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Antiatherogenic and plaque stabilizing effects of saffron ethanolic extract in atherosclerotic rabbits

Iman Nabilah Abd Rahim[1,2], Effat Omar[1,2], Suhaila Abd Muid[1,3] and Noor Alicezah Mohd Kasim[1,2*]

Abstract

Background Saffron, the dried stigma of the flower *Crocus sativus* L., has been shown to have therapeutic effects on cardiovascular diseases. Several studies have explored the impact of saffron on atherosclerosis. However, the mechanism underlying the plaque-stabilizing and antiatherosclerotic effects of saffron has not been widely studied. Therefore, this study aimed to investigate the mechanism of the antiatherosclerotic and plaque-stabilizing effects of saffron ethanolic extract in experimentally induced atherosclerotic rabbits.

Methods New Zealand White rabbits were fed a 1% high-cholesterol diet (HCD) for 8 weeks to induce established atherosclerosis. The rabbits were then treated with 50 or 100 mg/kg/day saffron ethanolic extract (SAF), simvastatin (2.5 mg/kg/day) or placebo for another 8 weeks. Body weight, lipid profile, percentage of atherosclerotic lesions, immunohistochemical analysis, and quantitative real-time polymerase chain reaction were performed at baseline, after high-cholesterol diet feeding, and after the intervention.

Results The results showed that SAF had no significant effect on body weight. However, treatment with both doses of SAF markedly attenuated the levels of low-density lipoprotein (LDL) and total cholesterol (TC) in atherosclerotic rabbits. Higher doses of SAF markedly reduced atherosclerotic lesions in rabbit aortas. Additionally, SAF suppressed the tissue and gene expression of adhesion molecules and pro-inflammatory biomarkers in the aorta. SAF also reduced MMP-9 tissue expression in the aortas of atherosclerotic rabbits, thereby increasing plaque stability.

Conclusions Our findings suggest that saffron ethanolic extract exhibits therapeutic potential in rabbits with HCD-induced atherosclerosis. This effect may be associated with the modulation of inflammatory pathways, leading to reduced expression of pro-inflammatory cytokines, endothelial activation markers, and matrix metalloproteinases. The observed reduction in vascular inflammation and endothelial activation may contribute to improved lipid profiles, decreased atherosclerotic lesion severity, and enhanced plaque stability. While these findings highlight the potential of saffron ethanolic extract as an adjunctive treatment for atherosclerosis, further studies are warranted to clarify its direct effects on lipid metabolism and underlying molecular mechanisms.

Clinical trial number Not applicable.

Keywords Atherosclerosis, Cardiovascular disease, Saffron, Matrix metalloproteinases, Plaque stability, Rabbits

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Background

Atherosclerosis, characterized by the formation of fibrofatty lesions in the artery wall, is a major cause of morbidity and mortality worldwide. It causes the vast majority of myocardial infarctions, strokes, and debilitating peripheral artery disease [1]. Oxidative stress or elevated intracellular levels of reactive oxygen species (ROS) stimulate endothelial cell activation. This results in the upregulation of adhesion molecules (such as vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), and e-selectin), an increase in vascular permeability, and a decrease in bioavailable nitric oxide (NO), leading to the recruitment of circulating monocytes into the intimal layer [2]. Monocytes then differentiate into macrophages and subsequently engulf oxidized low-density lipoprotein (ox-LDL) particles in the intima, leading to foam cell formation [3]. The accumulation of foam cells leads to the development of fatty streaks, which are the earliest visible lesions in atherosclerosis [4].

Eventually, macrophages and the apoptosis of foam cells promote the release of various pro-inflammatory cytokines (such as nuclear factor kappa B (NF-κB), interleukin-6 (IL-6), and interleukin-8 (IL-8)) [5]. This process transforms the fatty streak into a fibrous plaque, which is recognized as the hallmark of established atherosclerosis.

Maintaining a stable plaque is crucial for preventing complications such as myocardial infarction and stroke. Matrix metalloproteinase-9 (MMP-9), which is released by macrophages, neutrophils, and fibroblasts, facilitates the migration of vascular smooth muscle cells through the internal elastic lamina, thereby contributing to plaque instability [6]. In contrast, tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) functions as an antagonist of MMP-9, counteracting its activity. This action of TIMP-1 helps to inhibit the degradation of extracellular matrix proteins and ultimately contributes to the stabilization of atherosclerotic plaques [7].

Atherosclerosis is a chronic and complex disease caused by the interplay of various contributing factors, including dietary habits, lifestyle choices, genetic predispositions, environmental influences, and systemic factors [8]. Appropriate lifestyle management and dietary habits are fundamental to the success of managing atherosclerosis. However, many people struggle to maintain such a healthy lifestyle. Statin therapy has been the mainstay in the treatment of atherosclerosis. Nevertheless, increasing amounts of clinical data support that long-term statin therapy may increase atherosclerotic calcification [9]. Additionally, statins are linked to a greater risk of diabetes mellitus and elevated hepatic transaminase levels [10]. Myalgia or muscle pain is a commonly reported symptom among patients who consume statins [11]. Hence, adjunctive therapies could be considered to current antiatherosclerotic agents, offering minimal to no toxic side effects [12].

Saffron, derived from the stigma of the *Crocus sativus* L. flower, possesses significant therapeutic potential, particularly in cardiovascular health. Its cardioprotective effects are primarily attributed to its major bioactive compounds, including crocin, crocetin, safranal, and picrocrocin, which have been shown to reduce oxidative stress and inflammatory responses. Additionally, the presence of carotenoids, flavonoids, and other bioactive components further enhances its antioxidative and anti-inflammatory properties, providing protection against cardiovascular diseases [13–15].

Saffron extract has been studied extensively for its potential health benefits and safety profile [16]. Research has revealed both its therapeutic potential and associated risks at varying doses [17]. In a previous clinical trial, several healthy adults experienced side effects after consuming 14 mg of a standardized saffron extract. These side effects included vivid dreams, increased muscle pain, and increased thirst. However, the adverse effects were mild, and no participants dropped out due to them [18]. In a toxicity study on BALB/c mice, the LD50 value of saffron was determined to be 4120 ± 556 mg/kg [19]. Another study involving subchronic exposure to high doses of saffron extract (4000 and 5000 mg/kg) for five weeks in BALB/c mice revealed significant decreases in red blood cell and white blood cell counts and hemoglobin levels, along with increases in blood urea nitrogen and creatinine levels and liver enzyme activity, including alanine transaminase and aspartate aminotransferase, as confirmed by histopathological findings [20]. Importantly, however, these doses were very high. In contrast, saffron extract at lower doses had protective effects on atherosclerotic rabbits. For example, administering saffron ethanolic extract at 50 and 100 mg/kg/day orally for 8 weeks reduced serum urea levels, thereby decreasing renal injury caused by a high-cholesterol diet [12].

Saffron extract has demonstrated significant hypolipidemic effects in various studies, indicating its potential as an alternative or adjunct therapy for managing lipid profiles. Systematic reviews of in vivo studies have confirmed that saffron extract significantly reduces TC, LDL, and triglycerides (TG), while increasing HDL in hyperlipidemic animal models [21]. Experimental studies on Sprague Dawley rats have shown that serum cholesterol levels decreased after treatment with hydromethanolic saffron extract for fourteen days [22]. Another in vivo study further demonstrated that saffron and its active constituent, crocin, could reduce TG and TC in hyperlipidemic rats [23]. The study by Samarghandian (2014) also found that saffron extract reduced the risk of hyperlipidemia in diabetic encephalopathy rats [24]. In high-fat-fed obese rats, saffron extracts significantly decreased serum

TC, TG, and LDL while increasing HDL, thus reducing atherosclerosis and insulin resistance [25].

Saffron has also shown antiatherogenic properties. In a previous study, saffron extract has been shown to enhance levels of adiponectin, a protein with antiatherogenic properties, and decrease lipoprotein (a), a marker of cardiovascular risk, in diabetic rats [26, 27]. Moreover, a previous study demonstrated saffron's direct antiatherogenic effects, where plaque accumulation in HFD-fed mice decreased in a dose-dependent manner following treatment with saffron aqueous extracts [28]. Additionally, the same study found that saffron exhibited plaque-stabilizing effects by reducing MMP-2 and enhancing TIMP-2 tissue and gene expression in the aortas of mice [28]. These beneficial effects of saffron extract are likely due to its antioxidant and anti-inflammatory properties. While previous studies have investigated saffron's plaque-stabilizing effects, various markers and types of saffron extraction have been employed.

In our study, we assessed the impact of saffron ethanolic extract on MMP-9 and TIMP-1 expression. A previous study reported that elevated serum levels of MMP-9 and TIMP-1 indicate instability in carotid artery atherosclerotic plaques [29]. Furthermore, several studies have indicated that high MMP-9 levels can predict atherosclerotic plaque instability and that excessive MMP-9 expression may contribute to this instability [30, 31]. Conversely, overexpression of TIMP-1 has been shown to significantly reduce plaque progression and enhance the stability of vulnerable plaques in murine vein grafts [32]. While multiple studies have explored the effects of saffron and its bioactive compounds on atherosclerosis [28, 33, 34], these studies primarily focused on different biomarkers, including MMP-2, pancreatic lipase, and VCAM-1, rather than MMP-9 and TIMP-1. Our study is novel in utilizing saffron ethanolic extract instead of aqueous extracts or isolated compounds such as crocin or crocetin and specifically evaluating MMP-9 and TIMP-1 as key markers of plaque stability. Rabbits were selected due to their LDL-dominant lipoprotein metabolism, which closely resembles that of humans, and their high sensitivity to a cholesterol-enriched diet, making them an ideal model for studying atherosclerotic plaque formation and progression [35]. Nevertheless, research on the mechanism underlying the antiatherosclerotic effects of saffron ethanolic extract in rabbits is limited.

Therefore, this study aimed to investigate the mechanism of the antiatherosclerotic and plaque-stabilizing effects of saffron ethanolic extract in experimentally induced atherosclerotic rabbits. We also postulated that the effects of saffron ethanolic extract on atherosclerosis could be mediated by its impact on inflammatory agents and matrix metalloproteinases (MMPs).

Methods

Animals

Thirty-six male New Zealand White rabbits were purchased from A Sapphire Enterprise, Malaysia. After the rabbits arrived at the animal laboratory, they were quarantined for 2 weeks and caged individually in an environmentally controlled clean air room with a temperature of 22 + 2 °C and a 12-h light/12-h dark cycle with a relative humidity of 60 + 5%. This study was approved by the Universiti Teknologi MARA Committee on Animal Research & Ethics with ethical approval number UiTM CARE: 326/2020. All experiments carried out in this study were compliant with the ARRIVE guidelines.

Preparation of saffron ethanolic extract

Saffron, the dried stigma of the flower *Crocus Sativus* L., was purchased from World Care Groups Sdn. Bhd., the supplier of SaharKhiz Saffron Co. (Mashhad, Iran) in Malaysia. The flowers were harvested in Khorasan Razavi Province, located in northeast Iran, in compliance with the guidelines established by the Plant Protection Organization, Ministry of Jihad-e Agriculture, Islamic Republic of Iran. The stigma of each flower was carefully picked and processed and then air-dried in the shade prior to extraction. The samples were identified and confirmed by a botanist from The National University of Malaysia and deposited at the herbarium of this department with voucher specimen number ID006/2021 for future reference.

The dried stigma of the saffron plant was extracted via the maceration method. In brief, 100 g of dried, ground saffron stigma was soaked in 1500 mL of 80% ethanol (v/v) with a magnetic stirrer for three days at room temperature. The mixture was then filtered and concentrated under reduced pressure at 40 °C via a rotary evaporator to remove the ethanol. The resulting extract was then stored overnight at -80 °C, lyophilized via a freeze dryer, and stored at -80 °C until further use. The percentage yield of the extract was 50% (w/w). Chromatographic analysis of bioactive compounds in a saffron extract from a previous study revealed that a 1-gram sample of the dried saffron extract contained 29% crocins and 1.9% safranal [36]. An ethanol extract was used in this study because it yields relatively high concentrations of bioactive compounds of saffron, such as crocin, picrocrocin, and safranal [37]. Additionally, the total flavonoid content, total phenolic content, and antioxidant activity of the ethanolic extract were greater than those of other solvents, such as water and methanol [38].

