

The effect of chrysin on ischemia-reperfusion injury in the rat epigastric artery skin island flap

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ABSTRACT

BACKGROUND: The return of oxygenated blood by reperfusion after ischemia triggers paradoxical tissue damage known as ischemia-reperfusion (I/R) injury. This study aims to investigate the effect of chrysin (C) on I/R injury in epigastric island flaps in rats.

METHODS: Thirty male Wistar albino rats were randomly divided into five groups (n=6): the sham control group (Group I), the flap I/R-untreated group (Group II), the I/R + C 10 mg/kg/day group (Group III), the I/R + C 50 mg/kg/day group (Group IV), and the I/R + C 100 mg/kg/day (Group V). Chrysin was administered orally for seven days before and after surgery. Flap elevation was performed on the eighth day, followed by eight hours of induced ischemia. Flap survival rate, as well as biochemical and histopathological parameters, were evaluated.

RESULTS: Oral administration of chrysin significantly reduced fibroblast activity at all treatment doses in rats subjected to I/R injury. Although improvements were observed in flap survival rate, oxidative stress index (OSI), and vascular proliferation at 10 mg/kg/day and 50 mg/kg/day doses, as well as in total oxidant status (TOS), tumor necrosis factor-alpha (TNF- α), active inflammation, chronic inflammation and ulceration across all treatment doses, these changes did not reach statistical significance. Total antioxidant capacity (TAC) values, consistent with existing literature, did not appear to influence the positive outcomes.

CONCLUSION: This study examined the effects of chrysin treatment on ischemia-reperfusion injury using a rat inferior epigastric artery skin island flap model. Although favorable molecular changes were observed, these did not translate into significant improvements in clinical outcomes.

Keywords: Chrysin; reperfusion injury; ischemia.

INTRODUCTION

Paradoxically, the restoration of blood flow following ischemia can worsen tissue damage, a phenomenon known as ischemia-reperfusion (I/R) injury.^[1] The harmful effects of I/R injury are encountered in clinical scenarios such as crush injuries, circumferential burns, and traumatic amputations, as well as in surgical procedures including fasciotomy, revascularization, replantation, free tissue transfer, and face or limb transplantation.^[2,3]

The pathophysiology of I/R injury involves reactive oxygen species (ROS), polymorphonuclear leukocytes (PMNL), the complement system, and endothelial cells. I/R injury remains one of the most significant causes of total and, particularly partial, flap loss, despite recent advancements in flap surgery. Maintaining a balance between oxidants and antioxidants is crucial for preventing such loss.^[3,4]

Flavonoids are specialized metabolites found in many plants that benefit human health due to their biological properties.^[5] Chrysin (5,7-dihydroxyflavone) is a flavone naturally present in

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honey, propolis, and blue passionflower. It exhibits antioxidant activity by scavenging ROS,^[6] has vasodilatory effects,^[7,8] and demonstrates anti-inflammatory properties.^[9,10] Studies have shown that chrysin prevents I/R injury in various tissues, including the heart,^[11,12] brain,^[9,13-18] liver,^[19] kidney,^[19,20] gastric mucosa,^[21] ovary,^[22] and testis.^[23]

However, no studies have examined the role of chrysin in I/R injury in skin flaps. In this study, we investigated the effect of chrysin on I/R injury in inferior epigastric artery skin island flaps in rats.

MATERIALS AND METHODS

Ethical Approval

This experimental study was approved by the Health Sciences University Local Ethics Committee for Animal Experiments at our university (Protocol number: 2022-27). All surgical procedures were conducted at the same center in accordance with the guidelines for the care of laboratory animals published by the United States National Institutes of Health.

Animal Model

Thirty male Wistar albino rats, weighing between 250 and 550 grams and aged 6-12 months, were housed individually in standard rat cages at a controlled temperature of 21-24°C with 50% humidity maintained throughout the day. All rats had ad libitum access to food and water, and a 12-hour automatic light/dark cycle was maintained.

Sample Size Estimation

Sample size calculation for one-way Analysis of Variance (ANOVA) was performed using the G*Power Version 3.1.9.4 software. With the α error set at 0.05 and the β error at 80%, the effect size coefficient (Cohen's *f*) was considered large (0.70), resulting in a total sample size of 30. The number of animals per group was determined based on previously conducted studies.^[1,21,24]

Experimental Protocol

The rats were randomly divided into five groups, with six rats in each group:

- Group I (Sham Control): The rats were fed ad libitum. Their flaps were raised but not subjected to ischemia. The duration of anesthesia for the sham group was identical to that of the other groups, in which eight hours of ischemia were applied.
- Group II (Vehicle Control): The rats received 1 mL of a control agent (distilled water and propylene glycol) via oral gavage once daily, in addition to ad libitum feeding, for seven days preoperatively and seven days postoperatively. On the eighth day of the experiment, the flaps were raised, and ischemia was induced for eight hours by placing microclamps on the inferior epigastric artery and vein.



Figure 1. Treatment of rats via oral gavage.

- Groups III, IV, and V (Experimental Groups): These groups received 10 mg/kg/day, 50 mg/kg/day, and 100 mg/kg/day of chrysin, respectively. The treatment agent was administered once daily at the appropriate dose by oral gavage in a volume of 1 mL for seven days preoperatively and seven days postoperatively. Ischemia was induced for eight hours using microclamps on the eighth day of the experiment.

Photographs of the flaps were taken for macroscopic measurement of flap survival rate, and two 15×15 mm tissue biopsies were collected from the distal part of the flap for biochemical and histopathological examinations.

