



The Novel Role of *Crocus sativus* L. in Enhancing Skin Flap Survival by Affecting Apoptosis Independent of mTOR: A Data-Virtualized Study



Zahra Habibi¹ · Mahmood Hoormand² · Majid Banimohammad³ ·
Marjan Ajami⁴ · Gholamreza Amin⁵ · Mohsen Amin¹ · Hamidreza Pazoki-Toroudi³

Received: 22 May 2022 / Accepted: 25 July 2022

© Springer Science+Business Media, LLC, part of Springer Nature and International Society of Aesthetic Plastic Surgery 2022

Abstract

Background Despite the improvements to enhance skin flap viability, the effects of ischemia-reperfusion (IR), oxidative stress, necrosis, and apoptosis are still challenging. *Crocus sativus* L. (Saffron) is highly noticeable due to its tissue-protective and antioxidant properties. So, we aimed to investigate its effects on skin flap viability, oxidative stress, apoptosis markers, histopathological changes, and mTOR/p-mTOR expression.

Materials and Methods 40 Sprague-Dawley rats, weighing 200–240 g, were divided into four groups including: (1) Sham (8 × 3 cm skin cut, without elevation); (2) Flap Surgery (8 × 3 cm skin flap with elevation from its bed); (3) Saffron 40 mg/kg + Flap Surgery; and (4) Saffron

80 mg/kg + Flap Surgery. Saffron was administrated orally for 7 days. At day 7, flap necrosis percentage, histopathological changes, malondialdehyde level, Myeloperoxidase and superoxide dismutase activity, Bax, Bcl-2, mTOR, and p-mTOR expression were measured. Protein expressions were controlled by β -Actin.

Results Saffron administration decreased flap necrosis percentage ($p < 0.01$), which was not dose-dependent. Treatment groups showed significant histological healing signs (Neovascularization, Fibroblast migration, Epithelialization, and Epithelialization thickness), decreased MDA content ($p < 0.01$), increased SOD ($p < 0.01$) and decreased MPO activity ($p < 0.01$). Bax and Bcl-2 expression, decreased and increased respectively in treated groups ($p < 0.0001$). mTOR and p-mTOR expression were not changed significantly in Saffron treated groups.

Conclusion Saffron could increase skin flap viability, alleviate necrosis, decrease oxidative stress and decrease apoptotic cell death, after skin flap surgery, but it acts independent of the mTOR pathway. So, Saffron could potentially be used clinically to enhance skin flap viability.

No Level Assigned This journal requires that authors assign a level of evidence to each submission to which Evidence-Based Medicine rankings are applicable. This excludes Review Articles, Book Reviews, and manuscripts that concern Basic Science, Animal Studies, Cadaver Studies, and Experimental Studies. For a full description of these Evidence-Based Medicine ratings, please refer to the Table of Contents or the online Instructions to Authors www.springer.com/00266. <https://www.springer.com/00266>

Keywords Skin flap · *Crocus sativus* L., Saffron · Oxidative stress · Reactive oxygen species (ROS) · Ischemia-reperfusion (IR)

Zahra Habibi and Mahmood Hoormand are equally contributed in this study and are considered as first author.

✉ Mohsen Amin
m-amin@tums.ac.ir

✉ Hamidreza Pazoki-Toroudi
pazoki49@gmail.com

¹ Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

² Department of Pharmacology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

³ Department of Physiology and Physiology Research Center, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

⁴ Department of Food and Nutrition Policy and Planning, National Nutrition and Food Technology Research Institute, School of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Science, Tehran, Iran

⁵ Department of Pharmacognosy, Faculty of pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Introduction

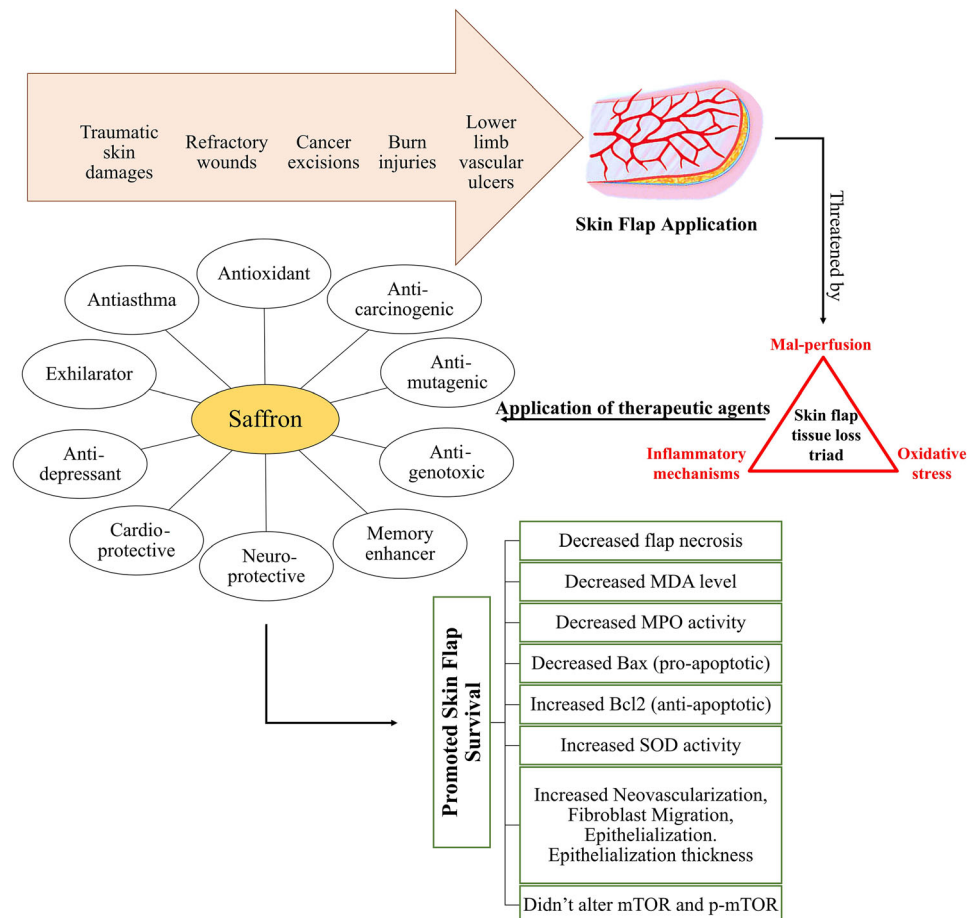
Skin flaps are a widely-used major strategy of plastic, aesthetic, and reconstructive surgeries for treatment of skin defects. This method is used to overcome traumatic skin damages, refractory wounds, cancer excisions, burn injuries, and lower limb vascular ulcers due to its convenience and resilience [1–4]. Nevertheless, rate of flap tissue loss, as the main challenging issue, varies from 25 to 50% of flap length [5]. Skin flap survival (SFS) is threatened by a triad including mal-perfusion, inflammatory mechanisms, and oxidative stress (Fig. 1) [6, 7]. Mal-perfusion triggers extensive damage of cell membrane in ischemic flap tissues which results in increased levels of arachidonic acid metabolites. It promotes recruitment of inflammatory cells, substantially the neutrophils. Immune cells produce more reactive oxygen species (ROS) and inflammatory mediators in ischemic tissue. ROS threatens tissue viability, causing DNA and cell membrane destruction, lipid peroxidation, cell apoptosis, and protein changes. In addition, ischemia–reperfusion injury (IRI) occurs due to consequent neovascularization. Blood reperfusion triggers a burst of ROS is the cardinal culprit of IRI pathogenesis which is

accompanied by impaired cellular redox homeostasis and extensive cellular apoptosis. ROS accumulation and apoptosis of functional cells are major factors resulting from necrosis of skin flaps [8–10].

Systemic or local administration of various therapeutic agents with the aim of preventing distal flap necrosis, has recently attracted growing attention. Treatments include angiogenesis promotion, oxidative stress/apoptosis/inflammation attenuation, administration of agents such as blood viscosity diminishers, calcium channel blockers (CCB), vasodilators, anti-thrombotics, anti-coagulants, anti-inflammatories, anti-oxidants, steroids and hyperbaric oxygen therapy. However, Surgical delay could be used to control distal necrosis but it requires at least one additional surgery [11–14].

Nowadays, there is a special focus on reducing oxidative stress and increasing tissue defense mechanisms to deal with ROS injury. Major antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione (GSH) are important for cellular protection due to their ability to detoxify free radicals, such

Fig. 1 Skin flaps are used as a major technique in reconstructive surgery but the survival of flaps decreases under the influence of threatening factors. Saffron increases the survival of skin flaps and has the potential for clinical use



as ROS. At the same importance of ROS elimination, apoptosis control is noticeably mentioned enhance SFS. It would be a challenging issue that except decrease in ROS effects, necrosis, and inflammation, we could increase anti-apoptotics and decrease pro-apoptotics. Bcl-2 is an important protein of BCL2 family that prevent cell death through apoptosis. This family also contains pro-apoptotics such as Bax. By decreasing Bax, or increasing Bcl-2, SFS could be promoted (Fig. 2) [15, 16].

Autophagy as intracellular degradation/recycling of dysfunctional proteins and organelles, has a great control on cell signaling, homeostasis, cell survival, and apoptosis prevention. It can alleviate ROS production and suppress apoptosis via the mTOR signaling pathway. It is reported that autophagy has attenuated liver IRI, inhibited apoptosis induced by intestinal ischemia-reperfusion, and had important role in disc degeneration due to alleviating mitochondrial dysfunction. As a consequence, research suggest that autophagy induction could enhance angiogenesis, decrease apoptosis and oxidative stress. Altogether it might be beneficial for skin flaps survival (Fig. 2) [17, 18].

Dried stigmas of *Crocus sativus* L. (Saffron), a worthy plant of Iridaceae family is mainly made up of carbohydrates including starch, reducing sugars, gums, pectin, pentosans, and dextrans. It is a plant with a vast range of pharmacological properties such as anti-carcinogenic, anti-mutagenic, and anti-genotoxic [19, 20], anti-oxidant [21], memory enhancer [22], neuro-protective [23, 24], anti-depressant, exhilarator, antiasthma, and cardio-protective effects (Fig. 1) [25]. Even it is declared that Saffron is a

potential drug-supplement for severe acute respiratory syndrome coronavirus (COVID) management [26, 27]. Components such as Crocin (digentiobiosyl8, 8'-diapocrotene-8, 8'-oate; $C_{44}H_{64}O_{24}$) [28] and Crocetin are more responsible for cardio-protective and anti-atherosclerotic properties [29, 30]. Saffron has been used as an effective factor on neuro-degenerative disease and related memory impairment, ischemic retinopathy and age-related macular degeneration, coronary artery disease, blood pressure abnormalities, acute and chronic inflammatory disease, mild to moderate depression, seizure, Parkinsonism [31, 32]. It is reported that oral administration of Saffron extract, has inhibited cisplatin, cyclophosphamide, mitomycin-C and urethane induced genotoxicity in male Swiss albino mice [33, 34].

