https://doi.org/10.31925/farmacia.2026.4.15 **ORIGINAL ARTICLE**

SAFFRON PETALS AS A POTENTIAL THERAPEUTIC AGENT IN OBESE WISTAR RATS: A MULTI-TARGET APPROACH TO LIPID METABOLISM AND REDOX HOMEOSTASIS

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Abstract

The petals of the saffron (*Crocus sativus* L.) are the main by-product of the saffron industry. They are a source of several bioactive compounds, such as polyphenols, flavonoids, and carotenoids, which have a significant effect in preventing various diseases. This study aimed to investigate the anti-obesity effects of Algerian Saffron petal extract (SPE) through an *in vivo* study. An obese rat model was orally administered SPE at different doses (20, 40, and 100 mg/kg b.w./day). Then plasma and tissue lipid parameters, lipase activities, glycemia, and redox biomarkers were assessed. Our results showed that SPE treatment significantly reduced body weight, prevented adiposity, hepatic steatosis and modulated the activities of lipid metabolism-related enzymes. Furthermore, SPE alleviated hyperglycemia, dyslipidemia, and oxidative stress, and importantly, enhanced the antioxidant defenses in obese rats. In conclusion, our findings highlight the therapeutic potential of this saffron by-product against obesity and its related metabolic disorders.

Rezumat

Petalele de șofran (*Crocus sativus* L.) reprezintă principalul subprodus al industriei șofranului. Acestea constituie o sursă de compuși bioactivi, precum polifenoli, flavonoide și carotenoide, care exercită un efect semnificativ în prevenirea diverselor afecțiuni. Acest studiu și-a propus să investigheze efectele extractului din petale de șofran algerian (SPE) printr-un studiu *in vivo*. Un model de șobolan obez a primit SPE pe cale orală, în doze diferite (20, 40 și 100 mg/kg corp/zi). Ulterior, au fost evaluați parametrii lipidici plasmatici și tisulari, activitățile lipazice, glicemia și biomarkeri redox. Rezultatele obținute au arătat că tratamentul cu SPE a redus semnificativ greutatea corporală, a prevenit adipozitatea, steatoza hepatică și a modulat activitatea enzimelor implicate în metabolismul lipidic. În plus, SPE a ameliorat hiperglicemia, dislipidemia, stresul oxidativ și a potențat mecanismele antioxidante de apărare la șobolanii obezi. În concluzie, rezultatele obținute evidențiază potențialul terapeutic al acestui subprodus al șofranului în tratarea obezității și a tulburărilor metabolice asociate.

Keywords: saffron petals extract, rat model, obesity, oxidative stress

Introduction

Obesity is the result of a long-term imbalance between energy intake and expenditure. It is the leading cause of early mortalities worldwide as it drives comorbidities such as type 2 diabetes, dyslipidemia, hypertension, hepatic steatosis and cardiovascular disease. These conditions can be exacerbated by a variety of factors, such as sedentary lifestyles, high-calorie diet and genetic predisposition (1, 2).

First-line obesity management mainly involves behavioural modifications such as energy-restricted diets and physical activity. Its persistent prevalence highlights the need for novel therapeutic strategies (3). Using “quick fix” treatments, including surgical and medical therapies, has shown significant weight loss; however, they are often associated with side effects, health risks, and high costs (4, 5).

It has been proposed that natural products can serve as biological agents for the treatment and prevention of obesity through functional foods and nutraceuticals derived from them (6-8). Using medicines derived from plants or herbs, known as the oldest form of healthcare in human history and referred to as “phytotherapy”, appears to be one of the safest and

most effective strategies for managing obesity (9, 10).

Saffron (*Crocus sativus* L.), commonly referred to as "red gold", is a stemless perennial herb belonging to the Iridaceae family, which comprises 85 to 100 species distributed worldwide, mainly in the Mediterranean region, Europe, and Western Asia. The valuable spice known as "saffron" is obtained from the dried stigma of *Crocus sativus* L., where its nickname "red gold" highlights its vibrant red colour, high economic value and widespread use in global cuisine and the pharmaceutical field due to its organoleptic and therapeutic properties (11).

The petals of *C. sativus* constitute the main by-product of saffron processing. Although they are produced in large quantities, they go unused and are often discarded. As a result, increasing attention is drawn towards their potential applications. Several studies have shown that these petals possess several active components, including anthocyanins, flavonoids, mineral matter, starch, amino acids, and vitamins (riboflavin and thiamine) (12-14). Furthermore, recent reports indicated that saffron petal extract has antioxidant, anti-inflammatory, hepatoprotective, neuroprotective, cardioprotective, and antimicrobial properties (15-18). These findings highlight the potential of saffron petal extract in managing conditions associated with oxidative stress and metabolic imbalance. Since metabolic disorders characterise obesity, the use of SPE seems to be an excellent therapeutic approach.

This study aimed to optimise the extraction process for saffron petals and to perform a phytochemical screening to identify the phytochemical classes present in the petal extract. Then, systematically, the potential of the extract treatment was studied both *in vitro* and *in vivo*. The *in vitro* biological activities of the extract were evaluated by assessing its cytotoxic, anti-hemolytic, anti-inflammatory, and lipid peroxidation-inhibitory effects. Subsequently, an *in vivo* study was conducted to investigate the effect of SPE supplementation on obesity-related disorders, particularly dyslipidemia and oxidative stress. In order to assess the impact of SPE supplementation, the body weight, lipid profiles, serum biochemical parameters and oxidative stress were investigated, and a histopathological study was carried out in cafeteria diet-induced obese rats.

Materials and Methods

Preparation of petal extract

In this study, *Crocus sativus* L. petals were harvested between October 15th and November 15th, 2025, during the full flowering stage (anthesis), from plants grown in the Djebel Zaafran area of the Ain Fezza Commune (latitude: 34°52′36″ N; longitude: 01°12′55″ W; altitude: 863 m; Tlemcen Province, Algeria). Botanical identification of the plant material was authenticated by Prof. F. Hassani from the Laboratory of Ecology and Management of Natural Ecosystems, University of Tlemcen, Algeria. A voucher specimen was deposited at the Laboratory of Natural Products (Department of Biology, University of Tlemcen) under the accession number *Crocus* L. 224. In order to prepare the hydromethanolic extract, the collected petals were shade-dried at room temperature and then milled into a fine powder. An amount of 5 g of this powder was then macerated in 100 mL of 70% v/v (Methanol/ distilled water) for 24 hours in a dark place. The mixture was brewed, filtered using Whatman No.1 filter paper and oven-dried at 40° for 24 hours until it was completely dry. The extraction yield (w/w) of the hydromethanolic extract of petals was 55%.

Phytochemical screening

The petal extract was subjected to preliminary phytochemical screening to assess the presence of secondary metabolites. Phytochemical tests were carried out according to standard procedures. The extract was tested for the presence of saponins, tannins, flavonoids, quinones, anthraquinones, coumarins, reductive compounds, terpenoids, and alkaloids. Various tests have been conducted qualitatively to determine the presence or absence of bioactive compounds (19, 20).

In vitro biological activities study of SPE

Human red blood cell (RBC) suspension preparation

Blood samples (8 mL) were collected in Lithium Heparin tubes from the peripheral veins of the arm of a healthy human volunteer. Blood collection was performed with approval from the Ethics Committee of Tlemcen University Hospital, and written informed consent was obtained from the volunteer in accordance with the Declaration of Helsinki. The collected blood samples were centrifuged at 3000 rpm for 10 min to isolate the erythrocytes. Then, the RBC pellet was washed with an equal volume of iso-saline solution (0.9% NaCl). This washing step was repeated three times until the supernatant was clear. After final aspiration of the supernatant, the remaining pellet was diluted 1:10 (v/v) in iso-saline solution to obtain a 10% erythrocyte suspension.

Cytotoxicity test

Cytotoxicity of SPE was evaluated using a hemolytic test according to Bulmus *et al.* (2003) (21). 0.4 mL of RBC suspension (10%) was maintained in contact with 1.6 mL of the extract and/or gallic acid at different concentrations (156, 312, 625, 1250, 2500, 5000 µg/mL (w/v)). The reaction mixtures were incubated at 37°C for 30 min, then centrifuged at 3000 rpm for 10 min, and hemolysis was quantified by measuring absorbance at 560 nm.

The percentage of Hemolysis of Human RBC was calculated using the following formula (1):

$$Hemolysis\% = \frac{\text{Abs}_{560\text{nm}}\ \text{Test solution}}{\text{Abs}_{560\text{nm}}\ \text{Control solution}} \times 100$$

Distilled water was used as the positive solution, and physiological saline water as the negative solution.

Anti-hemolytic activity test

A human RBC membrane stabilisation assay was carried out to evaluate the *in vitro* anti-hemolytic activity of saffron petal extract.