Experimental groups

After a 2-week acclimatization period, 36 NZW rabbits were randomly assigned to two main groups: the baseline group ($n = 12$) and the treatment group ($n = 24$).

(1) Baseline Group: This group was divided into two subgroups:

A1 ($n=6$): Rabbits were fed a normal diet (ND) for 2 weeks. This subgroup served as the healthy control, representing normal conditions without induced atherosclerosis.

A3 ($n=6$): Rabbits were fed 50 g/kg/day of a 1% high-cholesterol diet (HCD) for 8 weeks to induce established atherosclerosis. This subgroup served as the atherosclerotic control subgroup for comparison with the treatment interventions.

(2) Treatment Group ($n=24$): Rabbits were fed the same high-cholesterol diet (HCD) for 8 weeks to induce established atherosclerosis and further divided into four subgroups ($n=6$ each) for different treatment interventions:

Group I (B2S50): Received 50 mg/kg/day saffron ethanolic extract (SAF).

Group II (B2S100): Received 100 mg/kg/day SAF.

Group III (B2statin): Received 2.5 mg/kg/day simvastatin.

Group IV (B2DW): Received distilled water as a control.

The dosing regimen for saffron ethanolic extract (SAF) was adapted from previous studies in rats, where oral doses of 100 and 200 mg/kg/day were utilized [39]. The doses were then converted from rats to rabbits by the conversion formula from a previous study [40]. Simvastatin was administered as a positive control at a dosage of 2.5 mg/kg/day, based on a previously established study [41].

The baseline group provides a reference for normal and atherosclerotic conditions without any treatment, whereas the treatment group can be used to evaluate the effects of different interventions on atherosclerosis. A normal diet was given to the rabbits during the treatment period. The experimental design of the study is depicted in Fig. 1.

Saffron extract in powdered form was dissolved in 1 mL of distilled water and administered to the rabbits via force-feeding using a 1 mL syringe to ensure precise and consistent dosing. The rabbits were gently wrapped in a blanket, leaving only their heads exposed. To prevent aspiration, the syringe was filled with the appropriate dose of saffron extract and checked for air bubbles. The syringe was then placed into the diastema, the large space between the incisors and premolars, angled back into the mouth, and the plunger was pressed slowly. Small amounts of the extract were given at a time, allowing the rabbits to rest and show signs of chewing and swallowing between doses. This process was repeated until all the extract had been administered, ensuring slow feeding to avoid aspiration.

At the end of the experiment, the rabbits were anaesthetized before being sacrificed to ensure they were unconscious and did not experience pain. A total of 5 mg/kg Xylazine followed by 35 mg/kg Ketamine were administered intramuscularly according to standard protocols, and the animals were maintained under anaesthesia until the absence of pain reflexes was confirmed. Euthanasia was then performed via intravenous injection of 100 mg/kg sodium pentobarbital, following the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals. This approach was selected to minimize suffering and ensure humane treatment in accordance with ethical standards.

Body weight analysis

Body weight (g) was measured in the rabbits at baseline, pretreatment (post-HCD feeding), and posttreatment in all the groups.

Biochemical analysis

Blood samples were collected from the marginal ear vein or the central auricular artery of the rabbits via plain tubes. The serum was then separated by high-speed centrifugation at 4000 rpm for 10 min and stored

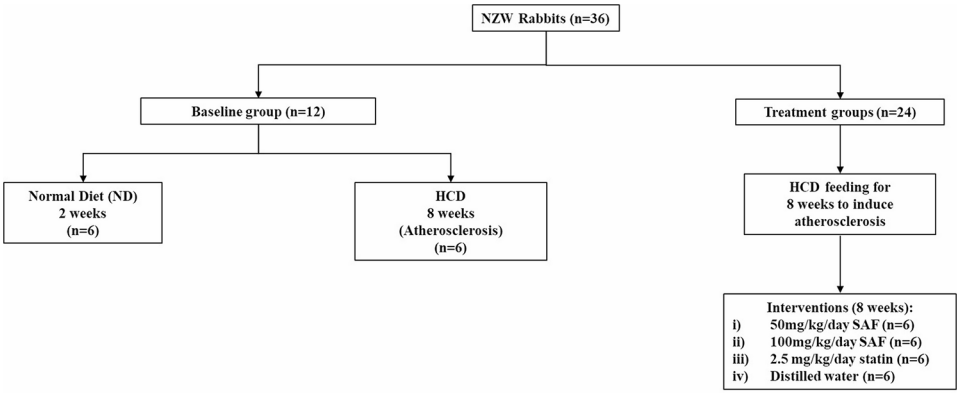

Fig. 1 Experimental design of the study

at − 80 °C until analysis. Low-density lipoprotein (LDL), total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) cholesterol levels were measured via an automated Roche Cobas c501 analyzer (Basel, Switzerland).

Quantification of the atherosclerotic lesion area

After dissection, the whole length of the aorta from the ascending aorta down to the bifurcation of the iliac arteries was obtained to evaluate the atherosclerotic lesions. A 5 mm section of each aorta specimen ($n = 6$ per group) was removed from the aortic arch of each rabbit, the primary site of atherosclerosis, for gene expression analysis. The remaining part of the aorta underwent quantification of atherosclerotic lesions by examining the intimal surface through Sudan IV staining, following the method described in a previous study, for the assessment of lesion size and lipid content [42].

The aortas were gently rinsed with normal saline and then longitudinally opened to expose the lumen. The opened aortas were pinned flat on cardboard in a stainless-steel tray and fixed in 10% neutral buffered formalin overnight. After fixation, the aortic vessels were washed with 70% ethanol and immersed in Herxheimer solution (5 g of Sudan IV in 500 mL of 70% ethanol and 500 mL of acetone) for 15 min at room temperature. They were then rinsed under running tap water for one hour. The stained aortic vessels were placed against a smooth background for imaging, and photographs were taken with a C-740 Ultra Zoom digital camera. Quantitative analysis was performed via ImageJ software (National Institute of Health) to determine the percentage of Sudan IV-stained atherosclerotic regions, which was then used to calculate the lesion area relative to the total aortic area. Finally, the aortas were immersed in 10% formalin for immunohistochemical analysis.

Immunohistochemical analysis

For immunohistochemical evaluation, samples were obtained from three specific regions of the aortic tissue: 10 mm from the aortic arch, 10 mm from the upper thoracic region, and 10 mm above the iliac bifurcation. Each section was 15 mm long. The fixed aortic sections were then embedded in paraffin blocks. After epitope retrieval, the aortic tissue Sects. (4–5 μm thick) were stained with the following primary antibodies: intracellular adhesion molecule-1 (ICAM-1) (1:100 dilution, MBS2111559; MyBioSource, San Diego, CA, USA), vascular cell adhesion molecule-1 (VCAM-1) (1:150 dilution, sc-18864; Santa Cruz Biotechnology, Dallas, TX, USA), e-selectin (1:150 dilution, sc-137054; Santa Cruz Biotechnology, Dallas, TX, USA), interleukin-6 (IL-6) (1:100 dilution, MBS2032018; MyBioSource, San Diego, CA, USA), interleukin-8 (IL-8) (1:100 dilution, MBS2025703; MyBioSource, San Diego, CA, USA), nuclear factor kappa B p65 (NF-κB p65) (1:150 dilution, sc-8008; Santa Cruz Biotechnology, Dallas, TX, USA), matrix metalloproteinases-9 (MMP-9) (1:100 dilution, sc-13520; Santa Cruz Biotechnology, Dallas, TX, USA) and the tissue inhibitor of matrix metalloproteinases 1 (TIMP-1) (1:100). The sections were then washed with wash buffer and incubated with an anti-mouse immunoglobulin biotinylated secondary antibody (Agilent Technologies, Santa Clara, CA, USA) for one hour, followed by incubation with streptavidin-peroxidase for another hour. The sections were then stained with DAB according to the manufacturer's protocol, and the nuclei were counterstained with hematoxylin. Immunostained sections were observed under a histopathology microscope (Olympus BX61 Upright Fluorescence Microscope), and pictures of each aortic vessel were saved as TIFF images. The immunohistochemical staining was scored semiquantitatively as 0: (no staining), + 1: (0–10% staining), + 2: (10–25% staining), or + 3: (> 25% staining) by two blinded observers.

qRT–PCR analysis

The process of extracting RNA was performed following the manufacturer's instructions. Total RNA was isolated from the aortas of atherosclerotic rabbits via the RNA extraction kit NucleoSpin® RNA Plus (Macherey Nagel, Düren, Germany). Reverse transcription was carried out via an Applied Biosystems™ Veriti™ 96-Well Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) to synthesize complementary DNA (cDNA). One hundred nanograms of template RNA was used for cDNA synthesis in a final volume of 20 μL following the manufacturer's instructions. Real-time quantitative reverse transcription polymerase chain reaction (qRT−PCR) was performed on a real-time PCR system CFX96 Touch™ Real-Time PCR Detection System using BlasTaq™ 2X qPCR MasterMix (Applied Biological Materials, Vancouver, Canada) according to the manufacturer's protocols. The target genes were inflammatory markers (IL-8, IL-6, and NF-κB p65) and endothelial activation markers (ICAM-1, VCAM-1, and e-selectin). The sequences of the primers used for each gene are shown in additional file 6. All the PCRs were performed in triplicate, and the relative expression levels of different groups were calculated via normalization to the mRNA expression levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), β-actin, and hypoxanthine phosphoribosyl transferase 1 (HPRT-1) via the $2^{-\Delta\Delta CT}$ or Livak methods [43].

Statistical analysis

Data normality was verified via the Shapiro−Wilk test. The results are expressed as the mean ± standard error of the mean (SEM). Within groups, changes were analyzed by paired samples t tests. Comparisons between

groups were conducted via one-way analysis of variance (ANOVA) with the Bonferroni post hoc correction. Categorical parameters for immunohistochemical analysis were compared via Fisher's exact test. Differences with a p value of <0.05 were considered statistically significant. The data were analyzed via SPSS software version 27.0 (Chicago, IL, USA).

Results

Bodyweight analysis

The body weight of New Zealand White rabbits (NZWRs) significantly increased after being fed a 1% high-cholesterol diet (HCD) for 8 weeks compared with the baseline body weight in all groups ($p<0.05$). However, statins, 50 and 100 mg/kg saffron ethanolic extract (SAF), and the placebo had no significant effect on the body weight of NZWR throughout the experimental period compared with that in the pretreatment group ($p>0.05$) (Fig. 2 and additional file 1).

Biochemical analysis

LDL and TC significantly increased in all the groups at the pretreatment level compared with the baseline level ($p<0.01$). TG levels were significantly lower in the B2S50, B2S100 and B2DW groups than in the baseline group ($p<0.05$).

Interestingly, posttreatment with 50 and 100 mg/kg/day SAF and statins markedly decreased TC and LDL in all the groups compared with those in the pretreatment and B2DW groups ($p<0.01$). TG significantly increased after treatment with 50 mg/kg/day SAF ($p<0.05$), whereas HDL decreased after treatment with 100 mg/kg/day SAF ($p<0.01$) (Fig. 3 and additional file 2).