Skin flap survival is a process that depends heavily on micro-environment of the tissue. Considering this issue and the properties of Saffron, it seems to be possible to manage oxidative stress and cell death of skin flap by Saffron administration. Saffron have received growing attention as potential preventive agent that scavenges ROS and detoxify them, inhibit inflammatory pathways, and finally decrease cell death. The present study was designed to investigate the modifying effects of Saffron extract on survival, antioxidant status, inflammation, apoptotic markers, histopathologic changes, and its probable effect on mTOR pathway of skin flap model in Sprague-Dawley albino rats.

Materials and Methods

Animals and Ethical Considerations

Male Sprague-Dawley rats ($N = 40$, weight = 200–240 g), were kept in 22–25 centigrade degrees and 12-h light, 12-h dark cycles. They were divided into four groups including the (1) Sham group, (2) Flap Surgery (Control) group, (3) Saffron 40 mg/kg, and (4) Saffron 80 mg/kg. After a one-week adaptation, animals were anaesthetized with 50 mg/kg ketamine hydrochloride 10% (i.p.) and 4 mg/kg xylazine 2% (i.p.). A method similar to McFarlane et al. [35] was used to lift the skin flap and assess the necrotic area. Animals were shaved, and the site was disinfected with alcohol. In the Sham group, skin was cut in 8×3 cm without skin elevation. In the three other groups, after providing two parallel 8 cm incisions, they were connected caudally with a 3 cm incision and skin was gently separated from its bed using a scalpel, then a sterile plastic film was placed between elevated skin and its supporting fascia. All incisions were sutured. Finally, rats were returned into separate cages. Saffron is administrated orally in flap surgery day and the next six days. Group 3 received Saffron

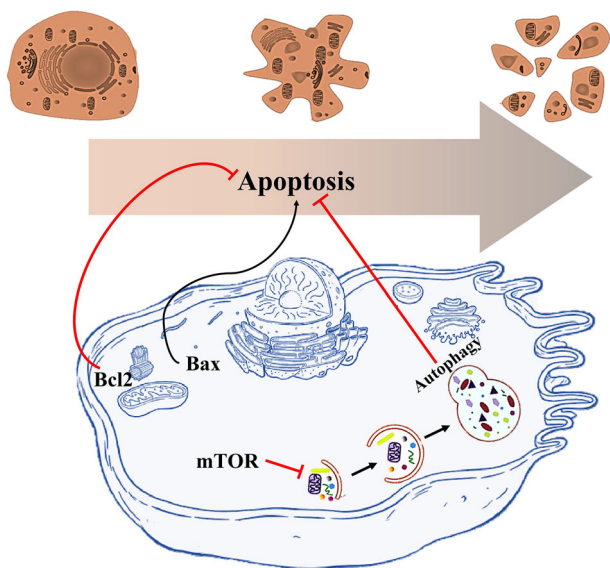


Fig. 2 Apoptosis promotes by increasing the Bax/Bcl-2 ratio. mTOR (especially mTORC1) inhibits autophagy. During autophagy, dysfunctional parts degrades and this results in apoptosis alleviation

extract at 40 mg/kg/day dosage and group 4 received Saffron extract at 80 mg/kg/day dosage. Then, on day seven after surgery, the animals were sacrificed by injecting ketamine at a dose of 150 mg/kg. Tissue sampling was performed by excising a full-thickness skin from the intermediate margin between the necrotic area and the grossly healthy area [36]. The flaps were divided into two parts by a precise longitudinal incision, one component used to measure Mean MDA level, MPO and SOD activity, Bax, Bcl-2, mTOR, and p-mTOR expression (kept at -80°C). The other part used to determine pathological damage and parameters (preserved in formalin 10%), Fig. 3.

The study protocol was approved by Research Ethics Committees of School of Medicine—Tehran University of Medical Sciences (Tehran, Iran), Approval ID: IR.TUMS.MEDICINE.REC.1400.1013. All applicable institutional and/or national guidelines for the care and use of animals were followed.

Measurement of Flap Necrotic Area

The percentage of the necrotic area in each flap was calculated on day seven after flap surgery. For this purpose, the flap necrosis rate was computed using the paper pattern method. The boundary between living tissue and necrosis was determined by markers of viability (including skin color, skin softness, warmth, and hair) and signs of necrosis (including dark skin, stiffness, coolness, and hair loss). A mold was drawn from the entire flap, and a transparent paper of equal size was cut and weighed to Calculating the percentage of necrosis. Then, the amount of necrosis was drawn on transparent paper and cut and weighed. By dividing the weight of the part of the transparent paper that showed flap necrosis by the total weight of the translucent paper, the percentage of flap necrosis was obtained.

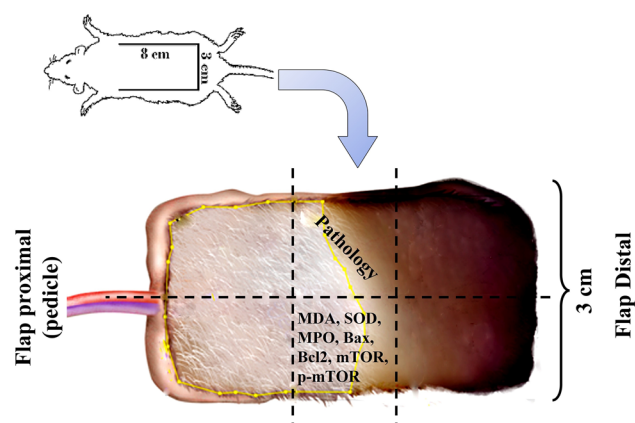


Fig. 3 Skin flap location. Tissue sampling was performed by excising a full-thickness skin from the intermediate margin between the necrotic area and the grossly healthy area

$$\begin{aligned} & \text{Percentage of flap necrosis (\%)} \\ &= \frac{\text{Weight of flap necrotic area transparent paper}}{\text{Total weight of the translucent paper}} \\ & \times 100 \end{aligned}$$

MDA Content, MPO and SOD Activity in the Flap Tissues

Tissue samples were obtained from each flap, weighed and then were homogenized in Tris-ethylenediamine ether acetic acid buffer (pH = 7.0 and 4°C) and diluted to 10% (v/v) in an ice bath. SOD enzyme activity was determined by using the kit method (ab65354, ab83464, Abcam), which was based on the method of Muthuraman et al. [37]. MDA was measured by determining the thiobarbituric acid reactive species (TBARS). The absorbance of the resultant product was measured at 534 nm (UV-Vis spectrophotometer). MPO activity in the flap tissue homogenates was determined based on commercially available MPO activity kit (Sigma-Aldrich; MAK069).

Western Blot Analysis to Determine Bax, Bcl-2, mTOR and P-mTOR Expression in the Flap Tissues

Mice were anesthetized at day 7. Immediately skin flaps were removed and frozen in liquid nitrogen and maintained at -80°C until used for western blot. We homogenized tissues in ice-cold RIPA lysis buffer (#9806, Cell Signaling Technology, Italy), containing protease inhibitor, to extract total protein. Later all samples were centrifuged at 13000 g for 20 min at 4°C , and then the supernatant was removed and protein concentration was quantified by spectrophotometer. Protein samples were boiled for 5 min at 95°C in sodium dodecyl sulfate loading buffer. Proteins (20 mg) were separated through 10% polyacrylamids SDS gels and transferred onto a nitrocellulose membrane. All membranes were incubated with primary antibodies for β -actin (Abcam; β -actin (ab8226), as loading control). For Bcl-2 analysis, we used rabbit polyclonal anti-rat Bcl-2 (ab59348), and for Bax analysis, we used anti-rat Bax (ab53154). We used mTOR (#2972, Rabbit polyclonal antibody for mTOR Cell Signaling Technology, Italy) and P-mTOR (#2974, Rabbit polyclonal antibody for Phospho-mTOR (Ser2481), Cell Signaling Technology, Italy) for mTOR and P-mTOR analyses. Then membranes were incubated with HRP-conjugated anti-mouse secondary antibody (#7072, Cell Signaling Technology, Italy). Membranes were developed with ECL plus reagent, and protein bands were visualized by exposing X-ray films, and finally quantified and analyzed by the lab work software.

Histological Evaluation

After day 7, flap tissues were collected, and consequently, the tissue preparation procedure for H&E staining was started. Tissue sections have been prepared by H&E staining on day seven and studied by light microscopy. Two expert pathologists blinded to the treatments, have evaluated all slides and they reported the results for Neovascularization, Fibroblast Migration, Epithelialization, and Epithelialization thickness. Scores were defined as follows: 0 = normal, 1 = mild, 2 = moderate, and 3 = severe for Neovascularization, Fibroblast Migration, Epithelialization, and Epithelialization thickness was reported by + or ++.

Statistical Analysis

All analysis was performed using GraphPad Prism 8 (GraphPad Software, San Diego, California, USA). One-way analysis of variance (ANOVA) and Post-Hoc analysis (Tukey test) were applied for comparing the biochemical data between groups. Data are presented as mean \pm SD, including 99% confidence interval (CI) ($\alpha = 0.01$).

Results

Percentage of the Flap Necrosis

Skin flap necrosis percentage in all flap samples determined at day seven after flap surgery and average of the necrotic of each group has been reported in Table 1, Supplementary Table 1, and illustrated in Fig. 4A. Sham group had the least flap necrosis (under 1%). Flap elevation led to a significant necrosis ($39.71 \pm 4.94\%$) in Flap Surgery (Control) group ($P < 0.0001$ vs. Sham). Saffron administration in 40 mg/kg/day dosage group, significantly decreased the necrotic percentage of the flap tissue to $23.47 \pm 3.54\%$ ($P < 0.0001$ vs. Flap Surgery group). Also, in 80

mg/kg/day dosage group, necrosis percentage significantly decreased to $19.77 \pm 4.60\%$ ($P < 0.0001$ vs. Flap Surgery group). We did not observe a significant difference in the flap necrosis percentage between Saffron 40 mg/kg/day and 80 mg/kg/day treated groups ($P = 0.162$). Difference between group means is illustrated in Fig. 4B. As shown in this figure, difference of the mean between the Sham group and Flap Surgery group is the most negative one, which indicates that flap surgery had an increasing effect on the flap necrosis percentage. In the other hand, the most positive differences of the means are related to the difference between Flap Surgery group and groups treated with Saffron, which indicates that Saffron treatment had a reducing effect on the flap necrosis percentage. Difference between Flap Surgery group mean, with Saffron 80 and Saffron 40 is 19.94% and 16.23%, respectively. Also, the difference between the two groups of Saffron 40 and Saffron 80 is almost close to the zero line, which shows that the results of these two groups are not significantly different.

