RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2025, **15**, 28313

Maturation-stage effects of *Ziziphus spina-christi* fruits and leaves on digestive, inflammatory, antioxidant, and glucose metabolism enzymes in obese rats with type 2 diabetes

Abeer Ayed Alshammari, [a] Nujud A. Almuzaini [a] and Sami Abbès *[b]

Ethnopharmacological relevance: *Ziziphus spina-christi* (Sidr/Nabag) is a medicinal fruit widely used in traditional Middle Eastern remedies. Rich in polyphenols, it offers potent therapeutic and nutritional benefits. *Aim of the study*: this study investigates the effects of *Ziziphus spina-christi* (ZSC) fruit and leaf extracts at different maturation stages in obese rats with type 2 diabetes (OB/T2D). *Materials and methods*: ZSC fruits (ZFU, ZFHR, ZFR) and leaves (ZL) were analyzed by DAD-HPLC. Their effects were evaluated in OB/T2D rats on digestive enzymes, insulin signaling, and metabolic pathways. *Results*: DAD-HPLC analysis of ZSC fruits and leaves identified 13 phenolic compounds. Phenolic acids decreased from 69% in unripe to 50.6% in ripe fruits, while polyphenolic content increased. In OB/T2D rats, administration of ZSC extracts restored pancreatic function by reducing pancreatic MPO activity. The extracts suppressed the activities of inflammatory enzymes and decreased oxidative stress markers, with the most remarkable effects observed in OB/T2D rats treated with ZFHR. Specifically, MPO activity was reduced by 58%, PLA2 by 46%, ELA by 62%, and PGS by 69%. Additionally, antioxidant defenses were strengthened, demonstrated by reductions in pancreatic H_2O_2 by 64%, TOS by 61%, and TBARS by 62%, along with an increase in TAS levels by 121%, compared to untreated OB/T2D rats. Ingestion of ZSC extracts inhibits intestinal α-amylase and lipase, slowing glucose and lipid absorption. The ZFHR showed the strongest inhibition, reducing α-amylase and lipase activities by 67 and 56%, respectively. This treatment also enhanced by a 68% increase in hepatic glycogen level. Additionally, administration of these extracts suppressed liver glucose anabolic enzymes G6PDH, G6P, FBP, and PEPCK; while stimulating glucose catabolic enzymes (HK, PK, LDH, PFK) and Krebs cycle enzymes (IDH, SDH, MDH). All extracts reduced BW and blood glucose levels, with the greatest effect in the ZFHR group, showing decreases of 30, 52, and 47% in BW, blood glucose, and HbA1c, respectively, followed by ZFU, ZFR, and ZL. These findings suggest that the semi-ripe fruit extract of ZSC may serve as a promising candidate for managing OB/T2D, due to its potent bioactivity and simultaneous richness in phenolic acids and polyphenolic compounds.

Received 12th May 2025
Accepted 23rd July 2025

DOI: 10.1039/d5ra03339j

rsc.li/rsc-advances

1. Introduction

Bioactive compounds derived from fruits and plants offer promising therapeutic potential.[1–4] These natural sources provide bioactive compounds with preventative, therapeutic, and health-promoting properties and often do not have some of the adverse side effects that are sometimes observed for synthetic drugs (*e.g.* impaired reproductive health, weakened immunity, and hepatic and renal toxicity).[5–9] Among these, *Ziziphus spina-christi*, a lesser-studied medicinal plant, is widely found in arid and Saharan regions. Belonging to the Rhamnaceae family, it has been traditionally used in folk medicine to treat digestive disorders, liver diseases, obesity, urinary issues, diabetes, skin infections, fever, diarrhea, and insomnia. In fact, the fruit of *Ziziphus spina-christi*, commonly known as Sidr or Nabag in Arabic and Christ's Thorn in English, is a thorny fruit tree highly resilient to extreme temperatures, soil salinity, and drought.[10] It is widely distributed across the Middle East and Arabia and has significant medicinal and nutritional value. Rich in bioactive compounds such as polyphenols, organic acids, and polysaccharides, it exhibits powerful biological activities. Traditionally, its fruits and leaves are used in medicine and as food, consumed fresh or processed into products like candies, jams, powders, and syrups.[11] The fruit's maturity stage influences its nutritional composition, with white-ripe fruits preferred for fresh consumption and red-ripe ones for drying.[12] Harvesting at inappropriate stages can lead to physiological

[a]*Department of Biology, College of Science, University of Ha'il, Ha'il, Saudi Arabia*
[b]*University of El Manar, Tunisia. E-mail: samirabbes579@gmail.com*

changes and reduced shelf life.[13] Studies indicate that ZSC extracts have therapeutic effects against, skin infections, and respiratory and urinary diseases, in addition to possessing strong anti-inflammatory and antimicrobial properties.[14–16] Moreover, this plant is well known in traditional medicine for its therapeutic effects against gastric ulcers. However, research on this topic remains limited.

Inflammation is the major factor responsible for the development of various chronic diseases, including obesity, type 2 diabetes, and gastric ulcers. Obesity is associated with chronic inflammation affecting the pancreas and various other organs, leading to insulin resistance and metabolic disorders. Similarly, type 2 diabetes is characterized by pancreatic inflammation, contributing to β-cell dysfunction and impaired glucose metabolism.[15–17] Obesity and type 2 diabetes represent major public health challenges worldwide, with prevalence steadily increasing. In 2022, around 16% of adults globally were obese, and this trend is expected to continue, with projections suggesting that by 2050, more than half of adults and a third of youth could be overweight or obese. As for diabetes, the number of affected individuals increased from 200 million in 1990 to 830 million in 2022, with over 90% of cases being type 2 diabetes, often linked to lifestyle factors. According to the International Diabetes Federation, by 2045, nearly 783 million people will live with diabetes, a 46% increase.[18] These alarming statistics highlight the need to adopt healthier lifestyles and improve access to healthcare, particularly in low- or middle-income countries where treatment access remains limited.