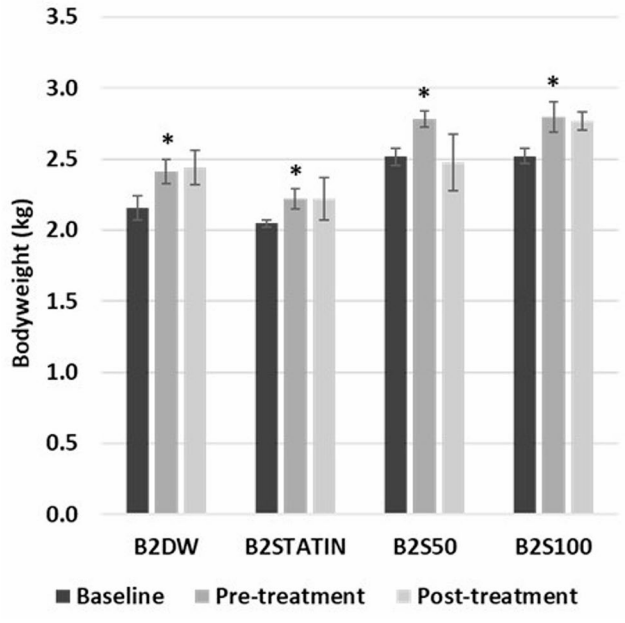

Fig. 2 Effects of SAF on the body weight of NZWR at baseline, pretreatment, and posttreatment. The data are presented as the means ± SEMs ($n=6$). Significance from baseline is represented as * at $p<0.05$. B2S50: Rabbits given 50 mg/kg/day SAF for 8 weeks; B2S100: Rabbits given 100 mg/kg/day SAF for 8 weeks; B2statin: Rabbits given 2.5 mg/kg/day simvastatin for 8 weeks; B2DW: Rabbits given distilled water for 8 weeks

Quantification of atherosclerotic lesions in the aorta

After an 8-week intervention with HCD, the A3 group presented a significant increase in atherosclerotic lesions in the aorta (11.88 ± 2.09%) compared with baseline ($p<0.01$). Following HCD intervention, the rabbits were subjected to various treatments for 8 weeks. Among them, those administered statins showed the greatest percentage reduction in the aortic lesion area, reaching 3.10 ± 0.77%, followed by those given 100 mg/kg/day SAF (4.08 ± 0.52%) and 50 mg/kg/day SAF (9.33 ± 0.57%). Compared with the other groups, the placebo group presented the highest percentage of aortic lesions (14.15 ± 0.57%) (Fig. 4 and additional file 3).

Immunohistochemical findings

VCAM-1, ICAM-1, and E-selectin expression significantly increased in the endothelium after 8 weeks of a high-cholesterol diet (HCD) ($p<0.01$). Following treatment with 50 or 100 mg/kg/day SAF, the expression of these adhesion molecules in the endothelium significantly decreased ($p<0.05$), although the decrease in E-selectin was not significant. Additionally, posttreatment with both doses of SAF significantly reduced VCAM-1 and E-selectin expression in macrophages ($p<0.05$). While lower ICAM-1 expression was observed in the macrophages of the B2S100 and B2statin groups than in those of the A3 group, this reduction was not significant ($p>0.05$) (Fig. 5 and additional file 4).

As anticipated, there was a significant increase in IL-6 and NF-κB expression within the endothelium following 8 weeks of HCD intervention ($p<0.05$). Additionally, a significant increase in endothelial IL-8 expression was observed in the placebo group (B2DW) compared with the A1 group ($p<0.05$). Compared with A3 and B2DW, treatment with 100 mg/kg/day SAF or statins significantly reduced NF-κB and IL-6 endothelial expression ($p<0.01$) (Fig. 6 and additional file 4).

Plaque stability effect of saffron extract

After 8 weeks of HCD intervention, there was a notable increase in MMP-9 and TIMP-1 expression in the endothelium ($p<0.05$) compared with that in the A1 group ($p<0.01$). Compared with the A3 group, the 100 mg/kg/day SAF ($p<0.01$) and statin ($p<0.05$) groups presented significantly reduced endothelial MMP-9 expression. Additionally, following statin treatment, TIMP-1 expression in the endothelium was significantly greater than that in the B2DW group ($p<0.05$) (Fig. 7 and additional

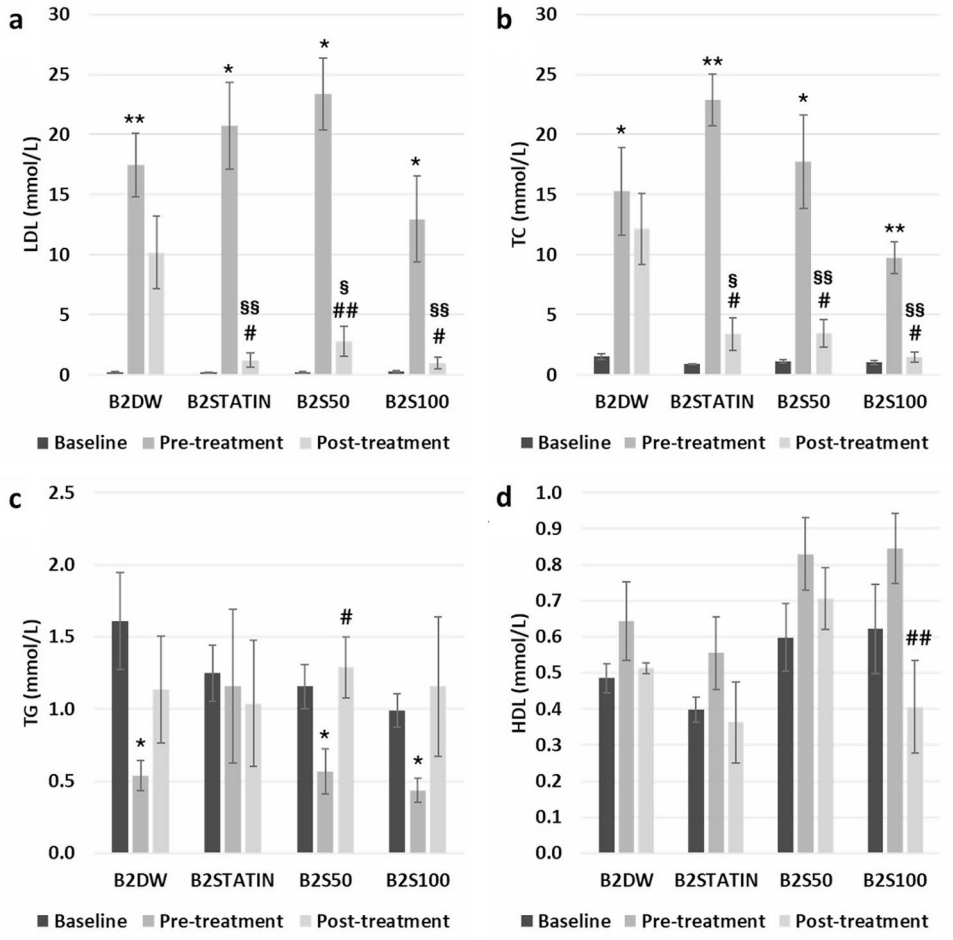

Fig. 3 Effects of SAF on the serum lipid profile of rabbits at baseline, pretreatment, and posttreatment. The data are presented as the means ± SEMs ($n=6$). Significance from baseline is represented as * at $p<0.05$. Significance from pretreatment is represented as follows: # at $p<0.05$ and ## $p<0.01$. Significance from B2DW is represented as follows: § at $p<0.05$ and §§ $p<0.01$. B2S50: Rabbits given 50 mg/kg/day SAF for 8 weeks; B2S100: Rabbits given 100 mg/kg/day SAF for 8 weeks; B2statin: Rabbits given 2.5 mg/kg/day simvastatin for 8 weeks; B2DW: Rabbits given distilled water for 8 weeks

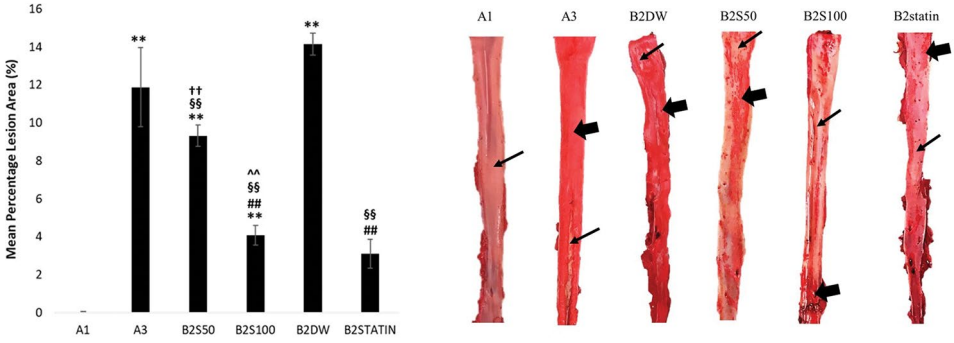

Fig. 4 Effects of SAF on the percentage of atherosclerotic lesions in the aortas of NZWRs. The thick arrow indicates the lesion area, whereas the thin arrow denotes the aorta without lesions. The values are expressed as the means ± SEMs of six rabbits. ** $p<0.01$ versus A1. ## $p<0.01$ versus A3. §§ $p<0.01$ versus B2DW. †† $p<0.01$ versus B2statin. ^^ $p<0.01$ versus B2S50. A1: Rabbits given normal diet for 2 weeks; A3: Rabbits given HCD for 8 weeks; B2S50: Rabbits given 50 mg/kg/day SAF for 8 weeks; B2S100: Rabbits given 100 mg/kg/day SAF for 8 weeks; B2statin: Rabbits given 2.5 mg/kg/day simvastatin for 8 weeks; B2DW: Rabbits given distilled water for 8 weeks

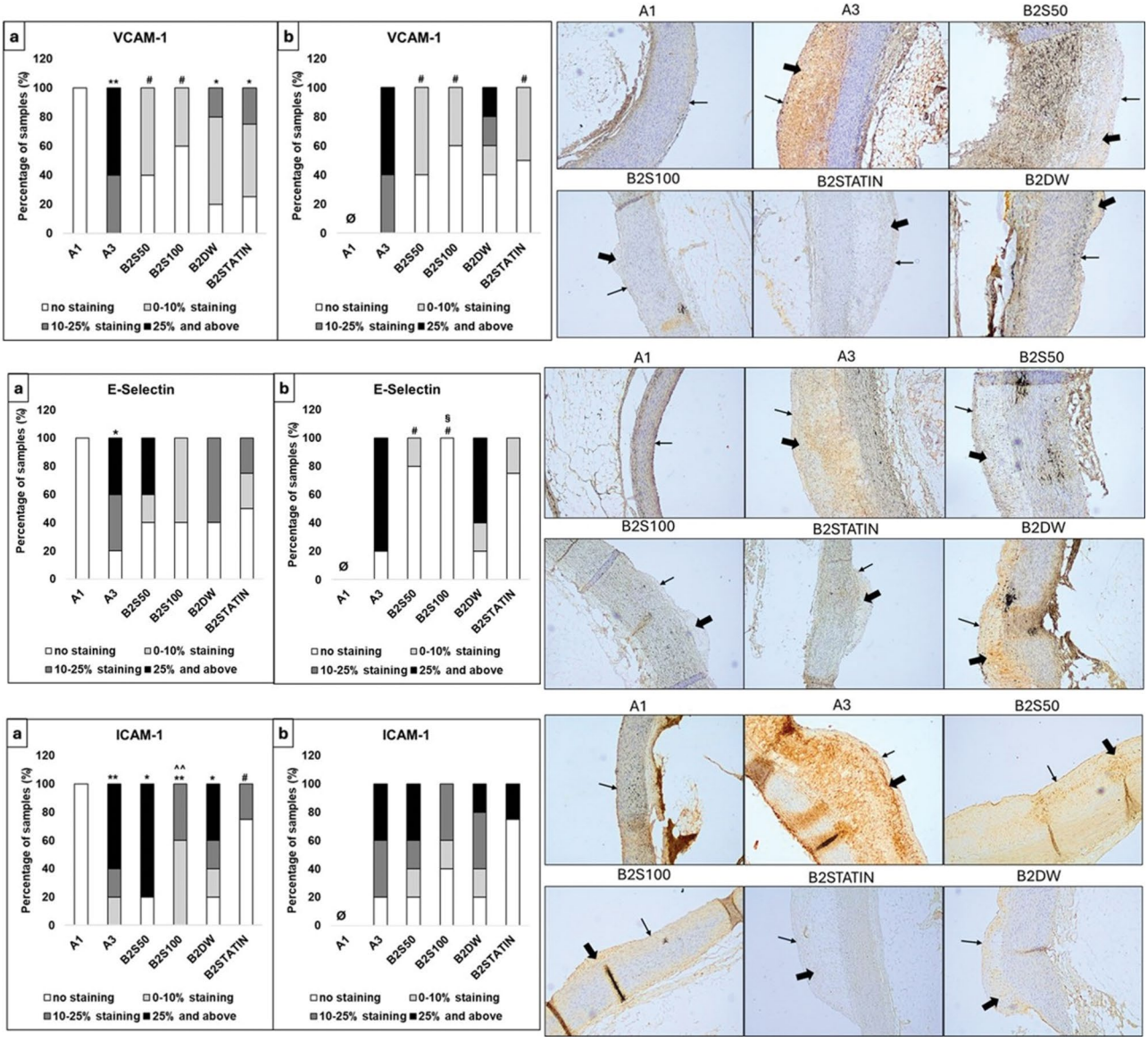

Fig. 5 Percentage of samples and representative images of aortas demonstrating positive immunohistochemical expression of VCAM-1, ICAM-1, and E-selectin in (a) the endothelium (thin arrow) and (b) macrophages (thick arrow). All depositions identified via immunohistochemistry on the endothelium and macrophages were semiquantitatively scored as follows: 0 (no staining), + 1 (0–10% staining), + 2 (10–25% staining), or + 3 (> 25% staining). * $p < 0.05$ versus A1. ** $p < 0.01$ versus A1. # $p < 0.05$ versus A2. § $p < 0.05$ versus B1DW. ^^ $p < 0.01$ versus B1S50. A1: Rabbits given normal diet for 2 weeks; A3: Rabbits given HCD for 8 weeks; B2S50: Rabbits given 50 mg/kg/day SAF for 8 weeks; B2S100: Rabbits given 100 mg/kg/day SAF for 8 weeks; B2statin: Rabbits given 2.5 mg/kg/day simvastatin for 8 weeks; B2DW: Rabbits given distilled water for 8 weeks

file 4). A more detailed presentation of the results for each immunohistochemistry biomarker can be found in additional file 4.

