound healing and to compare with its base, olive oil pretreatment in rabbits' palatal mucosa**.**

2. Materials and Methods

2.1.*Preparation and identification of components of Hypericum perforatum Oil*

The flowering tops of fresh plants (100 g), collected from Manisa region in Turkey, were inserted into a 500-ml glass bottle without pressure and the bottle was filled topical application. The components of the preperation were analyzed by high-performance liquid chromatography/diode-array detection (HPLC/DAD), which were summarized in our previous study [14].

2.2.Experimental groups and surgical procedure

In this experimental study, 36 healthy male New Zealand white rabbits (2.0–2.8 kg; 8 months old) were kept in a separate cages with a standard laboratory diet and water. The rabbits were divided into two groups randomly (n=18): p- HP oil (test) and p- olive oil (control). The control group received the same olive oil which was base of the HP oil. The protocol of this study was approved by Local Ethical Committee on Animal Experiments, Bolu Abant Izzet Baysal University (decision number: 2015/13).

In this study, each groups of rabbits were topically pretreated with either HP or olive oil separately. The applications were initiated 3 (n=12), 7 (n=12) and 14 (n=12) days before the surgery for each groups and continued until the end of the study. Topical administrations were applied as follows: 0.1 ml, 30 seconds and twice a day. A 16 mm2 (4 × 4 mm) in size at a depth of 1-2 mm experimental wound was surgically made using stainless steel blade on the rabbits' palatal mucosa under xylazine/ketamine HCl (5/35 mg/kg) anesthesia (SG). The wound was located 1 mm behind the incisive papilla and between the two incisors. The animals also received topical applications of both the test and control material via cotton pellets immediately after the surgery, and continued up to the end of study (SG) [14]. A 6 × 6 mm2 gingival tissue biopsy containing epithelium + connective tissue was excised from the relevant field on days 3, 7 and 14 (test: n = 2, control: n = 2) for the 3, 7 and 14 days pretreatment groups. The animals of each groups were not sacrified at the indicated days. To increase the number of data per each pretreatment groups, they were assembled and considered as the pretreatment administration groups instead of analyzing the 3, 7 and 14 days pretreatments separately. As a consequence, a total of 6 samples were analyzed per group for the evaluated time periods (on 3rd, 7th and 14th days).

2.3. Wound healing activity

The anterior–posterior (AP) and mesial-distal (MD) dimensions of the wound area were determined with digital caliper on days 3, 7, and 14 under general anesthesia. The researcher (OAK) who was blinded to the treatment groups performed and recorded all the measurements. The clinical wound healing activity including erythema, epithelial confluence and general samples were embedded in a paraffin block and serial wound appearance was scored as well [20]. These clinical measurements were performed by the same single calibrated and blinded examiner (OAK).

2.4. Histopathology (H&E) and immunohistochemical analysis

with olive oil. After 30 days of maceration with sunlight, the mixture became dark red and was ready to use for

The tissue samples were fixed in neutral 10% formalin for 24–72 hours. Following routine fixation procedures, sections at a thickness of 5 μm in the sagittal direction were obtained. Subsequently, the samples were deparaffinized and stained with hematoxylin and eosin (H&E). RE and GTM were analyzed based on the Li, Diao [21] method by the same pathologist. New blood vessel formation and fibroblast cell numbers were determined immunohistochemically by VEGF and FGF-2 analysis in five different areas at 40 x magnification.

2.5. Biochemical analysis

Malondialdehyde (MDA) and catalase (CAT) were analyzed to evaluate oxidative stress. Therefore, gingival tissue samples (70–90 mg) were homogenized, and levels of MDA and CAT were measured using enzyme-linked immunosorbent assay (ELISA).

2.6. Statistical analysis of the data

Statistical analyses were carried out using the SPSS program. The normality of the data was verified using Shapiro–Wilk test. The differences between groups and within each groups were analyzed by analysis of variance (ANOVA) and Tukey's test for normally distributed parameters: Kruskal–Wallis and Mann–Whitney U test for non normally distributed parameters. P value < 0.05 was considered as statistically significant.

3. Results

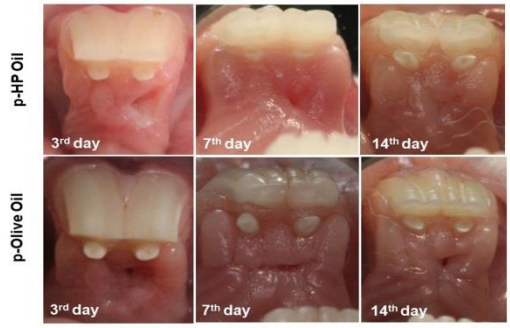
3.1. Clinical wound healing

During the experimental study, one animal in the p-HP oil group was omitted from the study on the 14th day due to the need of systemic antifungal treatment.

