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Use of Systemic *Rosmarinus Officinalis* to Enhance the Survival of Random-Pattern Skin Flaps

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Background: Skin flaps are commonly used in soft-tissue reconstruction; however, necrosis can be a frequent complication. Several systemic and local agents have been used in attempts to improve skin flap survival, but none that can prevent flap necrosis have been identified.

Aims: This study aims to determine whether the use of systemic *Rosmarinus officinalis (R. officinalis)* extract can prevent flap necrosis and improve skin flap recovery.

Study Design: Animal experimentation.

Methods: Thirty-five Wistar albino rats were divided in five groups. A rectangular random-pattern flaps measuring 8×2 cm was elevated from the back of each rat. Group I was the control group. In Group II, 0.2 ml of *R. officinalis* oil was given orally 2h before surgery. *R. officinalis* oil was then applied orally twice a day for a week. In Group III, *R. officinalis* oil was given orally twice a day for one week before surgery. At the end of the week, 0.2 mL of R. officinalis oil was given orally 2 h before surgery. In Group IV, 0.2 mL of *R. officinalis* oil was injected subcutaneously 2 h before surgery. After the surgery, 0.2 mL *R. officinalis* oil was injected subcutaneously twice a day for one week. In Group V, 0.2 mL *R. officinalis* oil was injected subcutaneously twice a day for one week prior to surgery. At the end of the week, one last 0.2 mL *R. officinalis* oil injection was administered subcutaneously 2 h before surgery. After the surgery, 0.2 mL *R. officinalis* oil was injected subcutaneously twice a day for one week.

Results: The mean percentage of viable surface area was significantly greater ($p<0.05$) in Groups II, III, IV, and V as compared to Group I. Mean vessel diameter was significantly greater ($p<0.05$) in Groups II, III, IV, and V as compared to Group I.

Conclusion: We have determined that, in addition to its anti-inflammatory and anti-oxidant effects, R. officinalis has vasodilatory effects that contribute to increased skin flap survival.

Keywords: *Rosmarinus officinalis*, flap survivability, tissue defect, rosemary

Skin flaps are commonly used in soft tissue reconstruction; however, necrosis can be a frequent complication. Several systemic and local agents have been used to improve skin flap survival, but none that can prevent flap necrosis has been identified (1-4).

Rosmarinus officinalis (R. officinalis) has been used in the prevention of flap necrosis (5); it is known to exert antimicrobial (6-10), antioxidant (11-18), anti-inflammatory (19,20), antifungal (6,9,10), antidiabetic (15), anticarcinogenic (13,19), antiplatelet (21), antimetastatic (13,19), antiproliferative (16) and antimutagenic (22) effects.

A previous study investigating the effects of *Rosmarinus officinalis* on the survival of skin flaps reported that the average ratio of viable surface area to total flap area was 28%

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in control groups; this value increased to 59% in groups that were treated with *R. officinalis* extract (5). The viable surface area was further increased to 68% by topical application of the oil for a week before flap elevation. The authors claimed that in addition to the anti-inflammatory and antioxidant effects, *R. officinalis* had vasodilatory effects that contributed to the increased flap survival.

To further investigate the effects of *R. officinalis* on flap survival, this study investigated whether the systemic administration of *R. officinalis* extract could prevent flap necrosis and improve skin flap recovery.

MATERIALS AND METHODS

Female Wistar albino rats, 12-14 weeks old and weighing 243-310 g, were used and placed in five groups, each containing seven rats. The experiments were performed in accordance with the guidelines for animal research from the National Institutes of Health and the Committee on Animal Research. The study was approved by Necmettin Erbakan University Meram School of Medicine Ethics Committee. The modified McFarlane rat model was used (23,24). In all groups, the backs of the rats were shaved. For anesthesia, 50 mg/kg of ketamine hydrochloride (Ketalar vial, Pfizer; İstanbul, Turkey) and 5 mg/kg of xylazine hydrochloride (Rompun vial, Bayer; Leverkusen, Germany) were intraperitoneally administered before surgery. No dressing was applied after surgery.

Group I was the control group. A rectangular random-pattern flaps measuring 8×2 cm and including the panniculus carnosus was elevated from the back of each rat. Electrocautery or hemostatic agents were not used to control bleeding. The flaps were then sutured to the location from where they were elevated, and 0.2 mL of physiological serum was orally administered twice a day for a week.

In Group II, 0.2 mL of *R. officinalis* oil (Doğa İlaç; İstanbul, Turkey) was orally administered 2 h before surgery. Subsequently, flaps were elevated and replaced as described for Group I. *R. officinalis* oil was then orally applied twice a day for a week.