Gene expression in the aorta

VCAM-1 gene expression in the aorta of rabbits in the A3 group was significantly greater than that in the A1 group (8.9-fold, $p < 0.01$). Posttreatment with both SAF and statins significantly decreased VCAM-1 gene expression in the aorta compared with that in the A3 and B2DW groups ($p < 0.01$).

E-selectin gene expression in the aortas of rabbits in the A3 group was significantly greater than that in the A1 group (24.8-fold, $p < 0.01$). Compared with A3 and B2DW, posttreatment with both doses of SAF and statins significantly decreased E-selectin gene expression in the aorta ($p < 0.01$).

ICAM-1 gene expression in the aorta of rabbits in the A3 group was significantly greater than that in the A1 group (11.3-fold, $p < 0.01$). Compared with the A3 and B2DW groups, the SAF and statin groups presented

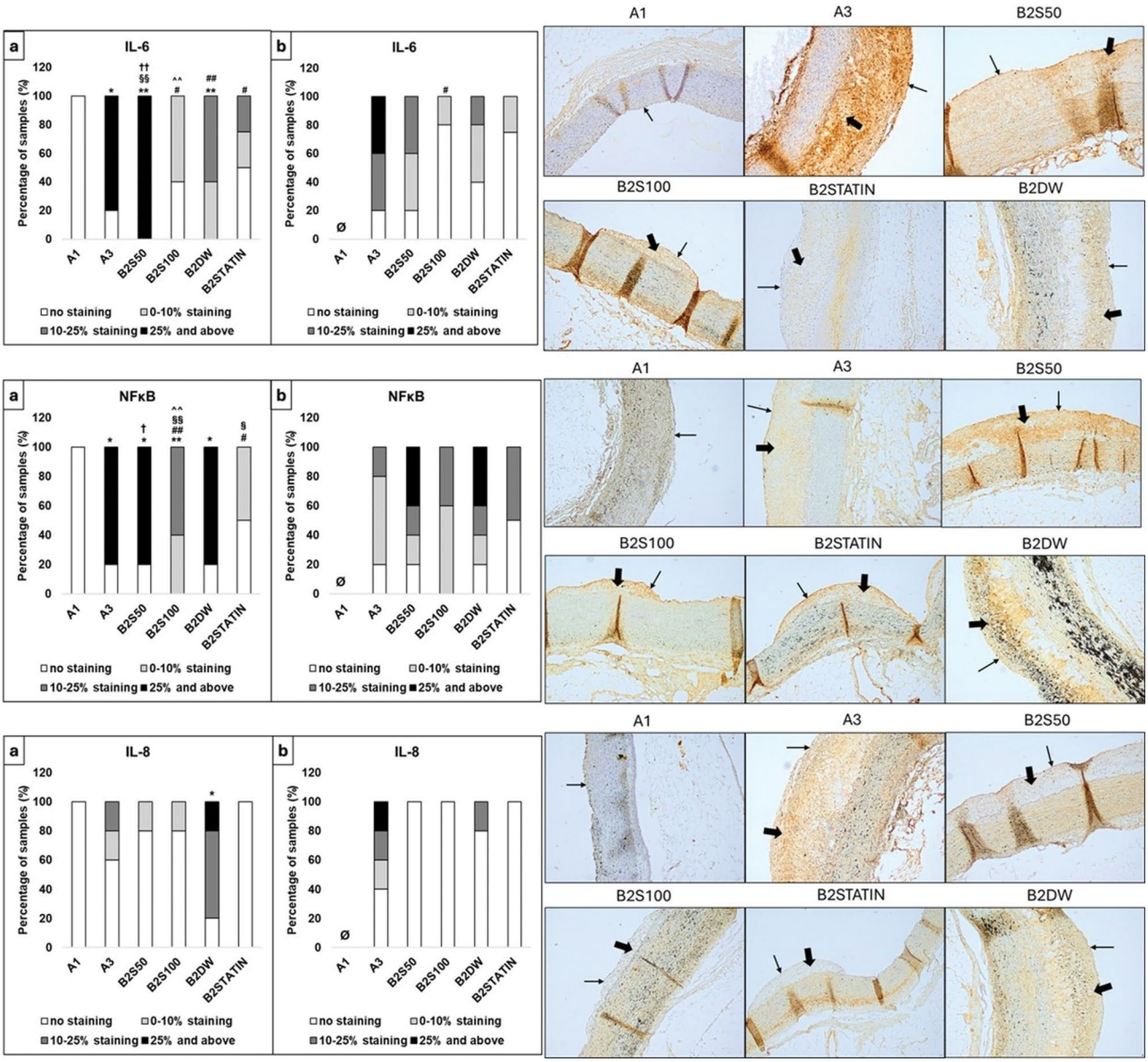

Fig. 6 Percentage of samples and representative images of aortas demonstrating positive immunohistochemical expression of IL-6, IL-8 and NF-κB p65 in (a) the endothelium (thin arrow) and (b) macrophages (thick arrow). All depositions identified via immunohistochemistry on the endothelium and macrophages were semi-quantitatively scored as follows: 0 (no staining), + 1 (0–10% staining), + 2 (10–25% staining), or + 3 (> 25% staining). * $p < 0.05$ versus A1. ** $p < 0.01$ versus A1. # $p < 0.05$ versus A3. ## $p < 0.01$ versus A3. § $p < 0.05$ versus B2DW. §§ $p < 0.01$ versus B2DW. † $p < 0.05$ versus B2statin. †† $p < 0.01$ versus B2statin. ^^$p < 0.01$ versus B2S50. A1: Rabbits given normal diet for 2 weeks; A3: Rabbits given HCD for 8 weeks; B2S50: Rabbits given 50 mg/kg/day SAF for 8 weeks; B2S100: Rabbits given 100 mg/kg/day SAF for 8 weeks; B2statin: Rabbits given 2.5 mg/kg/day simvastatin for 8 weeks; B2DW: Rabbits given distilled water for 8 weeks

significantly decreased ICAM-1 gene expression in the aorta ($p < 0.01$).

Compared with that in the A1 group, IL-6 gene expression in the aorta of the A3 group was significantly increased (up to 13.5-fold) ($p < 0.01$). Conversely, the aortas of rabbits treated with 50 and 100 mg/kg/day SAF and statins presented a notable reduction in IL-6 gene expression compared with those of the A3 and B2DW groups ($p < 0.01$).

IL-8 gene expression in the A3 group significantly increased up to 17-fold compared with that in the A1 group in the established atherosclerosis model ($p < 0.01$). Conversely, the B2S50, B2S100, B2statin, and B2DW groups presented notable decreases in IL-8 gene expression compared with the A3 group ($p < 0.01$). Notably, the B2S100 and B2statin groups presented significantly lower IL-8 gene expression than the B2DW group did ($p < 0.01$).

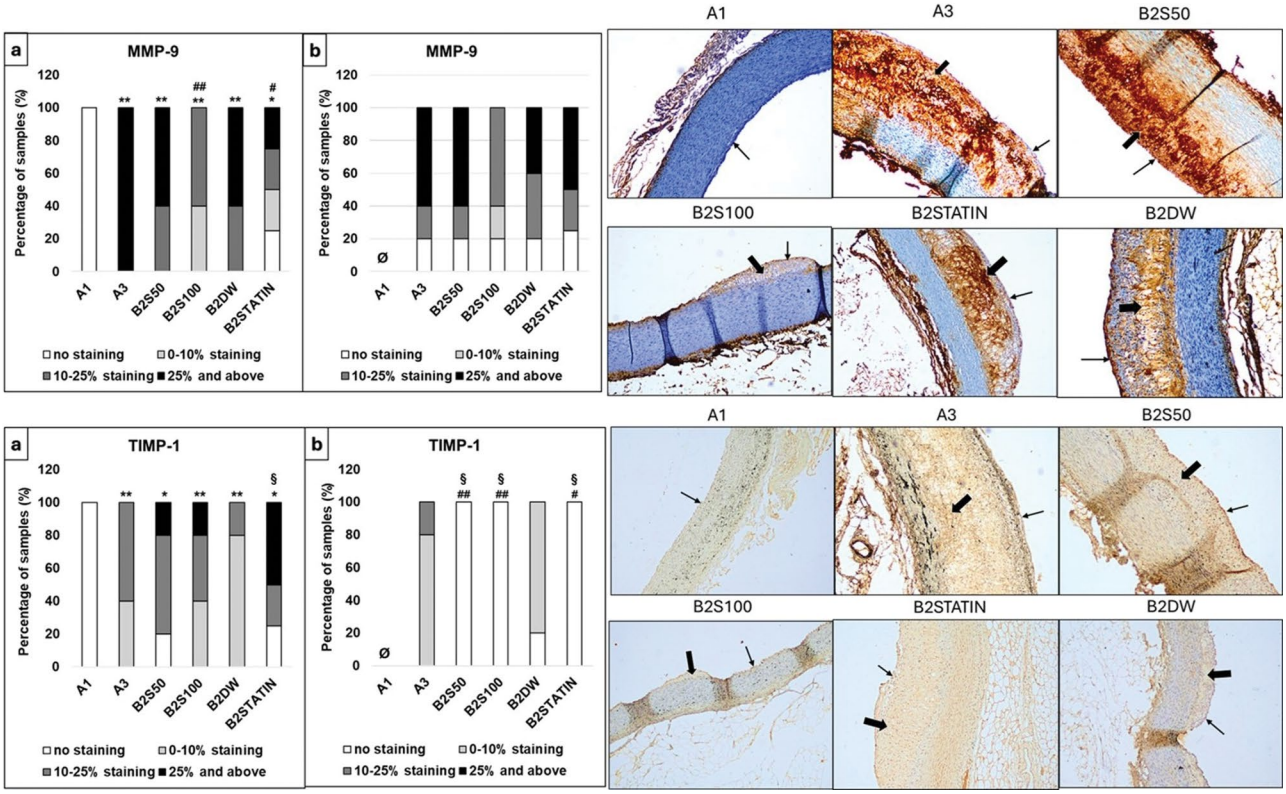

Fig. 7 Percentage of samples and representative images of aortas demonstrating positive immunohistochemical expression of MMP-9 and TIMP-1 in (**a**) the endothelium (thin arrow) and (**b**) macrophages (thick arrow). All depositions identified via immunohistochemistry on the endothelium and macrophages were semi-quantitatively scored as follows: 0 (no staining), + 1 (0–10% staining), + 2 (10–25% staining), or + 3 (> 25% staining). * $p < 0.05$ versus A1. ** $p < 0.01$ versus A1. # $p < 0.05$ versus A3. ## $p < 0.01$ versus A3. § $p < 0.05$ versus B2DW. A1: Rabbits given normal diet for 2 weeks; A3: Rabbits given HCD for 8 weeks; B2S50: Rabbits given 50 mg/kg/day SAF for 8 weeks; B2S100: Rabbits given 100 mg/kg/day SAF for 8 weeks; B2statin: Rabbits given 2.5 mg/kg/day simvastatin for 8 weeks; B2DW: Rabbits given distilled water for 8 weeks

Compared with that in the A1 group, NF-κB gene expression in the A3 group notably increased up to 6.5-fold ($p < 0.01$). Compared with A3 and B2DW, both SAF and statin significantly decreased NF-κB gene expression in the aorta ($p < 0.01$) (Fig. 8 and additional file 5).