Obesity is the leading cause of serious diseases such as heart disease, hypertension, and strokes due to the accumulation of fats in the arteries and tissues, which increases pressure on the heart and blood vessels. Moreover, obesity is a major risk factor for type 2 diabetes, as excess body fat leads to insulin resistance.[2,19,20] Additionally, obesity promotes joint problems, such as osteoarthritis, by exerting excessive pressure on the joints, particularly the knees and hips. Obese individuals are also more likely to suffer from respiratory disorders, such as sleep apnea, due to the excess fat blocking the airways.[21] From a digestive standpoint, obesity can lead to liver diseases, such as non-alcoholic fatty liver disease,[22] and worsen gastroesophageal reflux disease.[23] Furthermore, obesity affects mental health, causing issues such as depression and anxiety due to social stigma and hormonal changes. Many conventional treatments for obesity come with significant side effects. Medications used to treat obesity can lead to various side effects and toxic impacts on health. For example, orlistat (Xenical) inhibits fat absorption, causing gastrointestinal issues such as diarrhea and abdominal pain, as well as nutritional deficiencies in fat-soluble vitamins. Anorectic medications, like sibutramine, reduce appetite but can increase blood pressure, heart rate, and cause psychological issues such as anxiety and irritability.[24,25] Phentermine affects the central nervous system, leading to headaches, insomnia, and potential dependence. Liraglutide (Saxenda) can cause nausea, abdominal pain, and increase the risk of pancreatitis. Bupropion-naltrexone (Contrave) may cause sleep disturbances, suicidal thoughts, and hypertension, while topiramate (Qsymia) can lead to cognitive issues and kidney stone risks.[26,27] These side effects highlight the importance of careful medical supervision during the use of these treatments. As a result, there is a pressing need to develop alternative treatments based on natural products that offer broad biological activity, enhanced efficacy, and a safer overall profile.[5,8,9,28–32]

In this context, the primary objective of this study is to investigate how different stages of fruit maturation influence the phenolic composition and biological activity of ZSC. Particular emphasis is placed on understanding how the maturation process affects the levels of phenolic acids and polyphenolic compounds in the fruits, as determined by DAD-HPLC analysis. This study also aims to assess, for the first time, the combined effects of fruit ripeness and leaf composition on key metabolic and inflammatory pathways in OB/T2D rat. The biological impact of fruits at three maturation stages unripe, semi-ripe, and fully ripe along with leaf extracts, will be evaluated on enzymes associated with obesity (lipase), and type 2 diabetes (α-amylase). The maturation-dependent modulation of enzymes involved in glucose anabolism, catabolism, and the Krebs cycle will also be explored. Moreover, the study will assess how fruit maturity influences pancreatic function, through histological analysis and evaluation of inflammatory markers (MPO, PLA2, ELA, PGS), as well as antioxidant status *via* TAS, TOS, H_2O_2, and TBARS levels. These analyses aim to clarify whether the stage of fruit maturation enhances or diminishes the therapeutic potential of ZSC.

2. Materials and methods

2.1 ZSC recolt

The fruits and leaves of ZSC at different maturation stages were collected in February and July 2024 from 20 year-old trees in the Hail region, Saudi Arabia. The fruits were classified into three maturation stages: unripe (light green), half-ripe (>20% red-brown surface), and ripe (>80% red-brown surface). The leaves were harvested when green in February. The experiment followed a completely randomized design with three replications per stage. Thirty fruits and leaves were pitted and ground for biochemical analyses, including soluble solids content, titratable acidity, moisture content, individual and total phenolic compounds, total flavonoids, and antioxidant capacity.

2.2 Phenolic profile characterization of ZSC fruits and leaves using HPLC

Five grams of each fruit stage or leaves, after removing the seeds, were ground and extracted in 100 mL of 70% ethanol (HPLC grade) overnight. The mixture was then centrifuged for 10 minutes. The phenolic profile was determined as previously reported by Hamden *et al.*[33] The presence and concentration of phenolic compounds in the 70% ethanol extracts of fruits at different maturation stages and leaves were analyzed using reversed-phase HPLC with a binary gradient elution on a waters autopurification system equipped with a binary pump (Waters 2525, Germany), a UV-vis diode array detector (190–600 nm, Waters 2996), and a PL-ELS 1000 ELS detector (Polymer

Laboratory). Chromatographic separation was performed using a Kromasil C18 column (250 × 4.6 mm, 5 μm, Thermo) maintained at 40 °C. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), with a total run time of 60 minutes under the following elution conditions: 0–30 min, 20–50% B; 30–35 min, 50% B; 35–45 min, 50–100% B; 45–55 min, 100% B; 55–60 min, 100–20% B. The column was washed and reconditioned for 10 min with 20% B. The flow rate was set at 0.6 mL min^{-1}, and the injection volume was 50 μL. Major phenolic compounds were identified by comparing their retention times and UV spectra with pure standards when available or with literature-reported data [16,17]. To confirm peak identities, LC-MS analysis was performed using an Agilent 1100 LC system consisting of a degasser, binary pump, autosampler, and column heater, with the column outlet coupled to an Agilent MSD Ion Trap XCT mass spectrometer equipped with an electrospray ionization (ESI) source. Data acquisition and analysis were conducted using ChemStation software under chromatographic conditions identical to those in HPLC. The MS parameters included electrospray ionization in positive ion mode with a capillary voltage of 3.5 kV, a drying temperature of 350 °C, a nebulizer pressure of 40 psi, a drying gas flow of 10 L min^{-1}, a maximum accumulation time of 50 ms, a scan speed of 26 000 m/z s^{-1} (ultra-scan mode), and a fragmentation time of 30 ms. Compounds were identified by comparing their retention times and mass spectra with those of reference standards when available.

2.3 Obesity induction

2.3.1 Animals. Forty-two male Wistar rats, weighing 135 g ± 9 and aged 8 weeks, were used for this study. The animal study protocol was approved by the National Medical Experimentation Ethics Committee, in accordance with international guidelines for animal experimentation (Approval No. MU-86/609/EEC). The rats were housed individually in stainless-steel wire mesh cages with ad libitum access to food and water. Environmental conditions were maintained at 55 ± 5% humidity, a temperature of 25–30 °C, and a 12 hour light/dark cycle.

2.3.2 Induction of obesity. Obesity induction was carried out following the method described by Tiss *et al.*[33] The HFF diet used for the rats consisted of 60% standard food, 15% sheep fats, 12% fructose, and 0.5% cholic acid. The rats had ad libitum access to this diet, and food and liquid intake were monitored weekly. Obesity and type 2 diabetes were induced in the rats, evidenced by a significant increase in body weight and blood glucose levels ≥2 g L^{-1}, measured by glucometer from tail blood samples.

