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https://doi.org/10.31925/farmacia.2025.2.10 **ORIGINAL ARTICLE**

EXPLORING THE THERAPEUTIC POTENTIAL OF SOUR CHERRY LEAF EXTRACT *(PRUNUS CERASUS* L.*)*: A COMPREHENSIVE *IN VITRO* AND *IN VIVO* EVALUATION

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Abstract

Sour cherry leaves *Prunus cerasus* (PC) are a valuable natural source of bioactive compounds. This study assessed the anti-inflammatory and anti-obesity properties of sour cherry aqueous extract. The phenolic profile was evaluated by high-performance liquid chromatography (RP-HPLC-PDA). The studied extract has a total phenolic content of 7.13 ± 0.12 mg GAE *per* gram and exhibits a notable antioxidant effect determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. Adding PC extract and orlistat (reference drug) to the high-fat diet (HFD) ameliorates the serum's lipid profile and reduces body gain by 9.84% and 15.61%, respectively. The histological sections demonstrate the effectiveness of the extract. The PC extract had no cytotoxic effect on RAW 264.7 cells and macrophages and inhibited nitrite production in a dose-dependent manner with an IC_{50} of nitric oxide inhibitory activity (NO) of 58.25 ± 0.36 µg/mL. The carrageenan-induced rat paw oedema model confirms the extract's anti-inflammatory potential in reducing paw thickness and improving oxidative stress parameters. In summary, sour cherry leaves could potentially be interesting for therapeutic use in controlling obesity and the treatment of inflammation.

Rezumat

Frunzele de vișin (*Prunus cerasus* – PC) reprezintă o sursă naturală valoroasă de compuși bioactivi. În acest studiu au fost evaluate proprietățile antiinflamatorii și antiobezitate ale extractului apos de vișin. Profilul fenolic a fost analizat prin cromatografie lichidă de înaltă performanță (RP-HPLC-PDA). Extractul analizat prezintă un conținut total de compuși fenolici de 7,13 ± 0,12 mg echivalenți de acid galic (GAE) *per* gram și un efect antioxidant semnificativ, determinat prin testele DPPH și FRAP. Administrarea extractului de PC, precum și a orlistatului (medicament de referință), în cadrul unei diete bogate în grăsimi (HFD), îmbunătățește profilul lipidic și scade rata de creștere în greutate cu 9,84%, respectiv 15,61%. Analiza histologică a evidențiat eficacitatea extractului. De asemenea, extractul de PC nu a prezentat efect citotoxic asupra celulelor RAW 264.7 și a macrofagelor și a inhibat producția de nitriți într-o manieră dependentă de doză, cu o valoare IC_{50} pentru inhibarea oxidului nitric (NO) de 58,25 ± 0,36 µg/mL. Modelul de edem indus cu caragenan la șobolani confirmă potențialul antiinflamator al extractului, prin reducerea grosimii labei și îmbunătățirea parametrilor de stres oxidativ. În concluzie, frunzele de vișin ar putea reprezenta o opțiune prospectivă pentru utilizarea terapeutică în controlul obezității și în tratamentul inflamației.

Keywords: sour cherry leaves, antioxidant, anti-obesity, anti-inflammatory

Introduction

Inflammation is the immune system's defensive response against various pathogens and cellular danger signals, mediated primarily by immune cells such as macrophages. Acute inflammation is the activation of complex enzymes, the secretion of free radicals, and the release of various inflammatory and pro-inflammatory mediators, which are frequently characterised by redness, swelling, pain, heat, and loss of tissue function [1, 2]. Steroidal and nonsteroidal anti-inflammatory medicines (NSAIDs) are the most widely prescribed pharmaceuticals for treating

inflammation; however, these drugs may have unfavourable effects on the cardiovascular, haematologic, renal, and hepatic systems [3]. Chronic low-grade inflammation plays a role in a variety of chronic diseases, such as obesity [4]. The latter has become a serious health problem, which is caused by an imbalance in energy intake and expenditure, leading to excessive fat formation and consequently to obesity-related complications [5]. Diet is a main contributing element to the development of obesity [6]. Consequently, patients are administered a series of pharmacotherapeutic regimens in anticipation of rapid and effective outcomes [7]. However, drugs for obesity treatment like orlistat could lead to gastrointestinal diseases, abdominal discomfort and flatulence [8, 9]. Furthermore, obesity is associated with low-grade inflammation [10, 11]. Consequently, several plants are commonly used to induce weight loss and manage or treat metabolic disorders due to the presence of secondary metabolites [12, 13]. Among the primary, and secondary metabolites, polyphenols, which have several effects providing substantial health benefits [14]. Polyphenols have been proposed as a therapeutic strategy for their potential to reduce inflammation [15] and prevent obesity [5]. Managing these health issues requires discovering polyphenols from plants with high efficacy and few adverse effects.

