Kocatepe Veterinary Journal

Kocatepe Vet J. (2024):17(4):325-333
DOI: 10.30607/kvj.1471008

RESEARCH ARTICLE

Stereological Investigation of the Effects of *Hypericum Perforatum* L. Plant on Skin Tissue in Second Degree Burns in Rats

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ABSTRACT

The aim of this study was to examine stereologically the effect of *Hypericum perforatum* L. oil on skin injuries resulting from burns. Experimental groups were selected 18 healthy male rats with an average weight of 250-300 grams as material. The rats were divided into 3 groups: control, burn, and burn+treatment, with 6 in each group. In the control group, second-degree burns were created and the rats were perfused. Control group rats were kept in formaldehyde for a week. Burns were applied to the burn, and burn+treatment groups with 100ºC water. 4gr/kg of Hypericum perforatum L. oil was given via gavage to the burn+treatment group only for 21 days. The burn group was not intervened. At the end of the process, rats in both the burn and burn+treatment groups were perfused. The skin of the animals, which were kept in formaldehyde for a week, and where second-degree burns occurred were dissected. After tissue tracking and tissue embedding procedures for all animals in the group, 5µm thick sections were taken to obtain 8-10 sections at a ratio of 1/75. Volume values were calculated using stereological methods from the sections photographed with an x4 objective. Calculations were made using the Shtereom I program in accordance with the Cavalieri's Principle. Non-parametric tests and Kruskal Wallis test were used in statistical evaluations. When the results were analyzed, it was observed that the administration of *Hypericum Perforatum* L. oil by gavage had an effect on the healing process of second degree burn wounds.
Key Words: Burn Wound, *Hypericum perforatum* L., Rat, Stereology, Volume.

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ÖZ

Sıçanlarda İkinci Derece Yanıklarda *Hypericum Perforatum* L. Bitkisinin Deri Dokusu Üzerine Etkilerinin Stereolojik Araştırılması

Yapılan bu çalışmada yanık sonucu oluşan deri yaralanmalarında *Hypericum perforatum* L. yağının etkisinin stereolojik olarak incelenmesi amaçlandı. Materyal olarak ortalama 250-300 gram ağırlığında sağlıklı 18 adet erkek rat seçilerek deney grupları oluşturuldu. Ratlar her grupta 6 adet olmak üzere kontrol, yanık ve yanık +tedavi olmak üzere 3 gruba ayrıldı. Kontrol grubunda ikinci derece yanık oluşturularak ratlar perfüze edildi. Bir hafta süreyle kontrol grubu ratlar formaldehitte bekletildi. Yanık ve yanık+tedavi gruplarına 100ºC'lik su ile yanık oluşturuldu. Sadece yanık+tedavi grubuna 4gr/kg *Hypericum perforatum* L. yağı gavaj yoluyla 21 gün süreyle verildi. Yanık grubuna müdahale edilmedi. Süreç sonunda hem yanık hem de yanık+tedavi grubundaki ratlar perfüze edildi. Bir hafta süreyle formaldehitte tutulan hayvanların ikinci derece yanık oluşturulan bölge derileri diseke edildi. Tüm gruptaki hayvanlar için doku takibi ve doku gömü işlemlerinden sonra 1/75 oranında 8-10 kesit elde edilecek şekilde ve 5µm kalınlığında kesitler alındı. 4'lük objektifle fotoğraflanan kesitlerden stereolojik yöntemlerle hacim değerleri hesaplandı. Hesaplamalar Cavalieri Prensibi'ne uygun olarak Shtereom I programı kullanılarak yapıldı. İstatistiksel değerlendirmelerde non-parametrik testler ve Kruskal Wallis testi kullanıldı. Sonuçlar analiz edildiğinde *Hypericum Perforatum* L. yağının gavaj yoluyla verilmesinin ikinci derece yanık yarasının iyileşme sürecinde etki ettiği gözlemlendi.
Anahtar Kelimeler: Hacim, *Hypericum perforatum* L., Rat, Stereoloji, Yanık Yarası