2.3.3 Experimental design and procedure. A total of 42 rats were randomly assigned to 5 experimental groups as follows: group 1 (Con) consisted of control rats fed a normal diet for 90 consecutive days. Group 2 (OB/T2D) included rats fed a high-fructose diet for 90 days. Groups 3, 4, 5, and 6 were treated with 200 mg per kg body weight[34] of *Ziziphus* fruit at different maturation stages unripe by gastric gavage route (OB/T2D-ZFU), half-ripe (OB/T2D-ZFHR), ripe (OB/T2D-ZFR), or leaves (OB/T2D-ZL) administered daily by gastric gavage.[35] Group 6 (OB/T2D-ORL) included rats fed a high-fructose diet and administered 10 mg kg^{-1} of orlistat by gastric gavage.[36] All treatments were administered for 90 days following a pretreatment with HFFD. Only rats with blood glucose levels ≥2 g L^{-1} were selected for this study. At the end of the study, rats were weighed and sacrificed by decapitation after anesthesia. Blood samples were collected, and serum was stored at −80 °C for biochemical analysis. Intestinal tissues were excised and washed with 0.9% NaCl solution, while pancreatic and intestinal mucosal tissues were homogenized, centrifuged, and stored at −80 °C for lipase and α-amylase assays.

2.4 Biochemical analysis

2.4.1 Pancreas biochemical analysis. Or this analysis, 1 g of pancreas tissue was homogenized in 10 mmol per L tris-HCl buffer (pH 7.4) at low temperature and subsequently centrifuged at 3000 × g for 15 min. MPO activity was measured as described by Queiroz *et al.*[37] based on the enzyme's ability to catalyze the oxidation of a chromogenic substrate (*o*-dianisidine) in the presence of hydrogen peroxide. The reaction produces a colored product, the intensity of which is proportional to MPO activity and was measured spectrophotometrically at 460 nm. ELA activity using a modified method from Chandra *et al.*,[38] based on the hydrolysis of the synthetic substrate *N*-succinyl-ala-ala-ala-*p*-nitroanilide (SANA), with absorbance measured at 410 nm and PLA2 activity following George *et al.*[39] which assesses enzyme activity based on the hydrolysis of phospholipid substrates, producing free fatty acids that can be quantified spectrophotometrically with absorbance measured at 414 nm. H_2O_2 levels were quantified using a colorimetric assay based on the reaction of hydrogen peroxide with a specific probe that produces a colored product. The intensity of the color, measured by absorbance at 450 nm, is proportional to the concentration of H_2O_2 in the sample,[40] while TOS and TAS were determined at 530 nm and 660 nm, respectively.[41,42] Lipid peroxidation was assessed using TBARS method by Buege and Aust,[43] and protein content was measured using the Bradford protocol.[44]

2.4.2 Liver biochemical analysis. Or this analysis, 1 g of liver or intestinal mucosa was homogenized in 10 mmol per L tris-HCl buffer (pH 7.4) at low temperature, followed by centrifugation at 3000 × g for 15 min. The resulting supernatant was collected and stored at −80 °C for subsequent analyses. Protein concentration was determined spectrophotometrically by quantifying the blue-violet complex formed from the reaction of peptide bonds in proteins with Cu^{2+} in an alkaline solution (Kit Biolabo, France ref. 80016). The activity of G6-PDH was quantified using a commercial kit from Bolabo, France (ref. 97089). The hepatic activities of glucose metabolism enzymes as HK, PK, G6P, and FBP were quantified using the protocols described by Brandstrup *et al.*,[45] Pogson & Denton,[46] Hikaru & Toshitsugu,[47] and Gancedo & Gancedo,[48] respectively. The liver glycogen content were determined using the protocols described by Leloir *et al.*,[49] respectively. Liver PFK activity was measured according to Castano *et al.*,[50] with modifications. Liver homogenates were prepared in tris–HCl buffer (50 mmol L^{-1}, pH 8.2) containing 10 mmol per L DTT, 5 mmol per L magnesium sulfate, and 1 mmol per L EDTA. After centrifugation (20 000 × g, 15 min, 3 °C), enzyme activity was determined by the generation of NAD at 340 nm. Results were

expressed as mmol h^{-1} mg^{-1} tissue protein. Liver PEPCK activity was measured spectrophotometrically at 340 nm using a reaction mixture containing phosphoenolpyruvate, tris–HCl, GDP, NAD, sodium bicarbonate, malate dehydrogenase, and $MnCl_2$. Results were expressed as mmol h^{-1} mg^{-1} tissue protein and described by Mommsen *et al.*[51] IDH activity was performed as described by Duncan and Fraenkel.[52] Liver homogenate supernatant was mixed with isocitrate, phosphate buffer, NAD, and manganese sulfate. The formation of NADH was measured spectrophotometrically at 340 nm. The optical density was used to calculate the enzyme activity, expressed as mmol h^{-1} mg^{-1} tissue protein. The liver SDH activity was performed as described by Veeger *et al.*[53] The supernatant from the liver homogenate was mixed with succinate, phosphate buffer, potassium ferricyanide, and bis(trimethylsilyl) acetamide. The reduction of potassium ferricyanide was measured spectrophotometrically at 420 nm. The optical density was used to calculate SDH activity, expressed as $mmol^{-1}$ h^{-1} mg^{-1} tissue protein. The MDH quantity was performed as described by Shonk and Boxer.[54] The supernatant from the liver homogenate was mixed with oxaloacetate, triethanolamine hydrochloride (pH 7.6), NADH, and ethylenediaminetetraacetic acid (EDTA). The optical density was measured at 340 nm relative to a blank and used to calculate MDH activity, expressed as $mmol^{-1}$ h^{-1} mg^{-1} tissue protein. Glycogen levels were measured following the method of Ong and Khoo.[55]

2.4.3 Intestinal mucosa biochemical analysis. For this analysis, 1 g of intestinal mucosa was homogenized in 10 mmol per L tris–HCl buffer (pH 7.4) at low temperature and subsequently centrifuged at $3000 \times g$ for 15 minutes. Intestinal α-amylase activity was determined colorimetrically by the conversion of CNPG3 into GNPG2 and glucose using a kit from Biolabo (ref. 99123). Lipase activity: lipase activity was determined using a kit from Biolabo (99881).

2.4.4 Biochemical plasma analysis of enzyme activities and metabolic markers. Glucose, and glycated hemoglobin levels were determined using commercial kits (Biolabo, ref. 87409 and 22010).

2.5 Statistical analysis

Data are presented as mean ± standard deviation (SD) for 6 rats per group. A total of 8 animals per group were included for statistical calculations. One-way analysis of variance (ANOVA) followed by Fisher's post hoc test (StatView) was used to assess differences between groups. Statistical significance was set at $p \leq 0.05$.