The mean dimensional changes of the wound area in both MD and AP directions for each group on distinct days are shown in Table 1. Accordingly, the changes in both dimensions between groups did not reach a statistically significant level ($p>0.05$). On the other hand, within the groups comparison showed that both the MD and AP dimensions on the 7th and 14th days were statistically smaller than that of the 3rd day in each group ($p<0.05$). Beside, the reduction of the AP dimensions in each evaluated time periods in both groups was significant ($p<0.05$).

In comparison to p-olive oil, p-HP oil treated group showed significantly higher epithelial confluence scores on the 7th day and general wound appearance scores on the 7th and 14th day of the treatment ($p<0.05$). On the other hand, erythema scores did not differ between groups at any time point ($p>0.05$) (See Table 2, Figure 1).

Figure 1: Clinical appearance of the study groups.



p: Pretreatment, HP: Hypericum perforatum

Table 1. Dimensional changes of surgical wound region in the test and control group by day (mm).

	HP oil (test)	***Olive oil (control)***	***P****	***HP oil (test)***	***Olive oil (control)***	***P****
3rd day	**MD**			**AP**		
n	6	6		6	6	
Mean±Sd	2,57±0,72	2,45±0.48		2,67±0.68	3,02±0.78	
Min-max	1,90-3,93	1,69-2,89		1,79-3,24	1,77-3,92	
Median	2,46	2,64		3,03	3,13	
7th day						
n	6	6		6	6	
Mean±Sd	1,44±0.44[a]	1,57±0.73[a]	0.830	1,87±0.34[a]	1,97±0.62[a]	0.359
Min-max	0,70-1,89	0,80-2,63		1,37-2,25	0,80-2,59	
Median	1,49	1,56		1.95	2,05	
14th day						
n	5	6		5	6	
Mean±Sd	0,68±0.19[a]	0.77±0.40[a]		0,97±0.21[ab]	1,02±0,13[ab]	
Min-max	0.54-1.01	0.34-1,46		0,66-1,20	0,76-1,12	
Median	0.59	0.70		1,03	1,07	
$p^{\#}$	**0.000**	**0.000**		**0.000**	**0.000**	

*#ANOVA-Tukey, p<0.05, n:number of samples.
* : difference between groups, #: difference within groups by day.
a: difference from 3rd day, b: difference from 7th day), HP:hypericum perforatum, MD:mesial-distal, AP:anterior-posterior, Sd:standart deviation, Min:minimum, Max:maximum

Table 2. Erythema, Epithelial confluence and general wound appearance scores of test and control groups

	Erythema			Epithelial confluence			General wound appearance		
	HP oil (test)	***Olive oil (control)***	***p****	***HP oil (test)***	***Olive oil (control)***	***p****	***HP oil (test)***	***Olive oil (control)***	***p****
3rd day									
n	6	6		6	6		6	6	
Mean±Sd	2,33±0,51	3.00±0,63	0.075	0.50±0,54	0,16±0,40	0.241	1,33±0,51	1,16±0.40	0.523
Min-max	2.00-3.00	2.00-4.00		0.00-1.00	0.00-1.00		1.00-2.00	1.00-2.00	
Median	2.00	3.00		0.50	0.00		1.00	1.00	
7th day									
n	6	6		6	6		6	6	
Mean±Sd	0.00±0.00[a]	0.50±0.54[a]	0.056	3,16±0,75[a]	2,16±0.40[a]	**0.023**	2.50±0.54[a]	1.50±0.54	**0.019**
Min-max	0.00-0.00	0.00-1.00		2.00-4.00	2.00-3.00		2.00-3.00	1.00-2.00	
Median	0.00	0.50		3.00	2.00		2.50	1.50	
14th day									
n	5	6		5	6		5	6	
Mean±Sd	0.00±0.00[a]	0.00±0.00[a]	1.000	4.00±0.00[ab]	3.66±0.51[ab]	0.174	3,20±0.44[a]	2,33±0,51[ab]	**0.024**
Min-max	0.00-0.00	0.00-0.00		4.00-4.00	3.00-4.00		3.00-4.00	2.00-3.00	
Median	0.00	0.00		4.00	4.00		3.00	2.00	
p^{Ω}	**0.000**	**0.001**		**0.001**	**0.000**		**0.003**	**0.010**	

Kruskal Wallis- Mann Whitney U test p<0.05. n:number of samples.
*: difference between groups, Ω: difference within groups by day.
(a: difference from 3rd day, b: difference from 7th day)
(HP:Hypericum perforatum, Sd:standart deviation, Min:minimum, Max:maximum)

3.2. Histopathology

The RE and GTM scores were summarized in Table 3. Even though the epithelialization was completed earlier and the RE scores were higher in the p-HP oil than that of the p-olive oil group, the results did not reach significance (p>0.05). When we compare the GTM scores between groups, the results were only significant on the 7th day of the treatment (p<0.05).