MDA Level

Lipid peroxidation as an index of oxidative stress in the flap samples was determined based on mean MDA level in the tissue at day seven after flap surgery. MDA level of each group has been reported in Table 1, Supplementary Table 2, and illustrated in Fig. 5A. Sham group had the least MDA level (13.90 ± 10.58 nmol/mg protein). After flap elevation we observed a significant increase in the flap tissue MDA level in Flap Surgery group ($P < 0.0001$ vs. Sham group) and MDA level reached 69.83 ± 7.63 nmol/mg protein in this group. Saffron administration in 40 mg/kg/day dosage group, significantly decreased the MDA level of the flap tissue to 36.46 ± 10.00 nmol/mg protein ($P < 0.0001$ vs. Flap Surgery group). Also, in Saffron 80 mg/kg/day dosage group, MDA level significantly decreased to 36.34 ± 10.34 ($P < 0.0001$ vs. Flap Surgery group). We did not observe a significant difference in the MDA level between Saffron 40 mg/kg/day and 80 mg/

Table 1 Measured necrosis percentage, MDA level, SOD and MPO activity, Bax, Bcl-2, mTOR, and p-mTOR expression

	Unit	Sham	Flap Srg	Saffron 40	Saffron 80
Necrosis Percentage \pm SD	%	0.219 ± 1.512	39.714 ± 4.948	23.479 ± 3.545	19.770 ± 4.607
Mean MDA level \pm SD	n mol/mg protein	13.903 ± 10.587	69.838 ± 7.638	36.469 ± 10.005	36.345 ± 10.341
SOD Activity \pm SD	U/mg protein	69.943 ± 16.887	11.843 ± 3.692	66.963 ± 11.085	70.734 ± 8.578
MPO Activity \pm SD	U/mg protein	0.878 ± 0.158	1.962 ± 0.331	1.200 ± 0.180	1.256 ± 0.336
Bax Expression	Relative protein expression	0.515 ± 0.176	1.648 ± 0.405	0.791 ± 0.256	0.621 ± 0.239
Bcl-2 expression	Relative protein expression	1.599 ± 0.373	0.436 ± 0.116	1.709 ± 0.348	1.910 ± 0.247
mTOR expression	Relative protein expression	0.951 ± 0.213	1.048 ± 0.491	0.891 ± 0.310	0.921 ± 0.290
P-mTOR expression	Relative protein expression	1.199 ± 0.357	1.236 ± 0.161	1.309 ± 0.380	1.210 ± 0.271

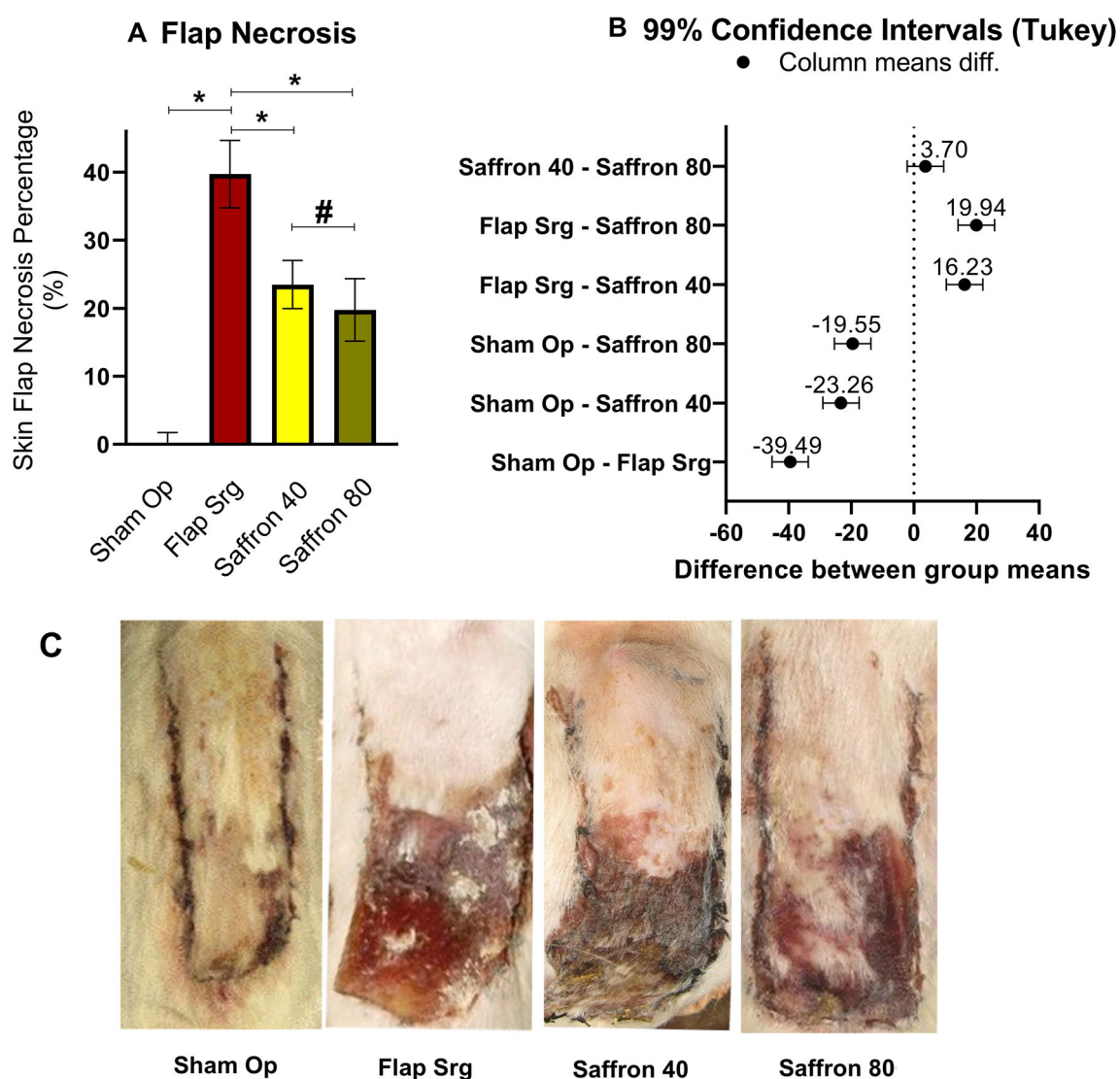


Fig. 4 **A** Flap necrosis percentage 7 days after surgery. Data are represented as Mean \pm SD. (*) $P < 0.0001$ and significant. (#) $P = 0.162$ and non-significant. **B** Difference between group means

kg/day treated groups ($P = 0.999$). Difference between group means is illustrated in Fig. 5B. As shown in this figure, difference of the mean between the Sham group and Flap Surgery group is the most negative one, which indicates that flap surgery had an increasing effect on the MDA level. In the other hand, the most positive differences of the means are related to the difference between Flap Surgery group and groups treated with Saffron, which indicates that Saffron treatment had a reducing effect on the MDA level. Difference between Flap Surgery group mean, with Saffron 80 and Saffron 40 is 33.49 nmol/mg protein and 33.36 nmol/mg protein, respectively. Also, the difference between the two groups of Saffron 40 and Saffron 80 is almost close to the zero line, which shows that the results of these two groups are not significantly different.

SOD Activity

SOD activity was used as an indicator of the antioxidant capacity of the flap tissue. SOD activity of each group has been reported in Table 1, Supplementary Table 3, and illustrated in Fig. 6A. Flap Surgery group had the least SOD activity (11.84 ± 3.69 U/mg protein) and we observed a significant decrease in the flap tissue SOD activity in this group ($p < 0.0001$ vs. Sham group). Saffron administration in 40 mg/kg/day dosage group, significantly increased SOD activity of the flap tissue to 66.96 ± 11.08 U/mg protein ($P < 0.0001$ vs. Flap Surgery group). Also, in Saffron 80 mg/kg/day dosage group, SOD activity significantly increased to 70.73 ± 8.57 U/mg protein ($P < 0.0001$ vs. Flap Surgery group). We did not observe a significant difference in the SOD activity between Sham, Saffron 40

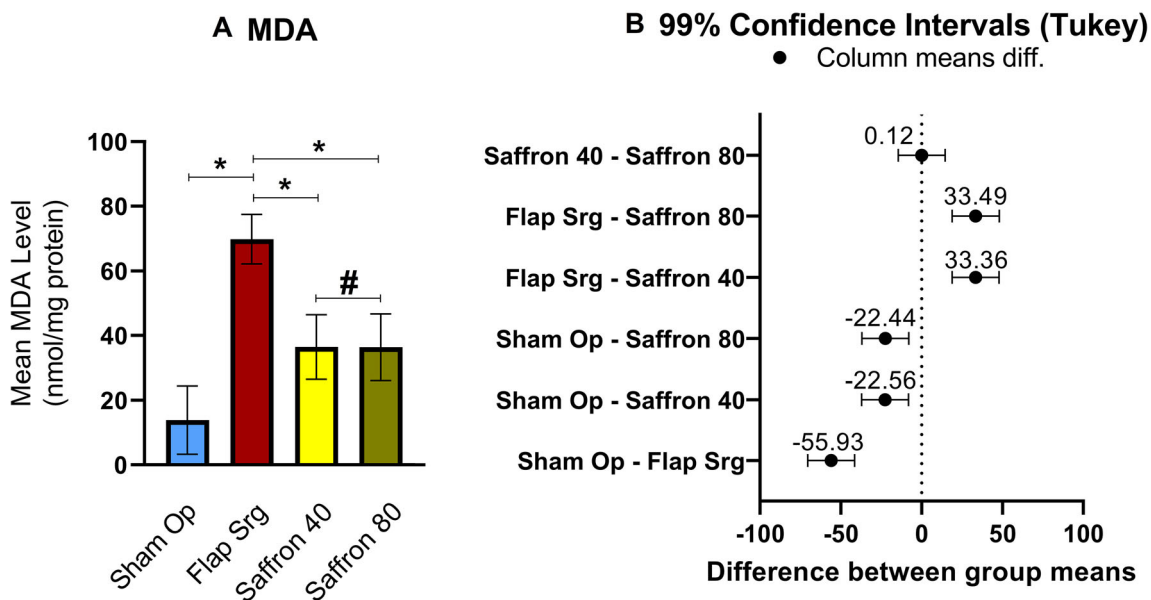


Fig. 5 **A** MDA level seven days after surgery. Data are represented as Mean ± SD. (*) $P < 0.0001$ and significant. (#) $P = 0.999$ and non-significant. **B** Difference between group means