To cite this article: COŞKUN A.Ş. ÇAKMAK G. STEREOLOGICAL INVESTIGATION OF THE EFFECTS OF HYPERICUM PERFORATUM L. PLANT ON SKIN TISSUE IN SECOND DEGREE BURNS IN RATS. Kocatepe Vet J. (2024):17(4):325-333

Submission: 19.04.2024 **Accepted**: 11.09.2024 **Published Online**: 05.10.2024
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INTRODUCTION

The skin consists of three layers, which from the outermost to the deepest are: epidermis, dermis, and hypodermis. It constitutes 15% of the total body mass in adult individuals. The areas where the epidermis layer is thickest are the soles of the feet and the palms of the hands. The thinnest is the epidermis of the eyelids. The dorsal region is an area where the epidermis is thick. Additionally, the epidermis is thick in the hip and belly areas (Öztürk 1999; Olcer and Gonul 2002; Aksoy 2013).

The burn is tissue damage that occurs as a result of exposure of part or all of the body to various conditions such as heat, radiation, chemicals or electricity. Infection may develop in the early stages of the burn. It can protect the integrity of skin and subcutaneous tissue in burns up to 40°C. At temperatures of 45ºC and above, the integrity of the skin and subcutaneous tissues may be disrupted due to irreversible denature of cell proteins. Destruction of tissues is defined as burn. The degree of denaturation is proportional to the contact surface area, temperature, and exposure time of the material causing the damage (Değerli 2006; Kaymaz 2018).

Burn degrees are classified depending on the cause, depth and width of the injury. In burn cases, treatment begins by first determining the depth of the burn area and the percentage of tissue. In burn injuries, the length of time it takes for injuries to heal is related to the size of the wound (Hettiaratchy and Papini 2004). Additionally, the age of the patient is also a factor in the recovery period. Especially in the elderly, delays may occur in the inflammation phase of wound healing due to the slowing down of body metabolism. Disruptions in the degranulation of lymphocytes and macrophages are the most important factor. It has been reported that the decrease in the synthesis of collagen and the decrease in tissue epithelialization, which are among the delaying factors in wound healing and are related to the mechanical resistance of the tissue (Witte and Barbul 1997). Second and 3rd degree burn injuries and inhalation burns are the most difficult burn injuries to heal. (Theoret 2005). Immunological balance and wound healing phases are negatively affected by sepsis, shock or inflammation that may occur in the body (Nursal et al. 1999).

Plants of the genus *Hypericum*, also known as St. John's Wort, Hypericaceae, occurs naturally on almost all continent in the world except Antarctica. 469 species of this genus have been identified. These plants can be found in the form of shrubs, trees or herbs. They are found in different habitats, such as high mountains in tropical or temperate regions, avoiding hot, salty, and extremely dry regions (Crockett and Robson 2011). *Hypericum perforatum* L. has been known as a plant with medicinal properties for centuries. It is known that oil-containing *Hypericum perforatum* L. preparations are used externally in the treatment of minor burns, wounds and inflammatory conditions. Its internal use has been preferred in the treatment of depressive and anxiety diseases (Greeson et al. 2001).

The term stereology is derived from the Greek word "stereos", which means three-dimensional object (Akalan and Demirkan 2013). It can be defined as the branch of science that allows interpretation of the actual three-dimensional properties of the object by using data obtained from two-dimensional sections (Mayhew and Gundersen 1996). In other words, stereology is reported as a method that uses two-dimensional cross-sections of an object to obtain quantitative information about its geometric structure (Cruz-Orive 1993).

The aim of this study is to reveal the effects of *Hypericum perforatum* oil applied by gavage method on the skin of rats with second-degree burns, on skin healing, stereologically by volumetric value calculations. Thus, scientific contributions will be made to the fields of anatomy, histology, surgery, and stereology.