Table 3. Re-epithelization and granulation tissue maturation scores of test and control groups.

	Re-epithelization			***Granulation Tissue Maturation***		
	HP oil (test)	***Olive oil (control)***	***p****	***HP oil (test)***	***Olive oil (control)***	***p****
3rd day						
n	6	6		6	6	
Mean±Sd	0.33±0.81	0.00±0.00	0.317	1.00±0.00	1.33±0.51	0.138
Min-max	0.00-2.00	0.00-0.00		1.00-1.00	1.00-2.00	
Median	0.00	0.00		1.00	1.00	
7 th day						
n	6	6		6	6	
Mean±Sd	4.00±0.00[a]	3.50±0.54[a]	0.056	3.66±0.51[a]	2.16±0.75	**0.007**
Min-max	4.00-4.00	3.00-4.00		3.00-4.00	1.00-3.00	
Median	4.00	3.50		4.00	2.00	
14th day						
n	5	6		5	6	
Mean±Sd	4.00±0.00[a]	3.66±0.51[a]		3.80±0.44[a]	3.33±0.51[ab]	
Min-max	4.00-4.00	3.00-4.00	0.174	3.00-4.00	3.00-4.00	0.140
Median	4.00	4.00		4.00	3.00	
p^{Ω}	**0.000**	**0.002**		**0.001**	**0.003**	

Kruskal Wallis- Mann Whitney *U* test p<0.05. n:number of samples.
*: difference between groups, $^{\Omega}$: difference within groups by day.
(**a**: difference from 3th day, **b**: difference from 7th day)

(HP:Hypericum perforatum, Sd:standart deviation, Min:minimum, Max:maximum)

Table 4. Tissue levels of FGF-2 and VEGF in the study groups

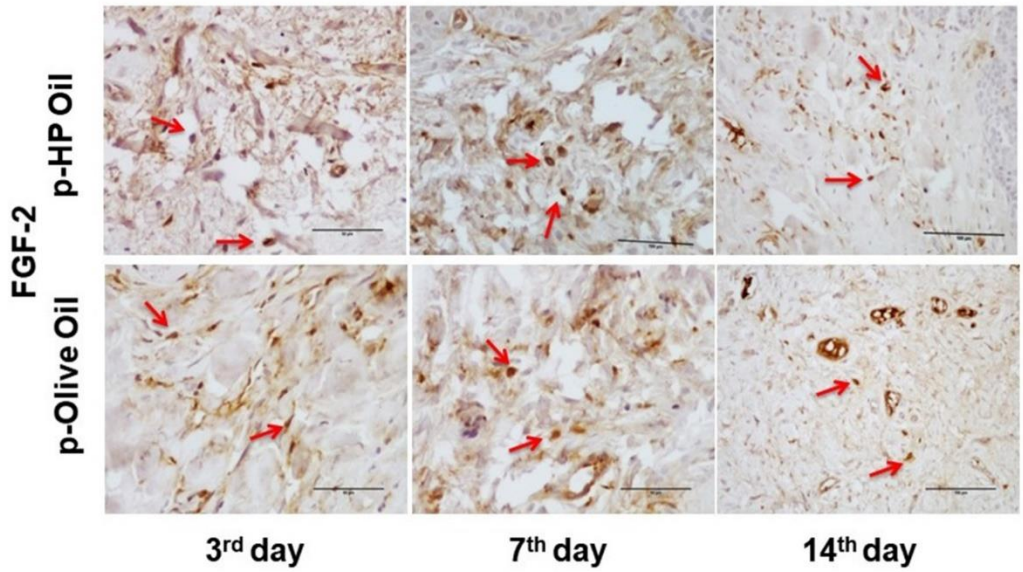
	FGF-2			***VEGF***		
	HP oil (test)	***Olive oil (control)***	***p****	***HP oil (test)***	***Olive oil (control)***	***p****
3rd day						
n	6	6		6	6	
Mean±Sd	27,86±5,00	15,83±6,63	**0.005**	1,25±0,48	1,74±1,28	0.396
Min-max	18,60-32,40	9,20-27,40		0,83-2,00	0,33-3,83	
Median	28,60	14,20		1,08	1,58	
7 th day						
n	6	6		6	6	
Mean±Sd	22,56±7,01	15,33±1,99	**0.035**	1,52±0,58	1,69±0,57	0.627
Min-max	14,00-33,80	12,80-18,00		1,00-2,66	1,00-2,50	
Median	21,70	15,40		1,41	1,58	
14th day						
n	5	6		5	6	
Mean±Sd	16,20±4,22[a]	14,00±4,27	0.415	1,51±0,19	1,48±0,11	0.793
Min-max	11,40-21,60	7,60-19,80		1,33-1,77	1,33-1,67	
Median	16,80	13,50		1,47	1,50	
$p^{\#}$	**0.014**	0.787		0.538	0.847	