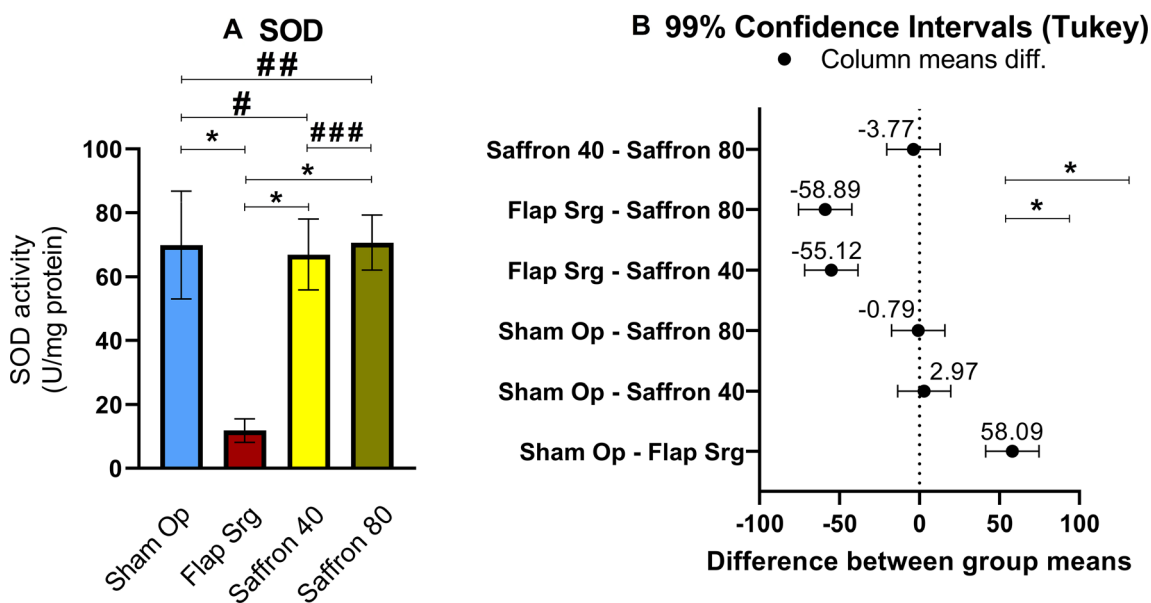


Fig. 6 **A** SOD activity seven days after surgery. Data are represented as Mean ± SD. * $P < 0.0001$ and significant. (#) $P = 0.931$, (##) $P = 0.998$, (###) $P = 0.872$ and non-significant. **B** Difference between group means

mg/kg/day and 80 mg/kg/day treated groups (Sham vs. Saffron 40 group: $P = 0.931$, Sham vs. Saffron 80 group: $P = 0.998$, Saffron 40 group vs. Saffron 80 group: $P = 0.872$). Difference between group means is illustrated in Fig. 6B. As shown in this figure, difference of the mean between the Sham group and Flap Surgery group is the most positive one, which indicates that flap surgery had a reducing effect on the SOD activity. In the other hand, the most negative differences of the means are related to the difference between Flap Surgery group and the groups

treated with Saffron, which indicates that Saffron treatment had an enhancing effect on the SOD activity. Difference between Flap Surgery group mean, with Saffron 80 and Saffron 40 is -58.89 U/mg protein and -55.12 U/mg protein, respectively. Also, the difference between the two groups of Saffron 40 and Saffron 80 is almost close to the zero line, which shows that the results of these two groups are not significantly different.

MPO Activity

Leucocyte infiltration to the flap tissue was measured by determining MPO activity levels in the flap tissue. MPO activity of each group has been reported in Table 1, Supplementary Table 4, and illustrated in Fig. 7A. Sham group had the least MPO activity (0.87 ± 0.15 U/mg protein). After flap elevation we observed a significant increase in the flap tissue MPO activity in Flap Surgery group ($P < 0.0001$ vs. Sham group) and MPO activity reached 1.96 ± 0.33 U/mg protein in this group. Saffron administration in 40 mg/kg/day dosage group, significantly decreased the MPO activity of the flap tissue to 1.20 ± 0.18 U/mg protein ($P < 0.0001$ vs. Flap Surgery group). Also, in Saffron 80 mg/kg/day dosage group, MPO activity significantly decreased to 1.25 ± 0.33 U/mg protein ($P < 0.0001$ vs. Flap Surgery group). We did not observe a significant difference in the MPO activity between Saffron 40 mg/kg/day and 80 mg/kg/day treated groups ($P = 0.964$). Difference between group means is illustrated in Fig. 7B. As shown in this figure, difference of the mean between the Sham group and Flap Surgery group is the most negative one, which indicates that flap surgery had an increasing effect on the MPO activity. In the other hand, the most positive differences of the means are related to the difference between Flap Surgery group and groups treated with Saffron, which indicates that Saffron treatment had a reducing effect on the MPO activity. Difference between Flap Surgery group mean, with Saffron 80 and Saffron 40 is 0.70 U/mg protein and 0.76 U/mg protein, respectively. Also, the difference between the two groups of Saffron 40

and Saffron 80 is almost close to the zero line, which shows that the results of these two groups are not significantly different.

Bax and Bcl-2 Expression

Expression of the Bax and Bcl-2 proteins were measured by western blotting. Bax is as a pro-apoptotic indicator and Bcl-2 is as an anti-apoptotic indicator. On each sample the expression of the Bax or Bcl-2 was normalized to the expression of the control protein beta-actin. Bax expression of each group has been reported in Table 1, Supplementary Table 5, and illustrated in Figs. 8A, 10A and 11. Sham group had the least Bax expression (0.51 ± 0.17). After flap elevation we observed a significant increase in the flap tissue Bax expression in Flap Surgery group ($P < 0.0001$ vs. Sham group) and Bax expression reached 1.64 ± 0.40 in this group. Saffron administration in 40 mg/kg/day dosage group, significantly decreased the Bax expression of the flap tissue to 0.75 ± 0.25 ($P < 0.0001$ vs. Flap Surgery group). Also, in Saffron 80 mg/kg/day dosage group, Bax expression significantly decreased to 0.62 ± 0.23 ($P < 0.0001$ vs. Flap Surgery group). We did not observe a significant difference in the Bax expression between Saffron 40 mg/kg/day and 80 mg/kg/day treated groups ($P = 0.540$). Difference between group means is illustrated in Fig. 8B. As shown in this figure, difference of the mean between the Sham group and Flap Surgery group is the most negative one, which indicates that flap surgery had an increasing effect on the Bax expression. In the other hand, the most positive differences of the means are related

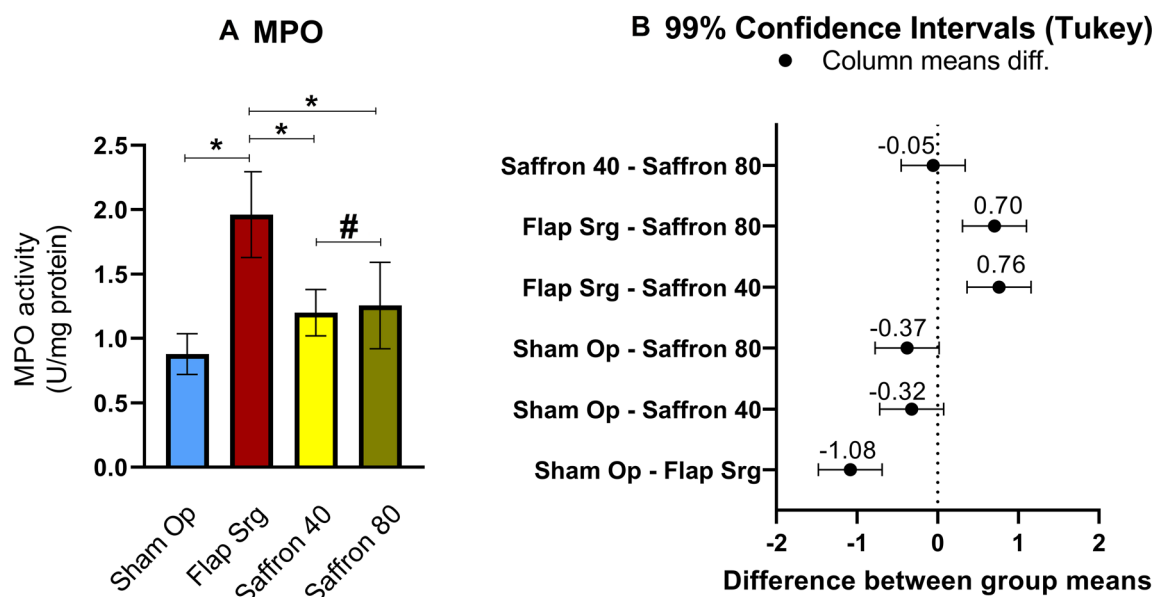


Fig. 7 **A** MPO activity seven days after surgery. Data are represented as Mean \pm SD. (*) $P < 0.0001$ and significant. (#) $P = 0.964$ and non-significant. **B** Difference between group means