Discussion

The present study is the first to provide evidence that saffron ethanolic extract ameliorates the development of atherosclerosis in rabbits, potentially through its anti-inflammatory effects. This is supported by the observed reduction in vascular endothelial markers, pro-inflammatory cytokines, and matrix metalloproteinases. These effects may contribute to improved lipid profiles, decreased atherosclerotic lesion severity, and enhanced plaque stability in the aorta.

Two doses of SAF (50 and 100 mg/kg/day) and simvastatin (2.5 mg/kg/day), which are 3 hydroxy-3-methylglutaryl-CoA reductase inhibitors, were tested. The groups initially received HCD alone for 8 weeks and then were treated orally with SAF or simvastatin for 8 weeks without HCD.

According to our previous study, an 8-week duration of 1% HCD feeding can lead to an increase in blood lipid levels and the development of atherosclerotic plaque formation [44]. In this study, we established atherosclerotic rabbit models via HCD feeding to explore the therapeutic effects of SAF on atherosclerosis. After 8 weeks of HCD intervention, the rabbits presented increases in body weight, hypercholesterolemia, and atherosclerotic plaque formation, as well as elevated protein expression associated with endothelial activation and pro-inflammatory biomarkers.

Obesity or excess body weight is an independent risk factor for atherosclerotic cardiovascular disease [45]. Obesity leads to more than 3.4 million deaths annually, primarily due to its role in chronic inflammation, which is a significant factor in the development of atherosclerosis [46]. In our study, the body weights of the rabbits significantly increased after HCD feeding. However, after treatment with both doses of SAF and statins, no significant reduction in body weight was observed. Consistent with a previous meta-analysis, no significant decrease in body weight was observed following saffron supplementation among obese patients [47]. Additionally, a previous

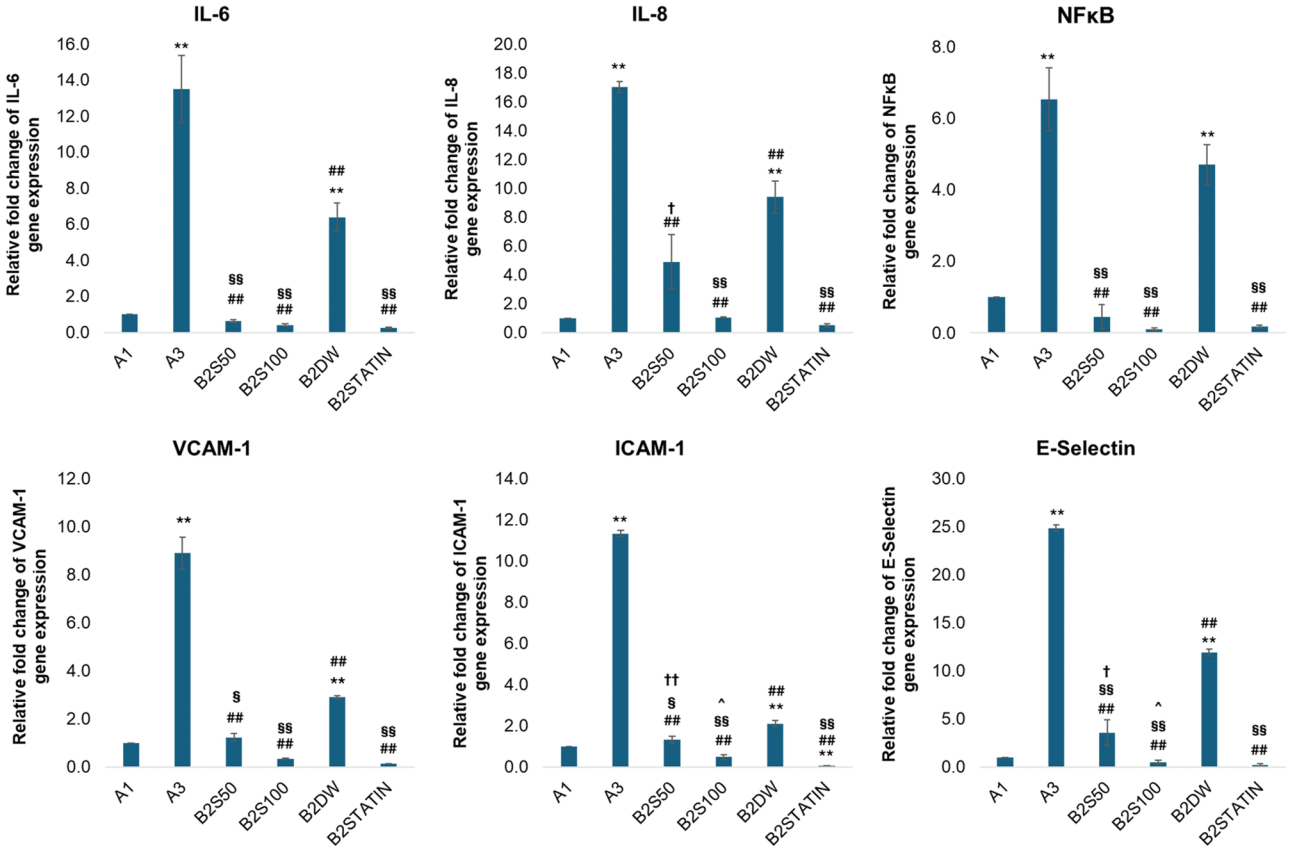

Fig. 8 Effects of SAF on VCAM1, ICAM-1, E-selectin, IL-6, IL-8 and NF-κB gene expression in the aortas of atherosclerotic rabbits. ** $p < 0.01$ versus A1, ## $p < 0.01$ versus A3, § $p < 0.05$ versus B2DW, §§ $p < 0.01$ versus B2DW, † $p < 0.05$ versus B2statin, †† $p < 0.01$ versus B2statin, ^ $p < 0.05$ versus B2S50. A1: Rabbits given normal diet for 2 weeks; A3: Rabbits given HCD for 8 weeks; B2S50: Rabbits given 50 mg/kg/day SAF for 8 weeks; B2S100: Rabbits given 100 mg/kg/day SAF for 8 weeks; B2statin: Rabbits given 2.5 mg/kg/day simvastatin for 8 weeks; B2DW: Rabbits given distilled water for 8 weeks

in vivo study using male Sprague Dawley rats as an animal model reported that the administration of 40 and 80 mg/kg saffron extract for 8 weeks led to an insignificant reduction in the body weight of the rats [36]. These results contradict those of previous studies that demonstrated a significant reduction in body weight in rats following the administration of saffron ethanolic extract [25, 48]. A recent study revealed that, compared with rats fed a chow diet, those fed 125 or 250 mg/kg saffron powder for 6 weeks presented a significant reduction in body weight [49]. In a clinical trial, saffron extract was shown to increase satiety and decrease appetite in overweight women, resulting in weight loss [50]. This inconsistency in results may be attributed to various factors, such as the differences in doses, durations and types of saffron extract used in the studies.

Assessing the lipid profile is a firmly established method for evaluating the risk of atherosclerosis [51]. Consequently, it has been extensively utilized as a cornerstone of cardiovascular disease prevention and as a focal point for pharmacological treatments in clinical practice for several decades [52]. Numerous prior in vivo and clinical trial investigations have validated that simvastatin effectively lowers LDL and TC levels in hyperlipidemic subjects [53–56], potentially by increasing LDL receptor expression on the surface of the liver, which leads to increased uptake of LDL from the blood into the liver, subsequently decreasing the levels of other ApoB-containing lipoproteins, such as LDL, in the bloodstream [57]. This effect was further supported by our study, which demonstrated the hypolipidemic effects of SAF and simvastatin in atherosclerotic rabbits. Although several studies have explored the effects of saffron administration on lipid profiles, the findings are inconsistent. In some studies, saffron supplementation significantly reduced lipid profiles, whereas other studies failed to observe such significant effects [21, 58–61]. An interesting observation in the current study was the decrease in LDL and TC following intervention with both doses of SAF; however, there were no significant effects on TG or HDL levels. It is plausible that a longer duration of saffron treatment is necessary to increase HDL levels, as suggested by a previous meta-regression analysis study [59]. Several previous studies have shown that saffron

extracts significantly reduce TG levels [25, 62–64]. The insignificant reduction in TG levels observed in our study may be due to differences in extraction methods, dosages, and durations compared with those used in prior studies.

To the best of our knowledge, this is the third study indicating the direct antiatherosclerotic effects of saffron by assessing atherosclerotic plaques in vivo. The first study revealed that administering crocetin, one of the major bioactive compounds in saffron, to rabbits significantly reduced atherosclerotic lesions in the aortas of the rabbits [33]. Another study reported that saffron aqueous extract attenuates the progression of atherosclerosis and increases plaque stability in apo $E^{-/-}$ mice through the amelioration of inflammatory markers [28]. High levels of LDL and low levels of HDL in the blood contribute to an increased probability of atherosclerotic plaque development in the arteries [65]. Hence, our study suggests that the potential reduction in plaque development in the aorta following treatment with higher doses of SAF and simvastatin may be attributed to the lower levels of LDL and TC detected in the bloodstream of the rabbits.

Research has shown that the consumption of HCD and lipid peroxidation lead to an inflammatory response that triggers the production of reactive oxygen species (ROS) [66]. ROS play a significant role in atherogenesis by activating NF-κB in vascular smooth muscle cells [67]. Activated NF-κB increases the production of inflammatory cytokines, such as TNFα, IL-1β, IL-6, and IL-8. These cytokines contribute to the inflammatory response within the arterial wall by promoting endothelial dysfunction, promoting the release of adhesion molecules such as VCAM-1, ICAM-1, and e-selectin. These biomarkers play crucial roles in regulating leukocyte recruitment to inflammatory sites, subsequently promoting the development of atherosclerotic lesions [68]. To further elucidate the mechanism by which saffron reduces atherosclerotic lesion areas in the aorta of rabbits, we assessed endothelial activation and pro-inflammatory biomarker expression via immunohistochemistry. We confirmed in the present study that VCAM-1, ICAM-1, e-selectin, IL-6, IL-8 and NF-κB are nearly absent in healthy endothelium but are evidently induced in irregular endothelium covering atherosclerotic plaques in rabbits. Interestingly, all these biomarkers decreased after treatment with a relatively high dose of SAF or statins, although some changes were not statistically significant.

To corroborate the tissue expression findings, we performed gene expression analysis via qRT–PCR. The results from both the tissue and gene expression analyses in our study are in line with each other, demonstrating a significant decrease in the expression of endothelial activation genes (VCAM-1, ICAM-1, and e-selectin) and pro-inflammatory genes (IL-6, IL-8, and NF-κB) following treatment with SAF and statins.

Extensive research has consistently revealed that antioxidants inhibit the expression of adhesion molecules, thereby slowing the progression of atherosclerosis [69–71]. Research on the medicinal properties of saffron revealed that saffron has strong antioxidative activity, which is primarily attributed to the presence of its major bioactive compounds, such as crocin, crocetin and safranal [38]. A previous in vivo study reported that crocetin could decrease VCAM-1 in atherosclerotic rabbits by blocking the NF-κB signaling pathway [33]. In addition, a study revealed that the intraperitoneal injection of a hydroethanolic extract of saffron could reduce the mRNA expression levels of ICAM-1 in Wistar rats [72]. According to a prior in vitro study, saffron and crocin could downregulate the expression of e-selectin in lipopolysaccharide-stimulated human coronary artery endothelial cells [73]. These findings suggest that the antiatherosclerotic effects of SAF might be attributed, at least in part, to the antioxidant capacity of its major bioactive compounds [74].