Prunus cerasus L., commonly known as the sour cherry, is a deciduous tree in the *Rosaceae* family [16]. Their fruits have been used as a preventative treatment in traditional medicine, such as cardiovascular disease [17], central nervous system pathologies [18], inflammation [19], cancer [20], and diabetes [19]. These studies provide evidence for the potential health benefits of sour cherry fruit; few of them concern the leaves.

Research has shown that polyphenolic compounds found in cherry leaves are undervalued and could be applied as essential ingredients in pharmaceutical products [13, 18, 19]. These leaves have a good potential as a sustainable source of food additives, functional food ingredients, and even supplements for cosmetics [23, 24]. It is important to note that more research is needed to fully understand the effects and benefits of PC leaves.

Based on the above-mentioned information, we attempt to determine the phenolic profile by RP-HPLC-PDA and the antioxidant potential of PC leaf extract. To add novelty to our work, we evaluated the anti-obesity effectiveness *in vivo* using orlistat as a positive control. We also looked into histological impacts through investigation of the liver, kidney, and adipose tissues for the first time. The anti-inflammatory properties of macrophages (RAW 264.7 cells) *in vitro* and the rat paw oedema model caused by carrageenan *in vivo* were also investigated.

Materials and Methods

Materials

Plant material

In 2021, sour cherry leaves were collected from the Oued Lakhder region in Tlemcen (Algeria). The leaves were cleaned, shade-dried at room temperature, ground into fine powder, and stored at -4°C.

Preparation of aqueous extract

The infusion was prepared using 1 g of sample added to 100 mL of boiling water [25]. After 20 minutes of shaking, the mixture was centrifuged at 4000 rpm for 20 minutes, and the supernatant was gathered for further analysis [26].

RP-HPLC-PDA analysis

The phenolic components in sour cherry leaves extract were analysed using a Perkin Elmer Flexar system with a binary pump delivery system and a C18 column (150 mm × 4.6 µm, 5 µm). The mobile phase included the solvent A - Acetic acid (2%) and B - Acetonitrile. The gradient elution method comprised 5 minutes with 90% A, 25 minutes with 10% A, and 15 minutes with a linear gradient from 90% to 100% B, followed by 20 minutes of equilibration. The flow rate was set at 1 mL/min. The chromatograms were examined at 280 nm [27]. Compounds and peak assignments were identified based on their retention times and UV-Vis spectra compared with the standards used.

Total phenolic contents and evaluation of antioxidant activity

Total phenolic contents in the aqueous extract were determined by the Folin-Ciocalteu method [28]. Results were expressed as mg of gallic acid equivalent to dry matter (mg GAE/g). Antioxidant activity was estimated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) [29] and ferric-reducing antioxidant power (FRAP) assays [30]. Ascorbic acid and gallic acid were used as standard.

Experimental animals

The study was conducted on 25 healthy male Wistar rats (200–260 g) at the biology and chemistry laboratories of the University of Tlemcen, Algeria. The animals were kept in controlled conditions (23 ± 2°C, humidity 40%, and 12 h light/dark cycle). All animals were fed a standard diet and water *ad libitum* during acclimatisation. The experiment followed the ARRIVE criteria and was carried out in accordance with the United Kingdom's Animals (Scientific Procedures) Act, 1986, and related guidelines. The authorisation was obtained from the Institutional Ethical Committee for Animal Research.

Following two weeks of acclimatisation with a standard diet, the rats were randomly divided into two groups: Group I, rats receiving a normal diet (ND), and Group II, animals receiving a high-fat diet (HFD) for 20 weeks.

The HFD constituted 50% of the standard diet, 28% of lipids, 12% of protein (casein), 4% fructose and 5% starch. The weight of both Group I and Group II was monitored weekly. After 14 weeks of HFD supplementation, rats of Group I were divided into two groups for a period of 6 weeks; Group I_A continued to receive the standard diet (ND), and Group I_B received the PC extract along with the ND (ND + PC). Rats of Group II were further divided into three groups; Group II_A continued receiving HFD; Group II_B received PC extract along with HFD (HFD + PC); Group II_C received orlistat (30 mg/kg b.w,) [31] supplemented to HFD (HFD + Orl). Orlistat is an anti-obesity drug that acts as a potent inhibitor of gastric and pancreatic lipase, reducing dietary fat absorption [32]. Following a 14-week HFD, obesity was verified using the Lee index, which was calculated using the following formula:

$$Lee\ index = \frac{2\sqrt{Bodyweight\ (g)}}{naso - anal\ length(cm)} \times 100$$

Rats with an index above 310 were considered obese [33].

Sampling and chemical analysis

At the end of the experiment, the rats were anaesthetised after 12 hours of fasting. Whole blood was collected, and serum was obtained by centrifugation (3000 rpm for 15 minutes). Total cholesterol (TC), triglycerides (TG), creatinine (Crea), urea, alanine transaminase (ALT), and aspartate transaminase (AST) enzyme activities were measured using the Spinreact kit in the haematology laboratory of Tlemcen Hospital (Algeria).

Histological analysis

The liver, testes, adipose tissues, heart and kidneys were surgically removed, and wet weights were measured. The organ fragments were embedded in paraffin wax and treated in 10% formaldehyde for histological examination. They were then divided into histological sections that were 5 µm thick and stained with haematoxylin and eosin solution.