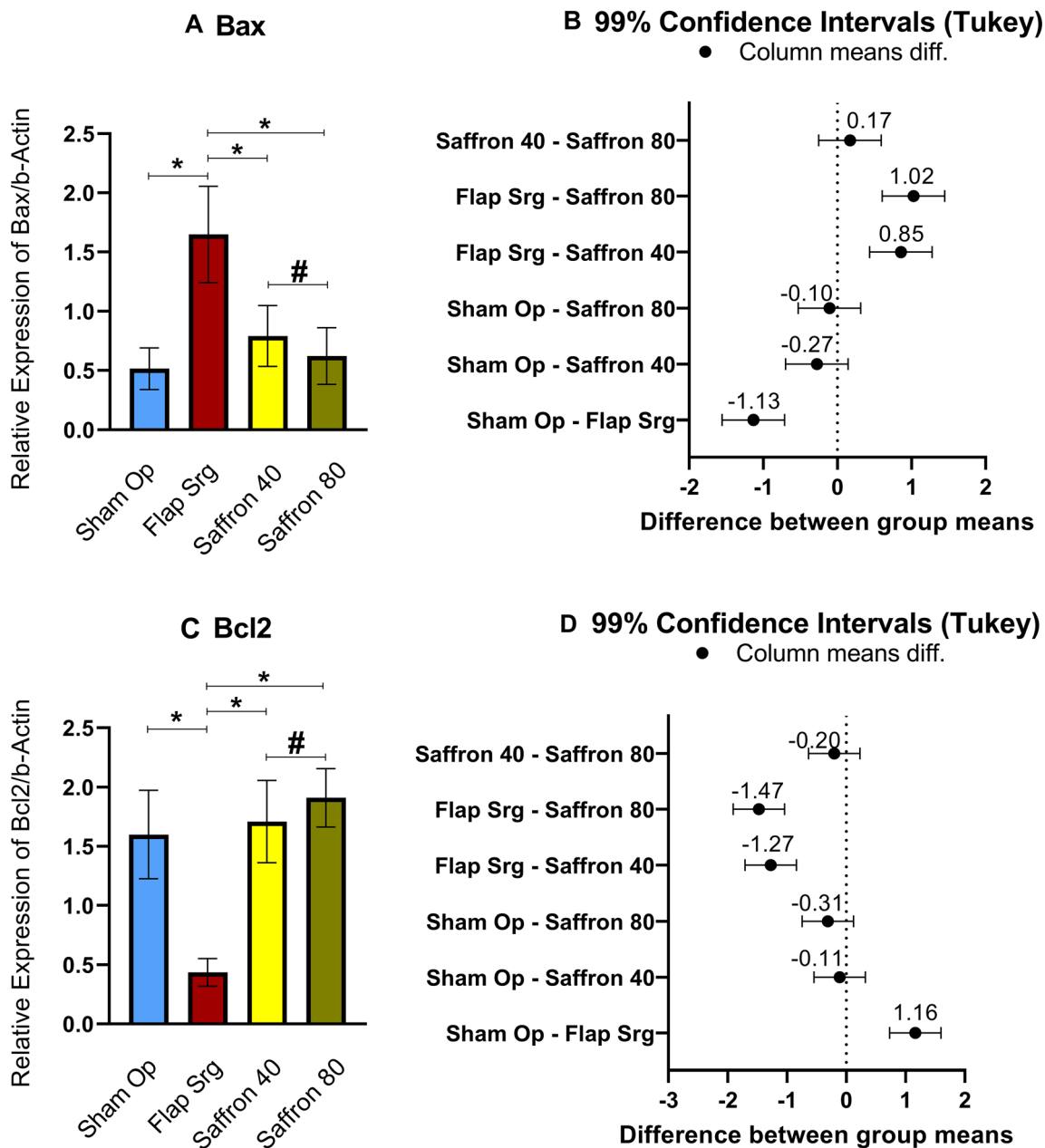


Fig. 8 Relative expression of protein/b-actin 7 days after surgery. Data are represented as Mean ± SD. **A** Bax expression. **C** Bcl-2 expression. (*) $P < 0.0001$ and significant. # in diagram **A** $P = 0.540$

and non-significant. # in diagram **C** $P = 0.418$ and non-significant. **B** and **D** Difference between group means

to the difference between Flap Surgery group and groups treated with Saffron, which indicates that Saffron treatment had a reducing effect on the Bax expression. Difference between Flap Surgery group mean, with Saffron 80 and Saffron 40 is 1.02 and 0.85, respectively. Also, the difference between the two groups of Saffron 40 and Saffron 80 is almost close to the zero line, which shows that the results of these two groups are not significantly different.

Bcl-2 expression of each group has been reported in Table 1, Supplementary Table 6, and illustrated in

Figs. 8C, 10A and 11. Flap Surgery group had the least Bcl-2 expression (0.43 ± 0.11) and we observed a significant decrease in the flap tissue Bcl-2 expression in this group ($p < 0.0001$ vs. Sham group). Saffron administration in 40 mg/kg/day dosage group, significantly increased Bcl-2 expression of the flap tissue to 1.70 ± 0.34 ($P < 0.0001$ vs. Flap Surgery group). Also, in Saffron 80 mg/kg/day dosage group, Bcl-2 expression significantly increased to 1.91 ± 0.21 ($P < 0.0001$ vs. Flap Surgery group). We did not observe a significant difference in the Bcl-2 expression

between Sham, Saffron 40 mg/kg/day and 80 mg/kg/day treated groups (Sham vs. Saffron 40 group: $P = 0.828$, Sham vs. Saffron 80 group: $P = 0.094$, Saffron 40 group vs. Saffron 80 group: $P = 0.418$). Difference between group means is illustrated in Fig. 8D. As shown in this figure, difference of the mean between the Sham group and Flap Surgery group is the most positive one, which indicates that flap surgery had a reducing effect on the Bcl-2 expression. In the other hand, the most negative differences of the means are related to the difference between Flap Surgery group and the groups treated with Saffron, which indicates that Saffron treatment had an enhancing effect on the Bcl-2 expression. Difference between Flap Surgery group mean, with Saffron 80 and Saffron 40 is -1.47 and -1.27 , respectively. Also, the difference between the two groups of Saffron 40 and Saffron 80 is almost close to the zero line, which shows that the results of these two groups are not significantly different.

mTOR and p-mTOR Expression

mTOR and p-mTOR expression were evaluated in flap tissues to determine whether Saffron affects via modifying this pathway or not. The results are prepared in Table 1, Supplementary Table 7 and 8, and illustrated in Figs. 9, 10B and 11. There were no significant differences between any of the experimental groups for both mTOR and p-mTOR expression. It is obvious that according to heat map of protein expression (Fig. 11), there is no significant change between expression of mTOR and p-mTOR in experimental groups.

Histopathologic Changes

Histologic sections of flap tissue are prepared in Table 2 and illustrated in Fig. 12. Neovascularization as an important effect of treatment has been investigated in samples. Flap Surgery group sections was fully necrotized. Saffron 40 group showed moderate epithelialization, fibroblast migration, and neovascularization. Saffron 80 group showed severe epithelialization, moderate fibroblast migration and neovascularization. Epithelialization thickness in Saffron 40 group and Saffron 80 group, was + and ++, respectively.

Discussion

Despite all scientific advances and the use of methods that increase skin flap survival, the maintenance of flap tissue against necrosis and tissue damage is still a major concern for surgeons and researchers [1, 38]. So, for the first time in the present study, we aimed to evaluate the effect of

Saffron (*Crocus sativus* L.) on Skin flap survival and investigate whether it affects via mTOR pathway. Our studies were performed at three levels: macroscopic, microscopic and molecular. Our results indicated that the use of Saffron extract significantly reduced tissue necrosis rate. We observed that MDA (as a marker of lipid peroxidation), MPO (as a marker of neutrophil infiltration), and Bax (pro-apoptotic) significantly decreased in Saffron treated groups. In the other hand, SOD (anti-ROS enzyme), Bcl-2 (anti-apoptotic), neovascularization, epithelialization, epithelialization thickness, and fibroblast migration increased consequent to Saffron administration. Saffron had also no effect on mTOR or p-mTOR expression.

Nowadays, increasing of skin flap survival is the challenging issue of research. The survival of skin flaps is threatened by mal-perfusion, mal-oxygenation, low excretion and retention of tissue waste, necrosis and apoptosis, ischemia-induced injury, reperfusion injury, ROS injury, and inflammatory processes. It seems much more useful to avoid the creation of flap threatening processes and factors, rather than confronting them such as prevention of tissue necrosis via reduction of ROS production, elimination of created ROS, increasing of total antioxidant capacity (TAC) of flap tissue, and even use of exogenous antioxidants [10, 15, 36, 39, 40].

Studies have demonstrated the protective effect of Saffron on oxidative stress in different tissues such as abdominal organs, kidney, testis, and endothelial cells [21, 41–46]. Crocin, safranal, carotene and crocetin as the most abundant constituents of Saffron stigmas, represent antioxidant effects. Noticeably, Saffron extract is mentioned as a potent anti-oxidant with high ability to scavenge ROS, especially superoxide anions and hydrogen peroxide (H_2O_2) resulting in inhibitory effect on lipid peroxidation [22, 29, 32, 41]. Also, Saffron is suggested in autoimmune encephalomyelitis, and cerebral ischemia [23, 47, 48]. In these ischemic models, Saffron or its active components, reduced MDA level and lipid peroxidation, increased the activity of SOD, GPx, and antioxidant capacity.

In our study, flap necrosis and MDA levels increased with skin flap surgery. MDA, as a stable metabolite of lipid peroxidation, indicates the amount of oxidative stress. Our results also showed that flap necrosis and the level of MDA in skin flap tissue was significantly reduced by administering Saffron. This directly confirms the antioxidant effect of Saffron on the skin flap tissue. By reducing oxidative stress, peroxidation and degradation of lipids are also reduced and less MDA is produced. By a decrease in lipid degradation, it can be expected that cell membranes remain undamaged which potentially prevents arachidonic acid-dependent inflammation. Considering that we have administered Saffron orally, it is possible to have a good assurance of the absorption and bioavailability of Saffron,