Preparation and Application of Chrysin

Chrysin with >97% purity^[9,16,18,20] (Sigma-Aldrich, USA) was mixed into a solution prepared with propylene glycol (Tekkim, Türkiye) and distilled water at a ratio of 20:80 by volume using a magnetic stirrer. The suspension was freshly prepared in 8 mL vials, each containing 1 mL of the treatment agent at the desired dose. Before administration, the suspension was homogenized by shaking and delivered using 1 mL injectors and an 18-gauge, 75 mm ball-tipped curved gavage probe (Fig. 1).

Surgical Procedures

All rats were operated on under general anesthesia by the same surgeon, who was blinded to group assignments. Ketamine (80-90 mg/kg; Ketalar, Pfizer Warner-Lambert, Türkiye) and xylazine (5-10 mg/kg; Alfazyne, Alfasan International BV, Netherlands) were administered intraperitoneally for general anesthesia after the rats were properly restrained. The abdominal, inguinal, and thigh areas were shaved, and the rats were fixed in the supine position on the operating table.

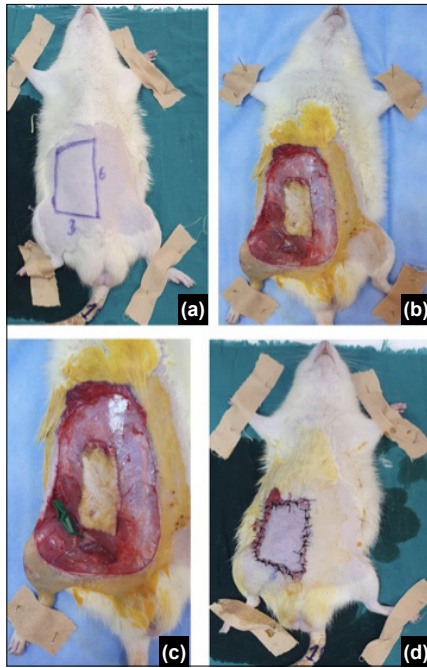


Figure 2. (a) The inferior epigastric flap was designed. (b) The flap was elevated. (c) The artery and vein of the flap were clamped to block blood flow. (d) The flap was inset in the same location.

Asepsis was ensured using 10% povidone-iodine. All surgeries were performed at an ambient temperature of $36 \pm 1^\circ\text{C}$ to prevent hypothermia. A 3×6 cm skin island flap with an axial pattern was designed based on the pedicle of the inferior epigastric artery and vein. The flap was bordered superiorly by the costal arch, laterally by the axillary line, inferiorly by the inguinal ligament, and medially by a vertical line from the xiphoid to the pubis (Fig. 2a). The flap was dissected from medial to lateral, including the panniculus carnosus. The epigastric artery and vein, branching from the femoral artery and vein, were identified and dissected free from surrounding tissue (Fig. 2b). Preparation and dissection of the epigastric artery island flap were performed according to the description by Petry and Wortham.^[25]

In Group I, the flap was returned to its original position without being subjected to ischemia. In Groups II, III, IV, and V, total flap ischemia was induced by clamping the pedicle with atraumatic microvascular clamps to block blood flow (Fig. 2c). The total ischemia time was eight hours. After clamp removal at the end of the eighth hour, arterial and venous flow was confirmed using the milking test, and the flaps were inset in the original position with 4-0 silk sutures (Silk, Dogsan Ltd., Türkiye) (Fig. 2d). Appropriate dressing was applied.

The rats were placed in individual cages. On the seventh postoperative day, after photographs were taken, tissue samples measuring 15×15 mm were collected under general anesthesia. At the conclusion of the experiment, the rats were euthanized by cervical dislocation. Only one animal was lost

in Group V during the experiment.

Macroscopic Measurement of Flap Survival Rate

After equidistant flap photography, the images were analyzed using the ImageJ program (LOCI, University of Wisconsin). Necrotic and viable areas were measured; the viable area was divided by the whole area, and the flap survival rate was expressed as a percentage. Suspicious areas within the flaps were considered necrotic.

Biochemical Analysis

Tissue samples collected for biochemical analyses were placed in Eppendorf tubes and stored at -80°C . (Samples were taken from the same location on all flaps.) The tissues were homogenized in a cocktail containing four times their weight of Triton X solution and protease inhibitors. After homogenization, the supernatant was collected from the homogenate following centrifugation at 6,000 rpm for five minutes. Levels of total oxidant status (TOS), total antioxidant capacity (TAC), and tumor necrosis factor- α (TNF- α) were measured using the micro-enzyme-linked immunosorbent assay (ELISA) method. The oxidative stress index (OSI) was calculated by dividing the TOS value ($\mu\text{mol/L}$) by the TAC value ($\mu\text{mol/L}$) and multiplying the result by 100.^[26-28]

Since oxidative stress is characterized by an imbalance between oxidant and antioxidant molecules, studying ischemia-reperfusion, a condition known for its pronounced oxidative stress effects, led us to investigate oxidant and antioxidant levels. Previous studies have demonstrated the use of oxidative stress markers such as superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA), myeloperoxidase (MPO), and catalase.^[3,4,29] However, measuring individual oxidants and antioxidants separately is often impractical due to cost, time constraints, and procedural complexities, as well as the cumulative nature of their effects. In conclusion, all reviewed studies agree on a crucial point: using oxidative stress markers as indicators offers greater sensitivity to the imbalance between oxidants and antioxidants compared to evaluating individual parameters. Therefore, we chose to measure total oxidant or antioxidant capacities and express them as a ratio, known as the oxidative stress index. To provide a comprehensive assessment of oxidative stress, we evaluated not only the individual levels of TOS and TAC but also their ratio.^[30] These markers have been increasingly employed in recent I/R injury research.^[1,19,24]