Evaluation of anti-inflammatory activity in vitro

Cytotoxicity Assay

An *in vitro* assessment of the cytotoxicity of the extract of sour cherry leaves was conducted using the murine macrophage Raw 264.7 cell line. RAW 267 macrophage. A 96-well culture plate was filled with 4 cells at 2-4 × 10^4 cells/well, and the cells were incubated for 24 hours at 37°C with 5% CO_2. For a whole day, cells were exposed to LPS and sour cherry aqueous extract (1 mg/mL and 10 mg/mL). A 100 µL solution containing 1 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) was then added, and it was incubated for 4 hours at 37 °C. After that, the MTT crystals were dissolved with DMSO (dimethyl sulfoxide) after the supernatant was discarded. The absorbance was then determined with a spectrophotometer at 570 nm [34].

The selectivity index (SI) was calculated using the following formula:

$$SI = \frac{CC50\ macrophage}{IC50\ parasite}$$

SI: Selectivity Index, CC_{50}: the treatment concentration causing 50% of cell death

Measurement of NO production

Griess reagent was used to measure the generation of nitrite (NO). In 96-well plates, macrophage RAW 264.7 cells were plated at a density of 3 × 10^6 cells/ well and allowed to adhere for an entire night at 37°C with a 5% CO_2 incubator. The aqueous extract was added to the cells in varying concentrations. Lipopolysaccharides (LPS) at 1 µg/mL (10 µL) were added to stimulate inflammation, and plates were incubated at 37 °C for 24h. Briefly, 100 µL of supernatants from cultured cells were combined with Griess reagent and incubated for 20 minutes at room temperature. The absorbance was then determined using a microplate reader (Synergy HT, BioTek) at 570 nm [35], A $NaNO_2$ solution was used as a standard to determine the NO concentration. Moreover, $IC_{50,}$ the concentration that reduced NO generation by 50%, was also possible to be determined. The positive control was N-nitro-L-arginine methyl ester (L-NAME).

Evaluation of anti-inflammatory activity in vivo

Sixteen healthy male and female Wistar rats (200 - 220g) were acquired from the animal house at Abou Bakr Belkaid University, Tlemcen, Algeria. Rats were housed in standard environmental conditions (humidity 40%, temperature 23 ± 2°C, and 12 h light/dark cycle). The experiment followed the ARRIVE criteria and was carried out in accordance with the United Kingdom's Animals (Scientific Procedures) Act, 1986, and related guidelines. The authorisation was emitted by the Institutional Ethical Committee for Animal Research.

The carrageenan-induced paw oedema model was used to evaluate the anti-inflammatory effect of PC leaf extract according to Winter's method [36] with modifications. A 0.1 mL suspension of 1% carrageenan in saline was injected into the plantar aponeurosis of the right paw of rats to induce acute oedema. An hour before the intraplantar injection of carrageenan, Wistar rats were given three different oral treatments: 100 mg/kg of sour cherry leaf extract, 50 mg/kg of diclofenac, and physiological water as the control group. The oedema was assessed before (0 h) and at 1, 2, 3, and 6 h following carrageenan injection. Paw thickness was measured with a digital caliper before carrageenan injection (0 hours) and then at regular intervals of 1, 2, 3, 4, 5 and 6 hours after the injection.

Paw oedema in the individual rodent was defined as:

$$Paw\ oedema = paw\ volume\ in\ analysed\ time\ point - innitial\ paw\ volume$$

The proportion of the inhibition of the inflammatory reaction was calculated using the following formula:

$$\%\ of\ inhibition = 1 - \frac{change\ of\ paw\ volume\ in\ analysed\ animal}{change\ of\ the\ paw\ volume\ in\ carragenan\ group} \times 100$$

Assessment of oxidative stress parameters

Blood pellets were utilised to prepare hemolysates to determine antioxidant properties. The total soluble protein content of the samples was initially measured according to [37] using 500 µL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) dissolved in 2.5 M HCl added to 200 µL of the sample, incubated for 1 h in the dark with vertexing, precipitated with TCA, washed with ethanol-ethyl acetate, and dissolved in guanidine hydrochloride. Glutathione (GSH) levels were then determined according to the method of [38]. A 1 mL sample was mixed with ice-cold water and TCA (50%). After 10 min stirring and centrifugation (1200 g for 15 min), 400 µL of the supernatant was added to Tris buffer (0.4 mol/L, pH 8.9) and DTNB (0.01 mol/L).

SOD activity was determined by monitoring the change in absorbance at 440 nm for 3 minutes in a specific buffer containing hemolysate and pyrogallol [39]. CAT activity was determined according to Sinha's method[40]. Homogenates and 0.03 mol/L H_2O_2 were mixed in phosphate buffer (0.066 mol/L, pH 7.0). After 5 minutes of incubation, $TiOSO_4$ was added, and the absorbance was read against a buffer blank. One CAT unit is 1 mmol H_2O_2 degraded *per* minute *per* mg of protein.