MATERIALS and METHODS

In order to conduct this study, the decision dated 25.11.2021 and numbered 2021/11-14 was obtained from Van Yuzuncu Yil University Animal Experiments Local Ethics Committee. This study was supported by Yuzuncu Yil University Scientific Research Projects Coordination Unit with the project number TYL-2022-10179. In this study, 18 healthy male albino rats with an average weight of 250-300 grams, obtained from Van Yuzuncu Yil University Experimental Medicine Application and Research Center. Animals were housed in individual cages that did not allow contact with each other, under standard conditions (22±1 ºC, 12-hour light/dark cycle) with unlimited water consumption and daily feed supply.

The study was conducted with 18 rats distributed in a control burn group, a burn group and a burn+treatment group, 6 animals in each group. In the control burn rats the damaged tissue was removed immediately after burning without any treatment. After the burn tissue boundaries were determined, it was extirpated with the help of a scalpel and forceps. On the first day of the study, animals in all groups were anesthetized intraperitoneally with a combination of 50 mg/kg ketamine and 15 mg/kg xylazine. The back region was preferred as the area where the burn wound would be created. Thus, the animal was prevented from scratching and licking the burn area (Kahkeshani

et al. 2013). The rats were placed in the position lying on their abdominal regions. The hair on the back areas was removed with the help of a razor as much as the area to be burned. The water was heated to 100°C.

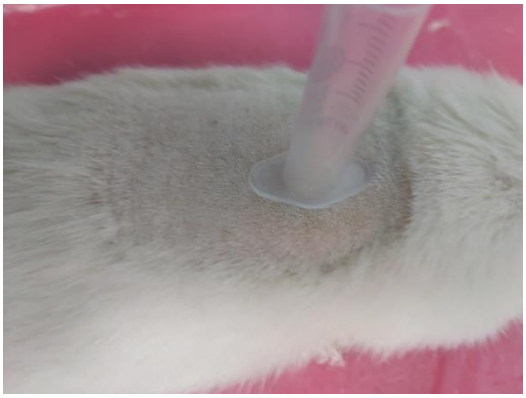

Figure 1. Stage of formation of burn wounds

From the first day of the study, 5mg/kg carprofen was administered subcutaneously to the burn+treatment and burn groups for analgesia for 7 days. Since the effect duration is 24 hours, the application was made every morning at 08.00. Rats in the burn+treatment group were given 1 ml of *Hypericum perforatum* L. oil by gavage through a tube placed in the esophagus every morning at 08:05 for 21 days.

The control group was sacrificed after the burn wound was created. For the sacrifice process, the rats were placed under general anesthesia. General anesthesia was achieved by intraperitoneal administration of 50 mg/kg ketalar and 15 mg/kg xylazine. After anesthesia rats were perfused. After perfusion, the rats were placed in 10% buffered formaldehyde and fixed. One week later, the skin areas where the burn wound was created were dissected. Tissue processing was applied to the dissected tissues from back region. Then, the tissues were blocked with paraffin. Sections were taken from the obtained blocks serially and parallelly, with a

Two independent areas with a diameter of 0.4 cm on the back of the rats were contacted with 100°C water during 20 seconds (Figure 1).

thickness of 5 µm, using sequential random sampling and the 1/75 stepping method. An average of 8-10 sections were obtained for each animal. The sections were stained with hematoxylin-eosin dye (Figure 2) The sections were examined by Olympus U-TV0, 5XC-3 (Tokyo, Japan). The resulting preparations were photographed under a microscope with a x4 objective The volume values of the burn wound areas were calculated using the Shtereom I program. This software uses stereological methods in a computer environment ensures its implementation. The program simultaneously records video of the microscope image. It is sent to the computer with the help of a camera and allows measurements to be made on it. Calculations can also be made on photographs transferred to the computer environment. Cavalieri's Principle was adhered to in the calculations by this program. After 21 days, the same procedures were repeated in the burn and burn+treatment groups. In order to strengthen the visual side of the work, shots with x10 and x40 objective were also obtained and included in the study.

Statistical Analysis

Four separate groups were created: "control and burn group", "control and burn+treatment group", "burn and burn+treatment group", "control, burn and burn+treatment group", and the Npar test was performed using Kruskal-Wallis and Mann-Whitney U test was applied. Statistical analyzes were completed in the study using SPSS version 24.0. The values were given in Table 1 , Table 2, Table 3, Table 4, Table 5, Table 6, Table 7.