Histopathological Analysis

A full-thickness tissue sample, including both healthy and necrotic areas, was fixed in 10% formalin solution at room temperature. Each sample was embedded in a paraffin block. Sections $4 \mu\text{m}$ thick were cut from the paraffin blocks using a microtome, parallel to the long axis of the tissue sample and including all skin folds. These sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome. Follow-

ing this, areas measuring 1 cm in length and 2 mm in thickness were selected from each preparation for histopathological evaluation. These areas were divided into five equal parts of 2 mm each, ensuring full-thickness representation. Evaluations were performed by counting cells and related structures within each of these areas.

Scoring was based on: PMNL count for active inflammation; plasma cell and lymphocyte count for chronic inflammation; ulcer area size for ulceration; vessel number and diameter for vascular proliferation; and fibrotic area assessment for fibroblast activity. H&E staining was used for the first four parameters, while Masson's trichrome staining was used for assessing fibroblast activity. Additionally, immunohistochemical staining was performed to evaluate vascularization. For this purpose, CD31 (CONFIRM anti-CD31 (JC70) Primary Antibody, Roche, Basel, Switzerland) and CD34 (CONFIRM anti-CD34 (QEnd/10) Primary Antibody, Roche, Basel, Switzerland) were used, along with stained slides (TOMO - 11/90 Microscope Slides, Tomo, Matsunami, Japan). All specimens were evaluated blindly by an experienced pathologist who was unaware of the group assignments.

Statistical Analysis

IBM SPSS version 21 (IBM SPSS Inc., Chicago, IL) was used for data analysis. For continuous data, descriptive statistics were presented as mean \pm standard deviation (SD). The conformity of continuous data to normal distribution was assessed using the Kolmogorov-Smirnov test, and the homogeneity of variances was evaluated using Levene's test. Ordinal parameters were analyzed using the Kruskal-Wallis test, followed by the Dunn-Bonferroni paired comparison test. For parametric variables, one-way analysis of variance (ANOVA) was applied. A p-value <0.05 was considered statistically significant.

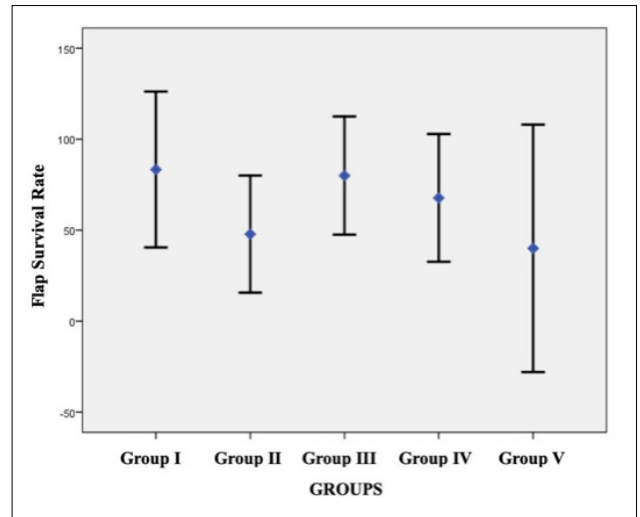


Figure 3. Error-bar graph illustrating the 95% confidence intervals for the mean flap survival rates.

RESULTS

One subject in Group V died during the study. No signs of infection, seroma, or hematoma were observed in any of the subjects during the postoperative period.

Flap Survival Rate

In Groups III and IV, the mean flap survival rate was lower than that of Group I, but higher than that of Group II. The value for Group V was lower than those of Groups I and II. These differences were not statistically significant ($p=0.262$) (Table 1, Fig. 3).

Biochemical Results

The mean TOS values of Groups III, IV, and V were higher than that of Group I and lower than that of Group II. However, the differences were not statistically significant ($p=0.395$) (Fig. 4).

Table 1. ANOVA results comparing the distributions of flap survival rates in the groups

Group	N	Mean	Standard Deviation	p-value
Flap Survival Rate				
Group 1 (Sham Control)	6	83.33%	40.82%	p>0.05*
Group 2 (Vehicle Control)	6	47.83%	30.69%	
Group 3 (10 mg/kg/day Chrysin)	6	80%	30.98%	
Group 4 (50 mg/kg/day Chrysin)	6	67.75%	33.45%	
Group 5 (100 mg/kg/day Chrysin)	6	40%	54.77%	

*Not statistically significant.

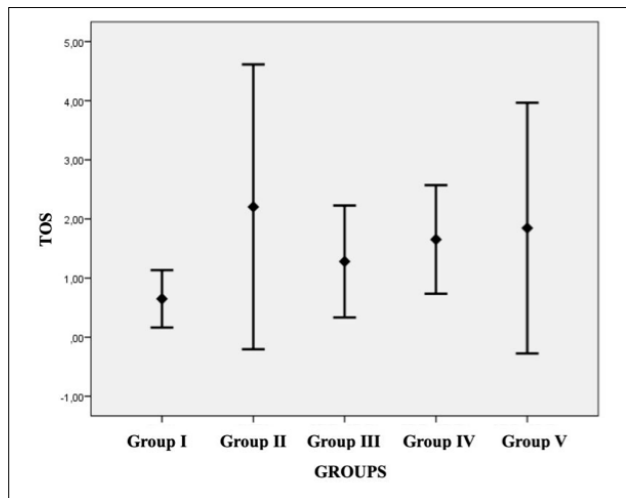


Figure 4. Error-bar graph illustrating the 95% confidence intervals for the mean total oxidant status (TOS) values.