*#ANOVA-Tukey, p<0.05, n:number of samples.
* : difference between groups, #: difference within groups by day.
(a: difference from 3rd day, b: difference from 7th day)
(HP:Hypericum perforatum, FGF: fibroblast growth factor, VEGF: vascular endothelial growth factor, Sd:standart deviation, Min:minimum, Max:maximum)

3.3. Immunohistochemistry

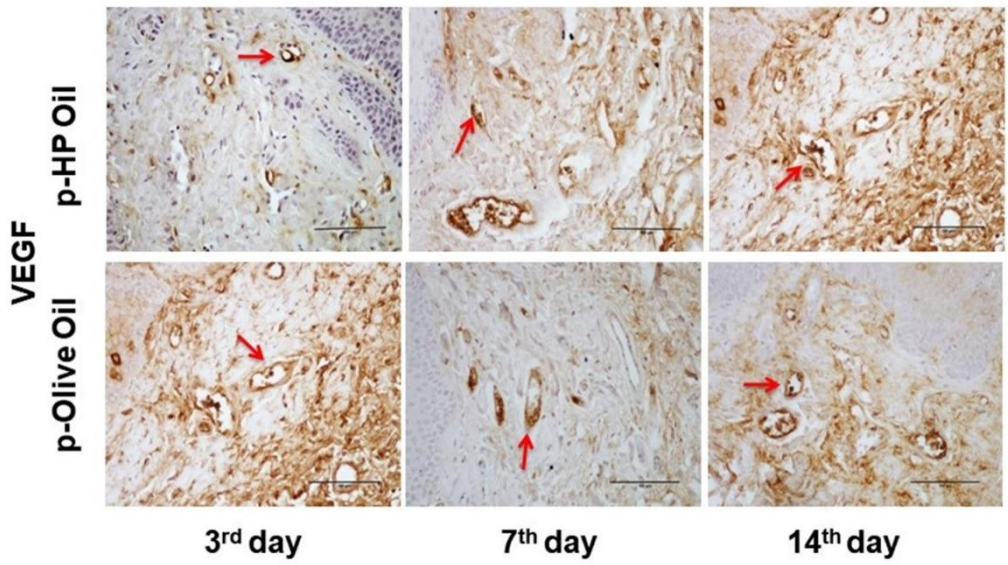
The number of FGF-2-stained cells were higher in the p-HP oil than that of the p-olive oil group on the 3rd and 7th day of the treatment ($p<0.05$), but the results did not differ between the groups on the 14th day ($p>0.05$). Intragroup comparison showed that FGF-2-stained cells were reduced on day 14 compared to day 3 in the p-HP oil group ($p<0.05$), and no significant changes were determined at any time interval in the p-olive oil group. The p-topical application with HP oil or olive oil did not alter the amount of VEGF ($p>0.05$) (See Table 4). The FGF-2 and VEGF-stained cells/vessels in the tissue sections are illustrated in Figures 2 and 3, respectively.

Figure 2: FGF-2 stained cells in the tissue sections.



p: Pretreatment, HP: Hypericum perforatum, FGF-2: fibroblast growth factor 2

Figure 3: VEGF stained vessels in the tissue sections.



p: Pretreatment, HP: Hypericum perforatum, VEGF: Vascular endothelial growth factor

3.4. Tissue CAT and MDA levels

Pretreatment with HP oil significantly increased the tissue CAT (ng/ml) levels at all time periods compared to the control group. When the tissue levels of CAT (ng/ml) within the groups are compared, it was determined that the levels were increased in the p-HP oil group ($p<0.05$). Conversely, p-olive oil treatment decreased the CAT activity and the results were statistically significant on the 14th day compared to the 3rd day. Compared with the control group (p-olive oil), MDA (nmol/ml) levels were decreased with p-HP oil treatment, but this difference was only significant on the 14th day ($p<0.05$). Pretreatment with olive oil did not alter the MDA (nmol/ml) levels within the groups by day. However, significant decreases were observed on day 14 compared to day 3 in the p-HP oil treatment group ($p<0.05$) (See Table 5).

Table 5. CAT (ng/ml) and MDA (nmol/ml) levels of the study groups.