Table 1. Non-parametric test results of burn, and burn+treatment groups.

Descriptive Statistics					
	Group Size	**Average**	**Standard Deviation**	**Minimum**	**Maximum**
Burn+Treatment	12	6328170833.500	2772592260.44741	3989541667.00	12805375000.00
Group	12	1.60	.516	1	2

Table 2. Mann-Whitney U test results of burn, and burn+treatment groups.

Rank				
	Group	**Group Size**	**Average Rank**	**Ranking Total**

Burn+Treatment	**Burn**	6	8.50	34.00
	Treatment	6	3.50	21.00
	Total	12		

Test Statistics	
	Burn+Treatment
Mann-Whitney U	.000
Wilcoxon W	21.000
Z-Score	-2.558
Approximate Significance (Bid-End)	.011

a. Grouping Variable: Groups1, b. Uncorrected for equations

Table 3. Non-parametric test results of control, and burn groups.

Descriptive Statistics					
	Group Size	**Average**	**Standard Deviation**	**Minimum**	**Maximum**
Control-Burn	12	7500750000.100	2044602671.469	5991916667.00	12805375000.00
Groups	12	1.40	.516	1	2

Table 4. Mann-Whitney U test results of control, and burn groups.

Rank				
	Groups	**Group Size**	**Average Rank**	**Ranking Total**
Control-Burn	**Control**	6	4.50	27.00
	Burn	6	7.00	28.00
	Total	12		

Test Statistics	
	Control-Burn
Mann-Whitney U	6.000
Wilcoxon W	27.000

Z-Score	-1.279
Approximate Significance (Bid-End)	.201
Definitive Analysis [2*(1-Undirected Analysis)]	.257b

a. Grouping Variable: Groups1, b. Uncorrected for equations

Table 5. Mann-Whitney U test results of control, and burn+treatment groups.

Rank				
	Groups 1	**Group Size**	**Average Size**	**Rank Total**
Control-Treatment	**Control**	6	9.50	57.00
	Treatment	6	3.50	21.00
	Total	12		

Test Statistics	
	Control-Treatment
Mann-Whitney U	.000
Wilcoxon W	21.000
Z-Score	-2.882
Approximate Significance (Bid-End)	.004
Definitive Analysis [2*(1-Undirected Analysis)]	.002b

a. Grouping Variable: Groups1, b. Uncorrected for equations

Table 6. Non-parametric test results of control, burn, and burn+treatment groups.

Descriptive Statistics					
	Group Size	**Average**	**Standart Deviation**	**Minimum**	**Maximum**
Anova	18	6424989583.4375	2163080871.10712	3989541667.00	12805375000.00
Groups 3	18	2.00	.894	1	3

Table 7. Kruskal-Wallis test results of control, burn, and burn+treatment groups.

Rank			
	Groups 3	**Group Size**	**Average Rank**
Anova	**Control**	6	10.50
	Burn	6	13.00
	Treatment	6	3.50
	Total	18	

Test Statistics A B	
	Anova
Chi-Square	11.250
Degrees of Freedom	2
Asymptotic Significance	.004
Asymptotıc Sıgnıficance	

A. Kruskal Wallis Test, B. Grouping Variable: Groups 3

RESULTS

Regional total volume values on the sections of control, burn, burn+treatment groups were obtained in cm^3 using stereological measurement methods. The values were given in Table 8.

According to Table 8 the burn area volume values in the control group were examined, it was observed that the highest volume value belonged to R1 with 0.3520 cm^3, while the lowest volume value was determined to be in R3 with 0.3050 cm^3. The mean burn area volume value in the control group was determined as 0.3168 cm^3. Additionally, according to Table 8 the highest CE value was found to be 0.041 in R3 and R4. The lowest CE value was observed at R1 as 0.038. The mean CE value of the control group was calculated to be 0.040. When the point numbers of the control group were examined, it was determined that the highest value was 704 and belonged to R1. It was determined that the lowest number of points belonged to R3 and R4 with 610. The mean number of points was calculated to be 633 (Table 8).