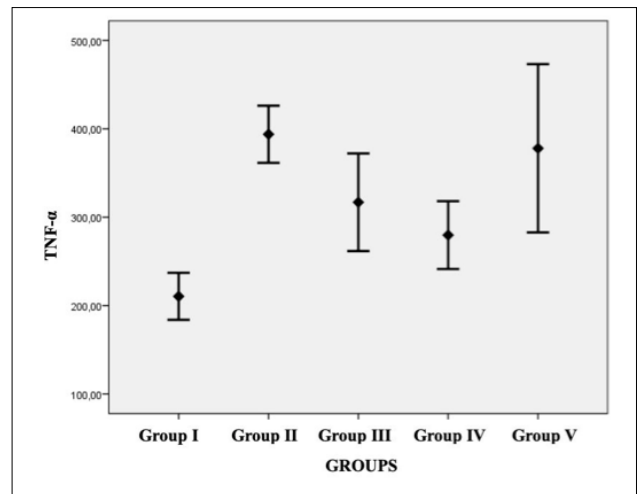


Figure 7. Error-bar graph illustrating the 95% confidence intervals for the mean tumor necrosis factor-alpha (TNF-alpha) values.

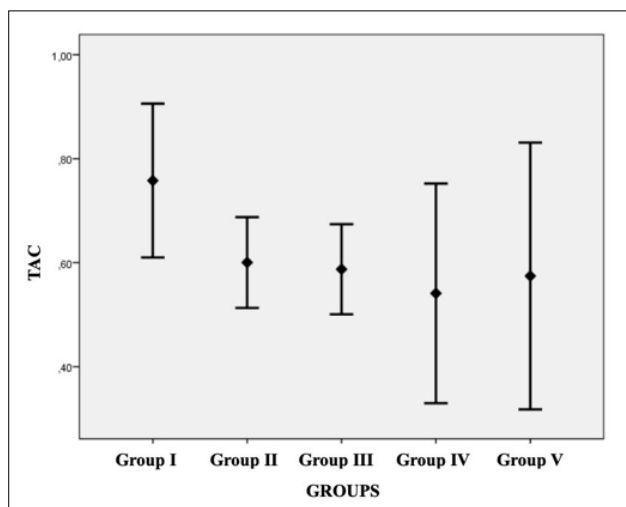


Figure 5. Error-bar graph illustrating the 95% confidence intervals for the mean total antioxidant capacity (TAC) values.

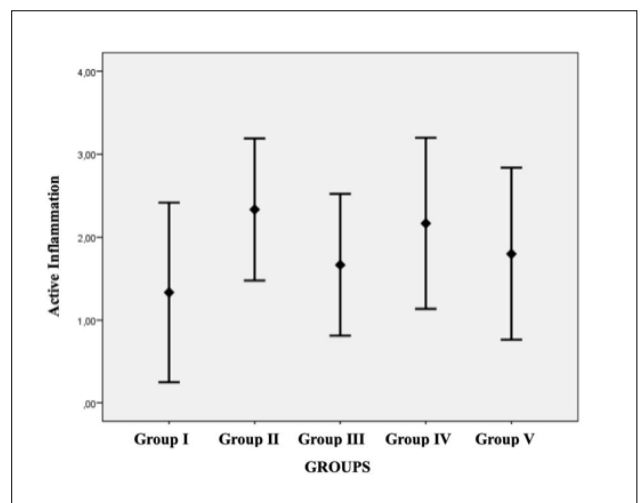


Figure 8. Error-bar graph illustrating the 95% confidence intervals for the mean active inflammation scores.

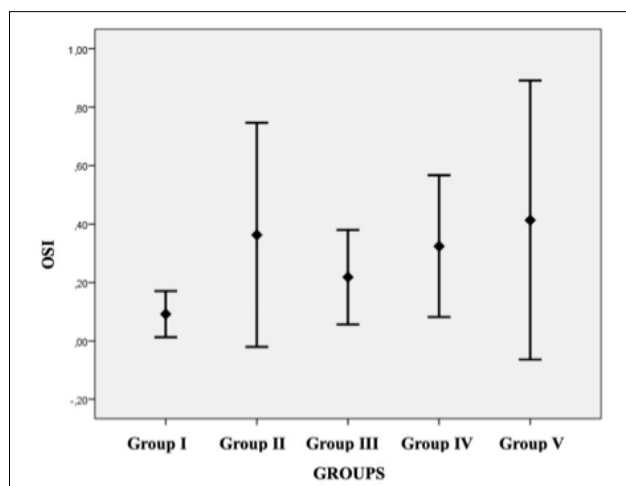


Figure 6. Error-bar graph illustrating the 95% confidence intervals for the mean oxidative stress index (OSI) values.

The mean TAC values of Groups III, IV, and V were lower than those of Groups I and II, with no statistically significant differences observed ($p=0.147$) (Fig. 5). The mean OSI values of Groups III and IV were higher than that of Group I and lower than that of Group II. The OSI value of Group V was higher than those of both Groups I and II. These differences were not statistically significant ($p=0.279$) (Fig. 6). The mean TNF- α values in Groups II, III, and V were significantly higher than the value in Group I ($p<0.05$). The value for Group IV was between those of Groups I and II, and the difference was not statistically significant (Fig. 7, Table 2).