Statistical analysis

The statistical analysis was carried out using the Graph Pad Prism 5.0 program. The data in this study were reported as means ± SEM. One-way ANOVA was used to evaluate multiple comparisons. The Tukey posthoc test was used to identify significant differences between groups, with a statistical significance threshold of $p < 0.05$.

Results and Discussion

RP-HPLC-PDA analysis

The results obtained by RP-HPLC-PDA analysis showed that sour cherry leaf extract is a rich source of polyphenols. The chlorogenic acid was the predominant phenolic compound in the sour cherry leaf extract. The p-coumaric acid and rutin were present with important concentrations, while gallic acid and quercetin were detected in small amounts. Similarly, chlorogenic acid was identified in the sour cherry leaf [41], sour cherry fruit [42, 43], sour cherry pomace [44], cherry laurel leaves [45] and sweet cherry leaves [46]. Additionally, p-coumaric acid and rutin were found in some of the previously mentioned investigations.

Total phenolic content and antioxidant activity

Table I summarises the total phenolic content and the antioxidant activity of sour cherry leaves by DPPH and FRAP assays. PC extract had a total phenolic concentration of 7.13 ± 0.12 mg GAE/g, which was higher than the 3.17 mg GAE/g obtained from the same extract in another study [41]. Compared the cherry laurel leaf aqueous extract (0.83 mg GAE/g) [47], our findings discovered a higher concentration. It was reported that sour cherry leaves are a valuable natural source of polyphenolic molecules [22]. Additionally, of all extraction techniques tested, the extraction by decoction in boiling water (100°C) for 15 minutes is the most appropriate as it ensures the highest amount of phenols [48].

Table I

Total polyphenol content and antioxidant activity of sour cherry leaf extract

Samples	**Total polyphenol**	**Antioxidant activity**	
	(mg GAE/g)	DPPH IC_{50} (mg/mL)	FRAP IC_{50} (mg/mL)
PC extract	7.13 ± 0.12	0.18 ± 0.22	0.0009 ± 0.05
Ascorbic acid	-	0.058 ± 0.14	-
Gallic Acid	-	0.0491 ± 0.24	0.0038 ± 1.87

GAE: gallic acid equivalents

The IC_{50} value of DPPH in PC extract (0.18 ± 0.22 mg/mL) is close to that of ascorbic and gallic acid, which are used as antioxidant references. These results seem better than those of sour cherry hydromethanolic stem extracts with IC_{50} values of 0.36 mg/mL[49], as lower IC50 values indicate higher antioxidant activity [50].

The FRAP test is based on the ability to reduce Fe^{+3} to Fe^{+2}, which is associated with an increase in sample concentration. There is a significant reducing power in the PC extract. The scientific literature claims that the antioxidant activity has already been verified in sour cherry fruit [22, 51, 52] and sweet cherry leaves [25]. As compared between the cherry variety, sour cherries *Prunus cerassus* exhibit higher

antioxidant activity (DPPH and ORAC) than sweet cherries *Prunus avium* [53]. Dziadek *et al.* also noted that leaves of sweet cherry were characterised by higher antioxidant activity than fruit [46]. Phenolic compounds present in sour cherry leaves have a notable antioxidant effect. It may be due to the phenolic compounds present in the studied extract that are capable of neutralising reactive oxygen species and protect against oxidative stress-related damage [54]. Therefore, higher antioxidant capacity is correlated with higher phenolic content [55].

Effect of sour cherry extract on body weight, weight of organs and Lee index

High-fat diet (HFD) promotes obesity and hyperlipidaemia [56]. Results confirmed that taking HFD increases body weight (b.w.), which is significantly decreased after the treatment with sour cherry leaf extract and orlistat (reference drug). Body weight was similarly reduced in rats fed a normal diet (ND) and the PC extract. Nonetheless, there were no variations in the rats' body weight gain when high fructose and high cholesterol diets were combined with sweet cherry fruits and leaves [57, 58]. HFD feeding may increase visceral fat deposition [59]. The level of obesity in the rodent model is determined by measuring Lee's index. The HFD group demonstrated a considerable increase in the Lee index. The b.w. of experimental rats and Lee index results are resumed in Table II.

Table II

The b.w. and Lee index of experimental rats throughout the diet and treatment by sour cherry leaf extract

Groups	Initial b.w. (g)	b.w. after 14 weeks of diet (g)	b.w. after 6 weeks of treatment (g)	Lee index
ND	163.66 ± 2.60	244.66 ± 2.91	250.00 ± 2.88	353.63 ± 4.59
ND+PC	$210.66 \pm 0.33^{***}$	$264.66 \pm 6.69^{*,+}$	$212.66 \pm 7.68^{**,+}$	$358.93 \pm 2.69^{+}$
HFD	$273.66 \pm 2.33^{***}$	$364.66 \pm 4.73^{***}$	$379.66 \pm 6.0^{***}$	$412.28 \pm 2.07^{***}$
HFD+PC	$269.66 \pm 0.88^{***,*}$	$315.66 \pm 4.91^{***,**}$	$284.00 \pm 3.06^{**,+}$	$363.02 \pm 4.73^{***,+}$
HFD+Orlistat	$274.66 \pm 0.67^{***}$	$333.00 \pm 1.73^{***,**}$	$281.66 \pm 1.20^{**,+}$	$365.67 \pm 2.08^{***,+}$