	CAT			***MDA***		
	HP oil (test)	***Olive oil (control)***	***p****	***HP oil (test)***	***Olive oil (control)***	***p****
3rd day						
n	6	6		6	6	
Mean±Sd	65,29±16,08	41,91±12,78	**0.019**	5,33±1,59	6,66±0,98	0.114
Min-max	44,35-89,83	26,54-64,95		3,23-7,62	5,37-8,40	
Median	62,52	39,01		5,24	6,55	
7 th day						
n	6	6		6	6	
Mean±Sd	83,31±12,17	29,90±6,81	**0.000**	4,26±1,02	5,89±1,69	0.072
Min-max	66,02-97,05	26,54-64,95		3,05-5,58	4,23-7,97	
Median	81,98	28,71		4,45	5,60	
14th day						
n	5	6		5	6	
Mean±Sd	108,78±6,79[ab]	25,56±11,03[a]	**0.000**	2,47±1,30[a]	5,38±1,08	**0.003**
Min-max	99,66-118,65	12,59-40,16		0,34-3,89	4,63-7,52	
Median	108,60	25,44		2,58	5,01	
p#	**0.000**	**0.043**		**0.011**	0.258	

*#ANOVA-Tukey, $p<0.05$, n:number of samples.

* : difference between groups, #: difference within groups by day.

(a: difference from 3th day, b: difference from 7th day)

(HP:Hypericum perforatum, CAT: Catalase, MDA: Malondialdehyde, Sd:standart deviation, Min:minimum, Max:maximum)

4. Discussion

In our previous study, we utilized a rabbit model to test the effect of topically applied HP oil on the palatal mucosa wound healing. The results have shown that HP oil administration did not have an additional curative, antiinflammatory or antioxidant features compared to its base olive oil [14]. Therefore in the present study, we wondered the probable wound healing characteristics of p-administration of HP oil before the surgery. It was determined that p-HP oil application provided early epithelial healing, and showed superior antioxidant features than p-olive oil. To the best of our knowledge, this is the first study and significant to present the therapeutic benefits and antioxidant characteristics of p-administration of HP oil topically.

It has been reported that HP could facilitate cesarean wound healing and minimized formation of scar and its pain [6]. Same results were shown by Lavagna et al. [7] using a mixture including 70% oily extract of HP and 30% oily extract of Calendula. Nayak et al. [22] investigated the wound healing effect of HP + petroleum jelly (1:1) in an excisional dermal wound model in rats. The researchers demonstrated that the ointment significantly reduced the wound area and increased the rate of wound healing through modulating the inflammatory and proliferative phases of the healing cascade. Furthermore, they observed that the experimental group had dense bundles of collagen fibers, fibroblast cells and new blood vessels than those of the control. In a study conducted by Tanideh, Namazi [13] reported that epithelization and antiinflammatory characteristics of both topical and systemic administration of HP increased in oral mucositis model. Furthermore, although topical HP could accelerate wound healing in a diabetic wound, it was suggested to use systemic HP administration rather than the topical usage [5]. These antiinflammatory feature of HP was attributed to quercetin, however hyperforin has played a key role through inhibition of lymphocyte reactions, cyclooxygenase-1 and 5- lipoxygenase [24] and proliferation of T lymphocytes [23]. Therefore, these authors claimed that HP could be a therapeutic material for the topical treatment of inflammatory skin disorders. Beside, Fuller and Muller-Goymann [25] determined reduced proliferation, migration activity and contraction ability of fibroblast, and advised to use hyperforin for treatment of hypertrophic scars rather than promoting wound healing. In this study, a secondary healing model

in the palatal keratinized mucosa has been utilized to mimic the healing of gingivectomy operation. Previously, topical treatment with HP oil could not demonstrate an additional benefit on wound healing [14]. Pretreatment with HP oil resulted better wound appearance and earlier epithelial healing than that of the control group in the current study. The enhanced wound healing might be explained by the increased antibacterial activity due to the possible increased hyperforin content in mucosa with pretreatment protocol. Furthermore, the application of HP oil before the injury might revealed systemic effect, thus the effect of topical application might be promoted. Although higher contraction of both in MD and AP dimensions of the wound with pretreatment with HP oil, the results could not reach significance. In addition, the higher RE and GTM scores in the p-HP oil treatment group, and incomplete epithelial confluence in the control group on the fourteenth day of healing are in agreement with the literature. Therefore, it can be suggested to use the olive oil maseration of HP topically before the injury, and proceed until the wound closure occurs.