3. Results

3.1 HPLC analysis of phenolic compounds in ZSC fruits at different maturation stages and leaves

The extraction yield of phenolic compounds using a hydroalcoholic mixture was 15.8 mg g^{-1} for ZLU, 14.9 mg g^{-1} for ZFHR, and 11.8 mg g^{-1} for ZFR, while the extraction yield for the leaves was the lowest at 10.3 mg g^{-1}. The extraction yield of phenolic compounds using a hydroalcoholic mixture was 13 mg g^{-1}. Our HPLC analysis of fruit extracts at different maturation stages and ZSC leaves reveals the presence of 13 phenolic compounds, including 8 dominant phenolic acids and 5 polyphenolic compounds. A comparative study of the fruits during maturation highlights the dominance of phenolic acids in unripe fruits, accounting for approximately 69%, while polyphenolic compounds represent 31%. As maturation progresses, a gradual decrease in acid content is observed, accompanied by an increase in polyphenolic concentration. Specifically, in half-ripe and ripe fruits, the proportion of phenolic acids decreases to 58.8 and 50.6%, respectively, while polyphenolic compounds increase to 41.2 and 49.4%. Regarding the leaves, we identified 9 phenolic compounds, with the major ones being trans-catechin (26.3%), rutin (21.3%), ferulic acid (20.8%), and syringic acid (9.16%) (Table 1).

3.2 *Ziziphus spina-christi*, lipase activity and body weight in obese rats

The results of this study show that HFFD ingestion increases intestinal lipase activity, leading to enhanced digestion, absorption, and lipid accumulation, as evidenced by significant weight gain and obesity. Oral administration of ZFU, ZFHR, ZFR, and ZL at a dose of 200 mg per kg body weight suppresses lipase activity by 36, 57, 32, and 28%, respectively, and reduces body weight by 19, 30, 16, and 24% compared to untreated obese rats. A key finding of this study is that the semi-ripe fruits (ZFHR) exhibit strong lipase–inhibitory activity and significantly reduce body weight in OB/T2D rats, likely due to their high content of bioactive compounds (Fig. 1).

3.3 Pancreatic inflammation in obese rats and protective effects of ZSC

The results of this study indicate that OB/T2D is associated with severe lymphocytic infiltration, as evidenced by a significant increase in pancreatic activity of lymphocyte infiltration enzymes such as MPO, and inflammation-promoting enzymes including PLA2, ELA, and PGS. In contrast, oral administration of *Ziziphus spina-christi* extracts ZFU, ZFHR, ZFR, and ZL at a dose of 200 mg per kg body weight significantly suppressed pancreatic lymphocytic infiltration. The most pronounced anti-inflammatory effect was observed in rats treated with semi-ripe fruits (ZFHR), as reflected by a reduction in the pancreatic activities of MPO, PLA2, ELA, and PGS by 58, 46, 62, and 66%, respectively, compared to untreated OB/T2D rats (Fig. 2).

3.4 ZSC and pancreatic oxidative stress in obese rats

The results of this study show that HFF diet and obesity are associated with severe oxidative stress in the pancreas, as evidenced by a significant reduction in TAS. This suppression of pancreatic antioxidant capacity leads to an increase in H_2O_2 and TOS, resulting in pancreatic damage, marked by a significant rise in TBARS levels. Oral administration of ZFU, ZFHR, ZFR, and ZL induces pancreatic antioxidant activity, as indicated by an increase in TAS by 74, 120, 97, and 103%, respectively, compared to untreated obese rats. This enhancement leads to a reduction in H_2O_2, TOS, and TBARS, with the strongest protective effect observed in ZFHR-treated obese rats, showing

Table 1 Phenolic profiling of the fractions ZFU, ZFHR, ZFR, and ZL of *Z. spina-christi* was conducted using DAD-HPLC. Our results show that fruit maturation leads to a reduction in the total acid content and an increase in polyphenolic compounds. Statistical analysis presented the results of three repetitions as mean ± SD. Significant differences were determined as follows: $^{\alpha}p \leq 0.05$ compared to ZFU, $^{\beta}p \leq 0.05$ compared to ZFHR, and $^{\Omega}p \leq 0.05$ compared to ZFR[a]

Compounds (μg per g FW)	ZFU	ZFHR	ZFR	ZL
Gallic acid	10.18 ± 0.72	7.06 ± 0.12$^{\alpha}$	6.31 ± 0.93$^{\alpha\beta}$	4.31$^{\alpha\beta\Omega}$
1,2-Dihydroxy benzene	5.48 ± 0.03	8.15 ± 0.22$^{\alpha}$	6.02 ± 0.78$^{\alpha\beta}$	ND
4-Hydroxy benzoic acid	7.51 ± 0.18	7.12 ± 0.18	3.04 ± 0.33$^{\alpha\beta}$	ND
Vanillic acid	5.45 ± 0.02	4.70 ± 0.03$^{\alpha}$	2.98 ± 0.03$^{\alpha\beta}$	6.17$^{\alpha\beta\Omega}$
Caffeic acid	5.33 ± 0.04	5.25 ± 0.0$^{\alpha}$	4.34 ± 0.07$^{\alpha\beta}$	1.86$^{\alpha\beta\Omega}$
Syringic acid	5.61 ± 0.03	3.74 ± 0.07$^{\alpha}$	3.03 ± 0.12$^{\alpha\beta}$	9.16$^{\alpha\beta\Omega}$
p-Coumaric acid	6.48 ± 0.21	4.25 ± 0.15$^{\alpha}$	6.14 ± 0.20$^{\beta}$	5.87$^{\alpha\beta\Omega}$
trans-Ferulic acid	5.43 ± 0.08	5.86 ± 0.11$^{\alpha}$	5.78 ± 0.13$^{\alpha}$	20.85$^{\alpha\beta\Omega}$
Salicylic acid	10.13 ± 0.57	5.81 ± 0.65$^{\alpha}$	6.92 ± 0.42$^{\alpha\beta}$	ND
Cinnamic acid	7.15 ± 0.22	6.91 ± 0.18$^{\alpha}$	6.06 ± 0.15$^{\alpha\beta}$	ND
Rutin	13.43 ± 0.93	13.85 ± 0.73$^{\alpha}$	15.72 ± 0.22$^{\alpha\beta}$	21.30$^{\alpha\beta\Omega}$
Catechin	9.66 ± 0.51	12.44 ± 0.45$^{\alpha}$	15.11 ± 0.16$^{\alpha\beta}$	26.34$^{\alpha\beta\Omega}$
Quercetin	7.52 ± 0.03	14.65 ± 0.09$^{\alpha}$	15.49 ± 0.09$^{\alpha\beta}$	2.73$^{\alpha\beta\Omega}$

[a] Abbreviations: ZFU (*Ziziphus spina-christi* fruit unripe), ZFHR (ZSC fruit half-ripe), ZFR (ZSC fruit ripe), and ZL (ZSC leaves). DAD-HPLC (diode-array detector high-performance liquid chromatography).