According to Table 8, it was observed that the highest volume value belonged to R1 with a value of 0.0644 cm^3, while the lowest volume value was determined in R4 with a value of 0.0358 cm^3. In addition, the average volume value of the burned area in the burn group was determined as 0.0503 cm^3. Considering Table 9 in terms of CE values, it was determined that the highest CE value belonged to R4 with 0.041, while the lowest CE value belonged to R1 with 0.034. The average CE value was found to be 0.036. When evaluated in terms of point number values, it was seen that the highest point number value of the burn area was 1052 and was detected in R1. It was also stated that the lowest point number value was 586 and was calculated in R4 (Table 8). The average number of points was found to be 822. When the calculations in Table 8 were evaluated in general, it was determined that the highest volume value belonged to R1, while the highest point number value was also calculated in R1. In addition to these values, the lowest CE value was also detected in R1. The fact that the volume and number of points values in R1 are parallel to each other and the CE value is low indicate a positive situation in terms of the reliability of the study. Because the number of points placed on the tissue whose volume will be determined and the volume value should be in an inverse proportional relationship with the CE value. In addition, when the CE and dot number values are examined in terms of

other rats, it is seen that among these three values, the dot number and volume values increase and the CE values decrease as these values increase, or vice versa. In other words, when the number of points and volume values decrease individually, the CE value also increases.

According to the values in Table 8, it was observed that the highest volume value belonged to R4 with 0.0317 cm^3, while the highest dot number value was determined to be 519 in R4. The average volume value was determined as 0.0272 cm^3. It was noted that the average CE value was 0.048 and the average number of points was calculated as 446. The highest CE value was detected in R2 as 0.056. In addition, the lowest volume value was determined to belong to R2 with 0.0214 cm^3, and in parallel with this, the lowest number of points was calculated in R2 with a figure of 351. It was determined that the lowest CE value belonged to R4 and R6, with 0.044. What is striking in Table 8 is that the volume and number of points values individually increased and decreased in parallel with each other. At the same time, it was determined that CE values decreased or increased inversely proportional to this increase or decrease. In Table 8, the average volume value was determined as 0.0272 cm^3. It was noted that the average CE value was 0.048 and the number of points was calculated as 446.

When the control, burn+treatment and burn groups were evaluated in terms of the average volume values of the dermis burn area, it was determined that the average volume value in the burn+treatment group decreased by approximately 45.92% compared to the burn group. This value was found by taking the difference between the average volume value of the burn group and the average volume value of the burn+treatment group and calculating that the percentage of the burn group was equal to this difference.

The coefficient of error (EC), obtained as a result of dividing the standard deviation to the arithmetic mean, was calculated separately for the burn area volume values of the control, burn and burn+treatment groups (Table 9). The results showed that this value was below 5%. This shows that the study is accurate both in terms of determining the number of animals and the number of sections to be taken, and also that it is reliable (Unal et al. 2002).

Table 8. Volume, CE and point number values of the burned skin area in the control, burn, burn+treatment groups

Burn Area Volumes (cm^3)/CE/Noise			
	VOLUME	**CE**	**NOISE**
	Control/Burn/Burn+treatment	**Control/Burn/Burn+treatment**	**Control/Burn/Burn+treatment**
R1	0.3520/0.0644/0.0292	0.038/0.034/0.047	704/1052/478
R2	0.3180/0.0466/0.0214	0.040/0.037/0.056	636/761/351
R3	0.3050/0.0477/0.0251	0.041/0.037/0.047	610/780/410
R4	0.3050/0.0358/0.0317	0.041/0.041/0.044	610/586/519
R5	0.3125/0.0576/0.0252	0.040/0.035/0.049	625/945/413
R6	0.3085/0.0497/0.0311	0.040/0.036/0.044	617/810/509
Average Value	0.3168/0.0503/0.0272	0.040/0.036/0.048	633/822/446

R: Rat, CE: Coefficient of Error, Noise: Number of points.

Table 9. Average values of coefficient of error (CE) of the volumes of the burn dermis regions of the groups.