All values are expressed as mean ± SEM (n = 5); p < 0.01 as compared to the normal group, $^{*}p < 0.05$ as compared to the normal group; $^{+}p < 0.05$ as compared to the HFD group

As Table III shows, the administration of HFD has a remarkable increase in the organ's weight compared to those of the ND group. However, the supplementation of PC extract and orlistat significantly decreased the weight of organs. It is well-known that orlistat is a potent inhibitor of gastric and pancreatic lipase, which reduces dietary fat absorption [60]. Besides, rodents fed ND with PC extract decreased the weight of all tested organs. It is important to take into account the study's limitations, which include the need to divide the rats into various distinct groups in order to deliver accurate doses of the plant extract [61].

Table III

The weight of the organs in the control and experimental rats

Organs	ND	ND + PC	HFD	HFD + PC	HFD + orl
Heart (g)	0.78 ± 0.03	0.74 ± 0.03	$1.11 \pm 0.05^{***}$	$0.77 \pm 0.18^{+}$	$0.96 \pm 0.03^{**,+}$
Liver (g)	6.11 ± 0.38	$5.41 \pm 0.63^{**}$	$10.13 \pm 0.50^{***}$	$7.95 \pm 0.41^{**,+}$	$8.37 \pm 0.38^{**,+}$
Kidney (g)	1.50 ± 0.08	$1.36 \pm 0.09^{**,+}$	$2.17 \pm 0.13^{***}$	$1.81 \pm 0.08^{**,+}$	$1.92 \pm 0.09^{***,+}$
Adipose tissue (g)	1.79 ± 0.07	$1.64 \pm 0.21^{**,+}$	$4.37 \pm 0.80^{***}$	$3.30 \pm 0.19^{***,+}$	$3.11 \pm 0.25^{***,+}$
Testicles (g)	4.04 ± 0.06	$3.68 \pm 0.21^{**,+}$	$4.96 \pm 0.14^{***}$	$4.19 \pm 0.12^{+}$	$4.24 \pm 0.14^{+}$

All values are expressed as mean ± SEM (n = 5); p < 0.01 as compared to the normal group, $^{*}p < 0.05$ as compared to the normal group; $^{+}p < 0.05$ as compared to the HFD group

Effect of sour cherry extract on biochemical parameters

Table IV shows the impact of sour cherry leaf extract on biochemical parameters. Rodents fed HFD increased TG and TC contents, which the addition of PC extract and orlistat could decrease. The supplementation of PC extract to the ND group similarly decreased these parameters compared to the ND group (Table III). Similar findings were obtained when 1% of sweet cherry leaves were administered with a high-fructose diet [58]. Previous studies highlight sour cherry fruit's effectiveness in combination with HFD in decreasing TC and TG levels [62]. This effect might be connected to PC extract's rutin and chlorogenic acid content, which have been shown to lower TG levels [63] and improve lipid metabolism [64, 65]. Creatinine and urea levels are serum markers of kidney function status [66]. Sour cherry extract enhances the creatinine and urea concentration. This may explain why the extract did not exert toxic effects on the kidneys. In contrast, elevated creatinine and Urea concentrations observed in rodents fed HFD and HFD + orl suggest a potential association with an increased risk of renal failure. Renal toxicity induced by orlistat drug has been previously demonstrated [67]. ALT and AST enzymes are markers for liver function. A notable

elevated ALT and AST were observed in rats fed HFD compared to ND-fed rats. Increased liver enzyme levels may indicate fatty liver, liver damage and/or oxidative stress [68]. The experimental groups receiving PC extract and orlistat presented a reduction in the level of serum marker enzymes. Sweet cherry leaves also have a favourable effect on lowering the activity of these enzymes [57].

Table IV

Blood parameters in the control and experimental rats

Groups	ND	ND + PC	HFD	HFD + PC	HFD + orlistat
TC (mg/dL)	47.60 ± 1.72	$45.4 \pm 1.44^{*,+}$	$92.80 \pm 2.08^{***}$	$62.4 \pm 1.03^{**,+}$	$64.0 \pm 0.88^{**,+}$
TGs (mg/dL)	48.00 ± 1.46	$44.2 \pm 2.76^{+}$	$78.00 \pm 3.12^{***}$	$37.2 \pm 1.2^{**,+}$	$56.00 \pm 5.39^{*,+}$
Urea (mg/dL)	5.76 ± 0.21	$5.27 \pm 0.12^{*,+}$	$6.66 \pm 0.05^{**}$	$6.52 \pm 0.21^{**,}$	$8.05 \pm 0.03^{***,+}$
CREA (mg/L)	27.80 ± 1.46	26.4 ± 1.12	$52.40 \pm 0.93^{***}$	$26.2 \pm 0.37^{+}$	$36.40 \pm 1.12^{*,+}$
AST (u/L)	72.00 ± 1.15	$77 \pm 3.64^{+}$	$124.00 \pm 1.15^{***}$	$80.33 \pm 8.89^{*,+}$	$91.66 \pm 0.88^{**,+}$
ALT (u/L)	36.66 ± 0.88	$44.33 \pm 5.29^{*,+}$	$92.00 \pm 1.15^{***}$	$42.33 \pm 5.57^{*,+}$	$49.00 \pm 0.58^{**,+}$