Wound healing is a complex and multi-cellular process that aims formation of new blood vessels and restoration of the epithelium and connective tissue after injury [26]. Fibroblast growth factor (FGF)-2 and vascular endothelial growth factor (VEGF) have been determined to promote angiogenesis synergistically, and therefore, have much caution in the field of wound healing [27]. It has been reported that topical administration of FGF-2 could enhance cell proliferation and migration, wound re-epithelialization and collagen deposition [28, 29]. In an animal model of surgically wounded submandibular glands, the use of collagen gels with FGF-2 improved salivary gland regeneration [30]. Furthermore it has been shown that periodontal tissue regeneration was promoted by using recombinant (rh) FGF-2 [31]. We have reported previously that topical HP oil treatment has not increased the FGF-2 positive stained cell number in the early period of acute wound healing [14]. On the other hand, the number of cells that presented positive staining for FGF-2 was superior in the p-HP oil than those in the p-olive oil on days 3 and 7 in this current study. Additionally, the intragroup comparison demonstrated that the high number of FGF-2 cells in the p-HP oil–treated group decreased gradually in line with the wound healing process however, the cell number did not differ in the p-olive oil- treated group. Consequently, it could be suggested that topical HP oil administration was not sufficient to promote FGF-2 expression and pretreatment and also continued administration of Hp oil is critical in the stimulation of fibroblast collagen production [4] to promote wound healing.

VEGF promotes the formation of new blood vessels, mediates vascular permeability and provides chemotactic factors for inflammatory cells [27]. In literature it has been demonstrated that hypericum essential oils had antiangiogenic properties [32] and inhibits tumor-related angiogenesis [33]. Furthermore, in an in vitro study, the reported results showed that Hyperforin was able to inhibit PMN chemotaxis and chemoinvasion without affecting their viability and chemokine- receptor expression. Furthermore Hyperforin blocked the inflammation-triggered angiogenesis by both local injection and daily systemic administration [34]. On the other hand, in contrast to other studies [33, 34], Tassone et al. [35] showed that hyperforin, an important component of HP, up-regulated the expression of VEGF in central nervous system tumour cells. Additionally in a diabetic rat model, both topical and systemic HP oil administration increased the number of vessel formation during dermal wound healing in the early period. On the other hand angiogenesis was found to be lower than the control group [5]. HP oil treatment decreased the VEGF levels in the early period of oral mucosal healing [14], while pretreatment with HP-oil did not have an additional effect on VEGF levels in the current study. Therefore, it could be concluded that pre- and proceeding administration of HP oil might have increased the hyperforin content in the oral mucosa so that this would be resulted with the increased antiangiogenic and anti-inflammatory characteristics of HP oil.

It has been demonstrated that HP possessed antioxidant activities including free radical scavenging capacity, and could be used for preventing and treating pathological conditions related to oxidative stress [36]. Suzen et al. [37] studied the protective effects of Hp and quercetin against ischemia/reperfusion (I/R) injury. They reported that MDA and nitric oxide (NO) levels were significantly lower, and total antioxidant status were significantly higher in the Hp group than those in the quercetin group. They concluded that although both Hp and quercetin had protective effects against I/R injury of the testes, the protective effect of Hp was found to be stronger than that of quercetin. In line with, Abd El Motteleb and Abd El Aleem [38] reported that HP caused dose-dependent reduction of elevated MDA in diabetic nephropathy and concluded that HP may have a renoprotective effect through reduction of oxidative stress and enhancement of antioxidant defense mechanisms. The lipid-lowering and antioxidative properties of HP was investigated by Ghosian et al. [39] in rats fed a cholesterol-rich diet. The researchers determined that markers of oxidative stress and lipid peroxidation induced by hyperlipidemia were significantly decreased by systemic HP administration. Furthermore, HP treatment reduced the MDA levels and increased the SOD, CAT, and GSH-PX activity in rats with kidney ischemia/reperfusion damage [40]. In a clinical study, Naziroglu et al. [41] investigated the effects of HP on oxidative stress in serum and leukocytes of patients with multiple sclerosis, and reported that the lipid peroxidation level in the HP-incubated neutrophil were markedly decreased in those patients. In an oral mucositis model, it has been reported that topical/systemic HP administration reduced the tissue MDA level. Besides this, in the systemic HP oil–treated group, tissue MDA levels were found to be higher than that of topical application group [13].

In contrast to aforementioned literature, the antioxidant characteristics of topical HP oil application could not be

confirmed in our recent study [14]. On the other hand, pre-treatment with HP reduced the tissue MDA levels on the 14th day of healing. Furthermore, the tissue MDA levels were decreased in the pre-treated HP group during the wound-healing period, but the results were same for the pretreatment with olive oil group. Additionally, the tissue levels of CAT in the p-HP oil were found to be higher than that of the p-olive oil group in each evaluated time periods. Moreover, while the levels of CAT were increasing during the wound healing period with p-HP-treatment, the levels were found to be decreased in the control. These results demonstrated that in addition to daily application, pretreatment with HP oil before the intraoral surgery might enhance the effect of topical application through systemic effect. Thus, pretreatment with HP oil may have protective features and may be considered as an essential phase to increase the antioxidant characteristic during mucosal wound healing.