Burn Area Average Values of Coefficient of Error		
Control	**Burn**	**Burn+Treatment**
0.040	0.036	0.048

DISCUSSION

Today, a wide variety of agents are used in wound treatment. Topical products widely used in burn wounds all over the world and in our country contain antimicrobial, antibiotic, and antiseptic substances.

Recently, the use of plant extract creams that increase epithelial formation and have antioxidant properties has been increasing rapidly (Unal 2006; Mehrabani et al. 2015; Afshar 2016).

In a study conducted by Soykan (2020), it was aimed to compare the changes in the wound area after creating a burn wound in rats and treating it with amniotic fluid, myrrh tree extract, and silver sulfadiazine, using pathological analysis and autocad. As a result of the data obtained, it was revealed that myrrh tree extract supports wound healing. Although there are similarities in this thesis study conducted by Soykan (2020), there are differences in terms of rat breed. In addition, in the study conducted by Soykan (2020), a total of four burn wounds were created in two different regions, while in this study, two separate burn wounds were created only in the back region for redundancy. Choosing the back area is to ensure that the animal can not reach that area and to prevent irritation. In this study, which was conducted to heal the burn wound, no application was made to heal the wound on its own in the group where the burn was created and left to heal on its own. Both studies are similar in terms of grouping in the study. However, the effects of different extracts were investigated in both studies used and the studies differ in this respect. In addition, in the study conducted by Soykan (2020), area calculation was made with the autocad program. However, in this thesis study, volume calculations were made, the results of which were closer to reality, and the Cavalieri's Principle was used by using the Shtereom I program. The part where the two studies overlap is that plants that are predicted to affect the healing process of burn wounds were used. According to the data obtained, the effect of myrrh tree on healing was calculated as 22%, while the effect of *Hypericum perforatum* L. oil on healing was measured as 45%. Kiyan et al. (2015) aimed to investigate the treatment of *Hypericum perforatum* L. (St. John's Wort) in thermal burns and compare it with silver sulfadiazine treatment. For this purpose, thirty-five Wistar-albino type rats were randomly selected and divided into five groups, with seven rats in each group. A second-degree thermal burn was created on the back of the rats by exposing them to 100 °C boiling water for 10 seconds in an area of 4x4 cm. All groups were irrigated with 50 cc saline solution for three minutes. No treatment was applied to the control group. Only irrigation was applied to the burn control group. Silver sulfadiazine was applied twice daily to the topical silver sulfadiazine group. *Hypericum perforatum* was applied to the topical *Hypericum perforatum* group four times a day, every six hours. Another topical substance used in the preparation of *Hypericum perforatum* was applied to the treatment group with ajan-gel, four times a day, every six hours. Tissues taken by biopsy were cut into 5µm-thick sections on a microtome, examined under a light microscope, and stained with hematoxylin-eosin in accordance with standard procedures. Biopsies taken from the groups were evaluated under a light microscope. Results were determined using the Verhofstad Histopathological Scoring system. The results obtained from this scoring were compared. It was then scored by taking the arithmetic average of the scores given. In addition, skin wound healing and epidermis thickness, inflammatory cell infiltration (neutrophils, monocytes, and macrophages), degenerative and total hair follicle number were also included in the histopathological evaluation. As a result, in the *Hypericum perforatum* treatment group, the collagen discoloration was localized in the lower part of the epidermal layer and did not progress to the depth of the dermis in the *Hypericum perforatum* treatment group compared to the other groups. It was determined that the group was protected according to the control group and was structurally closest to the control group. Epidermal thickness and number of vessels in the *Hypericum perforatum* group were found to be statistically significantly higher than the other groups ($p<0.05$). The number of degenerative hair follicles in the *Hypericum perforatum* group was found to be significantly less than the other groups ($p<0.05$). It was also determined that the total number of hair follicles increased significantly at the twenty-fourth hour ($p<0.05$). Kiyan et al. (2015) differs from our study in that *Hypericum perforatum* was applied topically and was compared with different substances. In this study, application was made using the gavage method. Instead, the volumetric effect of *Hypericum perforatum* oil on burn wound healing was examined. Kiyan et al. (2015) the experimental period is limited to 24 hours. In our study, the application period of both studies differs as the application was carried out for 21 days. Kiyan et al. (2015) is based on histological evaluations. However, this study is based on determining the healing rate of the tissue volumetrically using stereological methods.