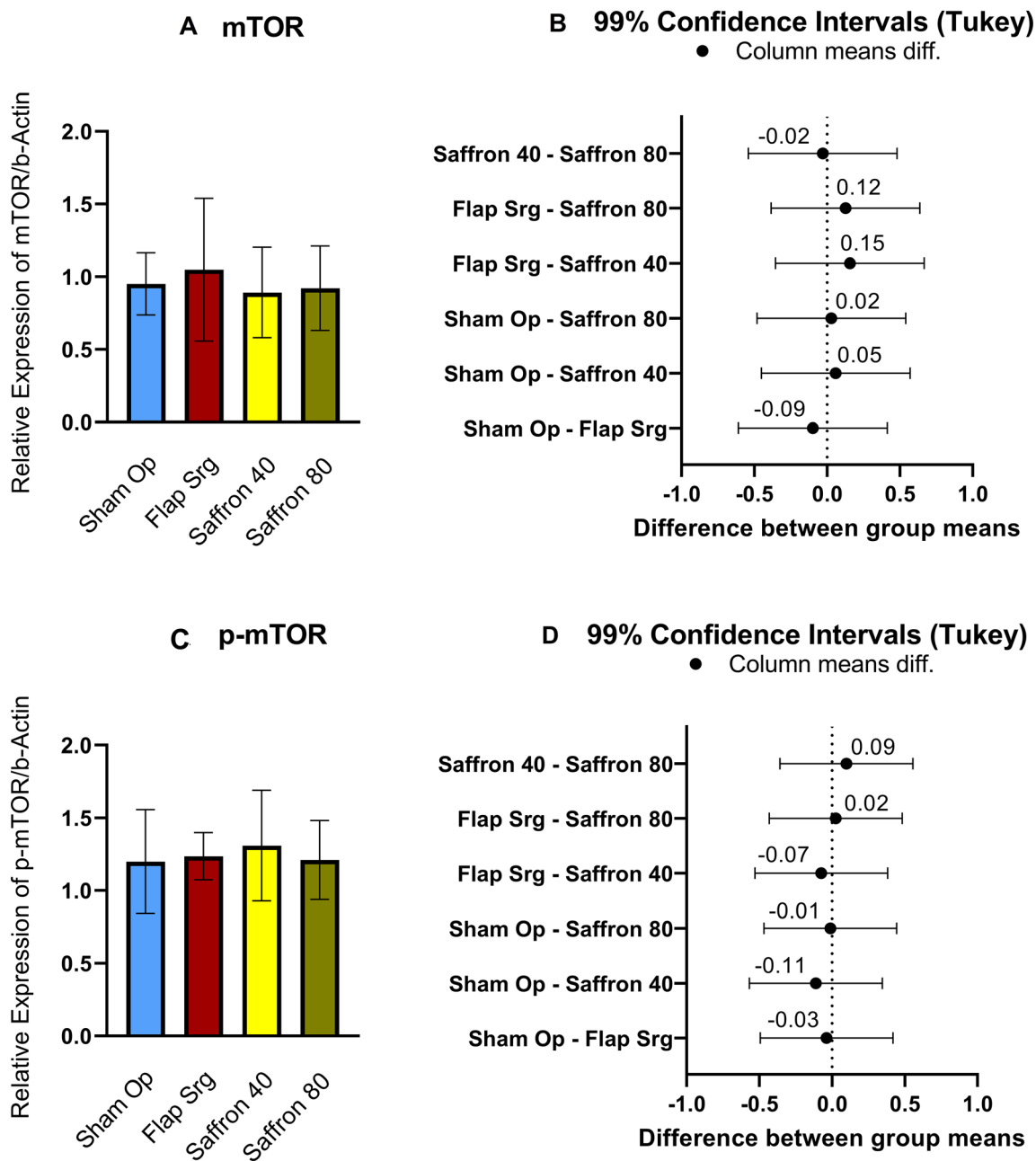


Fig. 9 Relative expression of protein/b-actin seven days after surgery. Data are represented as Mean \pm SD. **A** mTOR expression. **C** p-mTOR expression. Comparison of every group with another one

was non-significant for both mTOR and p-mTOR expression. **B** and **D** Difference between group means

because its effects have appeared in the skin tissue. Oral administration has numerous benefits, such as easily applicability, availability, and being more physiologic. All of these results are consistent with pervious findings which mentioned above [5, 49]. Another study [30] has evaluated Saffron effect on mitogen-activated protein kinase (MAPK) signaling pathways, in mediation of endothelial cell-survival against oxidative stress. The results of this study showed that Saffron administration has improved viability of cells in response to H₂O₂-induced toxicity and

has reduced production of ROS in H₂O₂-treated cells. It is a confirmation to our results.

Superoxide dismutase (SOD) is an enzyme that scavenges superoxide radicals and prevents their destructive effects. In our study, skin flap surgery significantly reduced SOD activity in the flap tissue. This means that flap surgery reduces ability of tissue to cope with oxidative stress. In the groups that treated with Saffron, the SOD activity increased significantly. Therefore, it can be concluded that Saffron has increased tissue ability to deal with reactive

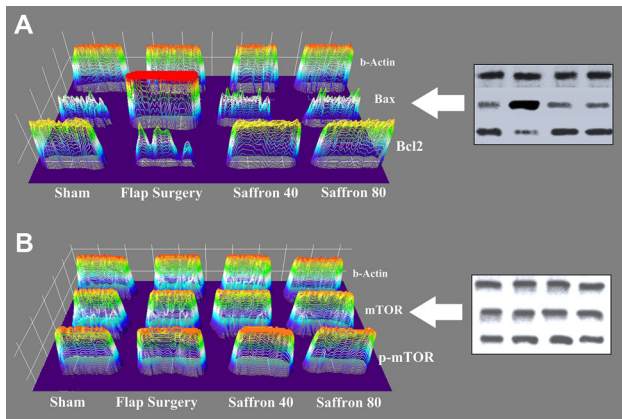


Fig. 10 3-Dimensional Western blot data virtualization of Bax, Bcl-2, mTOR, p-mTOR and b-actin expression

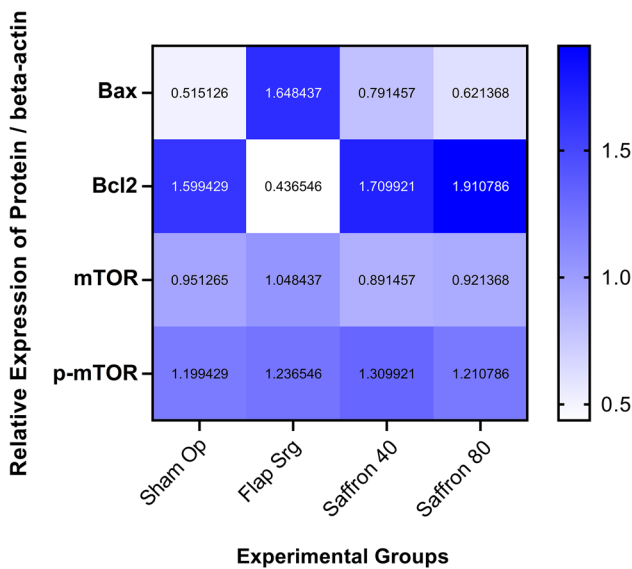


Fig. 11 Heat map of Bax, Bcl-2, mTOR, and p-mTOR expression in skin flap tissue

oxygen species. In recent research [50] about crocin effect on the viability of random pattern skin flaps, the authors have reported SOD increases after crocin application. This research and aforementioned ones [6, 11, 18, 28, 38, 42] is in line with our results and have reported that SOD increasing would result in tissue protection against ROS damage [16, 51].

Recent articles have also focused on anti-inflammatory properties of Saffron [52] and even declared that Saffron

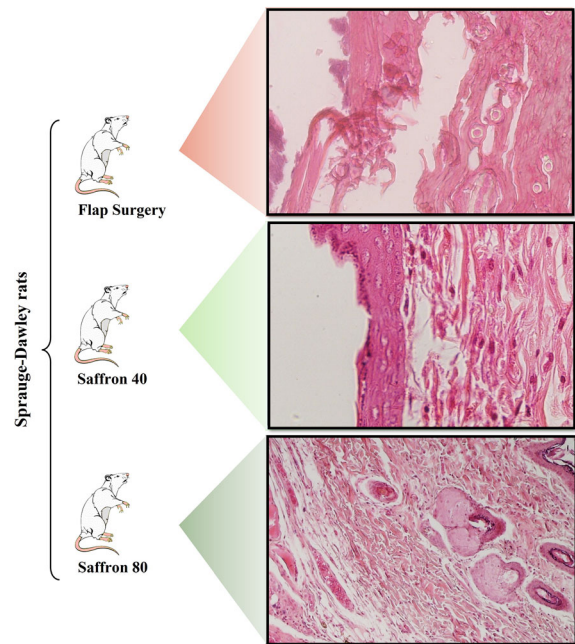


Fig. 12 Histopathologic study: H&E staining ($\times 100$ magnification) of Flap Surgery group, Saffron 40 group, and Saffron 80 group. In Saffron treated groups, healing level increased and necrosis decreased

represents a promising substance for toning down cytokine storm during COVID-19, as well as a potential preventive treatment for COVID-19 [26]. Scholars also mentioned that crocin anti-inflammatory properties results from effecting on cyclooxygenase pathway and inhibition of both cyclooxygenase1 (COX1), and cyclooxygenase 2 (COX2) enzymes, and inhibition of prostaglandin E2 (PGE -2) production, in a dose dependent manner [53]. Crocin has presented an inhibitory role on the high levels of bone joint exo-glycosidases, cathepsin-D and tartarate resistant acid phosphatases. Saffron and crocin are used to show relieving effects on arthritis by modifying cartilage deteriorating enzymes, inflammatory mediators, and matrix metalloproteinases MMPs [28]. The Myeloperoxidase (MPO) enzyme is active in tissue-infiltrated neutrophils, producing oxygen-reactive species so that neutrophils can fight their targets with these ROS. Therefore, the activity of MPO enzyme is a manifestation of inflammation and chemotaxis of neutrophils. In addition to other effects of inflammation, these ROS also cause damage to the skin flap tissue [1, 36, 49]. In our study, skin flap surgery increased MPO

Table 2 Results of histopathologic study

	Epithelialization	Epithelialization thickness	Fibroblast migration	Neovascularization
Grading	0–3	+/++	0–3	0–3
Flap surgery	Necrosis	Necrosis	Necrosis	Necrosis
Saffron 40	2	+	2	2
Saffron 80	3	++	2	2

activity, but in the groups that received Saffron, the enzyme activity level decreased significantly and resulted in an increase of skin flap survival. However, this effect did not change much with increasing the dose of Saffron in our experiment, and in both groups of Saffron 40 and Saffron 80, the rate of MPO activity decrease, was the same. Other studies [1, 49, 54] confirm our results about increasing the survival of skin flaps following a decrease in MPO activity.

In addition to tissue necrosis, apoptosis is mentioned as a noticeable process in tissue loss. Numerous mediators could co/regulate apoptosis pathways. It would be a challenging issue that except decrease in ROS, necrosis, and tissue inflammation, we could increase anti-apoptotics and decrease pro-apoptotics, which would directly result in enhancement of skin flap survival [16, 55]. Our study showed skin flap surgery results in increasing of Bax (pro-apoptotic) and decreasing of Bcl-2 (anti-apoptotic), meaning apoptosis promotion and consequent skin flap tissue loss. Saffron administration reversed this issue and resulted in decreasing of flap tissue loss and also expression of Bax, and increasing of tissue viability and Bcl-2. So, it might have concluded that Saffron moderates tissue loss via effecting on the expression intracellular apoptosis regulators. Also, a study [30] demonstrated that apoptosis has reduced significantly by Saffron administration in endothelial cells, which is similar to our results. Other studies on skin flap survival have confirmed that skin flap viability enhances with Bcl-2 increasing and Bax decreasing [16, 56, 57].