TC: total cholesterol, TG: triglyceride, CREA: Creatinine, ALT: alanine transaminase, AST: aspartate transaminase. All values are expressed as mean± SEM (n = 5); $p < 0.01$ as compared to the normal group, $^{*}p < 0,05$ as compared to the normal group; $^{+}p < 0.05$ as compared to the HFD group

Histological analysis

Fat cell formation, known as adipogenesis, is a differentiation process through which undifferentiated preadipocytes are converted into fully differentiated adipocytes. These adipocytes store energy [69]. Obesity is characterised by either hypertrophy (an increase in the size of adipocytes), hyperplasia (an increase in the number of adipocytes) or both [70, 71]. To our knowledge, no published study has investigated the histological effects of sour cherry leaves on obesity through tissue analysis. The histopathological sections of the adipose tissue of rats are shown in (Figure 1). Microscopy reveals similar-sized adipocytes in the ND group and the ND + PC extract. However, HFD consumption induced a considerable enlargement of adipose cells. It was reported that consumption of HFD resulted in an increase in the size of adipocytes [72]. The supplementation of PC extract and orlistat to HFD showed smaller adipose cell size than the HFD group. This may be due to the fact that polyphenols present in the studied extract control their differentiation and lipid metabolism [73].

The liver tissue of rats fed ND showed an intact central vein and normal hepatocytes with curved euchromatic nuclei radiating from the central vein (Figure 2a). The supplementation of PC extract to ND for 6 weeks showed liver histology similar to that of the ND-fed group (Figure 2b). However, the liver of obese rats revealed alterations due to portal inflammation (Figure 2c), which was markedly reduced by the supplementation of sour cherry leaves extract and orlistat (Figure 2d; Figure 2e). Furthermore, it has been reported that hyperlipidaemia is related to liver tissue damage, inflammation and dysfunction [74]. These findings suggest that PC extract may improve liver architecture.

The ND control group presented healthy kidneys with normal glomeruli, tubules, and interstitium (Figure 3a). The kidney of the (ND + PC) group exhibited almost the same kidney architecture as the ND control group. Eosinophilic polymorphism in the perirenal fat and infiltration of inflammatory cells are characteristics of the kidneys of obese rats and rats treated with orlistat. This suggests probable lipotoxicity, a syndrome defined by the storage of non-esterified fatty acids and their products in organs such as the kidney, thus inducing chronic inflammation [10].

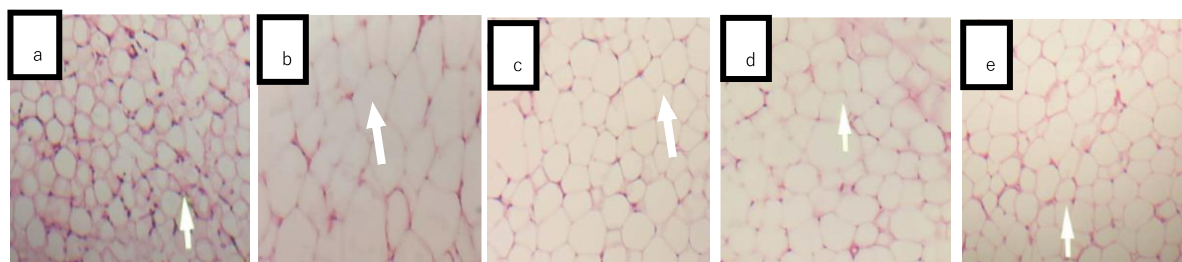

Figure 1.

Adipocytes of experimental group rats (haematoxylin and eosin, 40 X magnification)

The arrow shows the size of adipose cells.

a. normal diet (ND) similar-sized adipocytes; b. high-fat diet (HFD) enlargement of adipose cells; c. ND + PC (*Prunus cerasus* extract) small adipose cell size; d. HFD + PC with small adipose cell size; e. HFD + orlistat (standard drug) small adipose cell size