The reaction mixture was prepared by mixing 0.5 mL of SPE, gallic acid and sodium diclofenac at different concentrations (30, 75,150, 300 µg/mL (w/v)), 1.5 mL of sodium phosphate buffer (10 mM NaCl, pH 7.4) and 2 mL of hypo saline solution (0.36% NaCl). The mixture was incubated for 20 min at 37°C, then 0.5 mL of Human RBC suspension (10%) was added and incubated a second time for 1 hour at 56°C. After cooling, the tubes were centrifuged at 2500 rpm for 5 min, and the absorbance of the supernatant was measured at 560 nm.

Phosphate buffer and gallic acid were used as a negative control and a standard, respectively. The ability of SPE to stabilise RBC membranes was calculated in per cent by the formula (2):

$$Membrane\ stability\% = \frac{\text{Abs}_{560\text{nm}}\ \text{Negative control} - \text{Abs}_{560\text{nm}}\ \text{Test solution}}{\text{Abs}_{560\text{nm}}\ \text{Negative control}} \times 100$$

Gallic acid and sodium diclofenac were used as standards.

Anti-inflammatory activity test

The anti-inflammatory effect of SPE was tested using the Bovine Serum Albumin (BSA) denaturation inhibition assay (22). The following reaction mixture was prepared: 0.45 mL of BSA 5% and 0.05 mL of extract solution, sodium diclofenac at concentrations (3 µg/ mL, 30 ng/mL, 300 pg/mL) and adjusted to pH = 6.3 with a solution of HCl (1N). The mixture was incubated for 20 min at 37°C, then for 5min at 70°C. After cooling, 2.5mL of phosphate-buffered saline solution (pH 6.3) was added to the mixture, and the absorbance was measured at 416 nm.

The ability of the extract to inhibit protein denaturation was calculated using the following formula (3):

$$Inhibition\% = [1 - (\frac{\text{Abs}_{416\text{nm}}\ \text{Test solution} - \text{Abs}_{416\text{nm}}\ \text{Product control}}{\text{Abs}_{416\text{nm}}\ \text{Test control}})] \times 100$$

Distilled water was used as the negative control, and sodium diclofenac was used as the standard.

Lipid peroxidation inhibition assay

The inhibition of lipid peroxidation was determined by assaying thiobarbituric acid reactive substances (TBARS) in egg yolk, a rich source of lipids, according to the Method of Wong *et al*. (23).

Therefore, 500 µL of egg yolk (10% w/v of KCl solution 1.15% w/v) was mixed with 100 µL of SPE at different concentrations (300 pg/mL, 30 ng/mL, 3 µg/mL, 30 µg/mL, 75 µg/mL, 150 µg/mL) and completed to 1 mL with distilled water. Then, 1.05 mL of acetic acid 20% (pH 3.5) and 1.5 mL of TBA solution were added. The mixture was heated at 95°C for 1 hour. After cooling to room temperature, 5 mL of butanol was added, the mixture was agitated, and it was centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm.

The inhibition percentage of lipid peroxidation was calculated as follows (4):

$$\text{Inhibition}\% = [1 - \frac{\text{Abs}_{532\text{nm}}\ \text{Test solution}}{\text{Abs}_{532\text{nm}}\ \text{Negative control}}] \times 100$$

For the negative control, the extract was replaced by the egg yolk corresponding to 100% lipid peroxidation.

In vivo pharmacodynamic study of SPE

Animals and experimental protocol

All experiments were carried out in accordance with regulations and guidelines provided by the policies of the University of Tlemcen Institutional Animal Care and Use Committee (IACUC) (accreditation number: D01N01UN130120150006). A total of 48 male Wistar rats, 4 weeks old, weighing between 60 and 70 g, obtained from the Pasteur Institute (Algiers, Algeria), were used in this study. They were housed in separate cages (2–3 *per* cage) under controlled temperature (25 ± 2°C) and humidity (60 ± 5%) with a light regime identical to the natural photoperiod (12-h:12-h light/dark cycle). All rats had free access to food and water.

After one week of acclimation, the 48 rats were divided into two main groups of 24 animals each: one group was fed a normal diet (C) (65% carbohydrates, 25% proteins and 10% lipids (ONAB; Algeria)) and the other group was fed a cafeteria diet (O) for 8 weeks to induce obesity.

After these 8 weeks of diet feeding, each main group was further subdivided into four distinct subgroups (n = 6 rats *per* subgroup) and maintained for an additional 6 weeks, resulting in a total of eight experimental subgroups that were fed the appropriate diets for 14 weeks and co-administered SPE during the last 6 weeks as follows: C and O subgroups (C rats fed normal diets only and O rats fed cafeteria diet only), C20 and O20 subgroups (C20 rats fed normal diets and O20 rats fed cafeteria diet: both were supplemented with low dose of SPE (20 mg/kg b.w., once a day), C40 and O40 subgroups (C40 rats fed normal diets and O40 rats fed cafeteria diet: both were supplemented with medium dose of SPE (40 mg/kg b.w., once a day)), C100 and O100 subgroups (C100 rats fed normal diets and O100 rats fed cafeteria diet: both were supplemented with high dose of SPE (100 mg/kg b.w., once a day).

For oral gavage administration, the crude petal extract was dissolved in 0.9% sterile saline and adjusted to concentrations corresponding to doses of 20, 40, and 100 mg/kg body weight.

The rats in the C and O groups were fed 0.9% physiological saline to eliminate stress associated with the gavage procedure, and the treatment groups were fed petal extract once daily for 6 weeks. The body weight of rats was measured at the end of the treatment.

Blood and tissue samples

At the end of the experimental period (14 weeks), all rats were anaesthetised by an intraperitoneal injection and euthanised after an overnight fast. Blood samples were collected from the abdominal aorta into EDTA tubes and centrifuged at 4000 rpm for 10 min at 4°C. The resulting Plasma was collected and stored at −20°C until analysis. The liver, gastrocnemius muscle, and visceral adipose tissue were removed, rinsed with ice-cold 0.1 mol/L phosphate-buffered saline (PBS), quickly weighed, and stored at −80°C.

Preparation of erythrocyte lysate

Erythrocytes were lysed with ice-cold distilled water at a 10:1 ratio. After incubation for 15 min at 4°C, cell debris was removed by centrifugation at 2000 g for 15 min, and the supernatant was collected and stored at -20°C until analysis.

Preparation of homogenate

Three types of homogenates were prepared. For the first one, tissue aliquots were homogenised in 10 mM phosphate-buffered saline (PBS) at pH 7.4 containing 1% KCl. The samples were centrifuged at 6000 g for 15 min at 4°C, then the supernatant was collected and used to determine lipid and redox biomarker levels.

For the second homogenate, tissue aliquots (visceral adipose tissue and gastrocnemius muscle for lipoprotein lipase (LPL) activity, and liver tissue for hepatic lipase (HL) activity) were homogenised in ice-cold buffer containing 9% NaCl, 2% albumin and heparin (Sigma, St. Louis, MO, USA), pH 7.4. The samples were centrifuged at 10000×g for 15 min at 4°C. The collected supernatant was used to assess LPL and HL substrate-cleaving activities spectrophotometrically, following the established method (24).

The third homogenate, used for hormone-sensitive lipase (HSL) activity assays, was prepared by homogenising adipose tissue in ice-cold buffer containing 0.25 M sucrose, 1 mM dithiothreitol and 1 mM EDTA, pH 7.4, supplemented with 20 µg/mL leupeptin, 2 µg/mL antipain and 1 µg/mL pepstatin.

Oxidative stress analysis

Oxidative stress biomarkers were determined in erythrocyte, liver, adipose tissue and muscle. Malondialdehyde (MDA) and carbonyl protein (CP) levels were estimated using specific methods with the thiobarbituric acid and 2,4-dinitrophenylhydrazine reactions, respectively (MAK085-1KT and MAK094-1KT, Sigma-Aldrich kits; St. Louis, MO, USA). For tissue samples (liver, adipose tissue, and muscle), MDA and CP concentrations were normalised to the fresh tissue weight and expressed as nmol/mg of wet tissue. In contrast, erythrocyte levels were expressed relative to haemoglobin content as µmol/g Hb.

Catalase activity was measured by spectrophotometric monitoring of the decomposition rate of hydrogen peroxide (H_2O_2, 35 mM) at 240 nm using catalase enzyme (CAT100-1KT, Sigma-Aldrich). One unit (U) of Catalase activity was defined as the amount of enzyme required to decompose 1µmol of H_2O_2 *per* minute under the assay conditions. The enzymatic activity was expressed as U/mg wet tissue for organs and as U/g haemoglobin (Hb) for erythrocyte samples. Reduced glutathione (GSH) levels were measured by a colourimetric method based on the formation of 2-nitro-5-thiobenzoic acid *via* GSH-mediated reduction of 5,5-dithiobis-(2-nitrobenzoic) acid, according to the manufacturer's instructions (MAK364-1KT, Sigma-Aldrich, Saint Louis, MO, USA). GSH concentration was expressed as µmol/g of wet tissue for organs and µmol/g Hb for erythrocytes.