In Group III, *R. officinalis* oil was orally administered twice a day for a week before surgery. At the end of the week, 0.2 mL of *R. officinalis* oil was orally given 2 h before surgery. Skin flaps were elevated and replaced as described for Group I. *R. officinalis* oil was orally applied twice a day for a week.

In Group IV, 0.2 mL of *R. officinalis* oil was subcutaneously injected 2 h before surgery. Skin flaps were elevated and replaced as described for Group I. After the surgery, 0.2 mL of *R. officinalis* oil was subcutaneously injected twice a day for a week.

FIG. 1. The flaps in Group I a week after surgery

TABLE 1. Viable and necrotic regions of the flaps and vessel diameters in Group I.

Rat	Viable areas of the flaps (cm^2)	Necrosis areas of the flaps (cm^2)	Viable areas of the flaps (%)	Necrosis areas of the flaps (%)	Vessel diameters (μm)
1	4.26	10.74	26.62	73.38	190
2	4.22	10.78	26.37	73.63	180
3	4.24	10.76	26.50	73.50	185
4	4.23	10.77	26.43	73.57	185
5	4.27	10.73	26.68	73.32	191
6	4.25	10.75	26.56	73.44	185
7	4.21	10.79	26.31	73.69	179
Mean	4.24	11.76	26.56	73.34	185

In Group V, 0.2 mL of *R. officinalis* oil was subcutaneously injected twice a day for a week prior to surgery. At the end of the week, 0.2 mL of *R. officinalis* oil was subcutaneously injected 2 h before surgery. Skin flaps were elevated and replaced as described for Group I. After the surgery, 0.2 mL of *R. officinalis* oil was subcutaneously injected twice a day for a week.

The flaps were evaluated a week after elevation. All images were acquired using the same digital camera, and the viable and necrotic regions were outlined on a transparent paper. The surface area of the viable and necrotic regions was then calculated. To eliminate false-positive results caused by necrosis-induced contraction, only the viable surface area was measured (cm^2) on the transparent papers using a digital planimeter (Ushikata X-plan 360C+, Yokohama; Kanagawa, Japan). The surface area of the necrotic region was measured by subtracting the viable surface area from the total flap area (16 cm^2). A biopsy specimen was taken from the viable area of the flap, and specimens were examined under light microscope (Olympus BX 51; Tokyo, Japan). In each group, the diameter of the largest blood vessel in the proximal flap was measured.

FIG. 2. The flaps in Group II a week after surgery

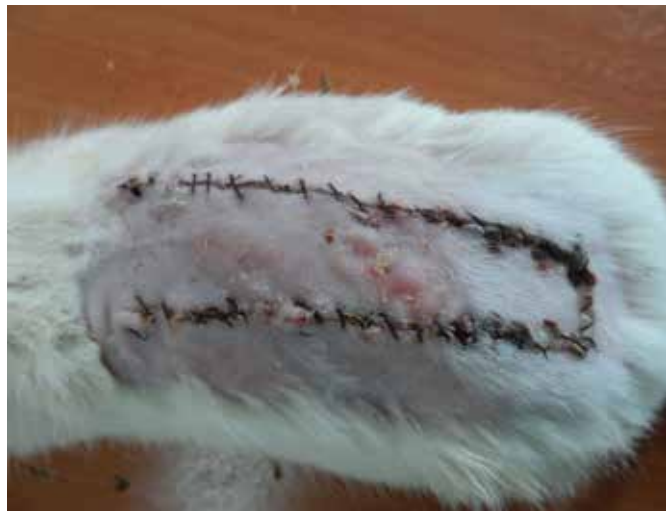

FIG. 3. The flaps in Group III a week after surgery

TABLE 2. Viable and necrotic areas of the flaps in Group II.

Rat	Viable areas of the flaps (cm^2)	Necrosis areas of the flaps (cm^2)	Viable areas of the flaps (%)	Necrosis areas of the flaps (%)	Vessel diameters (μm)
1	5.76	10.24	36.00	64.00	185
2	9.37	6.63	58.57	41.13	200
3	9.24	6.76	57.75	42.25	200
4	12.40	3.60	77.50	22.50	220
5	8.76	7.24	54.75	45.25	195
6	8.84	7.16	55.25	44.75	195
7	10.36	5.64	64.75	35.25	205
Mean	9.09	6.94	56.63	43.37	200

TABLE 3. Viable and necrotic areas of the flaps in Group III.