New research in recent years have suggested the role of autophagy in apoptosis, and declared that autophagy could decrease apoptosis, oxidative damage, and skin flap tissue loss. Moreover, the mTOR signaling pathway has been shown to act as an inhibitor of autophagy. We aimed to study the possible involvement of the mTOR pathway in the protective effects of Saffron. Studies have been performed on the effect of Crocin in liver cancer [58] and Crocetin in breast cancer [59] and their effects on autophagy have been reported, but the results of our study showed that Saffron has no effect on mTOR or p-mTOR expression in skin flap tissue. We did not observe significant mTOR and p-mTOR changes between groups. This indicates that Saffron effect on flap survival might be independent of mTOR and p-mTOR expression.

Histologically, neovascularization causes better perfusion and blood supply, so it prevents necrosis. Fibroblast migration helps tissue repair and epithelialization is as an indicator of healing process. In our study, Saffron treatment increased all of these items. Our results are consistent with previous studies [1, 6, 18, 50, 60–62].

Finally, it seems that Saffron has protective effect on skin tissue and could prevent skin flap tissue loss. Our study was an experimental study and all limitations of

experimental studies apply to ours. More research is needed to determine all the positive effects or toxicities of Saffron. To determine the greater extent of the impact of Saffron on the survival of the skin flap, it is suggested that researchers who will work on this issue in the future consider (1) measurement of skin flap survival while using mTOR inhibitors such as rapamycin to determine the contribution of this pathway to the rate of tissue loss, and (2) immunohistochemistry (IHC) examination of flap tissue for vascular growth factors (3) investigation of other molecular markers that are related to autophagy (4) measure markers at RNA level and protein level (by western blotting and IHC).

Conclusion

Firstly, according to the results, it could be concluded that Saffron has a great potential to be applied for increasing survival of skin flap tissue due to its ability to decrease apoptosis, oxidative stress and inflammatory-derived oxidative injury, and also enhancing flap survival via more effective neo-vascularization and epithelialization. Finally, we declare that all of aforementioned effects are independent of mTOR expression.

Secondly, this study gave an overall view to researchers and aesthetic surgeons about the positive effects of Saffron on random skin flap survival. Moreover, our study was experimental and it is not possible to use our method in skin flap surgeries immediately. Therefore, this is an opportunity for clinical researchers and aesthetic surgeons to design a clinical trial to approve our study and find a proper dose of Saffron for clinical use. All efforts are worthy to achieve enhanced life quality of patients.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00266-022-03048-6>.

Acknowledgement The authors would like to thank the Iran University of Medical Sciences, Tehran, Iran.

Author Contributions Study conception and design: Z.H., M.H., H.P., M.A., G.H. Acquisition of data: Z.H., H.P., M.B. Analysis and Interpretation of data: M.B., Z.H. Drafting of Manuscript: Z.H., M.B., H.P., M.H., G.H. Critical Revision: All authors

Funding The authors have no financial relationships relevant to this article to disclose. No funding source

Declarations

Conflict of interest The authors declare that they have no conflicts of interest to disclose.

Ethical Approval The study protocol was approved by Research Ethics Committees of School of Medicine - Tehran University of

Medical Sciences (Tehran, Iran), Approval ID: IR.TUMS.MEDICINE.REC.1400.1013. All applicable institutional and/or national guidelines for the care and use of animals were followed.

Informed Consent For this type of study informed consent is not required.

References

- Aryannejad A, Gandominejad A, Tabary M, Noroozi N, Abbasi A, Araghi F, Mohammad Jafari R, Dehpour AR (2021) Protective effect of modafinil on skin flap survival in the experimental random-pattern skin flap model in rats: The role of ATP-sensitive potassium channels and nitric oxide pathway. *J Plast Reconstr Aesthet Surg* 74(6):1346–1354
- Li D, Zhang R, Zhang Q, Xu Z, Xu F, Li Y, Chen X (2020) Clinical results of ear elevations in patients with microtia using skin grafts from three donor sites: a retrospective study. *Aesthet Plast Surg* 44(5):1545–1552
- Li Y, Cui C, Zhang R, Zhang Q, Xu Z, Xu F, Li D (2018) Anatomical and histological evaluation of the retroauricular fascia flap for staged auricular reconstruction. *Aesthet Plast Surg* 42(3):625–632
- Li Y, Zhang R, Zhang Q, Xu Z, Xu F, Li D (2017) An Alternative posterosuperior auricular fascia flap for ear elevation during microtia reconstruction. *Aesthet Plast Surg* 41(1):47–55
- Barati B, Zandi A, Asadi M (2021) Enhancement the skin flap survival in mice applying topical and systemic atorvastatine: a randomized clinical trial study. *Adv Oral Maxillofac Surg* 2(5):100049
- Ma X, Lin Y, Liu Y, Li W, He J, Fang M, Lin D (2021) Effects of Apigenin treatment on random skin flap survival in rats. *Front Pharmacol* 12:625733
- Souza RAC, Martinelli-Kläy CP, d’Acampora AJ, Bernardes GJS, Sgrott SM, Souza LAC, Lombardi T, Sudbrack TR (2021) Effects of sildenafil and tadalafil on skin flap viability. *Arch Dermatol Res*
- Li J, Bao G, Alyafeai E, Ding J, Li S, Sheng S, Shen Z, Jia Z, Lin C, Zhang C, Lou Z (2019) Betulinic acid enhances the viability of random-pattern skin flaps by activating autophagy. *Front Pharmacol* 10:1017
- Li Y, Jiang Q-L, van der Merwe L, Lou D-H, Lin C (2021) Preclinical efficacy of stem cell therapy for skin flap: a systematic review and meta-analysis. *Stem Cell Res Ther* 12(1):28
- Wu H, Chen H, Zheng Z, Li J, Ding J, Huang Z, Jia C, Shen Z, Bao G, Wu L, Mamun AA, Xu H, Gao W, Zhou K (2019) Trehalose promotes the survival of random-pattern skin flaps by TFEB mediated autophagy enhancement. *Cell Death Dis* 10(7):483
- Uslu AB (2020) Effect of dipyrindamole on random pattern skin flap viability in rats. *J Plast Surg Hand Surg* 54(4):240–247
- Mohammadi Z, Sharif Zak M, Majdi H, Mostafavi E, Barati M, Lotfimehr H, Ghaseminasab K, Pazoki-Toroudi H, Webster TJ, Akbarzadeh A (2019) The effect of chrysin-curcumin-loaded nanofibres on the wound-healing process in male rats. *Artif Cells Nanomed Biotechnol* 47(1):1642–1652
- Lou J, Zhang H, Qi J, Xu Y, Wang X, Jiang J, Hu X, Ni L, Cai Y, Wang X, Gao W, Xiao J, Zhou K (2022) Cyclic helix B peptide promotes random-pattern skin flap survival via TFE3-mediated enhancement of autophagy and reduction of ROS levels. *Br J Pharmacol* 179(2):301–321
- Asadi N, Pazoki-Toroudi H, Del Bakhshayesh AR, Akbarzadeh A, Davaran S, Annabi N (2021) Multifunctional hydrogels for wound healing: special focus on biomacromolecular based hydrogels. *Int J Biol Macromol* 170:728–750
- Xin D, Quan R, Zeng L, Xu C, Tang Y (2020) Lipoxin A4 protects rat skin flaps against ischemia-reperfusion injury through inhibiting cell apoptosis and inflammatory response induced by endoplasmic reticulum stress. *Ann Transl Med* 8(17):1086
- Zhou F, Zhang X, Jiang L, Li S, Chen Y, Wu J (2021) Pseudoginsenoside F11 enhances the viability of random-pattern skin flaps by promoting TFEB nuclear translocation through AMPK-mTOR signal pathway. *Front Pharmacol* 12:667524
- Wu H, Ding J, Li S, Lin J, Jiang R, Lin C, Dai L, Xie C, Lin D, Xu H, Gao W, Zhou K (2019) Metformin promotes the survival of random-pattern skin flaps by inducing autophagy via the AMPK-mTOR-TFEB signaling pathway. *Int J Biol Sci* 15(2):325–340
- Tu Z, Jiang X, Li Y, Yang S, Lin D, Shi Y, Mao C, Zhang X, Lin C (2021) Curcumin promotes the survival of ischemic random skin flaps via autophagy. *Am J Transl Res* 13(3):1337–1351
- Moradzadeh M, Sadeghnia HR, Tabarraei A, Sahebkar A (2018) Anti-tumor effects of crocetin and related molecular targets. *J Cell Physiol* 233(3):2170–2182
- Samarghandian S, Boskabady MH, Davoodi S (2010) Use of in vitro assays to assess the potential antiproliferative and cytotoxic effects of saffron (*Crocus sativus* L.) in human lung cancer cell line. *Pharmacogn Mag* 6(24):309–314
- Su X, Yuan C, Wang L, Chen R, Li X, Zhang Y, Liu C, Liu X, Liang W, Xing Y (2021) The beneficial effects of saffron extract on potential oxidative stress in cardiovascular diseases. *Oxid Med Cell Longev* 2021:6699821
- Ghadroost B, Vafaei AA, Rashidy-Pour A, Hajisoltani R, Bandedgi AR, Motamedi F, Haghghi S, Sameni HR, Pahlvan S (2011) Protective effects of saffron extract and its active constituent crocin against oxidative stress and spatial learning and memory deficits induced by chronic stress in rats. *Eur J Pharmacol* 667(1–3):222–229
- Azami S, Shahriari Z, Asgharzade S, Farkhondeh T, Sadeghi M, Ahmadi F, Vahedi MM, Forouzanfar F (2021) Therapeutic potential of saffron (*Crocus sativus* L.) in ischemia stroke. *Evid Based Complement Altern Med* 2021:6643950
- Zandi N, Pazoki B, Momeni Roudsari N, Lashgari N-A, Jamshidi V, Momtaz S, Abdolghaffari AH, Akhondzadeh S (2021) Prospects of saffron and its derivatives in Alzheimer’s disease. *Arch Iran Med* 24(3):233–252
- Su X, Li X-Y, Zhang Y-J, Liu Y-S, Yuan C, Yang F, Liu C, Chen H-W, Xiong X-J, Gao Y-H, Xing Y-W (2021) Cardioprotective effect of saffron total glycoside tablets in patients with breast cancer receiving anthracycline-based chemotherapy: study protocol for a multicentre, randomised, parallel, double-blind, placebo-controlled clinical trial. *Ann Palliat Med* 10(7):8283–8291
- Mentis A-FA, Dalamaga M, Lu C, Polissiou MG (2021) Saffron for “toning down” COVID-19-related cytokine storm: hype or hope? A mini-review of current evidence. *Metabol Open* 11:100111
- Husaini AM, Jan KN, Wani GA (2021) Saffron: A potential drug-supplement for severe acute respiratory syndrome coronavirus (COVID) management. *Heliyon* 7(5):e07068
- Bukhari SI, Manzoor M, Dhar MK (2018) A comprehensive review of the pharmacological potential of *Crocus sativus* and its bioactive apocarotenoids. *Biomed Pharmacother* 98:733–745
- Jam IN, Sahebkar AH, Eslami S, Mokhber N, Nosrati M, Khaledi M, Foroutan-Tanha M, Ghayour-Mobarhan M, Hadizadeh F, Ferns G, Abbasi M (2017) The effects of crocin on the symptoms of depression in subjects with metabolic syndrome. *Adv Clin Exp Med* 26(6):925–930
- Rahiman N, Akaberi M, Sahebkar A, Emami SA, Tayarani-Najaran Z (2018) Protective effects of saffron and its active