In a study conducted by Çelikkol (2015), the extract obtained by soaking the dried flowers and leaves of the plant *Hypericum perforatum* L. in olive oil caused experimental wounds in rats. *Hypericum perforatum* plant, was applied topically to the right one of these defects. Any application was made to the defects on the left. The groups were sacrificed on the fourth, seventh, fourteenth, and twenty-first days. At the end of the experiment, the effect of St. John's wort oil on wound healing was determined. According to the results of histopathological findings and immunohistochemical evaluations although a significant increase was observed in parameters affecting wound healing such as Fibroblast Growth Factor (FBF), Vascular

Endothelial Growth Factor (VEBF), and Epidermal Growth Factor (EBF), no significant difference was found in the degree of epithelialization, polymorphonuclear leucocytes (PMNLs) infiltration and the amount of macrophages. The effect of St. John's wort oil on the healing of the wound surface area was found to be significant on the fourth and seventh days in soft tissue defects compared to the non-applied groups. The study conducted by Çelikkol (2015) is similar to our study in terms of using animals of the same breed. Additionally, in the study conducted by Çelikkol (2015), the last time the study was terminated was the same as the last time the study was terminated. However, Çelikkol (2015) also determined groups to terminate the study on the 4th, 7th, and 14th days. However, this study was terminated on the 21st day. Additionally, both studies differ in terms of the way the extract is applied. Çelikkol (2015) found topical application of the extract appropriate in his study. In fact, this situation distances the study from systemic side effects. But it can prolong the duration of effect. In this study, the gavage method was used to achieve the most effective results in the shortest time. Both studies differ in this respect. In the aforementioned study, histopathological and immunohistochemical results were obtained. However, in this study, the results were evaluated through stereology and it was aimed to obtain realistic results. The fact that macrophage counting was performed makes Çelikkol's (2015) study valuable. Cell count was not preferred in this study, and it was thought that cell count could be evaluated by including it in the study in future studies.

The purpose of the study on burns by Kotsiou and Tesseromatis (2020) was to investigate whether *Hypericum perforatum* plant extract is effective in the treatment of burn wounds. In the study, 32 male Wistar rats were used and these rats were divided into 4 different groups. While burn wounds in the first group were treated with *Hypericum perforatum* plant extract, animals in the second group were treated with nitrofurazone cream. In the third group, only a burn wound was created and no treatment was applied. In the fourth group, a burn wound was created and treated with placebo cream. In histological evaluation, staining was done with haematoxylin and eosin. It was found that the collagen coloration of the *Hypericum perforatum* treatment group was localized in the lower part of the epidermal layer and did not go deeper into the dermis compared to other groups, and the epidermis, hair follicles and sebaceous glands were preserved. It was determined that the epidermal thickness and number of vessels in the group applied Nitrofurazone Ointment USP (0.2%) every 12 hours and the *Hypericum perforatum* group were significantly higher than the other groups ($p<0.05$). The number of degenerated hair follicles in the *Hypericum perforatum* applied group was found to be significantly lower than the other groups ($p<0.05$). It was determined that the total number of hair follicles increased significantly compared to the control group ($p<0.05$) and this number did not differ ($p>0.05$). The results showed that *Hypericum perforatum* plant extract had similar effects to nitrofurazone cream in healing burn wounds. The burn wounds of rats treated with both *Hypericum perforatum* plant extract and nitrofurazone cream healed faster than those in the placebo group. There was no statistical difference in wound healing times between groups treated with *Hypericum perforatum* plant extract and nitrofurazone cream. Both studies are similar in that the study is related to animal selection, material used, and wound healing. The use of *Hypericum perforatum* L. oil makes both studies similar. However, it differs in that Hypericin cream is applied four times a day. Additionally, in this study, the last time the study was terminated differs from the time our study was completed. In this study, topical application of the extract and application of Hypericin as a cream were found to be appropriate. In this study, the gavage method was used to achieve the most effective results in the shortest time. Both studies differ in this respect. Kotsiou and Tesseromatis (2020) reached histopathological and immunohistochemical results in their study. However, in this study, the results were evaluated through stereology and it was aimed to obtain realistic results. The prediction of metalloproteinases and the examination of plasma MMP-8 and -9 make the study of Kotsiou and Tesseromatis (2020) valuable. ELISA was not preferred in this study, which led to the idea that this method could also be taken into consideration in future studies.