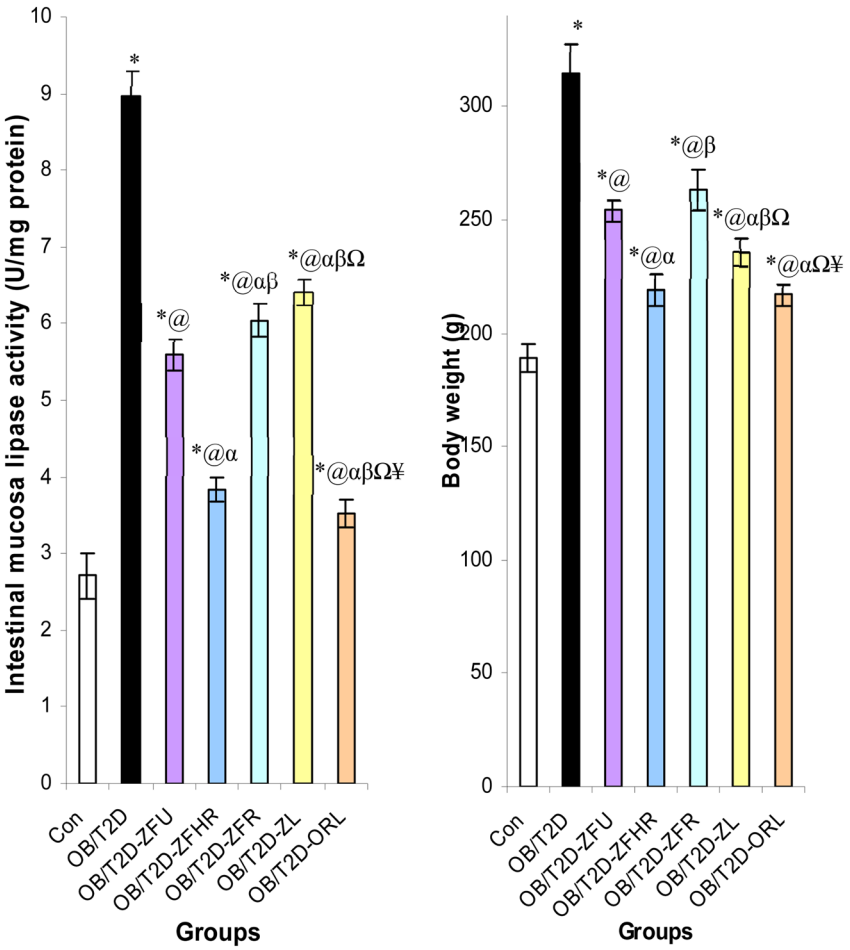

Fig. 1 Effect of OB/T2D, ZFU, ZFHR, ZFR, and ZL on intestinal lipase activity and body weight. The results show that a high-fat, high-fructose diet (HFFD) significantly increases intestinal lipase activity, enhancing lipid digestion and absorption, which leads to fat accumulation and the development of obesity. Oral administration of *Ziziphus spina-christi* extracts—unripe fruit (ZFU), semi-ripe fruit (ZFHR), ripe fruit (ZFR), and leaves (ZL)—inhibited intestinal lipase activity, with the most pronounced effect observed in the ZFHR-treated group, followed by ZFU. This inhibition was associated with a reduction in body weight. Data are expressed as mean ± SD from five replicates. Statistical significance was determined as follows: $^{*}p \leq 0.05$ *vs.* Con; $^{@}p \leq 0.05$ *vs.* OB/T2D; $^{\alpha}p \leq 0.05$ *vs.* ZFU-treated OB/T2D rats; $^{\beta}p \leq 0.05$ *vs.* ZFHR-treated OB/T2D rats; $^{\Omega}p \leq 0.05$ *vs.* ZFR-treated OB/T2D rats; and $^{¥}p \leq 0.05$ *vs.* ZL-treated OB/T2D rats.