Rat	Viable areas of the flaps (cm^2)	Necrosis areas of the flaps (cm^2)	Viable areas of the flaps (%)	Necrosis areas of the flaps (%)	Vessel diameters (μm)
1	15.68	4.32	98	2	260
2	15.80	0.20	98.75	1.25	265
3	15.77	0.23	98.56	1.44	255
4	15.83	0.17	98.93	1.07	265
5	13.90	2.10	86.87	13.13	225
6	15.88	0.12	99.25	0.75	270
7	14.70	1.30	91.87	8.13	230
Mean	15.38	0.62	96.12	3.88	252.8

Statistical analysis

Mean values were analyzed using Statistical Package for the Social Sciences version 13.0 for Window (SPSS Corp.; Chicago, IL, USA) statistical software. One-way analysis of variance and Tukey's post hoc test were used to compare the viable surface area and vessel diameter among the groups. P values of <0.05 were considered to be statistically significant.

RESULTS

No rats died during the experiments. No instance of infection was observed.

Measurements for Group I:

A week after flap elevation, the mean ratio of viable surface area to total flap area was 26.56%±0.323% (Figure 1). The mean diameter of the largest vessels in the proximal flap was 185±6 μm (Table 1).

Measurements for Group II:

A week after flap elevation, the mean ratio of viable surface area to total flap area was 56.63%±1.298% (Figure 2). The mean diameter of the largest vessels in the proximal flap was 200±20 μm (Table 2).

Measurements for Group III:

A week after flap elevation, the mean ratio of viable surface area to total flap area was 96.12%±2.102% (Figure 3). The mean diameter of the largest vessels in the proximal flap was 252±18 μm (Table 3).

Measurements for Group IV:

A week after flap elevation, the mean ratio of viable surface area to total flap area was 56.38%±1.299% (Figure 4). The mean diameter of the largest vessels in the proximal flap was 198±18 μm (Table 4).

Measurements for Group V:

A week after flap elevation, the mean ratio of viable surface area to total flap area was 57.06%±1.027% (Figure 5). The mean diameter of the largest vessels in the proximal flap was 202±18 μm (Table 5).

FIG. 4. The flaps in Group IV a week after surgery

FIG. 5. The flaps in Group V a week after surgery

TABLE 4. Viable and necrotic areas of the flaps in Group IV.

Rat	Viable areas of the flaps (cm^2)	Necrosis areas of the flaps (cm^2)	Viable areas of the flaps (%)	Necrosis areas of the flaps (%)	Vessel diameters (μm)
1	5.76	10.24	36.00	64.00	185
2	9.37	6.63	58.57	41.13	200
3	9.24	6.76	57.75	42.25	200
4	12.40	3.60	77.50	22.50	220
5	8.76	7.24	54.75	45.25	195
6	8.84	7.16	55.25	44.75	195
7	10.36	5.64	64.75	35.25	205
Mean	9.09	6.94	56.63	43.37	200

TABLE 5. Viable and necrotic areas of the flaps in Group V.

Rat	Viable areas of the flaps (cm^2)	Necrosis areas of the flaps (cm^2)	Viable areas of the flaps (%)	Necrosis areas of the flaps (%)	Vessel diameters (μm)
1	5.14	10.86	32.12	67.87	185
2	10.55	5.45	65.94	34.06	205
3	5.81	9.19	36.31	63.69	190
4	11.19	4.81	69.94	30.06	210
5	12.45	3.55	78.99	22.01	220
6	10.49	5.51	65.57	34.43	205
7	8.42	7.58	52.63	47.37	200
Mean	9.13	6.87	57.06	42.93	202.14

The mean percentage of viable surface area was significantly greater ($p<0.05$) in Groups II, III, IV, and V than in Group I, and it was significantly greater in Group III than in Groups II, IV, and V ($p<0.05$). The mean vessel diameter was significantly greater ($p<0.05$) in groups II, III, IV, and V than that in Group I, and it was significantly greater in Group III than in groups II, IV, and V ($p<0.05$) (Figure 6).

DISCUSSION

This study demonstrated that the viable surface area of *R. officinalis*-treated flaps was significantly greater in all treated groups as compared to control group. The oral administration of the oil for a week before surgery resulted in the greatest increase in flap survival (96%; Group III), and this group also showed the largest blood vessel diameters. We have therefore determined that in addition to its anti-inflammatory and antioxidant effects, *R. officinalis* has vasodilatory effects that contribute to increased skin flap survival.