Plasma Vitamin C was quantified using a colourimetric method that measures the formation of Fe^{+2} following Vitamin C-mediated reduction of Fe^{+3} (E-BC-K034-M, Elabscience) and was expressed as µmol/L.

Biochemical analysis

Plasma glucose and total protein concentrations were determined using commercial assay Kits (Spinreact). Plasma levels of total cholesterol (TC), triglyceride (TG), High-density lipoprotein cholesterol (HDL-C), and Low-Density lipoprotein cholesterol (LDL-C), and tissue levels of TC and TG, were determined using commercial assay Kits (Spinreact).

Histopathology study

Liver, visceral adipose tissue, and kidney were immediately fixed in 10% neutral-buffered formalin after rat sacrifice and subsequently embedded in paraffin before sectioning. The Hematoxylin/Eosin method was used to stain tissue sections (25,26), which were prepared for examination under a high-power microscope (100×), and photomicrographs were taken.

Statistical analysis

Results for *in vivo* assays are presented as means ± S.E.M. (n = 6 *per* group). For *in vitro* assays, results are expressed as means ± S.E.M. of three independent experiments, each performed in triplicate. After checking the normal distribution of the variables using the Shapiro-Wilk test, differences in means between the eight groups of rats (C, C20, C40, C100, O, O20, O40, O100) were assessed using the one -

way ANOVA test, followed by Tukey's post hoc test to identify pairwise differences between groups. Means labelled by different letters (a, b, c, d) differ significantly at $p < 0.05$. All statistical analyses and graphical representations were performed utilising GraphPad Prism 9 Software (GraphPad Software, San Diego, CA).

Results and Discussion

Qualitative analysis of phytochemicals of plants studied

Qualitative phytochemical screening of the hydro-methanolic extract of *Crocus sativus* Petal revealed the presence of tannins, flavonoids, quinones, anthraquinones, reductive compounds, and terpenoids (Table I). These findings align with the established bioactive profile of saffron floral parts. Furthermore, a detailed phenolic analysis using high-performance liquid chromatography coupled to a photodiode array and electrospray ionisation mass spectrometry (HPLC-PDA/ESI-MS) identified multiple flavonoid derivatives (including those of kaempferol, quercetin, isorhamnetin, and myricetin. This confirms that petal extracts are rich in flavonoids, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties (27).

A strongly positive reaction showed the presence of tannins. This result aligns with numerous reports indicating that C. sativus floral parts, particularly stigmas, are rich in both condensed and hydrolysable tannins (28). As phenolic compounds, tannins possess astringent properties and contribute to antioxidant and antimicrobial effects.

In addition, the detection of quinones and anthraquinones is significant; these compounds are known for their redox activity and can exhibit antimicrobial, anticancer, and cytotoxic effects in various plant extracts, including *Crocus sativus* tissues (28).

Furthermore, a positive test for reducing compounds typically reflects the presence of phenolics, which provide strong reducing power and antioxidant activity. Quantitative analyses confirm that total phenolic content, which correlates with reducing power, is higher in saffron petal extracts than in other floral parts, especially with alcoholic extraction (29).

Finally, terpenoids are detected; these compounds not only contribute to plant aroma and colour but also exhibit biological activities, including anti-inflammatory and antimicrobial effects. Consistent with this, a previous study analysed extracts from saffron flower petals and reported several secondary metabolites, including terpenoid-derived compounds such as safranal, picrocrocin, crocin, and crocetin (30).

Table I

Phytochemical screening of the Hydro-methanolic crude extract of *Crocus sativus* petal

Phyto. Name	Tests	Hydro-methanol extracts of *C. sativus* petals.
Saponins	Foam test	-
Tannins	$FeCl_3$	+++
Flavonoids	HCl + Mg^{++}	+++
Quinones	NaOH	++
Anthraquinones	NH_4OH	+
Coumarins	UV fluorescence	-
Reductive compounds	Fehling's liqueur	+++
Terpenoids	Chloroform +H_2SO_4	++
Alkaloids	Mayer	-
	Wagner	-

+++: High positive; ++: Moderate positive; +: Light positive; -: Negative.

In vitro cytotoxic, anti-hemolytic, anti-inflammatory and lipid peroxidation inhibitory effects of SPE

Establishing the biosafety of plant extracts is a critical step before proceeding to *in vivo* studies. This step ensures that the potential efficacy justifies the ethical implications of animal models for obesity management. Accordingly, we investigated the cytotoxicity, anti-hemolytic, anti-inflammatory, and lipid peroxidation-inhibitory effects of SPE.

The hemolytic assay was performed to assess the potential cytotoxicity of Crocus sativus Petal hydro-methanolic extract against HRBC *in vitro*. Hemolysis of RBCs is a crucial preliminary indicator of a compound's membrane-disrupting ability, which can cause cell rupture and haemoglobin release (31, 32). SPE showed a concentration-dependent effect on HRBC hemolysis. As shown in Table II, the extract exhibited lower hemolytic activity at concentrations of 156, 312, and 625 µg/mL, with hemolytic percentages of 11.86%, 12.21%, and 14.12%, respectively. However, the cytotoxic ability against HRBC increased significantly at high concentrations (1250, 2500, and 5000 µg/mL), reaching a hemolytic rate of 41.5% at the highest concentration tested. Moreover, at equivalent concentrations, our findings demonstrate that the extract studied exhibited lower hemolytic activity than gallic acid, the reference compound.

Table II

Effect of *Crocus sativus* petal hydro-methanolic extract on hemolysis of HRBCs

Treatment concentration (µg/mL)	Hemolysis%	
	Crocus sativus Petal hydro methanolic extract	Gallic acid
156	11.86 ± 1.06 ns	21.30 ± 1.72***
312	12.21 ± 1.12 ns	24.51 ± 1.80***
625	14.12 ± 1.22 ns	31.17 ± 2.00***
1250	32.53 ± 2.18**	36.50 ± 1.90***
2500	37.40 ± 2.20**	41.85 ± 2.10***
5000	41.50 ± 2.35**	46.88 ± 2.30***

ns = not significant ($p > 0.05$); **$p < 0.01$; ***$p < 0.001$ as compared to positive control.

Furthermore, we evaluated the extract's ability to stabilise the RBC membrane. Lysosomes are known to play a key role in the inflammatory process through tissue damage. Given that the lysosomal membrane shares structural similarities with RBC membranes, the extract's anti-hemolytic effectiveness on RBCs suggests an equivalent ability to stabilise the lysosomal membrane. Therefore, this anti-hemolytic activity indicates the extract's anti-inflammatory capacity (33, 34).

Our results showed that the extract exerted a concentration-dependent stabilisation effect on the erythrocyte membrane, comparable to that of sodium diclofenac, a standard anti-inflammatory agent (Table III). A moderate protective activity (28.65%) was observed at a concentration of 30 µg/mL of the extract, which increased to 61.19% and 69.2% at the highest concentrations of 150 and 300 µg/mL, respectively. In comparison, gallic acid showed slightly higher anti-hemolytic activity (59.5% and 63.44%) at lower concentrations (30 and 75 µg/mL). Still, it exhibited reduced efficacy at higher concentrations (150 and 300 µg/mL), with a drop to 35.12% at the highest dose. This non-monotonic, biphasic behaviour of gallic acid at high concentrations is a well-established phenomenon in redox biology, driven by a transitional switch from an antioxidant to a pro-oxidant state. At elevated concentrations and under thermal stress (56°C), phenolic compounds like gallic acid undergo rapid autoxidation, generating substantial amounts of hydrogen peroxide and phenoxyl radicals in the medium. These self-generated pro-oxidant species actively induce oxidative damage to the erythrocyte lipid bilayer, thus counteracting the initial protective effect and promoting membrane destabilisation.

These findings align with several studies demonstrating that saffron petal extracts are rich in phenolic compounds, such as flavonoids (27, 35), which were also confirmed in our study by primary phytochemical screening. The membrane-stabilising activity of the studied extract can be explained by the capacity of phenolic compounds to neutralise ROS generated in hypotonic media, thereby maintaining membrane integrity (36). Furthermore, Flavonoids exhibit anti-inflammatory effects through multiple pathways, including inhibition of the phospholipase A2 (37), COX-2 (38), and 5-LOX (38) enzymes, as well as inhibition of histamine release from mast cells (39).