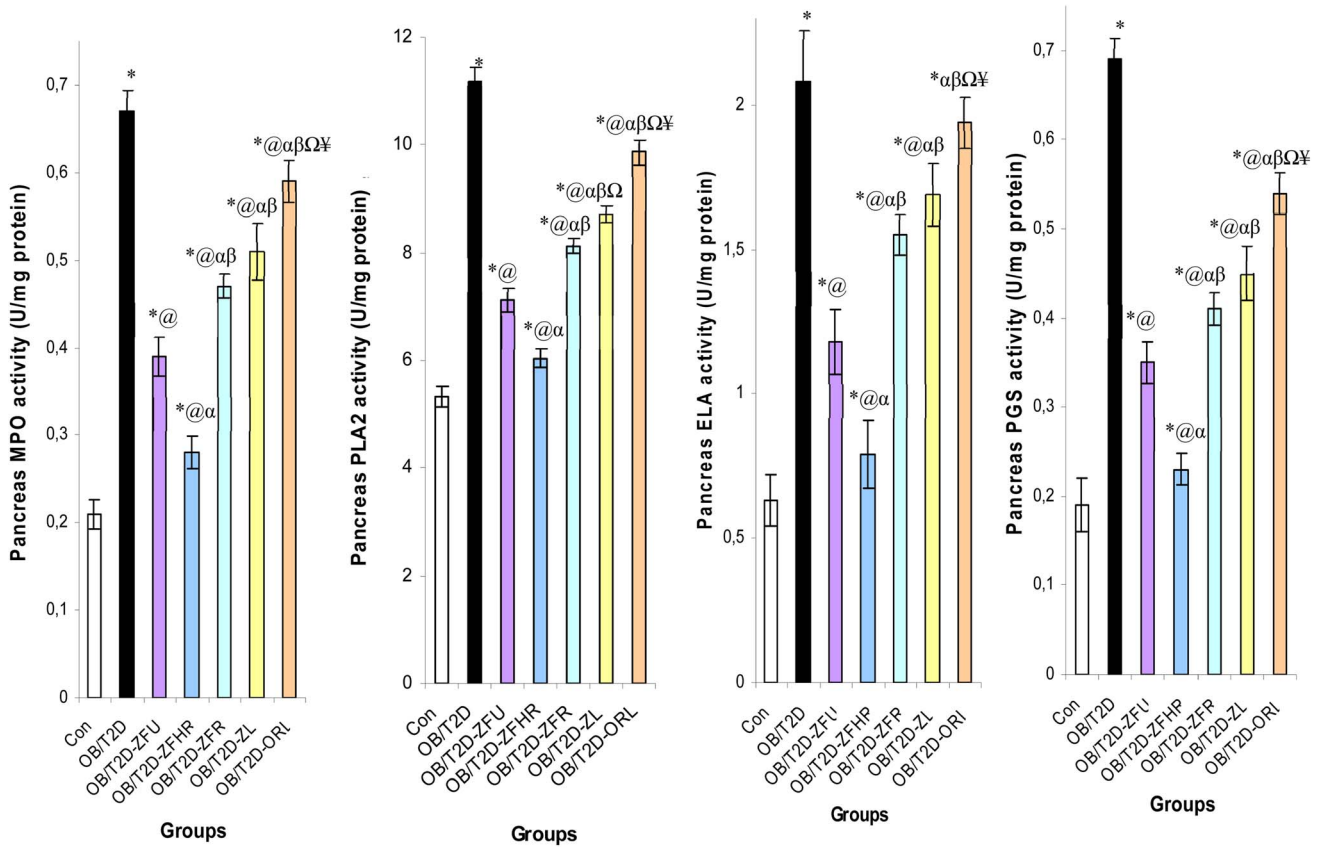

Fig. 2 Effect of ZFU, ZFHR, ZFR, and ZL on pancreatic neutrophil infiltration (MPO activity) and key inflammatory enzymes (PLA2, ELA, and PGS) in OB/T2D rats. Supplementation with ZFU, ZFHR, ZFR, and ZL led to a significant reduction in inflammation, as evidenced by decreased MPO activity and reduced levels of PLA2, ELA, and PGS. The most potent anti-inflammatory effect was observed in rats treated with semi-ripe fruits (ZFHR), followed by those receiving ZFU. Statistical analysis was performed as described in Fig. 1.

a reduction of 74, 61, and 62%, respectively, thereby preserving pancreatic function (Fig. 3).

3.5 *Ziziphus spina-christi*, obesity, and liver glucose anabolic enzyme activities

This study shows that OB/T2D caused pancreatic dysfunction causes suppression of insulin activity and a subsequent rise in the activity of glucose anabolic enzymes such as G6PDH, G6P, FBP, and PEPCK, leading to elevated blood glucose levels. Supplementation with FU, ZFHR, ZFR, and ZL promotes pancreatic function by inducing insulin signaling, resulting in the suppression of these glucose anabolic enzymes. The most potent effect was observed in obese rats with type 2 diabetes treated with ZFHR, showing a reduction in the activity of G6PDH, G6P, FBP, and PEPCK by 40, 42, 37, and 56%, respectively, compared to untreated OB/T2D (Table 2).

3.6 Liver glucose catabolic enzyme activities, Ob/T2D rats, and ZSC

Our study shows that Ob/T2D is associated with suppression of liver glucose catabolic enzyme activities such as HK, PK, LDH, and PFK. Ingestion of ZSC fruit extracts at different maturation stages, along with leaf extracts, induces the activity of these enzymes and protects against metabolic disorders, including the rise in blood glucose levels. The most potent effect was observed with ZFHR fruit extracts, which stimulated HK, PK, LDH, and PFK activities by 85, 88, 47, and 240%, respectively, compared to untreated OB/T2D rats (Table 2).

3.7 Liver Krebs cycle enzyme activities, Ob/T2D rats, and *Ziziphus spina-christi*

Our results show that the administration of FU, ZFHR, ZFR, and ZL reverses the decline in glucose metabolism within the Krebs cycle by stimulating the activity of IDH, SDH, and MDH, leading to enhanced glucose degradation into ATP and CO_2. In fact, supplementation with ZFHR in OB/T2D rats induced the activity of these enzymes by 88, 67, and 267%, respectively (Table 2). Furthermore, this study suggests that the semi-ripe fruits exhibit particularly strong activity in promoting carbohydrate catabolism and maintaining metabolic balance compared to other maturation stages and leaves, which may be attributed to their richness in bioactive compounds, including phenolic acids and polyphenolic compounds.

3.8 α-Amylase activity, liver glycogen, blood glucose, HbA1c levels, and OB/T2D

Our study shows that OB/T2D is associated with pancreatic dysfunction and type 2 diabetes, characterized by increased α-