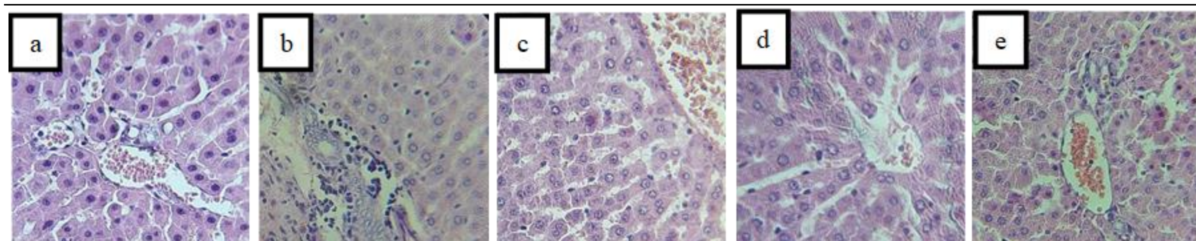

Figure 2.
Liver tissue of experimental group rats (haematoxylin and eosin, 40X magnification). a. ND, an intact central vein and normal hepatocytes; b. HFD, the presence of portal inflammation; c. ND + PC, standard liver architecture; d. HFD + PC, the absence of inflammation; e. HFD + Orl, the lack of portal inflammation

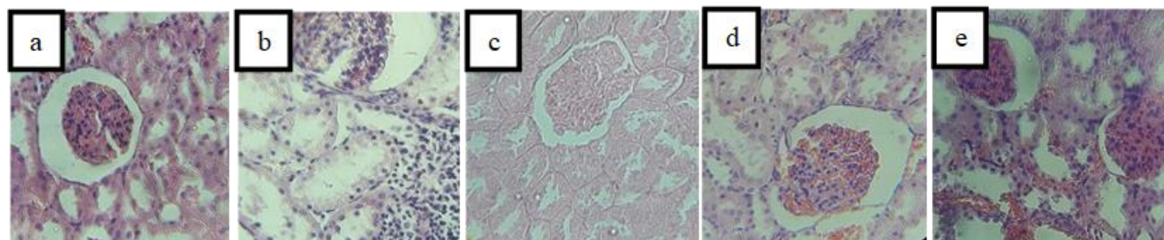

Figure 3.
Kidney tissue of experimental group rats (haematoxylin and eosin, 40X magnification). a. ND, normal glomeruli, normal tubules and normal interstitium; b. HFD eosinophilic polymorphism in the perirenal fat and an infiltration of inflammatory cells; c. ND + PC, standard kidney architecture; d. HFD + PC, the moderate and mild inflammation; e. HFD + orl, eosinophilic polymorphism in the perirenal fat and infiltration of inflammatory cells.

In vitro anti-inflammatory activity

Macrophages play a crucial role in the innate immune response. This study examined the potential anti-inflammatory properties of sour cherry leaves using RAW 264.7 cells as an *in vitro* inflammatory model. Table V presents the results of nitric oxide inhibitory activity (NO), cytotoxic effect and selectivity index (SI).

Table V

Nitric oxide inhibitory activity, cytotoxic effect, and selectivity index of PC extract

Samples	NO inhibition IC_{50} (µg/mL)	Cytotoxic effect LC_{50} (µg/mL)	Selectivity Index
Prunus cerasus	58.25 ± 0.36	62.82 ± 0.52	10.69
L-NAME	21.30 ± 0.24	332.41 ± 1.7	15.60

PC extract administration resulted in a dose-dependent reduction in NO production. Similar encouraging results in lowering inflammation were seen by the hydroethanolic extracts of sweet cherry leaves, which decreased NO generation by LPS-activated RAW 264.7 macrophages [75]. We can suggest that NO production was significantly inhibited by the tested extract. According to our research, sour cherry leaves have a high polyphenol content, which may explain why the PC extract has a greater effect on reducing NO accumulation. Furthermore, NO can combine with oxygen to form potent oxidants that cause oxidative damage to cells and tissues [76]. Consequently, by increasing ROS activity, NO production is inhibited [77].

The extract had a low cytotoxic effect on macrophage cells with an LC_{50} value of 622.82 ± 0.52 µg/mL. This value was similar to that of the positive control, L-NAME. The selectivity index (SI) is the ratio between cytotoxicity and biological activity, and it is generally considered that biological efficacy is not due to *in vitro* cytotoxicity when the SI ⩾ 10 [78]. A higher SI value indicates a safer and more effective compound [79]. The PC extract is in line with these hypotheses. Thus, the results highlight the efficacy of sour cherry leaves as anti-inflammatory agents and their harmlessness to macrophages (SI > 10).

In vivo carrageenan-induced rat paw oedema model

Carrageenan-induced oedema is suitable for identifying new anti-inflammatory agents [80]. In the control group, the orally administered 1% carrageenan solution caused visible inflammation within one hour of injection (oedema thickness was 6.52 mm), oedema gradually enhanced and reached 11.02 mm after 4 hours. However, PC extract and diclofenac reduced the oedema formation in rats against carrageenan-induced inflammation after 4 hours by 78.50 and 75.18 %, respectively. At the end of the experiment, there was no discernible difference between the inhibitory effect of diclofenac and sour cherry leaf extract (Figure 4).