Saffron and its main constituents also have the potential to decrease the levels of pro-inflammatory cytokines. One study demonstrated that ethanolic and aqueous saffron extracts relieved neuropathic pain in a chronic constriction injury model by inhibiting pro-inflammatory factors such as TNF-α, IL-6, and IL-1β [75]. A recent in vitro investigation revealed that crocin and crocetin reduced cell cytotoxicity and decreased the level of IL-8, an inflammatory cytokine, by blocking the NF-κB signaling pathway [13]. Another study reported that crocin at 50 mg/kg reduced nephropathy in mice and activated the Nrf2 signaling pathway, leading to the downregulation of NF-κB [76]. Nevertheless, our study is the first to report the effects of saffron extract on the abovementioned biomarkers in atherosclerotic rabbits.

To gain further insight into the plaque stabilizing mechanisms of SAF, we assessed the effects of both doses of SAF on the tissue expression of MMP-9 and its inhibitor, TIMP-1. To our knowledge, this is the first study to investigate the impact of saffron extracts on plaque stability by evaluating the tissue expression of MMP-9 and TIMP-1 in the aorta of atherosclerotic rabbits.

MMP-9 is an enzyme that breaks down extracellular matrix proteins and is involved in a variety of physiological and pathological processes, such as inflammation and tissue remodeling [6]. In the context of atherosclerosis, MMP-9 has been implicated in the development and progression of the disease. Research indicates that MMP-9 levels are elevated in vulnerable plaques compared with stable plaques. MMP-9 also serves as an indicator to predict the instability of atherosclerotic plaques and as a risk factor for future adverse cardiovascular events [77]. Therefore, MMP-9 is considered a potential biomarker

and therapeutic target in the management of atherosclerosis and related cardiovascular diseases.

Tissue inhibitors of metalloproteinases (TIMPs) are a family of naturally occurring primary physiological inhibitors of MMPs [78]. The presence of TIMP-1 activity within atherosclerotic plaques seems to be correlated with reduced MMP activity, leading to decreased matrix remodeling [79]. The preference for TIMP-1 over other TIMPs in this study is attributed to its high-affinity inhibition of MMP-9 [80, 81]. Maintaining an equilibrium between MMPs and TIMPs could be essential in extracellular matrix turnover, thereby modulating atherosclerotic plaque development [82].

In our study, high doses of SAF and simvastatin significantly reduced the tissue expression of MMP-9 in the endothelium and atherosclerotic plaque. Similar effects of saffron extract on MMP-9 have been presented in a previous study. A previous study revealed that saffron regulates various transcription factors, including NF-κB, which is associated with the production of MMP-9 [83, 84]. In a separate study, it was reported that treatment with saffron extract for one year decreased serum MMP-9 and increased serum TIMP-1 levels in patients with multiple sclerosis [85].

In contrast, in our study, the expression of TIMP-1 did not differ from that of MMP-9 in the treatment group. This disparity could be attributed to the prolonged presence of net MMP-9 activity in advanced plaques [86], resulting in inadequate endogenous levels of TIMP-1 to achieve complete inhibition [87, 88]. Intriguingly, a previous study revealed that statins inhibited MMP-9 secretion from rabbit smooth muscle cells and foam cells in a dose-dependent manner. However, no effect on TIMP-1 synthesis was observed, resulting in an imbalance in the MMP-9/TIMP-1 ratio [89]. In conclusion, the lack of impact of SAF and statins on TIMP secretion implies a selective posttranslational mechanism targeting MMPs rather than a general inhibition of protein synthesis. However, in our study, SAF still demonstrated plaque-stabilizing effects, albeit by inhibiting MMP-9 expression rather than increasing TIMP-1 expression in the endothelium and atherosclerotic lesion area of the rabbit aorta.

Despite the encouraging findings of our study, there are several limitations to consider. First, the assessment of plaque vulnerability in NZWRs fed a high-cholesterol diet (HCD) is typically indirect, as spontaneous plaque rupture is rarely observed in atherosclerotic plaques. However, atherosclerotic lesions in NZWRs share common characteristics with human unstable plaques, such as a thin fibrous cap and the presence of a necrotic core, and mimic some of the processes that lead to plaque rupture. Therefore, this animal model remains valid for studying plaque texture.

Nonetheless, it is crucial to proceed with caution when extrapolating our experimental findings to clinical contexts, as outcomes observed in controlled laboratory settings may not necessarily apply directly in clinical settings. However, the relative quantification of protein expression within the endothelium and macrophages via immunohistochemistry, along with the quantification of endothelial activation and pro-inflammatory genes via qRT−PCR, provides compelling evidence. Additionally, the statistically significant differences observed between the treatment groups and the baseline and control groups further support the inhibitory effects of the saffron ethanolic extract on inflammatory mediators. In this study, the levels of inflammatory cytokines such as TNFα, IL-1β, IL-6, and IL-8 in the plasma were not assessed. The focus was directed toward the localized inflammatory response within the aortic tissue, aiming for a more direct correlation with the observed pathological changes. While recognizing the potential value of measuring plasma cytokine levels, these assessments were outside the scope of the study. Thus, future research is essential to validate these findings further. Additionally, our study focused on analyzing MMP9 protein expression to understand molecular changes in aortic tissue. Although in situ zymography could have provided more comprehensive insight into the role of MMP9, technical limitations prevented its inclusion in this study. Nonetheless, previous research has demonstrated robust correlations between MMP9 protein levels and its activity [90–92]. Future studies should aim to incorporate assessments of both MMP9 protein expression and its enzymatic activity to enhance the understanding of its functions. Besides, this study primarily focused on the inflammatory and endothelial responses associated with atherosclerosis and did not investigate the direct molecular mechanisms underlying saffron ethanolic extract's impact on lipid metabolism. Future studies incorporating lipidomic profiling and mechanistic assays are warranted to fully elucidate its lipid-modulating effects.

In summary, our findings suggest that saffron ethanolic extract mitigates atherosclerosis progression and promote plaque stability in atherosclerotic rabbits, potentially through its anti-inflammatory effects. Our results demonstrate a reduction in endothelial activation markers, pro-inflammatory cytokines, and matrix metalloproteinases, which are associated with atherosclerosis progression. While this study highlights the vascular-protective effects of saffron ethanolic extract, its direct impact on lipid metabolism warrants further investigation. To the best of our knowledge, this is the first study to report the plaque-stabilizing effects of saffron extract by examining the tissue expression of MMP-9 and TIMP-1, supporting its potential as an adjunctive therapy for atherosclerosis.

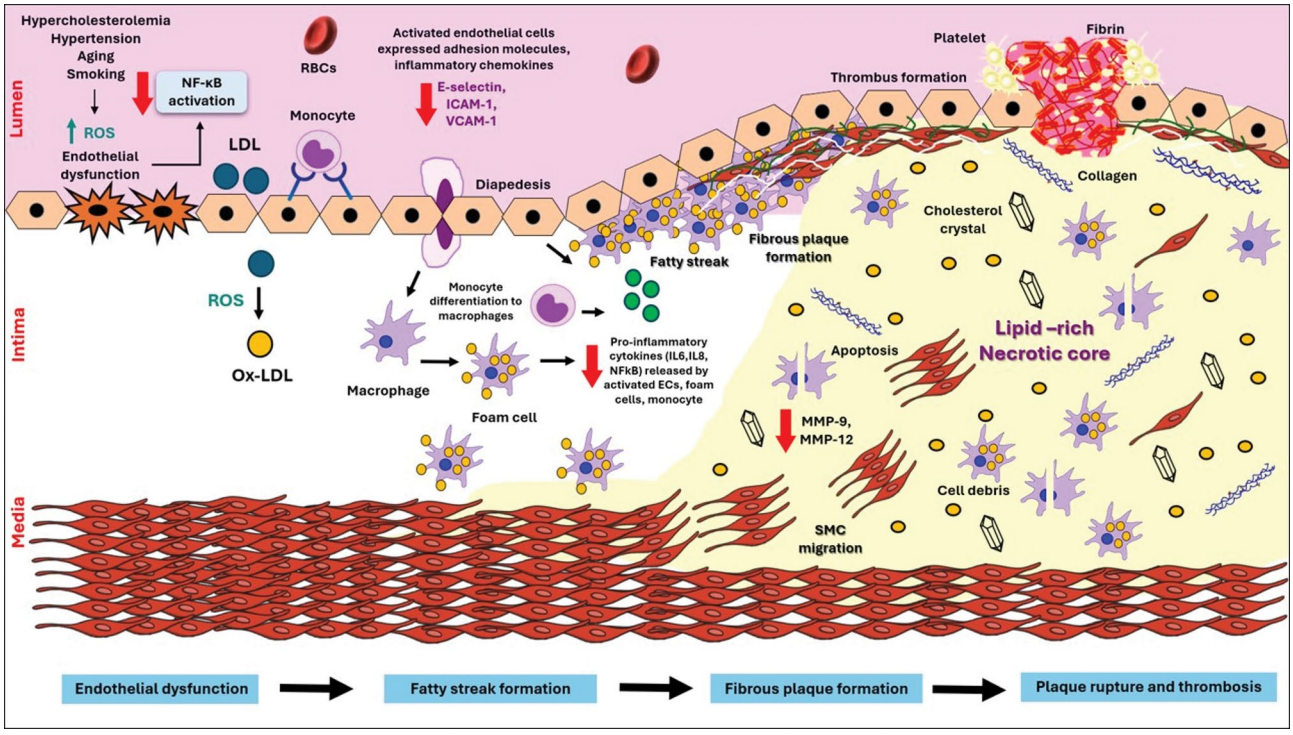

Fig. 9 The mechanism of saffron ethanolic extract in the treatment of HCD-induced atherosclerotic rabbits

Conclusion

In conclusion, as shown in Fig. 9, saffron ethanolic extract demonstrates a therapeutic effect in HCD-induced atherosclerotic rabbits, potentially through its anti-inflammatory properties. This is supported by the observed reduction in the tissue and gene expression of pro-inflammatory cytokines, endothelial activation biomarkers, and matrix metalloproteinases. These effects may contribute to improved lipid profiles, decreased severity of atherosclerotic lesions, and enhanced plaque stability in the aorta. While saffron ethanolic extract shows promise as an adjunctive treatment for atherosclerosis, further studies are warranted to elucidate its direct impact on lipid metabolism.

Abbreviations

cDNA	Complementary Deoxyribonucleic Acid
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
HCD	High-Cholesterol Diet
HDL	High-Density Lipoprotein Cholesterol
HPRT-1	Hypoxanthine Phosphoribosyl Transferase-1
ICAM-1	Intracellular Adhesion Molecule-1
IL-6	Interleukin-6
IL-8	Interleukin-8
LDL	Low-Density Lipoprotein Cholesterol
MMP-9	Matrix Metalloproteinases-9
MMPs	Matrix Metalloproteinases
NF-κB	Nuclear Factor kappa B
NO	Nitric Oxide
Nrf2	Nuclear factor erythroid 2–related factor 2
NZWR	New Zealand White rabbits
ox-LDL	Oxidized Low-Density Lipoprotein
qRT–PCR	Quantitative Real-Time Polymerase Chain Reaction
ROS	Reactive Oxygen Species
SAF	Saffron Ethanolic Extract
TC	Total Cholesterol
TG	Triglyceride
TIMP-1	Tissue Inhibitor of Matrix Metalloproteinases-1
TNF-α	Tumor Necrosis Factor-Alpha
VCAM-1	Vascular Cell Adhesion Molecule-1

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12906-025-04927-6.

Supplementary Material 1: Additional file 1.xls Mean body weight of rabbits at baseline, pretreatment and posttreatment. Additional file 2.xls Mean lipid profile of rabbits at baseline, pretreatment and posttreatment. Additional file 3.xls Percentages of atherosclerotic lesions in the aortas of New Zealand White rabbits. Additional file 4.xls Percentage of samples of aortas with positive immunohistochemical expression of endothelial activation proteins (VCAM-1, ICAM-1, and e-selectin), pro-inflammatory proteins (IL-8, IL-6, and NFkB p65), matrix metalloproteinases (MMP-9 and TIMP-1). Additional file 5.xls Relative fold changes in VCAM-1, ICAM-1, e-selectin, IL-6, IL-8 and NFkB gene expression in rabbits at baseline, pretreatment and posttreatment. Additional file 6.xls List of primers and annealing temperatures for the aorta in atherosclerotic rabbits.