Histopathological Results

The mean active inflammation scores of Groups III, IV, and V were found to lie between the scores of Groups I and II. These differences were not statistically significant ($p=0.359$) (Fig. 8). Similarly, the mean chronic inflammation scores of

Table 2. One-way analysis of variance (ANOVA) results comparing biochemical parameters among the groups

Group	N	Total Oxidant Status (TOS) (Mean ± SD, $\mu\text{mol H}_2\text{O}_2$ Equivalent/L)	Total Antioxidant Capacity (TAC) (Mean ± SD, mmol/L)	Oxidative Stress Index (OSI) (Mean ± SD)	Tumor Necrosis Factor-Alpha (TNF- α) (Mean ± SD, ng/L)
Group 1 (Sham Control)	6	0.649±0.462	0.758±0.141	0.091±0.075	210.447±25.331
Group 2 (Vehicle Control)	6	2.204±2.295	0.600±0.083	0.363±0.365	(393.868±30.817)*
Group 3 (10 mg/kg/day Chrysin)	6	1.280±0.901	0.587±0.083	0.218±0.154	(316.901±52.658)*
Group 4 (50 mg/kg/day Chrysin)	6	1.652±0.875	0.541±0.201	0.325±0.231	279.726±36.531
Group 5 (100 mg/kg/day Chrysin)	6	1.845±1.708	0.574±0.207	0.414±0.385	(377.988±76.727)*
p-value		p>0.05	p>0.05	p>0.05	p<0.05*

*Statistically significant.

Table 3. One-way analysis of variance (ANOVA) results comparing histopathological parameters among the groups

Group	N	Active Inflammation	Chronic Inflammation	Fibroblast Activity	Vascular Proliferation	Ulceration
Group 1 (Sham Control)	6	1.33±1.03	1.66±0.82	0.83±0.98	1.66±0.82	0.50±0.55
Group 2 (Vehicle Control)	6	2.33±0.82	2.66±0.52	(2.83±0.41)*	2.83±0.98	2.00±1.09
Group 3 (10 mg/kg/day Chrysin)	6	1.66±0.82	2.50±0.55	2.17±0.75	3.00±1.10	1.00±1.10
Group 4 (50 mg/kg/day Chrysin)	6	2.17±0.98	2.00±1.10	2.50±0.55	3.00±0.89	1.83±1.47
Group 5 (100 mg/kg/day Chrysin)	6	1.80±0.84	1.80±0.84	1.60±1.14	2.20±0.84	2.00±1.22
p-value		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05

*Statistically significant.

Groups III, IV, and V were also between the scores of Groups I and II, without statistical significance ($p=0.185$) (Fig 9). The mean fibroblast activity scores of Groups III, IV, and V were likewise between the scores of Groups I and II. The differences were statistically significant ($p<0.05$). Fibroblast activ-

ity was significantly higher in Group II compared to Group I ($p<0.05$) (Fig. 10). The mean vascular proliferation scores of Groups III and IV were higher than those of Groups I and II. The score for Group V was between those of Groups I and II. These differences were not statistically significant ($p=0.108$)

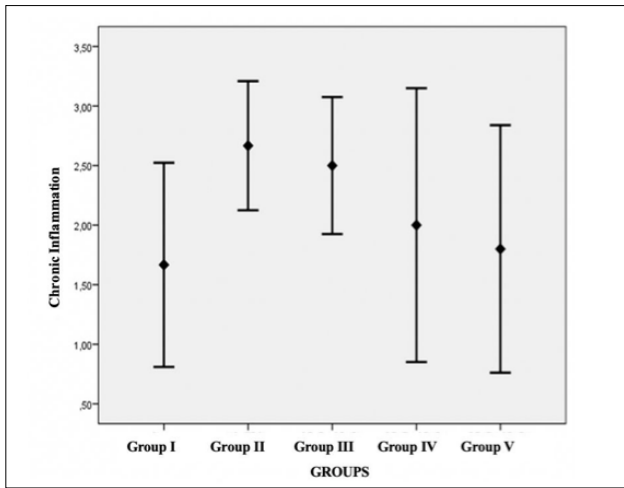


Figure 9. Error-bar graph illustrating the 95% confidence intervals for the mean chronic inflammation scores.

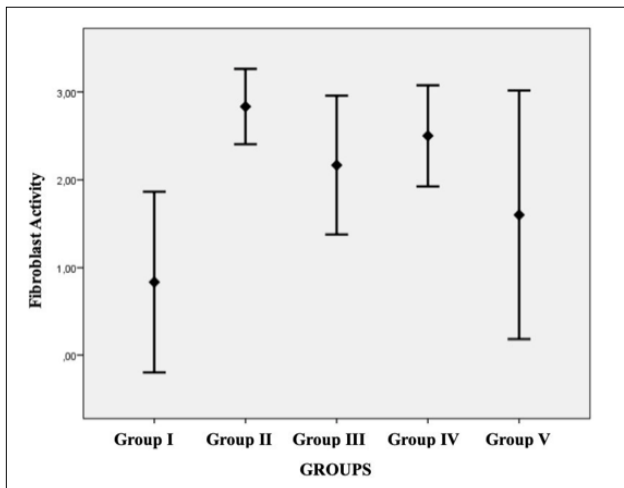


Figure 10. Error-bar graph illustrating the 95% confidence intervals for the mean fibroblast activity scores.