In a study conducted by Seyhan (2020); rats were divided into 3 equal groups of 8 animals each. These are Group A as the control group, Group B as the curcumin treatment group, and Group C as the *Hypericum perforatum* application group. A second-degree deep burn wound was created using a 2x2 cm diameter iron heating plate heated in boiling water for 5 minutes and placed on the skin by applying pressure for 20 seconds. Treatment was started 24 hours after the burn injury. Curcumin oil (2 cc) was applied to Group B and *Hypericum perforatum* oil was applied topically to Group C once a day for 20 days. Group A was considered as the control group and no drug treatment was applied. At the end of the experiment (day 21), all rats were sacrificed with an overdose of anesthetic and the burnt surface areas were removed for histopathological examinations. Tissues were embedded in paraffin wax and sections were cut at 5 µm thickness and stained with hematoxylin and eosin. At the end of the study, histological parameters, epithelialization, granulation tissue formation, inflammation and angiogenesis were evaluated in the

biopsy samples taken from the wound. Histological scores were made from 20 random fields per section from each sample at ×40 magnification. In Seyhan's (2020) study, second degree burn wounds were created with hot metal plates. In this study, boiled water at 100 ºC was preferred to create a second degree burn wound. There are method differences in both studies in terms of creating a burn wound. In addition, in the study conducted by Seyhan (2020), the last time the study was terminated is the same as the last time the study was terminated. The two studies are not similar in terms of the way the extract is applied. Seyhan (2020) found topical application of the extract appropriate in his study. In fact, this distances the study from systemic side effects. But it can prolong the duration of effect. In this study, the gavage method was used to achieve the most effective results in the shortest time. Both studies differ in this respect. In the aforementioned study, histological results were obtained. However, in this study, the results were evaluated through stereology and it was aimed to obtain realistic results.

CONCLUSION

Volumetric data have been obtained showing that the animals in which *Hypericum perforatum* oil was used supported faster recovery than the animals in which it was not used. As a result of this study, it was determined that there was a 45.92% difference in volume value between the treated group and the group without any treatment. We are of the opinion that this study may also lead to other studies investigating different histopathological parameters such as inflammatory cell infiltration (neutrophils, monocytes, and macrophages), degenerative and total hair follicle number, to be carried out at different durations and different burn degrees, and can be considered as a reference study.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: GÇ and AŞC contributed to the project idea, design and execution of the study. GÇ and AŞC contributed to the acquisition of data. GÇ analysed the data. GÇ and AŞC drafted and wrote the manuscript. GÇ reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical approval: This study was carried out at Van Yuzuncu Yil University Research Animals Application Center. This research was approved by The Ethics Committee of the Faculty of Veterinary Medicine, Van Yuzuncu Yil University (YUHADYEK, Ref No: 2021/11-14, Date: 25.11.2021).

Acknowledgement: In this study we would like to thank the Van Yuzuncu Yil University Scientific Research Projects Coordination Unit with the study entitled "Stereological Investigation of the Effects of *Hypericum Perforatum* L. Plant on Skin Tissue in Second Degree Burns in Rats", Project No: TYL-2022-10179, for their contributions to the present study and also thank the adminisration and staff of Van Yuzuncu Yil University Research Animals Application Center.

Explanation: This study was summarized from master's thesis of entitled "Stereological Investigation of the Effects of *Hypericum Perforatum* L. Plant on Skin Tissue in Second Degree Burns in Rats".

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