Table III

Effect of *Crocus sativus* petal hydro-methanolic extract on HRBCs membrane stability

Treatment concentration (µg/mL)	Membrane stability%		
	Crocus sativus Petal hydro methanolic extract	Gallic acid	Sodium Diclofenac
30	28.65 ± 2.48 ns	59.50 ± 3.80***	27.30 ± 2.20 ns
75	47.33 ± 3.15*	63.44 ± 3.50***	50.35 ± 3.40*
150	61.19 ± 3.85**	40.20 ± 3.10**	68.88 ± 4.10***
300	69.20 ± 4.10***	35.22 ± 2.80*	73.50 ± 4.50***

ns = not significant ($p > 0.05$); ***p* < 0.05; ****p* < 0.01; *****p* < 0.001 as compared to positive control.

To assess *in vitro* anti-inflammatory potential, the BSA denaturation inhibition assay was performed. Protein denaturation is a key marker of inflammation, in which proteins lose their structure and function due to high temperature or ROS generated during oxidative stress. In fact, denatured proteins act as autoantigens, triggering an immune response that promotes the release of inflammatory mediators (TNF-α, NO, IL-1β), thereby aggravating inflammation and leading to tissue damage and destruction (40,41). Our findings indicate that both the extract and sodium diclofenac showed concentration-dependent efficacy in protecting BSA from denaturation, with sodium diclofenac exerting slightly higher inhibitory activity than the extract (Table IV).

Table IV

Effect of *Crocus sativus* Petal hydro-methanolic extract on albumin denaturation

Treatment concentration	Albumin denaturation inhibition%	
	Crocus sativus Petal hydro methanolic extract	Sodium Diclofenac
300 pg/mL	8.45 ± 0.75 ns	9.22 ± 0.80 ns
30 ng/mL	9.62 ± 0.85 ns	12.30 ± 1.13 ns
3 µg/mL	11.95 ± 1.10 ns	19.22 ± 1.50*

ns = not significant ($p > 0.05$); *$p < 0.05$ as compared to positive control.

Notably, the extract exhibited a maximum inhibition of 11.95% at 3 µg/mL. Since phytochemical screening confirmed the presence of phenolic compounds in the extract, we suppose that the inhibitory activity of BSA denaturation may be attributed to these bioactive molecules. This hypothesis is supported by a previous study showing that tannins extracted from the bark of *Myricaria bracteata*, including tamarixinin A, are effective in reducing croton oil–induced ear oedema (69.8% inhibition at 200mg/kg). This efficiency is likely due to their antioxidant activity and capacity to scavenge free radicals (42, 43).

Although the tested concentrations of 300 pg/mL and 30 ng/mL showed non-significant inhibition in our trials, exploring such ultra-low doses is supported by the well-documented phenomenon of phytochemical hormesis and biphase dose-responses in the literature. Indeed, many plant-derived polyphenols exhibit distinct biological behaviours at picomolar or nanomolar scales, often acting as signalling modulators rather than direct chemical scavengers, which closely mimic the physiological circulating levels observed *in vivo* after metabolic degradation. Our preliminary screening across several orders of magnitude was therefore intended to capture any potential low-dose hormetic effects of SPE. Given that polyunsaturated fatty acids are incorporated into cell membranes and play essential roles, we conducted a lipid peroxidation assay to evaluate the extract's capacity to inhibit MDA formation, a product of polyunsaturated fatty acids generated by ROS. Inhibition of MDA formation reflects the extract's free-radical scavenging potential, thereby supporting membrane integrity. Our results indicate that SPE is efficient against lipid peroxidation in a concentration-dependent way (Table V). This efficiency is modest, very light at lower concentrations (300 pg/mL, 30 ng/mL, 3 µg/mL), not exceeding 16.98% of inhibitory activity.

In contrast, the protection rate increases gradually at higher concentrations (30 µg/mL, 75 µg/mL, and 150 µg/mL), reaching inhibition rates of 53.5%, 64.15%, and 74.2%, respectively. This protective effect is likely due to the extract's phenolic content. Studies have demonstrated that flavonoids and polyphenols contribute significantly to antioxidant and anti-inflammatory activities (44).

Table V

Effect of *Crocus sativus* Petal hydro-methanolic extract on lipid peroxidation

Treatment	Concentration	Lipid peroxidation inhibition%
***Crocus sativus petal hydro-methanolic* extract**	300 pg/mL	11.63 ± 0.94 ns
	30 ng/mL	12.22 ± 1.10 ns
	3 µg/mL	16.98 ± 1.45 ns
	30 µg/mL	53.50 ± 3.87***
	75 µg/mL	64.15 ± 4.28***
	150 µg/mL	74.20 ± 4.95***

ns = not significant ($p > 0.05$); ***$p < 0.001$ as compared to positive control.

Effects of SPE on body weight gain and absolute tissue weight in obese rats

Obesity is generally considered to result from an imbalance in energy metabolism, leading to overweight and commonly associated with a severely disturbed lipid profile (45, 46). Therefore, using SPE as a natural product containing bioactive compounds to alleviate obesity may be a potential approach (Figure 1a). Subsequently, in the present study, we induced obesity in male Wistar rats using a cafeteria diet. In fact, a cafeteria diet is a well-established model of diet-induced obesity commonly utilised in animal studies due to its close resemblance to human obesity (47). Rats were fed a cafeteria diet for 14 weeks and co-administered with three different doses of SPE (20, 40, and 100 mg/kg b.w./day) during the last 6 weeks of the experimental period. The results in Table VI showed that rats receiving a cafeteria diet exhibited significant, continuous weight gain from the first week onward compared with control rats. These findings agree with previous studies showing that cafeteria diet consumption induced adipose tissue accumulation and weight gain, ultimately leading to overweight and obesity (48), indicating, overall, a successful establishment of the obese animal model.

Table VI

Body weight, weight gain and *absolute tissue weight*

Items	C	C20	C40	C100	O	O20	O40	O100
Initial body weight (g)	$210.50^{b} \pm 12.77$	$210.60^{b} \pm 8.88$	$212.75^{b} \pm 6.34$	$208.00^{b} \pm 2.83$	$320.00^{a} \pm 8.21$	$318.00^{a} \pm 12.25$	$314.00^{a} \pm 12.45$	$318.25^{a} \pm 8.22$
Final body weight (g)	$276.00^{d} \pm 10.42$	$273.80^{d} \pm 9.71$	$273.25^{d} \pm 8.38$	$266.00^{d} \pm 5.48$	$442.50^{a} \pm 12.01$	$355.00^{b} \pm 9.23$	$335.00^{bc} \pm 11.77$	$329.00^{c} \pm 7.79$
Weight gain (g)	$65.50^{b} \pm 4.43$	$63.20^{b} \pm 5.17$	$60.50^{b} \pm 3.70$	$58.00^{b} \pm 2.94$	$122.50^{a} \pm 5.45$	$37.00^{c} \pm 5.02$	$21.00^{d} \pm 0.71$	$10.75^{e} \pm 0.50$
Liver (g)	$6.57^{c} \pm 0.46$	$6.56^{c} \pm 0.40$	$6.45^{c} \pm 0.53$	$5.94^{c} \pm 0.13$	$9.68^{a} \pm 0.49$	$7.58^{b} \pm 0.70$	$6.46^{c} \pm 0.30$	$6.11^{c} \pm 0.26$
Adipose tissue (g)	$4.30^{d} \pm 0.22$	$4.22^{d} \pm 0.33$	$4.23^{d} \pm 0.11$	$4.28^{d} \pm 0.17$	$7.71^{a} \pm 0.53$	$6.15^{b} \pm 0.32$	$5.01^{c} \pm 0.17$	$4.31^{d} \pm 0.26$
Skeletal muscle (g)	$4.45^{a} \pm 0.18$	$4.43^{a} \pm 0.43$	$4.50^{a} \pm 0.12$	$4.49^{a} \pm 0.26$	$4.41^{a} \pm 0.36$	$4.44^{a} \pm 0.28$	$4.50^{a} \pm 0.34$	$4.46^{a} \pm 0.19$

Values are presented as means ± SEM (n = 6). Different letters indicate significant differences between groups ($p < 0.05$).

After 6 weeks of administration, SPE showed no significant effect on body weight gain or tissue weights in control rats. In contrast, rats in the O20, O40, and O100 groups showed significantly lower body weight than the obese group. Moreover, liver and adipose tissue weight were significantly reduced in all SPE-treated groups compared with the O group, with the most pronounced effect observed at 100 mg/kg b.w/day (Table VI). These findings suggest a decrease in lipid storage. Our results are consistent with those of Mohaqiq *et al*. (49), who reported that oral administration of saffron petal extract at various concentrations reduced the mean weight of obese rats. Moreover, previous studies have suggested that saffron's bioactive compounds, such as crocin and safranal, could reduce body weight, food intake, and blood leptin levels (50,51). Given the significant improvement observed in the previous parameter following (reduction of body weight and tissue weight) SPE treatment and considering that our phytochemical screening result revealed the presence of these bioactive compounds in the prepared SPE, the observed improvement in O20, O40 and O100 groups is most likely related to the bioactive compounds present in the prepared SPE. Furthermore, it is worth noting that saffron stigma powder is well known to reduce food and calorie intake by minimising dietary fat absorption through the inhibition of pancreatic lipase and by enhancing satiety *via* the upregulation of neurotransmitters (52). Therefore, SPE and saffron stigma, both derived from the same plant, likely share several common bioactive compounds that contribute to similar anti-obesity mechanisms. Consistent with this, an *in vitro* study demonstrated that the n-butanol fraction of SPE inhibited lipase activity by 78% (53). Altogether, these findings highlight the potential of SPE to reduce body fat and promote weight loss through multiple mechanisms, in line with our results.