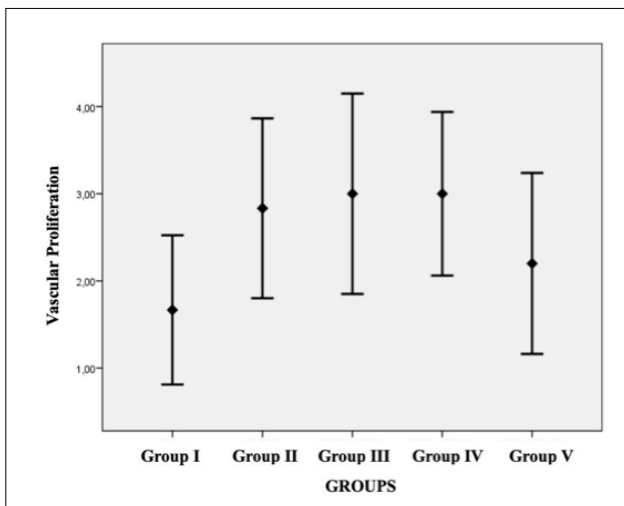


Figure 11. Error-bar graph illustrating the 95% confidence intervals for the mean vascular proliferation scores.

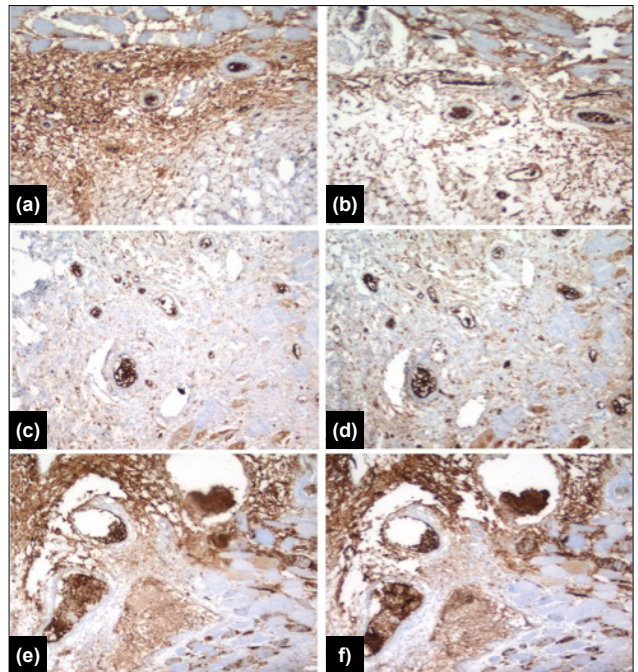


Figure 12. Immunohistochemically stained tissue samples using CD31 and CD34 antibodies for the evaluation of vascular proliferation. The samples on the left (a, c, e) were stained with anti-CD31, and those on the right (b, d, f) with anti-CD34. Samples in the first row (a,b) belong to the sham-control group; the second row (c,d) to the vehicle-control group; and the third row (e,f) to the 10 mg/kg/day chrysin treatment group (Group III). Vessel diameters and numbers increase progressively from the sham-control group to the treatment group.

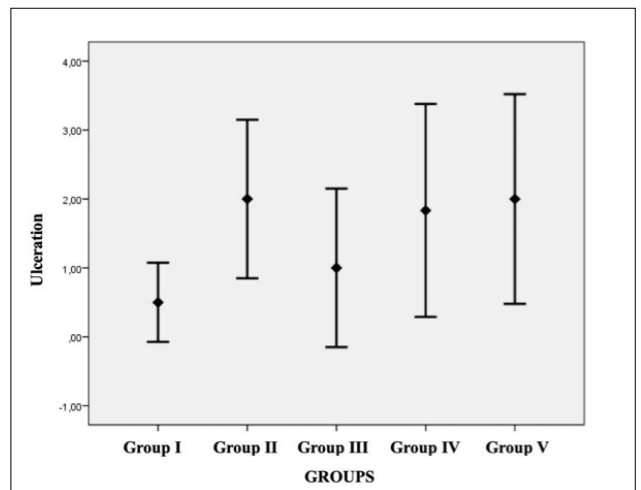


Figure 13. Error-bar graph illustrating the 95% confidence intervals for the mean ulceration scores.

(Figures 11 and 12). The mean ulceration scores of Groups III, IV, and V were found to be between the scores of Groups I and II. The differences were not statistically significant ($p=0.145$) (Fig. 13, Table 3).

DISCUSSION

Flaps are frequently used in reconstructive surgery to repair soft tissue defects and restore lost function.^[3,29] Blood flow in the distal part of the flap drops below 20% of normal levels within the first 6-12 hours,^[31] and the period during which permanent damage begins to occur is referred to as the critical ischemia time.^[32] Paradoxically, the return of oxygenated blood to tissue after ischemia paradoxically can exacerbate tissue damage, a phenomenon known as I/R injury. This pathophysiological process involves accumulation of ROS, aggregation of PMNL, and activation of the complement system and endothelial cells. Despite advancements in flap surgery, I/R injury is still a significant cause of flap failure.^[1,3,4]

In our study, we employed the experimental skin island flap I/R injury model described by Petry and Worham.^[25] This model is widely used in reconstructive surgery, particularly in free tissue transfer, where ischemia is intentionally induced and followed by reperfusion after vascular anastomosis. Numerous studies in the literature aim to prevent or reduce I/R injury, many of which use an ischemia duration of eight hours. To eliminate variability associated with individual vessel anastomosis patency and to standardize experimental conditions, arterial clamping of skin flaps has been adopted to simulate the ischemia-reperfusion state in vascular free graft transfer procedures.^[3,29]