Our findings align with those of others in the scientific literature regarding the reduction of paw oedema by the fruits and seeds of sour cherry [81]. The oedema size of the paw is a good indicator of the degree of inflammation and lasts for around five hours [82, 83]. Carrageenan-induced paw oedema development is a biphasic inflammatory phenomenon. The first phase lasted 1 to 2.5 hours and produced kinins, whereas the second phase lasted 2.5 to 6 hours and released prostaglandins. [84]. This finding suggests that the anti-inflammatory activity of *Prunus cerasus* leaf extract might inhibit the mediators of inflammation in both phases of oedema formation.

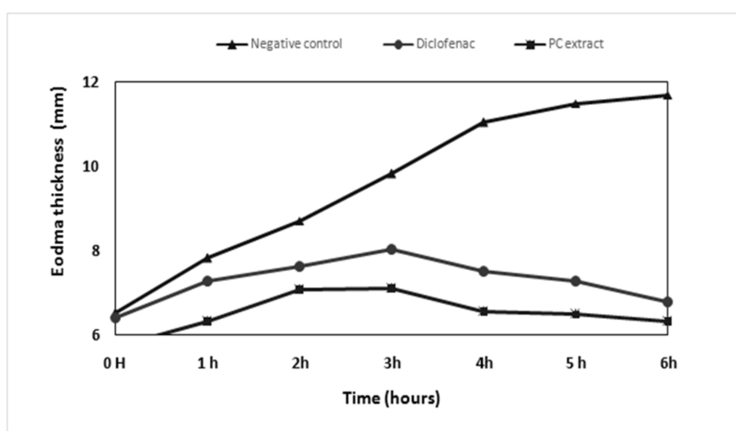

Figure 4.
Oedema paw thickness in experimental groups over six hours. Negative control group: visible inflammation and enhanced oedema; PC extract and diclofenac: reduced oedema formation in rats against carrageenan-induced inflammation.

Assessment of oxidative stress parameters
Glutathione (GSH) has the ability to protect important cellular components from damage caused by reactive oxygen species [85]. The GSH levels were somewhat lower in the carrageenan group than in the group that received diclofenac and aqueous extract, which increased the activity of this parameter (Figure 5a).

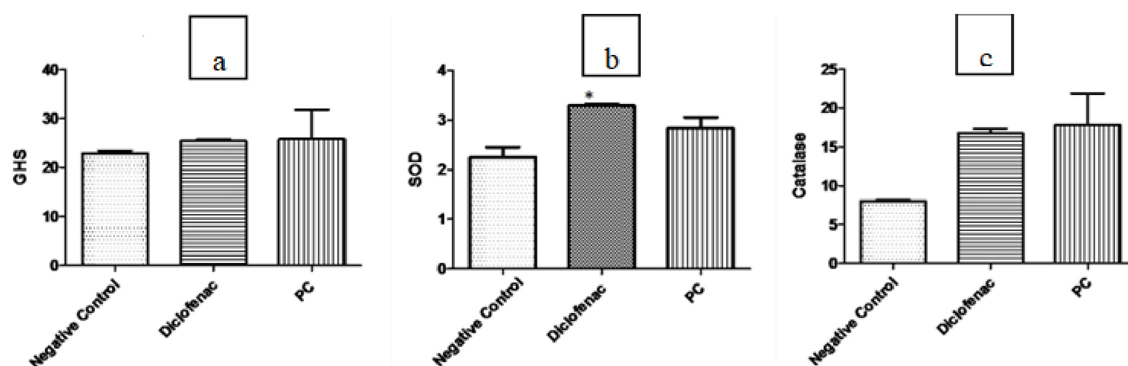

Figure 5.
The effect of the PC extract on the oxidative stress parameters A, GSH levels (mmol/mg protein); b. SOD levels (IU/mg protein); c, catalase levels (nmol/mg protein); negative control: carrageenan group
All values are expressed as mean ± SEM (n = 4); $^{**}p < 0.01$ as compared to the negative group; $^{***}p < 0.01$ as compared to the negative group; $^{*}p < 0.05$ as compared to the negative group.

Oxidative stress refers to an imbalance of the oxidant species and antioxidant species, such as CAT and SOD [86]. The oral administration of PC extract and standard drug increased significantly the level of SOD and CAT activities. However, these indices are decreased compared to the carrageenan group, as illustrated in Figures 5b and 5c. It has been reported that inflammatory conditions reduced the level and/or activity of antioxidant parameters such as CAT, SOD and GSH [87]. Sweet cherry vegetable parts (leaves stems, and flowers) have previously been shown to have a strong capacity to defend against oxidative stress and inflammation [25, 75]. The presence of secondary metabolites (polyphenols) in the examined plant may cause this outcome. Overall, the effect of sour cherry leaves on antioxidant parameters levels seems complex and

may depend on various factors. More research is needed to understand these dynamics.

Conclusions

The findings indicate that the phenolic compounds found in the aqueous extract of sour cherry leaves possess significant antioxidants, interesting anti-obesity, and notable anti-inflammatory effects. The results have shown the plant's potential as a natural source of bioactive compounds with promising pharmacological and therapeutic properties.

Conflict of interest

The authors declare no conflict of interest.

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