Effects of SPE on hyperglycemia and dyslipidemia in obese rats

The demonstrated efficacy of SPE in reducing body weight in obese rats led us to further investigate whether these benefits extend to hyperglycemia and dyslipidemia, two main characteristics of obesity-related metabolic disorders. In our study, obese rats exhibited significantly elevated plasma glucose, TC, and TG levels, along with increased LDL-C and decreased HDL-C levels, compared to control rats (Figure 1b, c, d, e). These results are consistent with previous studies reporting fluctuations in glucose and lipid metabolism in obesity (24, 54, 55). It is worth highlighting the strong association between these metabolic fluctuations and the onset of type 2 diabetes and cardiovascular diseases, in which dyslipidemia is the main cardiovascular risk factor (50, 56, 57).

As expected, SPE administration did not have a significant effect on glucose levels in the control group compared with the C group. In contrast, obese rats exhibited a significant decrease in plasma glucose levels in the O20 group and a highly significant decrease in the O40 group (Figure 1b). Numerous studies have demonstrated that the antihyperglycemic activity of SPE is largely attributable to its bioactive constituents such as crocin, crocetin and safranal (49, 58, 59)**.** Several mechanisms may explain this improvement in glycemia in SPE-treated obese rats, including reduced oxidative stress through protection of pancreatic β-cells from free radical damage induced by hydrogen peroxide, modulation of apoptotic pathways, and attenuation of pancreatic inflammation by reducing TNF-α and IL-1β levels (51, 60, 61).

Unexpectedly, despite the high dose of SPE (100 mg/kg b.w./day), obese rats in the O100 group showed a significant increase in plasma glucose levels compared to the O group, rather than the anticipated decrease. These findings may be explained by the carbohydrate content of saffron petals, which may contribute to this effect, and by the fact that, beyond a certain threshold, increasing the SPE dose can have the opposite effect on plasma glucose levels. Further investigations are therefore necessary to confirm these results and elucidate the potential dose-response relationship between the SPE treatment and glucose regulation. Regarding the impact of SPE treatment on dyslipidemia, plasma tri-

glyceride levels decreased in the treated-control groups compared with the C group, and in all SPE-treated obese groups compared with the O group (Figure 1d). Obese rats treated with 100 mg/kg b.w./day showed the most pronounced decrease in triglyceride levels. Meanwhile, the results showed a significant decrease in TC levels in the groups of rats treated with 20 mg/kg b.w./day and a highly significant decrease in those treated with 40 mg/kg b.w./day and 100 mg/kg b.w./day in both control and obese groups (Figure 1c).

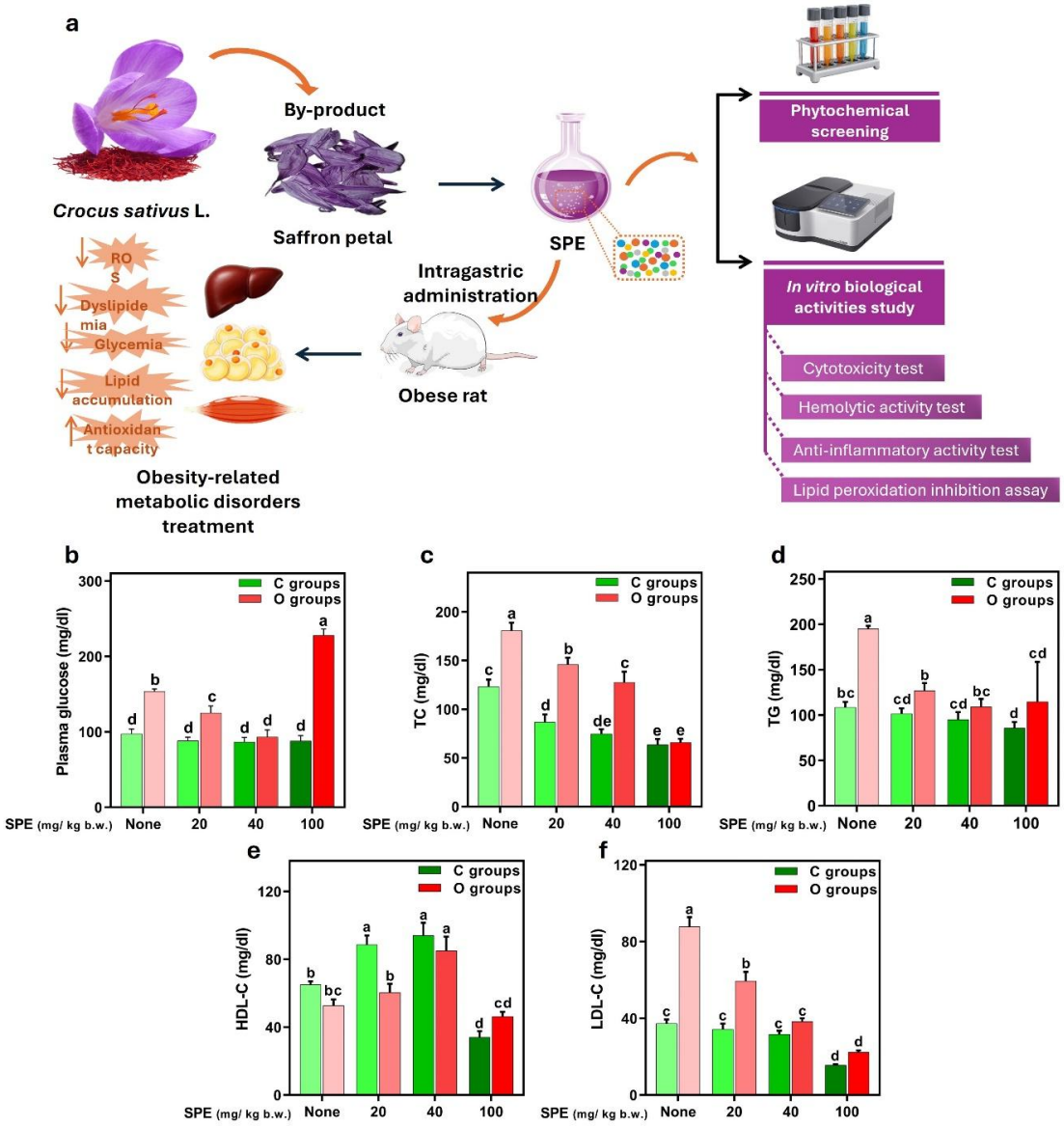

Figure 1.

Effects of SPE on hyperglycemia and dyslipidemia in obese rats. (a) Schematic illustration of SPE-based treatment for obesity in rats. (b) Plasma glucose levels; (c) Total cholesterol (TC); (d)Triglyceride (TG); (e) High-density lipoprotein cholesterol (HDL-C); (f) Low-density lipoprotein cholesterol (LDL C). Values are presented as means ± SEM (n = 6). Different letters indicate significant differences between groups ($p < 0.05$)

Furthermore, SPE treatment induced a significant increase in HDL-C in the C20 and C40 groups, whereas a highly significant decrease was observed in the C100 group compared with the C group. In obese rats, HDL-C was significantly elevated in the O20 and O40 groups but declined in the O100 group compared with the O group (Figure 1e). For LDL-C, control rats who received SPE treatment showed a significant reduction only in the C100 group. On the other hand, Obese rats treated with SPE showed a significant decrease in LDL-C levels in the O20, O40, and O100 groups, with the greatest decrease in the highest dose (O100 group) (Figure 1f).

Our results showed that the three different doses of petal extract significantly affect plasma lipid disorders associated with obesity. Our findings are

consistent with those of Mohaqiq *et al*. (49), who demonstrated that saffron petal extract reduced serum levels of LDL-C, TG, and TC and increased serum HDL-C levels in obese rats. This hypolipidemic effect could be attributed to the compounds crocin and crocetin (58). In fact, crocin has been shown to competitively inhibit pancreatic lipase, leading to malabsorption of fat and cholesterol and reduced lipid levels (62). Another study found that crocetin reduced blood TC levels, thereby reducing the risk of atherosclerosis and heart disease (58). To summarise, our results showed that 40 mg/kg b.w./day of SPE appears to be the optimal dose for preventing hyperglycemia and dyslipidemia characteristics of obesity-related metabolic disorders.