5. Conclusions

This paper revealed the antioxidant activity of p-HP oil. Furthermore, the antioxidant activity of p-HP oil administration may also have further contribution to secondary wound healing through early epithelial confluence, and enhanced FGF activity in the oral mucosa. However, its effects on angiogenesis during mucosal wound healing awaits for further clarification in terms of mechanism. The results of this study suggest that pretreatment should be required to accelerate intraoral secondary wound healing in conjunction with the daily topical administration of traditionally prepared HP oil. Further studies are also worthwhile to continue systematically for clarifying the exact mechanism.

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References:

1. Orhan, IE, Kartal, et al. Inhibitory effect of St. Johns Wort oil macerates on TNFalpha-induced NF-kappaB activation and their fatty acid composition, Journal of Ethnopharmacology, 2014, 155, 1086-1092.
2. Yadollah-Damavandi, S, Chavoshi-Nejad, et al. Topical Hypericum perforatum Improves Tissue Regeneration in Full-Thickness Excisional Wounds in Diabetic Rat Model, Evidence- Based Complementary and Alternative Medicine, 2015,article id:245328.
3. Saddiqe, Z, Naeem, I, Maimoona, A, A review of the antibacterial activity of Hypericum perforatum L, Journal of Ethnopharmacology, 2010, 131, 511-521.
4. Ozturk, N, Korkmaz, S, et al, Wound-healing activity of St. John's Wort (Hypericum perforatum L.) on chicken embryonic fibroblasts, Journal of Ethnopharmacology, 2007, 111, 33-39.
5. Altiparmak, M, Eskitascioglu, T, Comparison of Systemic and Topical Hypericum Perforatum on Diabetic Surgical Wounds, Journal of Investigative Surgery, 2018, 31, 29-37.
6. Samadi, S, Khadivzadeh, T, et al, The effect of Hypericum perforatum on the wound healing and scar of cesarean, The Journal of Alternative and Complementary Medicine, 2010, 16, 113-117.
7. Lavagna SM, Secci D, et al, B, Efficacy of Hypericum and Calendula oils in the epithelial reconstruction of surgical wounds in childbirth with caesarean section, IL Farmaco, 2001, 56, 451-453.
8. Mendonca, RJ, Coutinho-Netto, J, Cellular aspects of wound healing, Anais Brasileiros de Dermatologia, 2009, 84, 257-262.
9. Guo, S, Dipietro, LA, Factors affecting wound healing, Journal of Dental Research, 2010, 89, 219-229.
10. Dunnill, C, Patton, T, et al, Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process, International Wound Journal, 2017, 14, 89-96.
11. Oberoi, SS, Dhingra, C, et al, Antibiotics in dental practice: how justified are we, International Dental Journal, 2015, 65, 4-10.
12. Gunes, S, Tihminlioglu, F, Hypericum perforatum incorporated chitosan films as potential bioactive wound dressing material, International Journal of Biological Macromolecules, 2017, 102, 933-943.
13. Tanideh, N, Namazi, F, et al, Comparative assessment of the therapeutic effects of the topical and systemic forms of Hypericum perforatum extract on induced oral mucositis in golden hamsters, International Journal of Oral and Maxillofacial Surgery, 2014, 43, 1286-1292.
14. Gunpinar, S, Kilic, OA, et al, Evaluation of the Effect of Topical Hypericum perforatum Oil on Excisional Palatal Wound Healing in Rabbits, Journal of Investigative Surgery, 2018, 1-10.
15. Carr, AC, Maggini, S, Vitamin C and Immune Function, Nutrients, 2017, 9 (11), 1211.
16. Neiva, RF, Steigenga, J, et al, Effects of specific nutrients on periodontal disease onset, progression and treatment, Journal of Clinical Periodontology, 2003, 30, 579-589.
17. Slomiany, BL, Slomiany, A, Differential role of platelet-activating factor in gastric mucosal ulcer healing, Inflammopharmacology, 2003, 11, 237-248.
18. Slomiany, BL, Slomiany, A, Biphasic role of platelet-activating factor in oral mucosal ulcer healing, IUBMB Life, 2003, 55, 483-490.
19. Lawrence, T, Gilroy, DW, et al, Possible new role for NF-kB in the resolution of inflammation Nature Medicine, 2001, 7, 1291-1297.
20. Aliasl, J, Khoshzaban, F, et al, Comparing the Healing Effects of Arnebia euchroma Ointment With Petrolatum on the Ulcers Caused by Fractional CO2 Laser: A Single-Blinded Clinical Trial, Iranian Red Crescent Medical Journal, 2014, 16, e16239.
21. Li, K, Diao, Y, et al, Tannin extracts from immature fruits of Terminalia chebula Fructus Retz. promote cutaneous wound healing in rats, BMC Complementary and Alternative Medicine, 2011, 11, 86.
22. Nayak, SB, Isik, K, Marshall, JR, Wound-Healing Potential of Oil of Hypercium perforatum in Excision Wounds of Male Sprague Dawley Rats, Advances in Wound Care (New Rochelle), 2017, 6, 401-406.
23. Schempp, CM, Winghofer, B, et al, Topical application of St John's wort (Hypericum perforatum L.) and of its metabolite hyperforin inhibits the allostimulatory capacity of epidermal cells, British Journal of Dermatology, 2000, 142, 979-984.
24. Albert, D, Zundorf, I, et al, Hyperforin is a dual inhibitor of cyclooxygenase-1 and 5-lipoxygenase, Biochemical Pharmacology, 2002, 64, 1767-1775.
25. Fuller, J, Muller-Goymann, CC, Anti-proliferative and anti-migratory effects of hyperforin in 2D and 3D artificial constructs of human dermal fibroblasts - A new option for hypertrophic scar treatment?, European Journal of Pharmaceutics and Biopharmaceutics, 2018, 126, 108-114.
26. Barrientos, S, Brem, H, et al, Clinical application of growth factors and cytokines in wound healing, Wound Repair and Regeneration, 2014, 22, 569-578.
27. Przybylski, M, A review of the current research on the role of bFGF and VEGF in angiogenesis, Journal of Wound Care, 2009, 18, 516-519.
28. Zhang, X, Kang, X, et al, Stimulation of wound healing using bioinspired hydrogels with basic fibroblast growth factor (bFGF), International Journal of Nanomedicine, 2018, 13, 3897-3906.