Random-pattern skin flaps are widely used in plastic surgery. Although they are technically simple, flap necrosis can be seen as a complication. When flaps become necrotic, undesired complications such as additional surgical procedures, increased length of hospital stay and increased treatment costs are incurred. Thus, improving the survival of random-pattern skin flaps is an important goal in clinical practice (1-4).

There are several drugs that improve circulation in skin flaps. These may reduce the viscosity of the blood and increase the flexibility of erythrocytes. They may be axon blockers, alpha receptor blockers, or smooth muscle relaxants. Anti-inflammatory drugs and antioxidants have also been used to reduce the cellular effects of ischemia (25,26). The sequelae of these substances on flap viability rates vary between 68% and 83%. In our study, when *R. officinalis* was orally administered for a week before surgery, the flap survival rate increased to 96%. In comparison, the highest flap viability rate found in previous literature was 83% (25). In that study, the skin flap was 11×3 cm in size, and the average ratio of the viable surface area to the total flap area in the control group was 59%.

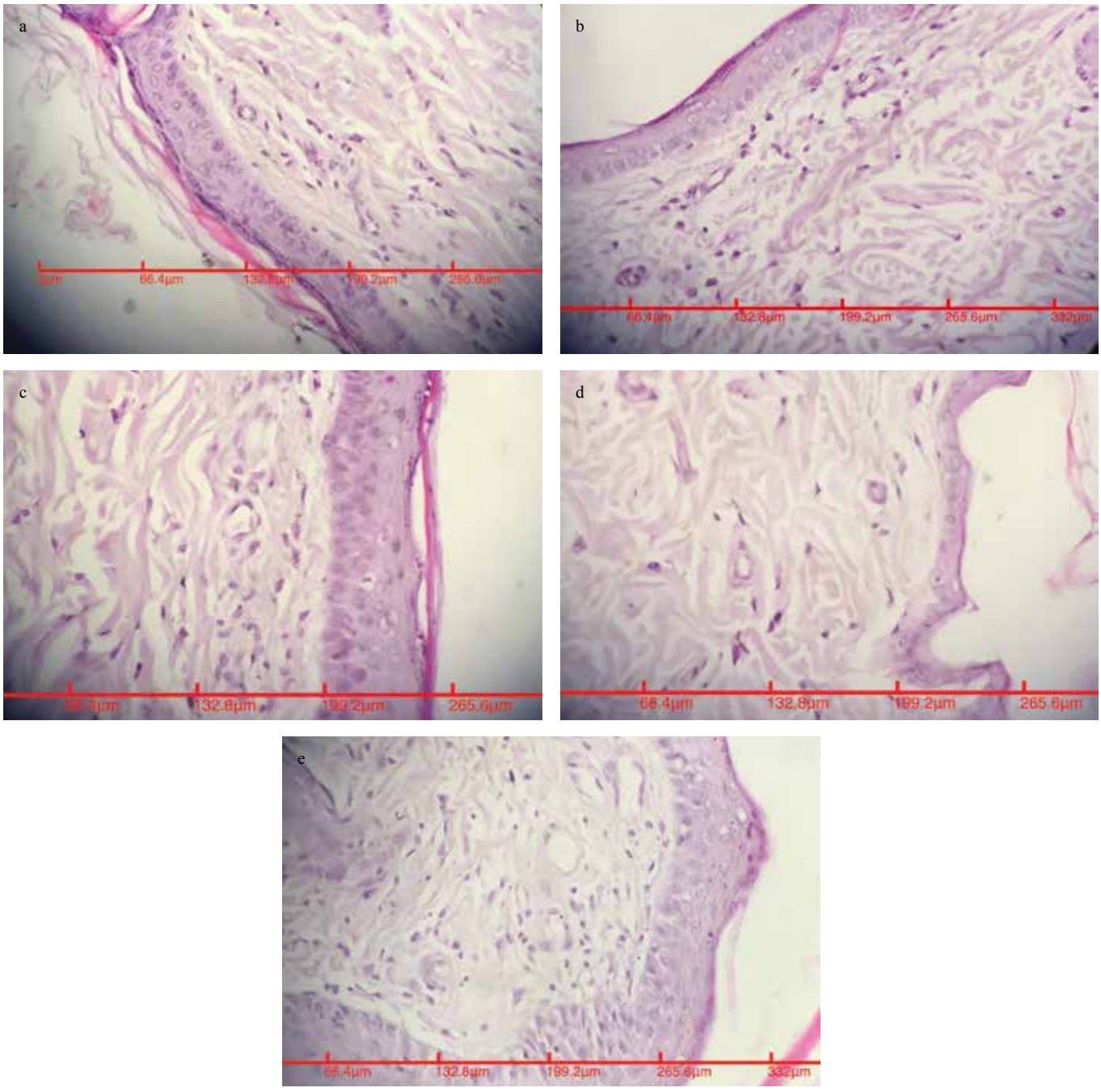

FIG. 6. a-e. x400, H&E; appearance of largest blood vessels in the proximal flap in Group I (a). x400, H&E; appearance of largest blood vessels in the proximal flap in Group II (b). x400, H&E; appearance of largest blood vessels in the proximal flap in Group III (c). x400, H&E; appearance of largest blood vessels in the proximal flap in Group IV (d). x400, H&E; appearance of largest blood vessels in the proximal flap in Group V (e)

The average ratio of viable surface area to total flap area was found to be highest in a study investigating the effects of the subdermal use of vascular endothelial growth factor (VEGF) on flap viability (27). The effect of VEGF on skin flap survival was studied in a rat transverse rectus abdominis musculocutaneous flap using a 3×8 cm skin paddle with the inferior epigastric vessels. VEGF was injected into the subcutaneous fascial layer in the area where the flap would be dissected, and the flap was then raised 7 days after injection. In this study, the average ratio of the viable surface area increased from 28% to 76% in the experimental group. This rate is lower than that in Group III, where a systemic dose of the *R. officinalis* oil for a week before surgery was administered.

While the average ratio of viable to necrotic surface area in control groups was 28% in the previous study on R. *officinalis*, this value was 27% in our study (5). Although our control group had lower success than previous studies, in our Group III, the viable surface area was greater. When both studies are considered together, we suggest that the bioavailability of *R. officinalis* is the greatest when orally administered.