Effects of SPE on lipid accumulation of liver, muscle and adipose tissue in obese rats

In obesity, lipid accumulation leads to hypertrophy of visceral adipose tissue and hepatic steatosis, which in turn contribute to muscle dysfunction, particularly through dysregulated adipokine production from adipose tissue. In addition, obesity is characterised by impaired lipolysis and reduced HSL expression in adipocytes (63, 64). Lipoprotein lipase, a key enzyme involved in triglyceride hydrolysis and an important marker of adipocyte differentiation, acts as a gatekeeper enzyme in the initiation and/or development of obesity, alongside reduced HSL activity (65).

To determine whether the improvement in dyslipidemia reflected reduced lipid accumulation in the liver, adipose tissue, and skeletal muscle after SPE treatment, we assessed the activities of key enzymes involved in lipid metabolism. In parallel, TC and TG levels in these organs were quantified. As shown in Figure 2 a, c, d, obese rats exhibited increased hepatic lipase and adipose tissue LPL activities, while adipose HSL activity was decreased compared to controls. These alterations in lipolytic enzyme activities are known to promote lipid accumulation, which likely explains the elevated levels of hepatic, adipose tissue and muscle TC and TG (Table VII) and the increased weight of tissues and body in obese rats. These results are consistent with the histological observations (Figure 3), which revealed excessive lipid accumulation characterised by both macrovesicular and microvesicular steatosis. This steatosis is accompanied by vascular congestion, indicating that the liver is having trouble draining blood normally, likely due to lipid accumulation. Moreover, the visceral adipose tissue of obese rats exhibited severe adipocyte hypertrophy and a disorganised architecture compared with the control group (Figure 3). However, supplementation with SPE reversed these trends. Obese rats treated with SPE showed reduced hepatic lipase and adipose tissue LPL activities and enhanced adipose HSL activity compared with untreated obese rats, with the most pronounced effects in the O100 group. Notably, SPE at 40 mg/kg b.w./day effectively restored the enzymatic activities of hepatic LPL, adipose tissue LPL, and HSL to normal levels (Figure 2 a, c, d).

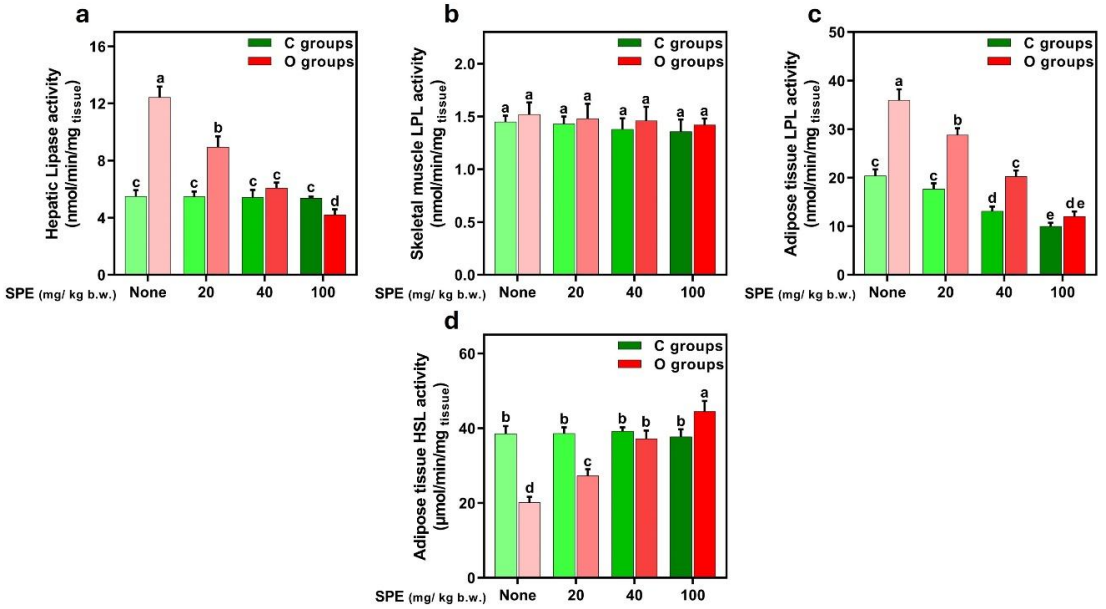

Figure 2.

Effects of SPE on Lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) activities in the liver, skeletal muscle, and adipose tissue of obese rats. (a) Hepatic Lipase activity; (b) skeletal muscle LPL levels; (c) Adipose tissue LPL levels; (d) Adipose tissue HSL levels. Values are presented as means ± SEM (n = 6). Different letters indicate significant differences between groups ($p < 0.05$)

Consistent with these enzymatic improvements, hepatic, adipose tissue, and muscle TC and TG levels were significantly reduced in SPE-treated obese rats, with more pronounced reductions observed at doses of 40 and 100 mg/Kg b.w./ day compared with 20 mg/Kg b.w./day (Table VII). These improvements were consistent with histological observations (Figure 3). Tissues from obese-treated groups showed moderate improvement, as evidenced by reduced lipid droplet accumulation in the liver, which appears to alleviate oxidative stress in blood vessels, thereby reducing vascular congestion. Additionally, adipose tissue showed changes in adipocyte size and a moderate recovery toward the morphology observed in the control group.

Table VII

Total cholesterol and triglyceride levels of liver, adipose tissue and skeletal muscle

Items	C	C20	C40	C100	O	O20	O40	O100
Total cholesterol levels (mg/g $_{tissue}$)								
Liver	$22.85^{cd} \pm 1.98$	$22.62^{cd} \pm 1.29$	$21.18^{d} \pm 1.86$	$19.26^{d} \pm 1.24$	$39.8^{a} \pm 1.94$	$34.42^{b} \pm 1.89$	$30.80^{b} \pm 1.76$	$26.26^{c} \pm 1.72$
Adipose tissue	$9.78^{c} \pm 0.67$	$7.63^{de} \pm 0.77$	$7.13^{e} \pm 0.74$	$6.86^{e} \pm 0.45$	$18.59^{a} \pm 1.07$	$16.06^{b} \pm 0.92$	$9.11^{cd} \pm 0.73$	$1.82^{f} \pm 0.16$
Skeletal muscle	$12.21^{b} \pm 0.69$	$10.04^{c} \pm 0.76$	$8.08^{d} \pm 0.27$	$6.07^{e} \pm 0.82$	$15.24^{a} \pm 1.03$	$13.76^{ab} \pm 0.72$	$8.14^{d} \pm 0.60$	$6.21^{e} \pm 0.30$
Triglyceride levels (mg/g $_{tissue}$)								
Liver	$66.63^{b} \pm 2.98$	$54.24^{c} \pm 3.47$	$48.03^{c} \pm 3.15$	$26.35^{d} \pm 2.30$	$92.71^{a} \pm 3.43$	$91.75^{a} \pm 2.72$	$87.38^{a} \pm 5.21$	$27.72^{d} \pm 2.05$
Adipose tissue	$95.24^{c} \pm 1.48$	$60.89^{e} \pm 2.12$	$76.54^{d} \pm 1.45$	$63.90^{de} \pm 4.96$	$167.71^{a} \pm 5.58$	$160.56^{a} \pm 8.74$	$131.54^{b} \pm 9.22$	$67.91^{de} \pm 3.93$
Skeletal muscle	$17.11^{a} \pm 0.78$	$16.50^{ab} \pm 0.82$	$14.65^{b} \pm 0.61$	$7.58^{c} \pm 0.72$	$18.05^{a} \pm 1.29$	$17.41^{a} \pm 0.68$	$16.44^{ab} \pm 1.60$	$9.53^{c} \pm 0.40$

Values are presented as means ± SEM (n = 6). Different letters indicate significant differences between groups ($p < 0.05$).

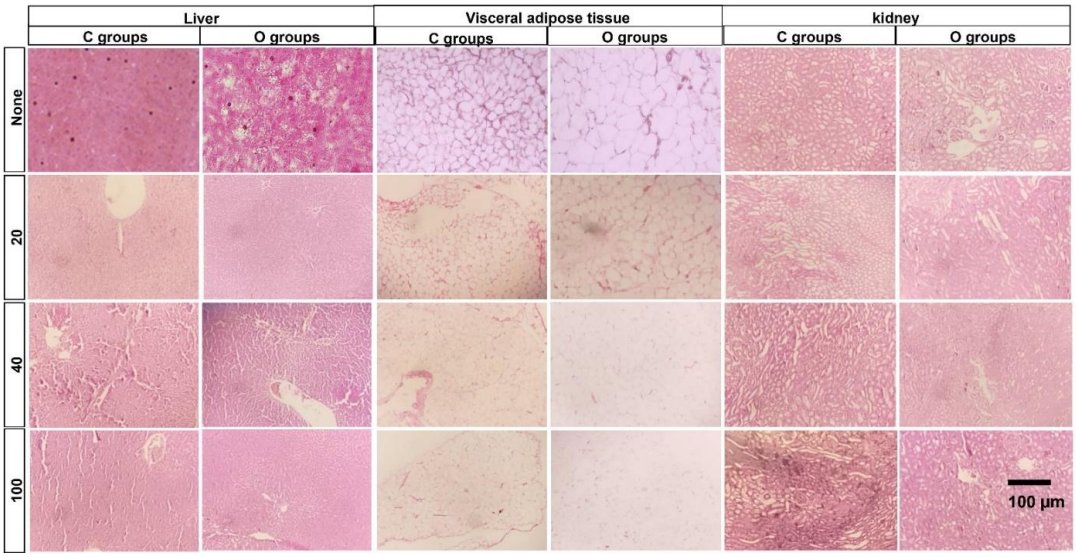

Figure 3.