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Author contributions
NAMK, EO and HMN designed the research study. INAR performed the research. NAMK, EO and SAM provided help and advice on conception, acquisition of data and supervision. INAR, NAMK and EO analyzed the data. INAR wrote the manuscript. EO and INAR performed histological examinations of the aorta. All the authors contributed to editorial changes in the manuscript. All the authors reviewed and approved the final manuscript.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
All the experiments were conducted in an appropriate animal experimentation facility. The animal experiments in this study were approved by and conducted in accordance with the rules and regulations of the Universiti Teknologi MARA Committee on Animal Research & Ethics (UiTM CARE). The ethical approval number was UiTM CARE: 326/2020.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Libby P, Buring JE, Badimon L, Hansson GK, Deanfield J, Bittencourt MS, Tokgözoğlu L, Lewis EF. Atherosclerosis. Nat Reviews Disease Primers. 2019;5(1):56.
2. Medina-Leyte DJ, Zepeda-García O, Domínguez-Pérez M, González-Garrido A, Villarreal-Molina T, Jacobo-Albavera L. Endothelial dysfunction, inflammation and coronary artery disease: potential biomarkers and promising therapeutical approaches. Int J Mol Sci 2021, 22(8).
3. Mehu M, Narasimhulu CA, Singla DK. Inflammatory cells in atherosclerosis. Antioxidants. 2022;11(2):233.
4. Jebari-Benslaiman S, Galicia-García U, Larrea-Sebal A, Olaetxea JR, Alloza I, Vandenbroeck K, Benito-Vicente A, Martín C. Pathophysiology of atherosclerosis. Int J Mol Sci 2022, 23(6).
5. Javadifar A, Rastgoo S, Banach M, Jamialahmadi T, Johnston TP, Sahebkar A. Foam cells as therapeutic targets in atherosclerosis with a focus on the regulatory roles of Non-Coding RNAs. Int J Mol Sci 2021, 22(5).
6. Olejarz W, Łacheta D, Kubiak-Tomaszewska G. Matrix metalloproteinases as biomarkers of atherosclerotic plaque instability. Int J Mol Sci 2020, 21(11).
7. Kremastiotis G, Handa I, Jackson C, George S, Johnson J. Disparate effects of MMP and TIMP modulation on coronary atherosclerosis and associated myocardial fibrosis. Sci Rep. 2021;11(1):23081.
8. Luk E, Gotlieb AI. Atherosclerosis. In: *Pathobiology of Human Disease.* edn. Edited by McManus LM, Mitchell RN. San Diego: Academic Press; 2014: 2970–2985.
9. Ngamdu KS, Ghosalkar DS, Chung HE, Christensen JL, Lee C, Butler CA, Ho T, Chu A, Heath JR, Baig M, et al. Long-term Statin therapy is associated with severe coronary artery calcification. PLoS ONE. 2023;18(7):e0289111.
10. Ruscica M, Ferri N, Banach M, Sirtori CR, Corsini A. Side effects of Statins: from pathophysiology and epidemiology to diagnostic and therapeutic implications. Cardiovascular Res. 2022;118(17):3288–304.
11. Newman CB, Preiss D, Tobert JA, Jacobson TA, Page RL, Goldstein LB, Chin C, Tannock LR, Miller M, Raghuveer G. Statin safety and associated adverse events: a scientific statement from the American heart association. Arterioscler Thromb Vasc Biol. 2019;39(2):e38–81.
12. Abd Rahim IN, Mohd Kasim NA, Omar E, Abdul Muid S, Nawawi H. Safety evaluation of saffron extracts in early and established atherosclerotic new Zealand white rabbits. PLoS ONE. 2024;19(1):e0295212.
13. Ghaffari S, Roshanravan N. Saffron; an updated review on biological properties with special focus on cardiovascular effects. Biomed Pharmacother. 2019;109:21–7.
14. Maqbool Z, Arshad MS, Ali A, Aziz A, Khalid W, Afzal MF, Bangar SP, Addi M, Hano C, Lorenzo JM. Potential Role of Phytochemical Extract from Saffron in Development of Functional Foods and Protection of Brain-Related Disorders. *Oxid Med Cell Longev* 2022, 2022:6480590.
15. Kianmehr M, Mahdizadeh F, Khazdair MR. The effects of Crocus sativus L.(Saffron) and its ingredients on dietary intakes in cardiovascular disease in Iranian population: A systematic review and meta-analysis. Front Nutr. 2022;9:890532.
16. Omidkhoda SF, Hosseinzadeh H. Saffron and its active ingredients against human disorders: A literature review on existing clinical evidence. Iran J Basic Med Sci. 2022;25(8):913–33.
17. Bostan HB, Mehri S, Hosseinzadeh H. Toxicology effects of saffron and its constituents: a review. Iran J Basic Med Sci. 2017;20(2):110–21.
18. Lopresti AL, Smith SJ. An examination into the mental and physical effects of a saffron extract (affron®) in recreationally-active adults: A randomized, double-blind, placebo-controlled study. J Int Soc Sports Nutr. 2022;19(1):219–38.
19. Hosseinzadeh H, Sadeghi Shakib S, Khadem Sameni A, Taghiabadi E. Acute and subacute toxicity of Safranal, a constituent of Saffron, in mice and rats. Iran J Pharm Res. 2013;12(1):93–9.
20. Muosa F, Al-Rekabi K, Askar S, Yousif E. Evaluation of the toxic effect of ethanolic extract of saffron in male mice after subchronic exposure. Donnish J Pharm Pharmacol. 2015;1:1–7.
21. Abd Rahim IN, Mohd Kasim NA, Isa MR, Nawawi H. A systematic review on the effect of saffron extract on lipid profile in hyperlipidaemic experimental animal models. Malays J Med Sci. 2022;29(4):14–27.
22. Arasteh A, Aliyev A, Khamnei S, Delazar A, Mesgari M, Mehmannavaz Y. Effects of hydromethanolic extract of saffron (Crocus sativus) on serum glucose, insulin and cholesterol levels in healthy male rats. J Med Plants Res. 2010;4:397–402.
23. Asdaq SMB, Inamdar MN. Potential of Crocus sativus (saffron) and its constituent, Crocin, as hypolipidemic and antioxidant in rats. Appl Biochem Biotechnol. 2010;162:358–72.
24. Samarghandian S, Azimi-Nezhad M, Samini F. Ameliorative effect of saffron aqueous extract on hyperglycemia, hyperlipidemia, and oxidative stress on diabetic encephalopathy in streptozotocin induced experimental diabetes mellitus. Biomed Res Int. 2014;2014:920857.
25. Hoshyar R, Hosseinian M, Rajabian Naghandar M, Hemmati M, Zarban A, Amini Z, Valavi M, Zare Beyki M, Mehrpour O. Anti-dyslipidemic properties of Saffron: reduction in the associated risks of atherosclerosis and insulin resistance. Iran Red Crescent Med J. 2016;18(12):22.
26. Elgazar AF, Rezq AA, Bukhari HM. Anti-hyperglycemic effect of saffron extract in alloxan-induced diabetic rats. Eur J Biol Sci. 2013;5(1):14–22.
27. Hemmati M, Zohoori E, Mehrpour O, Karamian M, Asghari S, Zarban A, Nasouti R. Anti-atherogenic potential of Jujube, saffron and barberry: anti-diabetic and antioxidant actions. EXCLI J. 2015;14:908.
28. Christodoulou E, Kadoglou NPE, Stasinopoulou M, Konstandi OA, Kenoutis C, Kakazanis ZI, Rizakou A, Kostomitsopoulos N, Valsami G. Crocus sativus L. aqueous extract reduces atherogenesis, increases atherosclerotic plaque stability and improves glucose control in diabetic atherosclerotic animals. Atherosclerosis. 2018;268:207–14.
29. Usmanova ZA. Relationship between the levels of mmp-9, timp-1, and zinc in biological samples of patients with carotid atherosclerosis. Int J Biomed. 2015;5(2):60–4.
30. de Nooijer R, Verkleij CJ, von der Thüsen JH, Jukema JW, van der Wall EE, van Berkel TJ, Baker AH, Biessen EA. Lesional overexpression of matrix metalloproteinase-9 promotes intraplaque hemorrhage in advanced lesions