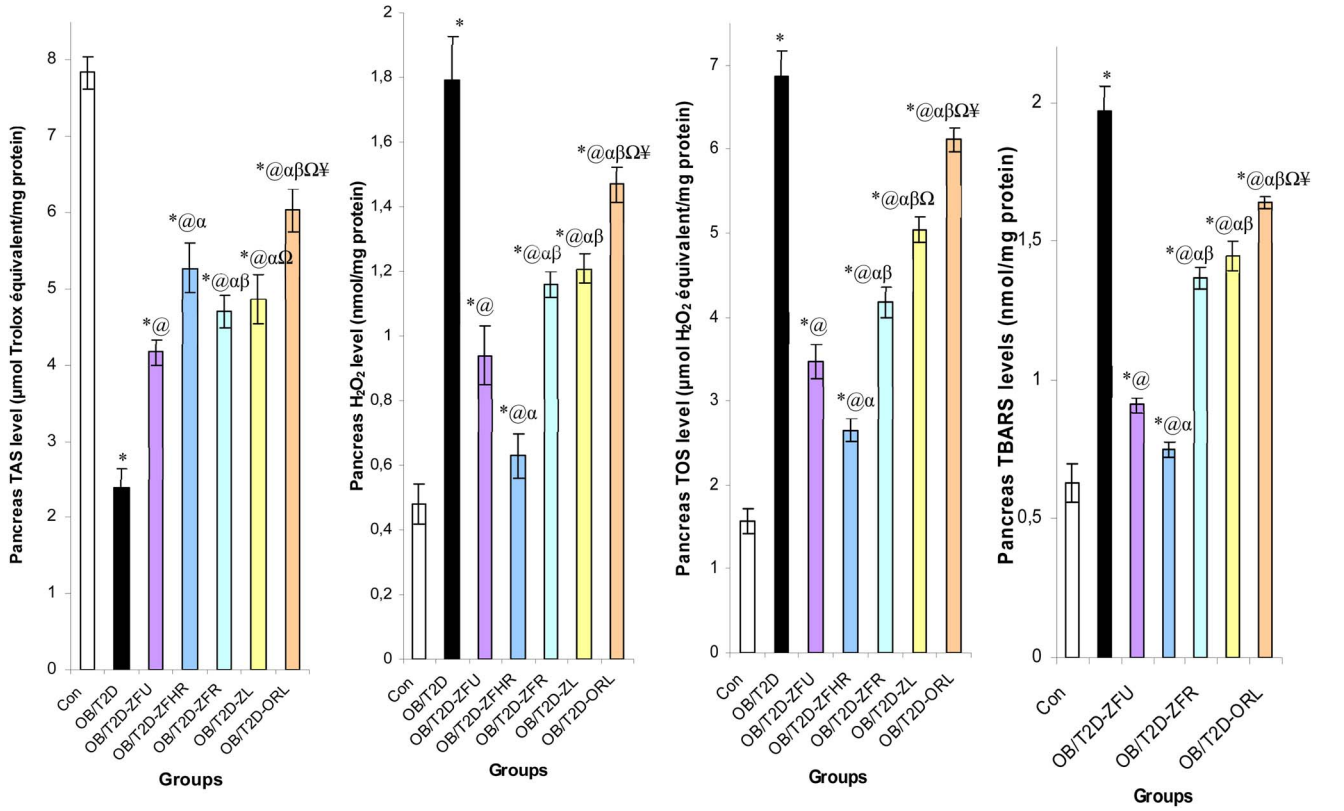

Fig. 3 Effect of OB/T2D and treatment with ZFU, ZFHR, ZFR, and ZL on pancreatic antioxidant capacity. This study demonstrates that ZFU, ZFHR, ZFR, and ZL provide strong protection against OB/T2D-induced oxidative stress in the pancreas, showing their antioxidant potential in restoring redox balance. Statistical analysis was performed as in Fig. 1.

Table 2 Effect of OB/T2D and treatment with *Ziziphus* fruits at different maturation stages (ZFU, ZFHR, ZFR) and leaves (ZL) on key metabolic enzyme activities. The activities of glucose anabolic enzymes (G6PDH, G6P, FBP, PEPCK), glucose catabolic enzymes (HK, PFK, LDH), and Krebs cycle enzymes (IDH, SDH, MDH) were evaluated. Oral administration of *Ziziphus* fruit and leaf extracts suppressed glucose anabolism and promoted glucose catabolism both in the plasma and through Krebs cycle activity, thereby contributing to improved glucose regulation, with the most pronounced protective effects against metabolic dysregulation observed in rats treated with semi-ripe fruits. Statistical significance was determined as follows: $^{*}p \leq 0.05$ *vs.* Con; $^{@}p \leq 0.05$ *vs.* OB/T2D; $^{\alpha}p \leq 0.05$ *vs.* ZFU; $^{\beta}p \leq 0.05$ *vs.* ZFHR; $^{\Omega}p \leq 0.05$ *vs.* ZFR; $^{¥}p \leq 0.05$ *vs.* ZL[a]

	Con	OB/T2D	OB/T2D-ZFU	OB/T2D-ZFHR	OB/T2D-ZFR	OB/T2D-ZL	OB/T2D-ORL
Liver glucose anabolic enzymes activities (U per mg protein)							
G6PDH	218 ± 7	489 ± 13*	369 ± 11$^{*@}$	286 ± 9$^{*@\alpha}$	342 ± 12$^{*@\alpha}$	409 ± 15$^{*@\alpha\beta\Omega}$	249 ± 9$^{*@\alpha\beta\Omega¥}$
G6P	987 ± 37	1897 ± 68*	1254 ± 43$^{*@}$	1098 ± 38$^{*@\alpha}$	1487 ± 54$^{*@\alpha\beta}$	1589 ± 67$^{*@\alpha\beta\Omega}$	1603 ± 75$^{*@\alpha\beta\Omega}$
FBP	412 ± 11	789 ± 23*	564 ± 19$^{*@}$	498 ± 22$^{*@\alpha}$	619 ± 27$^{*@\alpha\beta}$	668 ± 31$^{*@\alpha\beta}$	681 ± 26$^{*@\alpha\beta\Omega}$
PEPCK	87 ± 4	256 ± 11*	187 ± 7$^{*@}$	113 ± 5$^{*@\alpha}$	155 ± 6$^{*@\alpha\beta}$	203 ± 8$^{*@\alpha\beta\Omega}$	98 ± 4$^{*@\alpha\beta\Omega¥}$
Liver glucose catabolic enzymes activities (U per mg protein)							
HK	187 ± 6	87.9 ± 7*	134 ± 6$^{*@}$	163 ± 4$^{*@\alpha}$	123 ± 7$^{*@\beta}$	103 ± 3$^{*@\alpha\beta\Omega}$	96.4 ± 4$^{*\alpha\beta\Omega¥}$
PK	197 ± 7	91.2 ± 3*	142 ± 6$^{*@}$	172 ± 7$^{*@\alpha}$	112 ± 5$^{*@\alpha\beta}$	119 ± 5$^{*@\alpha\beta}$	110 ± 6$^{*@\alpha\beta}$
LDH	785 ± 23	478 ± 26*	598 ± 24$^{*@}$	709 ± 27$^{*@\alpha}$	642 ± 27$^{*@\beta}$	637 ± 29$^{*@\alpha\beta}$	643 ± 26$^{*@\alpha\beta}$
PFK	378 ± 22	107 ± 5*	323 ± 11$^{*@}$	364 ± 9$^{@\alpha}$	262 ± 8$^{*@\alpha\beta}$	207 ± 6$^{*@\alpha\beta\Omega}$	189 ± 7$^{*@\alpha\beta\Omega¥}$
Liver Krebs cycle enzymes activities (U per mg protein)							
IDH	3.78 ± 0.1	1.7 ± 0.08*	2.98 ± 0.1$^{*@}$	3.18 ± 0.1$^{*@\alpha}$	2.7 ± 0.1$^{*@\alpha\beta}$	2.89 ± 0.17$^{*@\alpha\beta}$	2.3 ± 0.09$^{*@\alpha\beta¥}$
SDH	2.87 ± 0.2	1.43 ± 0.1*	2 ± 0.23$^{@}$	2.4 ± 0.17$^{*@\alpha}$	2.1 ± 0.15$^{*@\beta}$	1.94 ± 0.16$^{*@\beta}$	1.7 ± 0.13$^{*@\alpha\beta\Omega}$
MDH	1.89 ± 0.2	0.4 ± 0.08*	1.3 ± 0.2$^{*@}$	1.6 ± 0.02$^{*@}$	1.2 ± 0.14$^{*@\beta}$	0.74 ± 0.1$^{*@\alpha\beta\Omega}$	0.6 ± 0.05$^{*@\alpha\beta\Omega}$

[a] Abbreviations: glucose-6-phosphate dehydrogenase (G6PDH), hexokinase (HK), phosphofructokinase (PFK), pyruvate kinase (PK), glucose-6-phosphatase (G6P), fructose-1,6-bisphosphatase (FBP), lactate dehydrogenase (LDH), phosphoenolpyruvate carboxykinase (PEPCK), isocitrate dehydrogenase (IDH), succinate dehydrogenase (SDH), and malate dehydrogenase (MDH).