Histopathology study demonstrated the restorative effect of SPE treatment on the liver, adipose tissue, and kidney of obese rats. H&E staining 100×

In treated control rats, low-dose SPE treatment had no significant effect on hepatic lipase (HSL) activity; however, it induced a significant reduction in adipose LPL activity at the medium and high doses. These enzymatic changes were accompanied by preserved hepatic and adipose tissue morphology compared with non-treated control groups, and by decreased levels of TC and TG in the liver, adipose tissue, and muscle in these rat groups.

A closer look at this dose-response profile in Table VII reveals that the dramatic near-halving of tissue cholesterol and triglycerides in healthy animals is restricted exclusively to the highest tested dose, 100 mg/kg (C100). This suggests that at a saturation threshold, the crude extract may induce overactivation of lipid-clearance mechanisms or excessive baseline inhibition of nutrient absorption, which might raise physiological safety concerns for non-obese subjects. Crucially, this over-activation is completely absent at the intermediate dose of 40 mg/kg (C40). In healthy rats, the 40 mg/kg dose preserves basal lipid homeostasis within a normal physiological window, while simultaneously achieving a powerful, statistically significant corrective effect in diet-induced obese rats (O40). Consequently, these data firmly establish 40 mg/kg as the optimal

therapeutic dose, balancing high anti-obesity efficacy with a safe homeostatic profile in healthy states, whereas the 100 mg/kg dose should be considered over effective and non-optimal.

Our study provides compelling evidence for an anti-obesity mechanism of SPE, involving modulation of lipase activity in both liver and adipose tissue. Importantly, our findings show that SPE treatment can restore dysregulated HSL and LPL activities and significantly reduce lipid accumulations in obese rats. This anti-obesity mechanism might be associated with the polyphenolic composition of SPE (7).

Recently, it has been shown that polyphenols play a crucial role in regulating LPL activity, thereby attenuating obesity and its related disorders. For instance, Hong Xu *et al*. (66) reported that phenolic compounds in the red-skin extract of lotus seeds could alleviate high-fat-diet-induced obesity in mice by regulating LPL activity in adipose tissue and muscle. Consistent with these findings, we confirmed that SPE is a valuable source of phenolic compounds and flavonoids, including kaempferol and quercetin, as well as carotenoids such as crocin (67, 68). These bioactive constituents are known to inhibit preadipocyte differentiation and reduce lipid accumulation in adipocytes (69, 70). Moreover, quercetin has been shown to prevent adipogenesis by up-regulating HSL expression and downregulating LPL expression (71). In addition, Crocin inhibits adipogenesis and stimulates lipolysis by activating AMP-activated protein kinase (AMPK) (70). Activation and phosphorylation of AMPK have been associated with decreased LPL activity in adipose tissue and increased LPL activity in skeletal muscle tissue (72). These mechanisms are fully consistent with our findings, in which SPE treatment restored normal levels of HSL and LPL activity and reduced lipid accumulation in obese rats.

Effects of SPE on oxidant status in obese rats

Oxidative damage is one of the obesity-mediated pathways that contributes to adipose tissue inflammation and systemic metabolic dysfunction, including impairments of glucose and lipid metabolism during obesity (8, 73). Since SPE contains bioactive compounds such as flavonoids, tannins, crocin, and kaempferol, which are known for their free-radical-scavenging and anti-inflammatory properties (17, 74), we evaluated the effect of SPE treatment on redox homeostasis during obesity by measuring lipid peroxidation and protein oxidation. To this end, we measured the levels of MDA and CP in erythrocytes, liver, adipose tissue and skeletal muscle. As shown in Figure 4, obese rats had elevated levels of these markers in all analysed tissues compared to the control group, indicating systemic oxidative stress. Our results are in accordance with numerous previous studies (75). This systemic oxidative stress may influence kidney functions (8), potentially explaining the histological findings in this study. Vascular congestion in hepatic and renal sections, alongside enlarged Bowman's space observed in kidney (Figure 3) may be due to the stress on blood vessels. Such vascular injury can compromise the filtration barrier, leading to a possible risk for progressive kidney disease.

Notably, SPE treatment at different doses significantly attenuated oxidative damage in obese rats. Specifically, significant reduction in MDA (Figure 4a, b, c, d) and CP (Figure 4e, f, g, h) levels were observed in erythrocyte, liver, adipose tissue and muscle in the O20 and O40 groups compared with the untreated O group, where the most remarkable antioxidant effect was observed at the 40 mg/kg b.w./day dose. The extract's alleviating effect on oxidative stress was evident in renal disorders. Histological observation (Figure 3) of kidney sections from treated obese rats showed overall preservation of Bowman's space and the absence of vascular congestion. Interestingly, results obtained in control groups further support the safety profile of SPE. Although the dose of 100mg/kg b.w./day induced increased levels of erythrocyte MDA and CP, this effect was not observed in liver and adipose tissue, where these markers were significantly reduced, nor in muscle, where no significant changes were detected (Figure 4). Overall, these findings provide compelling evidence that SPE treatment does not cause harmful side effects and effectively ameliorates oxidative statue in obese rats.

Effects of SPE on antioxidant capacity in obese rats

Several mechanisms could explain the link between obesity and oxidative stress, including inflammation, increased metabolic activity leading to excessive radical generation, and oxidative DNA damage in both plasma and key tissues involved in antioxidant defence. These processes eventually result in depletion of antioxidant defence capacity (76-79). Since SPE demonstrated significant effectiveness in reducing oxidative stress, the enhancement of antioxidant capacity in obese rats following SPE treatment was highly probable. To investigate this further, we assessed plasma vitamin C levels and measured Catalase and GSH in erythrocyte lysate, liver, adipose tissue and skeletal muscle. As expected, the results shown in Figure 5 indicates an increase and restoration of antioxidant parameters in obese rats after SPE treatment, with similar effects across the different doses. Conversely, untreated obese rats fail to activate adaptive and compensatory mechanisms of antioxidant systems due to the severity of oxidative stress. These findings demonstrate the capacity of SPE to attenuate obesity-related oxidative damage across multiple tissues.

Our results are consistent with previous studies showing that treatment with crocin and safranal promotes the antioxidant defence system and modulates

oxidative stress in diabetic rats (80) and that kaempferol and quercetin obtained from saffron petals could enhance the activity of antioxidant enzyme NQO1 and total antioxidant capacity to alleviate H_2O_2-induced oxidative damage in B16 cells (81).

In control groups, although GSH levels were slightly reduced in adipose tissue, liver, and muscle following treatment (Figure 5b, c, d), the results of plasma vitamin C and catalase in erythrocytes, liver, adipose tissue and muscle were maintained (Figure 5e, f, g, h, i). These findings indicate that SPE was not only safe due to its natural origin but also enhanced antioxidant systems due to its bioactive compound content.