but not at earlier stages of atherogenesis. Arterioscler Thromb Vasc Biol. 2006;26(2):340–6.
31. Jiang XB, Wang JS, Liu DH, Yuan WS, Shi ZS. Overexpression of matrix metalloproteinase-9 is correlated with carotid intraplaque hemorrhage in a swine model. J Neurointerv Surg. 2013;5(5):473–7.
32. de Vries MR, Niessen HW, Löwik CW, Hamming JF, Jukema JW, Quax PH. Plaque rupture complications in murine atherosclerotic vein grafts can be prevented by TIMP-1 overexpression. PLoS ONE. 2012;7(10):e47134.
33. Zheng S, Qian Z, Tang F, Sheng L. Suppression of vascular cell adhesion molecule-1 expression by Crocetin contributes to Attenuation of atherosclerosis in hypercholesterolemic rabbits. Biochem Pharmacol. 2005;70(8):1192–9.
34. Sheng L, Qian Z, Zheng S, Xi L. Mechanism of hypolipidemic effect of Crocin in rats: Crocin inhibits pancreatic lipase. Eur J Pharmacol. 2006;543(1–3):116–22.
35. Fan J, Kitajima S, Watanabe T, Xu J, Zhang J, Liu E, Chen YE. Rabbit models for the study of human atherosclerosis: from pathophysiological mechanisms to translational medicine. Pharmacol Ther. 2015;146:104–19.
36. Mashmoul M, Azlan A, Yusof BNM, Khaza'ai H, Mohtarrudin N, Boroushaki MT. Effects of saffron extract and Crocin on anthropometrical, nutritional and lipid profile parameters of rats fed a high fat diet. J Funct Foods. 2014;8:180–7.
37. Mohamadi Sani A, Mohseni S. Effect of type and concentration of solvent on chemical properties of saffron edible extract. Nutr Food Sci. 2014;44(1):2–5.
38. Rahaiee S, Hashemi M, Moini S, Shojaosadati SA, Razavi S. Comparison of phytochemical constituents and antioxidant activities of aqueous and alcoholic extracts of saffron. Qual Assur Saf Crops Foods. 2015;7(4):521–9.
39. Khayatnouri M, Safavi SE, Safarmashaei S, Babazadeh D, Mikailpourardabili B. The effect of saffron orally administration on spermatogenesis index in rat. Adv Environ Biol. 2011;5(7):1514–21.
40. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016;7(2):27–31.
41. Bocan TM, Mazur MJ, Mueller SB, Brown EQ, Sliskovic DR, O'Brien PM, Creswell MW, Lee H, Uhlendorf PD, Roth BD. Antiatherosclerotic activity of inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase in cholesterol-fed rabbits: a biochemical and morphological evaluation. Atherosclerosis. 1994;111(1):127–42.
42. Hamm T Jr, Kaplan J, Clarkson T, Bullock B. Effects of gender and social behavior on the development of coronary artery atherosclerosis in cynomolgus macaques. Atherosclerosis. 1983;48(3):221–33.
43. Livak KJ. TD Schmittgen 2001 Analysis of relative gene expression data using real-time quantitative PCR and the 2– ΔΔCT method. Methods 25 4 402–8.
44. Rahim INA, Kasim NAM, Omar E, Muid SA, Nawawi H. Evaluation on the Effectiveness of High Cholesterol Diet Feeding in Inducing Early and Established Atherosclerotic Lesions in New Zealand White Rabbits. *FBL* 2023, 28(4).
45. Limpijankit T, Vathesatogkit P, Matchariyakul D, Wiriyatanakorn S, Siriyotha S, Thakkinstian A, Sritara P. Causal relationship of excess body weight on cardiovascular events through risk factors. Sci Rep. 2022;12(1):5269.
46. Henning RJ. Obesity and obesity-induced inflammatory disease contribute to atherosclerosis: a review of the pathophysiology and treatment of obesity. Am J Cardiovasc Dis. 2021;11(4):504–29.
47. Tahmasbi F, Araj-Khodaei M, Mahmoodpoor A, Sanaie S. Effects of saffron (Crocus sativus L.) on anthropometric and cardiometabolic indices in overweight and obese patients: A systematic review and meta-analysis of randomized controlled trials. Phytother Res. 2022;36(9):3394–414.
48. Kianbakht S, Hashem Dabaghian F. Anti-obesity and anorectic effects of saffron and its constituent Crocin in obese Wistar rat. J Med Plants. 2015;14(53):25–33.
49. Aryaeian N, Alipour R, Jafari Karegar S, Soleimani M, Hosseini A, Hekmatdoost A. Saffron effects on liver enzymes, antioxidant capacity, insulin, inflammation and genes expression of lipolysis and lipogenesis in a rat model of non-alcoholic fatty liver disease. Clin Nutr Open Sci. 2024;53:95–107.
50. Gout B, Bourges C, Paineau-Dubreuil S. Satiereal, a Crocus sativus L extract, reduces snacking and increases satiety in a randomized placebo-controlled study of mildly overweight, healthy women. Nutr Res. 2010;30(5):305–13.
51. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, Benetos A, Biffi A, Boavida J-M, Capodanno D, et al. 2021 ESC guidelines on cardiovascular disease prevention in clinical practice: developed by the task force for cardiovascular disease prevention in clinical practice with representatives of the European society of cardiology and 12 medical societies with the special contribution of the European association of preventive cardiology (EAPC). Eur Heart J. 2021;42(34):3227–337.
52. Gaggini M, Gorini F, Vassalle C. Lipids in atherosclerosis: pathophysiology and the role of calculated lipid indices in assessing cardiovascular risk in patients with hyperlipidemia. Int J Mol Sci 2022, 24(1).
53. Stein EA, Lane M, Laskarzewski P. Comparison of Statins in hypertriglyceridemia. Am J Cardiol. 1998;81(4a):b66–9.
54. Collins R, Armitage J, Parish S, Sleigh P, Peto R. MRC/BHF heart protection study of cholesterol-lowering with Simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. Lancet. 2003;361(9374):2005–16.
55. Cavallini DCU, Bedani R, Bomdespacho LQ, Vendramini RC, Rossi EA. Effects of probiotic bacteria, isoflavones and Simvastatin on lipid profile and atherosclerosis in cholesterol-fed rabbits: a randomized double-blind study. Lipids Health Dis. 2009;8(1):1.
56. Hasler-Rapacz J, Kempen HJ, Princen HM, Kudchodkar BJ, Lacko A, Rapacz J. Effects of Simvastatin on plasma lipids and apolipoproteins in Familial hypercholesterolemic swine. Arterioscler Thromb Vasc Biol. 1996;16(1):137–43.
57. Morofuji Y, Nakagawa S, Ujifuku K, Fujimoto T, Otsuka K, Niwa M, Tsutsumi K. Beyond lipid-lowering: effects of Statins on cardiovascular and cerebrovascular diseases and cancer. Pharmaceuticals. 2022;15(2):151.
58. Asbaghi O, Soltani S, Norouzi N, Milajerdi A, Choobkar S, Asemi Z. The effect of saffron supplementation on blood glucose and lipid profile: A systematic review and meta-analysis of randomized controlled trials. Complement Ther Med. 2019;47:102158.
59. Rahmani J, Manzari N, Thompson J, Clark CCT, Villanueva G, Varkaneh HK, Mirmiran P. The effect of saffron on weight and lipid profile: A systematic review, meta-analysis, and dose-response of randomized clinical trials. Phytother Res. 2019;33(9):2244–55.
60. Bagur MJ, Alonso Salinas GL, Jiménez-Monreal AM, Serrano-Heras G, Martínez-Tomé M, Alonso GL. Effect of daily intake of a saffron infusion on blood cholesterol levels. Appl Sci. 2020;10(14):4763.
61. Kamalipour M, Akhondzadeh S. Cardiovascular effects of Saffron: an evidence-based review. J Tehran Heart Cent. 2011;6(2):59–61.
62. Dehghan F, Hajiaghaalipour F, Yusof A, Muniandy S, Hosseini SA, Heydari S, Salim LZA, Azarbayjani MA. Saffron with resistance exercise improves diabetic parameters through the GLUT4/AMPK pathway in-vitro and in-vivo. Sci Rep. 2016;6(1):25139.
63. Samarghandian S, Borji A. Anticarcinogenic effect of saffron (Crocus sativus L.) and its ingredients. Pharmacognosy Res. 2014;6(2):99.
64. Samarghandian S, Azimi-Nezhad M, Farkhondeh T. Immunomodulatory and antioxidant effects of saffron aqueous extract (Crocus sativus L.) on streptozotocin-induced diabetes in rats. Indian Heart J. 2017;69(2):151–9.
65. Hao W, Friedman A. The LDL-HDL profile determines the risk of atherosclerosis: A mathematical model. PLoS ONE. 2014;9(3):e90497.
66. Du Z, Yang Y, Hu Y, Sun Y, Zhang S, Peng W, Zhong Y, Huang X, Kong W. A long-term high-fat diet increases oxidative stress, mitochondrial damage and apoptosis in the inner ear of D-galactose-induced aging rats. Hear Res. 2012;287(1–2):15–24.
67. Gan J, Guo L, Zhang X, Yu Q, Yang Q, Zhang Y, Zeng W, Jiang X, Guo M. Anti-inflammatory therapy of atherosclerosis: focusing on IKKβ. J Inflamm. 2023;20(1):8.
68. Zhang J. Biomarkers of endothelial activation and dysfunction in cardiovascular diseases. RCM 2022, 23(2).
69. Weber C, Erl W, Pietsch A, Ströbel M, Ziegler-Heitbrock HW, Weber PC. Antioxidants inhibit monocyte adhesion by suppressing nuclear factor-kappa B mobilization and induction of vascular cell adhesion molecule-1 in endothelial cells stimulated to generate radicals. Arterioscler Thromb. 1994;14(10):1665–73.
70. Cominacini L, Garbin U, Pasini AF, Davoli A, Campagnola M, Contessi GB, Pastorino AM, Lo Cascio V. Antioxidants inhibit the expression of intercellular cell adhesion molecule-1 and vascular cell adhesion molecule-1 induced by oxidized LDL on human umbilical vein endothelial cells. Free Radic Biol Med. 1997;22(1–2):117–27.
71. Murphy N, Grimsditch DC, Vidgeon-Hart M, Groot PHE, Overend P, Benson GM, Graham A. Dietary antioxidants decrease serum soluble adhesion molecule (sVCAM-1, sICAM-1) but not chemokine (JE/MCP-1, KC) concentrations, and reduce atherosclerosis in C57BL but not ApoE*3 Leiden mice fed an atherogenic diet. Dis Markers. 2005;21:394152.
72. Mahmoudzadeh L, Najafi H, Ashtiyani SC, Yarijani ZM. Anti-inflammatory and protective effects of saffron extract in ischaemia/reperfusion-induced acute kidney injury. Nephrology. 2017;22(10):748–54.
73. Kasim NM, Abd Rahim I, Bakar NA, Ahmad R, Rahman TA, Froemming G, Nawawi HM. Saffron and its active compound, Crocin inhibit E-selectin in

LPS-stimulated human coronary artery endothelial cells. Atherosclerosis. 2020;315:e109.
74. Cerdá-Bernad D, Valero-Cases E, Pastor JJ, Frutos MJ. Saffron bioactives Crocin, Crocetin and Safranal: effect on oxidative stress and mechanisms of action. Crit Rev Food Sci Nutr. 2022;62(12):3232–49.
75. Amin B, Abnous K, Motamedshariaty V, Hosseinzadeh H. Attenuation of oxidative stress, inflammation and apoptosis by ethanolic and aqueous extracts of Crocus sativus L. stigma after chronic constriction injury of rats. Acad Bras Cienc. 2014;86(4):1821–32.
76. Qiu Y, Li Z. The hypoglycemic and renal protection properties of Crocin via oxidative stress-regulated NF-κB signaling in Db/db mice. Front Pharmacol. 2020;11:493233.
77. Li T, Li X, Feng Y, Dong G, Wang Y, Yang J. The Role of Matrix Metalloproteinase-9 in Atherosclerotic Plaque Instability. *Mediators Inflamm* 2020, 2020:3872367.
78. Brew K, Nagase H. The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. Biochim Biophys Acta. 2010;1803(1):55–71.
79. AB Z. Increased secretion of tissue inhibitors of metalloproteinases 1 and 2 from the aortas of cholesterol fed rabbits partially counterbalances increased metalloproteinase activity. Arterioscler Thromb Vasc Biol. 1999;19(7):1700–7.
80. Arpino V, Brock M, Gill SE. The role of timps in regulation of extracellular matrix proteolysis. Matrix Biol. 2015;44:247–54.
81. Papazafiropoulou A, Tentolouris N. Matrix metalloproteinases and cardiovascular diseases. Hippokratia. 2009;13(2):76.
82. Pussinen PJ, Sarna S, Puolakkainen M, Öhlin H, Sorsa T, Pesonen E. The balance of serum matrix metalloproteinase-8 and its tissue inhibitor in acute coronary syndrome and its recurrence. Int J Cardiol. 2013;167(2):362–8.
83. Zeinali M, Zirak MR, Rezaee SA, Karimi G, Hosseinzadeh H. Immunoregulatory and anti-inflammatory properties of Crocus sativus (Saffron) and its main active constituents: A review. Iran J Basic Med Sci. 2019;22(4):334–44.
84. Chrastina M, Póništ S, Dráfi F, Švík K, Khademnematolahi S, Pružinská K, Tchorbanov A, Bauerová K. Effect of saffron extract, Astaxanthin, and carnosic acid on the levels of matrix Metalloproteinase-9 and on body weight changes in arthritis experiments. Eur Pharm J, 69(2):26–33.
85. Ghasemi Sakha F, Azimi Saeen A, Moazzeni SM, Etesam F, Vaezi G. A randomized, Triple-blind Placebo-controlled trial to determine the effect of saffron on the serum levels of MMP-9 and TIMP-1 in patients with multiple sclerosis. Iran J Allergy Asthma Immunol. 2020;19(3):297–304.
86. Galis ZS, Sukhova GK, Libby P. Microscopic localization of active proteases by in situ zymography: detection of matrix metalloproteinase activity in vascular tissue. Faseb J. 1995;9(10):974–80.
87. Di Gregoli K, George SJ, Jackson CL, Newby AC, Johnson JL. Differential effects of tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 on atherosclerosis and monocyte/macrophage invasion. Cardiovasc Res. 2016;109(2):318–30.
88. Baker AH, Edwards DR, Murphy G. Metalloproteinase inhibitors: biological actions and therapeutic opportunities. J Cell Sci. 2002;115(Pt 19):3719–27.
89. Luan Z, Chase AJ, Newby AC. Statins inhibit secretion of metalloproteinases-1, -2, -3, and– 9 from vascular smooth muscle cells and macrophages. Arterioscler Thromb Vasc Biol. 2003;23(5):769–75.
90. Breviario S, Senserrich J, Florensa-Zanuy E, Garro-Martínez E, Díaz Á, Castro E, Pazos Á, Pilar-Cuéllar F. Brain matrix metalloproteinase-9 activity is altered in the corticosterone mouse model of depression. Prog Neuropsychopharmacol Biol Psychiatry. 2023;120:110624.
91. Rullman E, Norrbom J, Strömberg A, Wågsäter D, Rundqvist H, Haas T, Gustafsson T. Endurance exercise activates matrix metalloproteinases in human skeletal muscle. J Appl Physiol. 2009;106(3):804–12.
92. Yeghiazaryan M, Żybura-Broda K, Cabaj A, Włodarczyk J, Sławińska U, Rylski M, Wilczyński GM. Fine-structural distribution of MMP-2 and MMP-9 activities in the rat skeletal muscle upon training: a study by high-resolution in situ zymography. Histochem Cell Biol. 2012;138(1):75–87.

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