- components against oxidative stress and apoptosis in endothelial cells. *Microvasc Res* 118:82–89
31. Papandreou MA, Tsachaki M, Efthimiopoulos S, Cordopatis P, Lamari FN, Margarity M (2011) Memory enhancing effects of saffron in aged mice are correlated with antioxidant protection. *Behav Brain Res* 219(2):197–204
 32. Hosseinzadeh H, Modaghegh MH, Saffari Z (2009) *Crocus sativus* L. (Saffron) extract and its active constituents (crocin and safranal) on ischemia-reperfusion in rat skeletal muscle. *Evid Based Complement Altern Med* 6(3):343–350
 33. Premkumar K, Abraham SK, Santhiya ST, Gopinath PM, Ramesh A (2001) Inhibition of genotoxicity by saffron (*Crocus sativus* L.) in mice. *Drug Chem Toxicol* 24(4):421–428
 34. Premkumar K, Abraham SK, Santhiya ST, Ramesh A (2003) Protective effects of saffron (*Crocus sativus* Linn.) on genotoxins-induced oxidative stress in Swiss albino mice. *Phytother Res* 17(6):614–617
 35. McFarlane RM, Deyoung G, Henry RA (1965) The design of a pedicle flap in the rat to study necrosis and its prevention. *Plast Reconstr Surg* 35:177–182
 36. Tabary M, Aryannejad A, Noroozi N, Tavangar SM, Mohammad Jafari R, Araghi F, Dadkhahfar S, Dehpour AR (2021) Ivermectin increases random-pattern skin flap survival in rats: The novel role of GABAergic system. *J Surg Res* 259:431–441
 37. Pandurangan M, Veerappan M, Kim DH (2015) Cytotoxicity of zinc oxide nanoparticles on antioxidant enzyme activities and mRNA expression in the cocultured C2C12 and 3T3-L1 cells. *Appl Biochem Biotechnol* 175(3):1270–1280
 38. Rasti Ardakani M, Al-Dam A, Rashad A, Shayesteh Moghadam A (2017) Effect of systemic antioxidant allopurinol therapy on skin flap survival. *World J Plast Surg* 6(1):54–61
 39. Mehrjerdi FZ, Aboutaleb N, Pazoki-Toroudi H, Soleimani M, Ajami M, Khaksari M, Safari F, Habibey R (2015) The protective effect of remote renal preconditioning against hippocampal ischemia reperfusion Injury: role of KATP channels. *J Mol Neurosci* 57(4):554–560
 40. Pazoki-Toroudi HR, Ajami M, Habibey R (2010) Pre-medication and renal pre-conditioning: a role for alprazolam, atropine, morphine and promethazine. *Fundam Clin Pharmacol* 24(2):189–198
 41. Naderi R, Pardakhty A, Abbasi MF, Ranjbar M, Iranpour M (2021) Preparation and evaluation of crocin loaded in nanoniosomes and their effects on ischemia-reperfusion injuries in rat kidney. *Sci Rep* 11(1):23525
 42. Arjmand M-H, Hashemzehi M, Soleimani A, Asgharzadeh F, Avan A, Mehraban S, Fakhraei M, Ferns GA, Ryzhikov M, Gharib M, Salari R, Sayyed Hoseinian SH, Parizadeh MR, Khazaei M, Hassanian SM (2021) Therapeutic potential of active components of saffron in post-surgical adhesion band formation. *J Tradit Complement Med* 11(4):328–335
 43. Morvaridzadeh M, Agah S, Dulce Estêvão M, Hosseini AS, Heydari H, Toupchian O, Abdollahi S, Persad E, Abu-Zaid A, Rezamand G, Heshmati J (2021) Effect of saffron supplementation on oxidative stress parameters: a systematic review and meta-analysis of randomized placebo-controlled trials. *Food Sci Nutr* 9(10):5809–5819
 44. Sefidgar SM, Ahmadi-Hamedani M, Jebelli Javan A, Narenji Sani R, Javaheri Vayghan A (2019) Effect of crocin on biochemical parameters, oxidative/antioxidative profiles, sperm characteristics and testicular histopathology in streptozotocin-induced diabetic rats. *Avicenna J Phytomed* 9(4):347–361
 45. Vaez A, Mardani M, Razavi S (2014) Impact of saffron on rat sperm membrane integrity and spermatogenesis status. *Adv Biomed Res* 3:146
 46. Habibey R, Ajami M, Ebrahimi SA, Hesami A, Babakoochi S, Pazoki-Toroudi H (2010) Nitric oxide and renal protection in morphine-dependent rats. *Free Radic Biol Med* 49(6):1109–1118
 47. Abdel-Rahman RF, El Awdan SA, Hegazy RR, Mansour DF, Ogaly HA, Abdelbaset M (2020) Neuroprotective effect of *Crocus sativus* against cerebral ischemia in rats. *Metab Brain Dis* 35(3):427–439
 48. Zhong K, Wang R-X, Qian X-D, Yu P, Zhu X-Y, Zhang Q, Ye Y-L (2020) Neuroprotective effects of saffron on the late cerebral ischemia injury through inhibiting astrogliosis and glial scar formation in rats. *Biomed Pharmacother* 126:110041
 49. Javid Z, Norouzy A, Bangaleh Z, Shadnoush M (2018) Rosmarinus improved skin flap survival through mTOR dependent pathway. *Serbian J Dermatol Venereol* 10(4):113–118
 50. Cai L, Xie L, Dong Q (2020) Crocin enhances the viability of random pattern skin flaps: Involvement of enhancing angiogenesis and inhibiting oxidative stress. *Am J Transl Res* 12(6):2929–2938
 51. Cui H, Feng Y, Shu C, Yuan R, Bu L, Jia M, Pang B (2019) Dietary Nitrate protects against skin flap ischemia-reperfusion injury in rats via modulation of antioxidative action and reduction of inflammatory responses. *Front Pharmacol* 10:1605
 52. Zeinali M, Zirak MR, Rezaee SA, Karimi G, Hosseinzadeh H (2019) Immunoregulatory and anti-inflammatory properties of *Crocus sativus* (Saffron) and its main active constituents: a review. *Iran J Basic Med Sci* 22(4):334–344
 53. Hemshekhar M, Sebastin Santhosh M, Sunitha K, Thushara RM, Kemparaju K, Rangappa KS, Girish KS (2012) A dietary colorant crocin mitigates arthritis and associated secondary complications by modulating cartilage deteriorating enzymes, inflammatory mediators and antioxidant status. *Biochimie* 94(12):2723–2733
 54. Sun YD, Gao YS, Xu LW, Zhang YF, Cheng C, Wei KC, Lin J, Chen G, Liu CY, Li QF (2021) Protective effects of sodium ferulate on flap transplantation via anti-inflammatory modulation and oxidative stress inhibition. *Braz J Med Biol Res* 54(7):e10520
 55. Ajami M, Eghtesadi S, Razaz JM, Kalantari N, Habibey R, Nilforoushzadeh MA, Zarrindast M, Pazoki-Toroudi H (2011) Expression of Bcl-2 and Bax after hippocampal ischemia in DHA + EPA treated rats. *Neurol Sci* 32(5):811–818
 56. Ayatollahi SA, Ajami M, Reyhanfard H, Asadi Y, Nassiri-Kashani M, Rashighi Firoozabadi M, Davoodi SH, Habibi E, Pazoki-Toroudi H (2012) BCL-2 and Bax Expression in Skin Flaps Treated with Finasteride or Azelaic Acid. *Iran J Pharm Res* 11(4):1285–1290
 57. Zarch AV, Toroudi HP, Soleimani M, Bakhtiarian A, Katebi M, Djahanguiri B (2009) Neuroprotective effects of diazoxide and its antagonism by glibenclamide in pyramidal neurons of rat hippocampus subjected to ischemia-reperfusion-induced injury. *Int J Neurosci* 119(9):1346–1361
 58. Yao C, Liu B-B, Qian X-D, Li L-Q, Cao H-B, Guo Q-S, Zhou G-F (2018) Crocin induces autophagic apoptosis in hepatocellular carcinoma by inhibiting Akt/mTOR activity. *Onco Targets Ther* 11:2017–2028
 59. Zhang A, Li J (2017) Crocetin shifts autophagic cell survival to death of breast cancer cells in chemotherapy. *Tumour Biol* 39(3):1010428317694536
 60. Kushibiki T, Mayumi Y, Nakayama E, Azuma R, Ojima K, Horiguchi A, Ishihara M (2021) Photocrosslinked gelatin hydrogel improves wound healing and skin flap survival by the sustained release of basic fibroblast growth factor. *Sci Rep* 11(1):23094
 61. Habibey R, Pazoki-Toroudi H (2008) Morphine dependence protects rat kidney against ischaemia-reperfusion injury. *Clin Exp Pharmacol Physiol* 35(10):1209–1214

62. Pazoki-Toroudi HR, Hesami A, Vahidi S, Sahebjam F, Seifi B, Djahanguiri B (2003) The preventive effect of captopril or enalapril on reperfusion injury of the kidney of rats is independent of angiotensin II AT1 receptors. *Fundam Clin Pharmacol* 17(5):595–598

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.