Chrysin (5,7-dihydroxyflavone) is a flavone naturally found in honey, propolis, and blue passionflower. The average daily intake of flavonoids through a normal diet is 1-2 g, and they are generally non-toxic at doses between 0.5-3 g per day. Chrysin has a molecular weight of 254.238 Da and consists of 15 carbon atoms. It contains an unsaturated bond between the 2nd and 3rd carbon atoms and hydroxyl (OH-) groups at the 5th and 7th carbon positions.^[33,34] These structural features are essential for its ROS scavenging effect and antioxidant activity.^[6] Chrysin also exhibits vasodilatory effects by stimulating nitric oxide release.^[7,8] It modulates cytosolic calcium loading through activation of the peroxisome proliferator-activated receptor gamma/nuclear factor erythroid 2-related factor 2 (PPAR- γ /Nrf2) pathway and shows anti-inflammatory effects via activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling pathway.^[9,10] Additionally, it has been shown to lower TNF- α levels.^[12,14-17,23] It functions as an antioxidant by inhibiting xanthine oxidase and catalase,^[35] and it has been demonstrated to restore glutathione levels.^[14] Studies have identified chrysin as both an antioxidant and anti-apoptotic molecule,^[36] and it has been shown to prevent I/R injury in the tissues of the heart,^[11,12] brain,^[9,13-18] liver,^[19] kidney,^[19,20] gastric mucosa,^[21] ovary,^[22] and testis.^[23]

Although various agents have been investigated to mitigate I/R injury, the effects of chrysin on skin tissue remain unexplored. Chrysin possesses several ideal characteristics for therapeutic use: it is safe, affordable, readily available, and well-understood in terms of its mechanism of action. This

study aimed to evaluate the biochemical and histopathological effects of chrysin on I/R injury in skin flaps, contributing novel insights into its potential to reduce tissue damage.

According to the current literature, chrysin is typically administered orally after being dissolved in various solvents. Dimethyl sulfoxide,^[9,15,17,18] 1% Tween 80 solution,^[14] 5% sodium carboxymethyl cellulose,^[16] and corn oil^[13,22] have been used as solvents. Intraperitoneal administration was reported in one previous study.^[23] In our study, we opted to prepare oral suspensions, as most of the commonly used solvents have known toxic effects. Preoperative administration of chrysin for one week was chosen due to the low bioavailability of oral flavonoids, although beneficial effects have been observed with repeated dietary intake over several days.^[6,37-39] The dose range used in this study was selected to fall between the lowest and highest doses employed in previous research, as identified in the literature.^[18,19,39]

A previous study by Orsolić et al.^[39] demonstrated that administering chrysin orally at a dose of 50 mg/kg for one week in rats with Ehrlich ascites tumors enhanced the functional activity of macrophages. Durak et al.^[13] found that in mice subjected to global cerebral ischemia, induced by clamping the bilateral carotid arteries, oral administration of chrysin at a dose of 50 mg/kg/day for 10 days preoperatively and postoperatively resulted in significant neurodegenerative histological changes in the brain and significantly altered tissue levels of oxidants and antioxidants. Li et al.^[16] observed that in a model of cerebral ischemia induced by middle cerebral artery occlusion, preoperative administration of oral chrysin at a dose of 10 mg/kg/day for seven days suppressed inflammatory cytokines, including TNF- α , improved SOD activity, and histologically mitigated the effects of I/R injury. In their comprehensive review, Pingilli et al.^[19] reported that oral administration of chrysin at doses ranging from 25 to 100 mg/kg/day for six or seven days in mice and rats reduced TNF- α levels and upregulated nitric oxide (NO) in models of hepatic ischemia/reperfusion injury or hepatotoxicity induced by various agents. The review also highlighted that oral chrysin at doses of 10-50 mg/kg/day for 6-10 days in rats decreased inflammatory parameters, modulated oxidant and antioxidant levels favorably, and mitigated nephrotoxicity. However, in a study where nephrotoxicity was induced with gentamicin, the outcomes were not favorable. Xu et al.^[20] observed that in a model of renal ischemia-reperfusion injury induced by clamping the bilateral renal pedicles, administration of chrysin at a dose of 100 mg/kg/day for three consecutive days reduced levels of TNF- α and MPO. Additionally, it decreased apoptosis of tubular cells caused by renal ischemia-reperfusion.

In our study, oral administration of chrysin significantly suppressed fibroblast activity across all treatment doses in rats subjected to I/R injury. Although there were observable improvements in flap survival rates, OSI, and vascular proliferation at doses of 10 mg/kg/day and 50 mg/kg/day, as well as in TOS, TNF- α , active inflammation, chronic inflammation, and

ulceration parameters, these changes did not reach statistical significance. TAC values did not correlate with the observed positive outcomes, which is consistent with findings in the existing literature. Chrysin, classified as a low molecular weight antioxidant (molecular weight <1 kDa), primarily functions to reduce oxidant levels rather than to increase antioxidant levels.^[33,40]