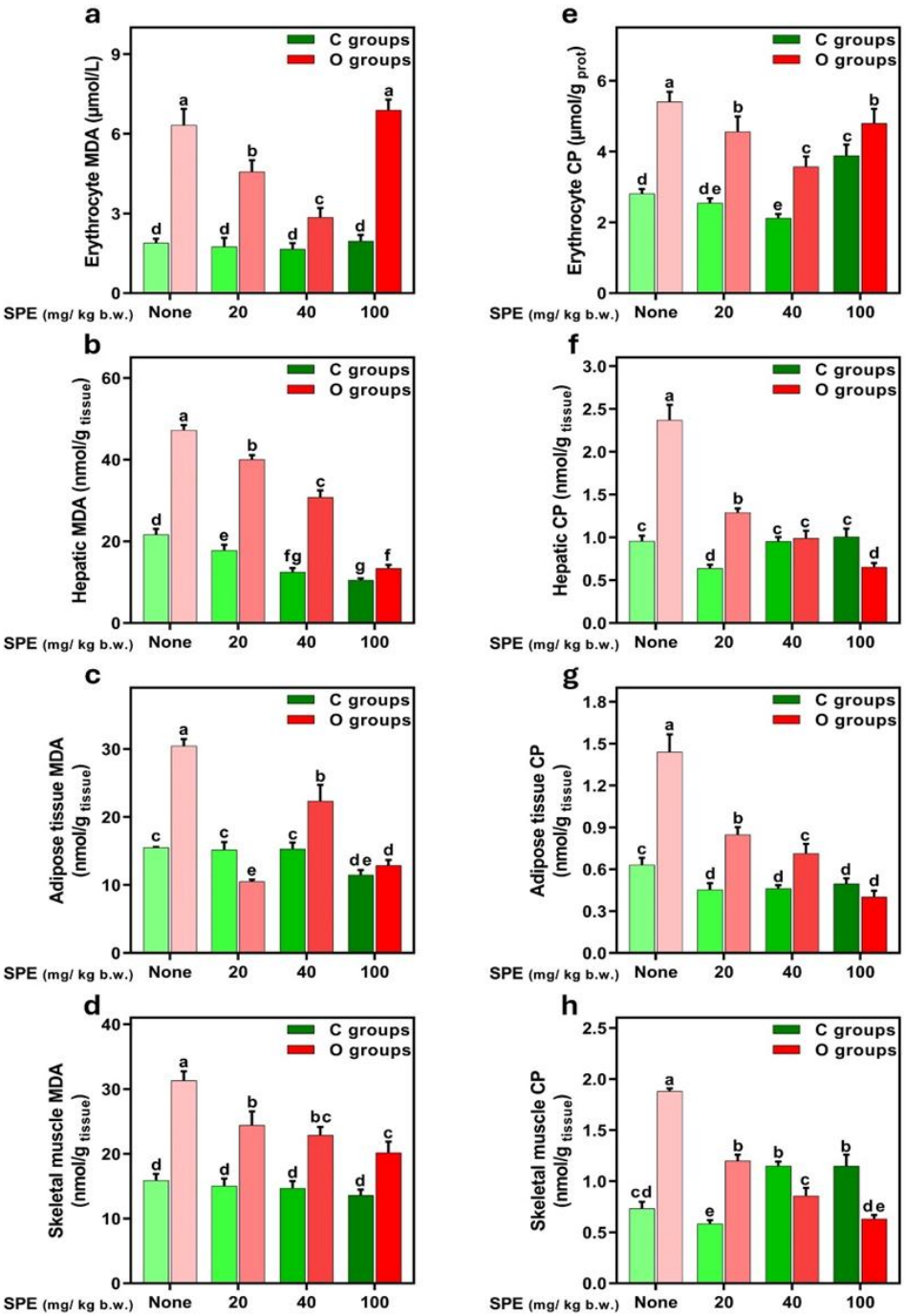

Figure 4.

Effects of SPE on oxidant status in obese rats. (a) Erythrocyte MDA levels; (b) Hepatic MDA levels; (c) Adipose tissue MDA levels; (d) Skeletal muscle MDA levels; (e) Erythrocyte CP levels; (f) Hepatic CP levels; (g) Adipose tissue CP levels; (h) Skeletal muscle CP levels. Values are presented as means ± SEM (n = 6). Different letters indicate significant differences between groups ($p < 0.05$)

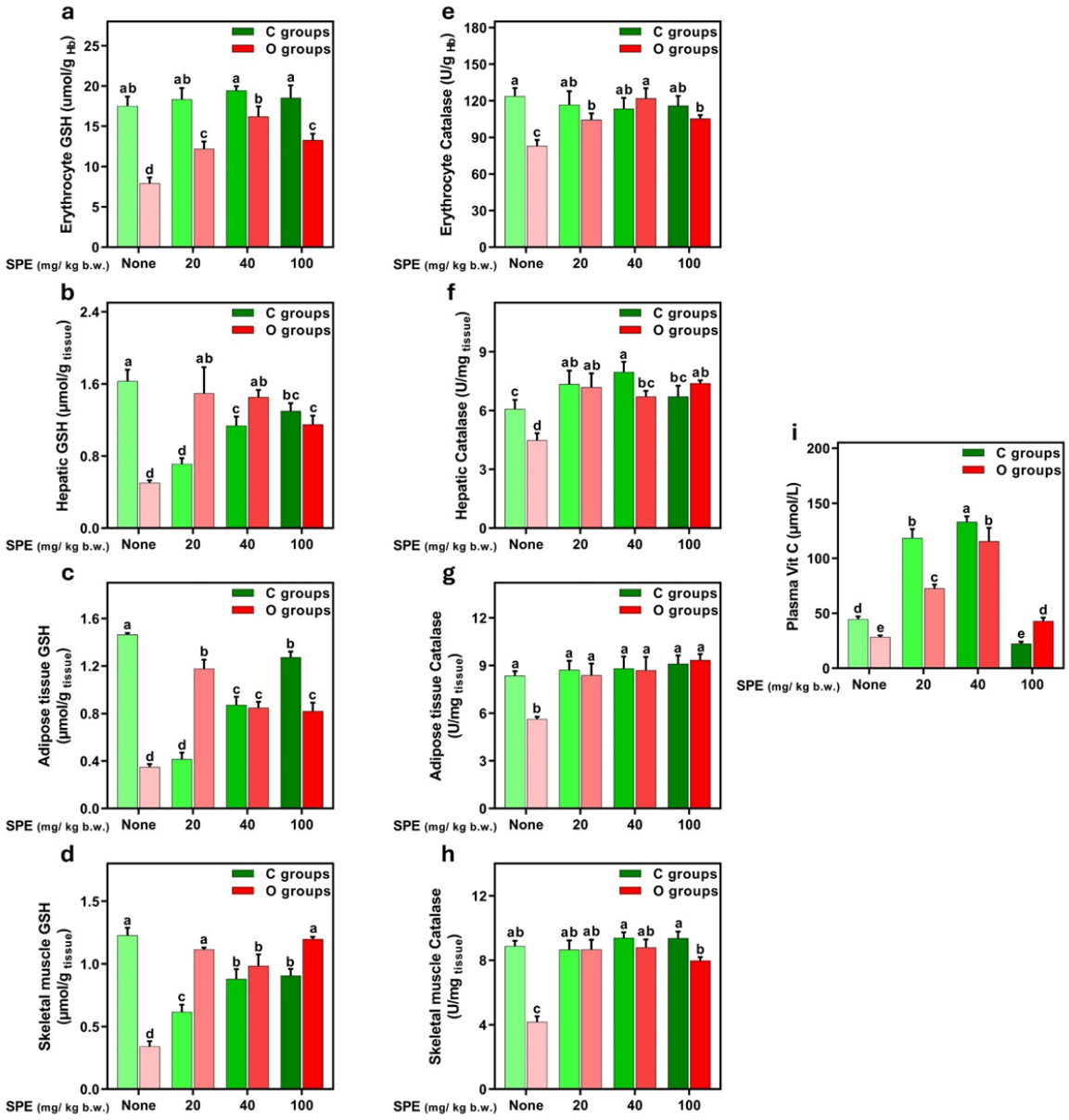

Figure 5.

Effects of SPE on antioxidant capacity in obese rats. (a) Erythrocyte GSH levels; (b) Hepatic GSH levels; (c) Adipose tissue GSH levels; (d) Skeletal muscle GSH levels; (e) Erythrocyte Catalase levels; (f) Hepatic Catalase levels; (g) Adipose tissue Catalase levels; (h) Skeletal muscle Catalase levels; (i) Plasma Vitamin C levels. Values are presented as means ± SEM (n = 6). Different letters indicate significant differences between groups ($p < 0.05$)

Conclusions

In conclusion, our results demonstrate that hydromethanolic saffron petal extract exerts protective effects against obesity-related metabolic disorders through its hypoglycemic, hypolipidemic, and antioxidative effects in cafeteria diet-induced obese rats. Notably, our findings revealed an additional anti-obesity mechanism of SPE through modulation of lipase activities in the liver and adipose tissue characterised by reduced adipogenesis *via* LPL inhibition. They enhanced lipolysis *via* HSL activation in obese rats. Among the tested doses, the 40 mg/kg b.w/day concentration consistently exhibited the highest efficacy across most measured parameters. Overall, the present study shows that saffron petals are a valuable by-product and a rich source of bioactive compounds and suggests supporting the potential development of SPE into functional foods for obesity management.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article. The raw datasets used and analysed in the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

All experimental procedures involving animals were carried out in accordance with the policies of the University of Tlemcen Institutional Animal Care and Use Committee (IACUC) (accreditation number: D01N01UN130120150006). For the *in vitro* assays involving human blood, the protocol was approved by the Ethics Committee of Tlemcen University Hospital, and written informed consent was obtained from the volunteer in accordance with the Declaration of Helsinki.

AI use disclosure

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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