29. Khan, S, Villalobos, MA, et al, Fibroblast growth factor and vascular endothelial growth factor play a critical role in endotheliogenesis from human adipose-derived stem cells, Journal of Vascular Surgery, 2017, 65, 1483-1492.
30. Kobayashi, F, Matsuzaka, K, Inoue T, The effect of basic fibroblast growth factor on regeneration in a surgical wound model of rat submandibular glands, International Journal of Oral Science, 2016, 8, 16-23.
31. Murakami, S, Takayama, S, et al, Recombinant human basic fibroblast growth factor (bFGF) stimulates periodontal regeneration in class II furcation defects created in beagle dogs, Journal of Periodontal Research, 2003, 38, 97-103.
32. Kiyan, HT, Demirci, B, et al, The in vivo evaluation of anti-angiogenic effects of Hypericum essential oils using the chorioallantoic membrane assay, Pharmaceutical Biology, 2014, 52, 44-50.
33. Billard, C, Merhi, F, Bauvois, B, Mechanistic insights into the antileukemic activity of hyperforin, Current Cancer Drug Targets, 2013, 13, 1-10.
34. Dell'Aica, I, Niero, R, et al, Hyperforin blocks neutrophil activation of matrix metalloproteinase-9, motility and recruitment, and restrains inflammation-triggered angiogenesis and lung fibrosis, The Journal of Pharmacology and Experimental Therapeutics, 2007, 321, 492-500.
35. Tassone, E, Maran, C, et al, Antidepressant hyperforin up-regulates VEGF in CNS tumour cells, Pharmacological Research, 2011, 63, 37-43.
36. Heinrich, M, Lorenz, P, et al, Lipid and Phenolic Constituents from Seeds of Hypericum perforatum L. and Hypericum tetrapterum Fr. and their Antioxidant Activity, Chemistry Biodiversity, 2017, 14 (8).
37. Suzen, A, Tekin, L, et al, Protective Effects of Hypericum perforatum and Quercetin in a Rat Model of Ischemia/Reperfusion Injury of Testes, European Journal of Pediatric Surgery, 2018, 28, 96-100.
38. Abd El Motteleb, DM, Abd El Aleem, DI, Renoprotective effect of Hypericum perforatum against diabetic nephropathy in rats: Insights in the underlying mechanisms, Clinical and Experimental Pharmacology and Physiology, 2017, 44, 509-521.
39. Ghosian, Moghaddam, MH, Roghani, M, Maleki M, Effect of Hypericum perforatum Aqueous Extracts on Serum Lipids, Aminotransferases, and Lipid Peroxidation in Hyperlipidemic Rats, Research in Cardiovascular Medicine, 2016, 5, e31326.
40. Cakir, M, Duzova, H, et al, The effect of hypericum perforatum on kidney ischemia/reperfusion damage, Renal Failure, 2017, 39, 385-391.
41. Naziroglu, M, Kutluhan, S, et al, Modulation of oxidative stress, apoptosis, and calcium entry in leukocytes of patients with multiple sclerosis by Hypericum perforatum, Nutritional Neuroscience, 2014, 17, 214-221.