The limitations observed in this study can be attributed to several factors, most notably chrysin's low oral bioavailability (estimated at ~1%), due to its poor absorption and rapid metabolism, which limits systemic circulation and delivery to skin tissue.^[33] Although the dosing was based on regimens successfully applied in brain, kidney, and liver models, those organ systems likely benefited from either enterohepatic recirculation (as in liver models) or extended daily dosing, which allowed sufficient systemic accumulation in organs such as the brain and kidney.^[13,16,19,20] In contrast, the skin, being less metabolically active and having different vascular characteristics, may require alternative delivery strategies or higher doses to achieve comparable bioactivity against I/R injury. Nanoparticle-based formulations, such as chrysin-loaded PLGA-PEG (poly(lactic-co-glycolic acid)-polyethylene glycol) nanoparticles, polymeric chrysin nanocapsules, and lipid-core nanocapsules, could enhance its delivery and therapeutic effect in skin tissues. Additionally, strategies involving folate-conjugated micelles or metabolic enzyme inhibitors such as sodium oleate may help mitigate rapid metabolism, thereby extending chrysin's active presence in circulation.^[41,42]

Moreover, the absence of plasma concentration measurements in our study limits our understanding of whether the administered doses achieved therapeutic levels in the skin. Future studies should include more direct assessments of bioavailability, such as measuring plasma drug concentrations, to confirm dose adequacy and chrysin's ability to reach effective levels in skin tissue.

Furthermore, the significantly higher standard deviation observed in Group V suggests considerable variability in chrysin's effects at elevated doses. This variability is likely attributable to individual metabolic differences among the animals, which may influence chrysin's systemic availability. This finding underscores the need for larger sample sizes in future studies to reduce variability and enhance the reliability of the results. Although our study was designed with an appropriate number of animals per group based on previous research, a larger sample size could have further improved the validity of the findings.^[21,24,25]

In conclusion, although our study provides encouraging preliminary evidence of chrysin's potential to mitigate oxidative stress and modulate histopathological parameters in I/R injury, its low bioavailability presents a significant barrier to therapeutic use. Overcoming these limitations through the use of advanced drug delivery systems and comprehensive pharmacokinetic monitoring, along with larger sample sizes,

will be essential to fully assess chrysin's therapeutic efficacy in the skin and other metabolically distinct tissues.

CONCLUSION

Oral administration of chrysin significantly suppressed fibroblast activity at all treatment doses in axial pattern skin island flaps subjected to I/R injury. Although positive changes were observed in flap survival rate, OSI and vascular proliferation at doses of 10 mg/kg/day and 50 mg/kg/day, and in TOS, TNF- α , active inflammation, chronic inflammation, and ulceration parameters across all treatment doses, these changes did not reach statistical significance. Consistent with existing literature, TAC values did not appear to contribute to the observed positive outcomes. To obtain more reliable and valid results, future studies should consider strategies to enhance chrysin's bioavailability, measure plasma drug concentrations, and increase sample size.

Ethics Committee Approval: This study was approved by the Health Sciences University Gülhane Animal Reserach Local Ethics Committee Ethics Committee (Date: 31.08.2022, Decision No: 22/27).

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DENEYSSEL ÇALIŞMA - ÖZ

Chrysin'in sıçan inferior epigastrik arter cilt ada flebinde iskemi reperfüzyon hasarına etkisi

AMAÇ: İskemi sonrası reperfüzyon ile oksijenize kanın dokuya geri dönüşü, iskemi-reperfüzyon (İ/R) hasarı olarak adlandırılan paradoksal doku yıkımını artıracak reaksiyonları başlatır. Çalışmamızın amacı sıçan inferior epigastrik arter cilt ada flep modelinde chrysin'in(C) İ/R hasarı üzerine etkisini ortaya koymaktır.

GEREÇ VE YÖNTEM: 30 erkek Wistar albino sıçan randomize beş gruba ayrıldı (n=6): sham-kontrol grubu (grup I), flep I/R ile tedavi edilmemiş (grup II) grup, I/R-C-10 mg/kg/gün grubu (grup III), I/R-C-50 mg/kg/gün grubu (grup IV) ve I/R-C-100 mg/kg/gün grubu (grup V). Chrysin preoperatif ve postoperatif yedi gün boyunca oral olarak uygulandı. Sekizinci günde flepler eleve edildi ve denekler sekiz saat boyunca iskemiye maruz bırakıldı. Flep sağkalım oranı, biyokimyasal ve histopatolojik parametreler değerlendirildi.

BULGULAR: Oral chrysin uygulaması, I/R hasarına maruz bırakılan sıçanlarda tüm tedavi dozlarında fibroblast aktivitesini önemli ölçüde baskılamıştır. Flep sağkalım oranı, oksidatif stres indeksi (OSİ) ve vasküler proliferasyon parametrelerinde 10 mg/kg/gün ve 50 mg/kg/gün dozlarında, total oksidan stres (TOS), tümör nekroz faktörü-alfa (TNF- α), aktif inflamasyon, kronik inflamasyon ve ülserasyon parametrelerinde ise tüm tedavi dozlarında olumlu değişiklikler gözlenirse de bu değişiklikler istatistiksel anlamlılığa ulaşmamıştır. Total antioksidan kapasite (TAK) değerlerinin, mevcut literatürle tutarlı olarak, olumlu sonucu etkilemediği görülmüştür.

SONUÇ: Çalışmamız, sıçan inferior epigastrik arter cilt adası flebinde iskemi-reperfüzyon (I/R) hasarına karşı chrysin tedavisinin etkilerini araştırmıştır. Moleküler düzeyde olumlu değişiklikler tespit edilmekle birlikte, bu bulguların klinik sonuçlara yansımadağı gözlemlenmiştir.

Anahtar sözcükler: Chrysin; iskemi; reperfüzyon hasarı.

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