In a previous study conducted to determine the safe dose rate of *R. officinalis*, ≤2 mg/kg was concluded to be safe for rats (28). With reference to this finding, we did not exceed this level in our study.

The proportions of the basic components of *R. officinalis* vary among genotypes. These components and their proportions are as follows: alpha-pinene (7.3-37.8%), kampen (0-12.1%), myrcene (0-6.6%), simolin (0-4.5%), limonene (0-7.2%), 1.8 cineol (13.6-67.3%), kamper (2.2-48.3%), and borneol (1.3-12.7%) (29). The rosemary oil used in the present study was obtained from rosemary plants grown in the Çukurova region (southern Anatolia). Although the genotype of this oil was not determined, it was believed to be the Kozan genotype, which is the most commonly found genotype in the region. The Kozan genotype contains alpha-pinene (13.5%), camphene (4.1%), mirsene (2.2%), simole (2.8%), limonene (2.4%), camphere (2.2%), bornele (5.3%), and 1.8 cineol (67.3%) and may be termed as the 1.8 cineol genotype because it contains the highest content of 1.8 cineol among other genotypes (29).

A previous study identified hesperetin, acacetin, diosmetin, ferulic acid, apigenin, luteolin, rosmarinic acid and caffeic acid as the active components in *R. officinalis* via capillary electrophoresis (30). *R. officinalis* oil was used as an oral and subcutaneous agent in our study. Although both applications of the oil increased skin flap survival, we did not determine the specific component responsible for the improvement. Further studies are required to clarify these effects.

The primary measure involved in preventing flap necrosis is increased blood circulation to the flap. Following our findings that *R. officinalis* significantly decreases the rate of flap necrosis, we suggest that this treatment is useful in patients with circulatory system problems and as an alternative therapy to increase blood circulation in flaps.

Furthermore, the antioxidant, antiproliferative, anti-inflammatory, and vasodilatory effects of *R. officinalis* may be useful in patients with chronic obliterative artery disease because of the compound's ability to increase blood flow and to serve as a prophylactic and therapeutic agent for diabetes-induced ulcers because of its antidiabetic, antimicrobial, and vasodilatory effects. This study demonstrated that *R. officinalis* can help prevent flap necrosis; however, further clinical studies are required.

Ethics Committee Approval: Ethics committee for this study was received from the ethics committee of Necmettin Erbakan University Meram School of Medicine.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - B.İ.; Design - B.İ.; Supervision - B.İ., M.D.; Resource - B.İ., F.B.; Materials - B.İ., F.B., S.K.; Data Collection and/or Processing - F.B., S.K.; Analysis and/or Interpretation - B.İ., F.B., A.Ö.G.; Literature Search - B.İ., F.B.; Writing - B.İ., F.B.; Critical Reviews - B.İ., A.Ö.G., M.D.

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