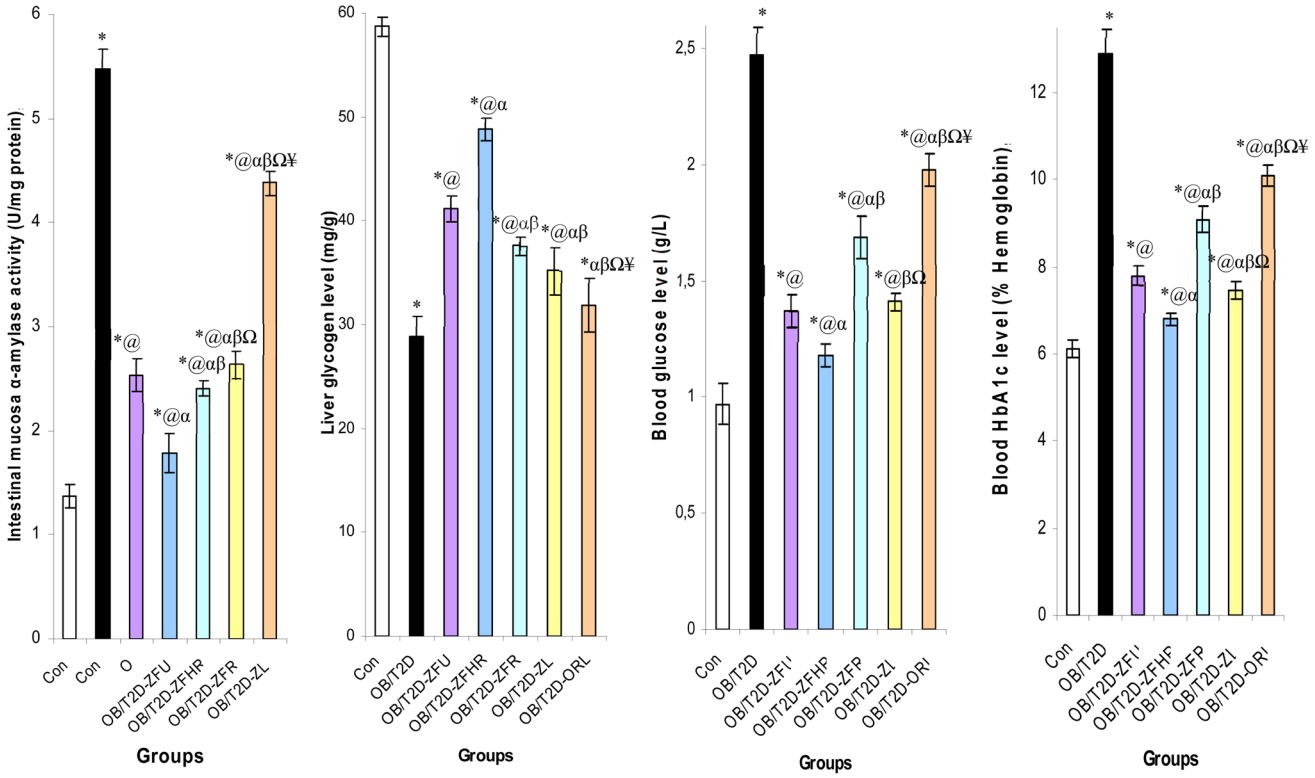

Fig. 4 Effect of OB/T2D and treatment with ZFU, ZFHR, ZFR, and ZL on intestinal α-amylase activity, liver glycogen content, and plasma glucose and HbA1c levels. Supplementation with ZFU, ZFHR, ZFR, and ZL protects against OB/T2D by inhibiting α-amylase activity, improving insulin sensitivity as indicated by increased liver glycogen levels, and reducing plasma glucose and HbA1c concentrations. The most potent effects were observed in rats treated with ZFU and ZFHR. Statistical analysis was performed as described in Fig. 1.

amylase activity, decreased liver glycogen content, and elevated blood glucose and HbA1c levels. Oral administration of ZFU, ZFHR, ZFR, and ZL resulted in significant suppression of α-amylase activity by 53, 67, 56, and 51%, respectively. Supplementation with these extracts also restored liver glycogen levels by 42, 68, 30 and 21% respectively, and further reduced blood glucose levels by 44, 52, 31, and 43%, as well as HbA1c percentages by 39, 47, 38, and 42%, respectively, compared to untreated obese rats. The strongest effect was observed in ZFHR-treated obese rats, which exhibited a 69% increase in liver glycogen content (Fig. 4).

4. Discussion

Our DAD-HPLC phenolic analysis of ZSC fruit extracts at different maturation stages and leaves identified 13 phenolic compounds, including 8 dominant phenolic acids and 5 polyphenols. Unripe fruits (ZFU) contain 69% phenolic acids and 31% polyphenols, with gallic acid and salicylic acid (10% each) being the most abundant, followed by cinnamic acid, 4-hydroxybenzoic acid, and *p*-coumaric acid (~7%), while other acids, including 1,2-dihydroxybenzene, vanillic acid, caffeic acid, syringic acid, and *trans*-ferulic acid, are present at ~5%. Polyphenols such as rutin (13%), catechin (9%), and quercetin (7%) are also detected. Phenolic acid levels decrease (58.8%) in half-ripe ZFHR and 50.6% in ripe (ZFR), while polyphenols increase (41.2% and 49.4%, respectively). Maturation is marked by declining levels of gallic, syringic, *p*-coumaric, and salicylic acids, stable concentrations of 4-hydroxybenzoic, caffeic, and *trans*-ferulic acids, and rising catechin, quercetin, and 1,2-dihydroxybenzene. In ripe fruits, phenolic acids account for 44%, with *p*-coumaric, *trans*-ferulic, salicylic, and cinnamic acids (~6% each), while polyphenols represent 46%. Leaves (ZL) contain 9 key phenolic compounds, notably *trans*-catechin (26.3%), rutin (21.3%), ferulic acid (20.8%), and syringic acid (9.16%). These results are qualitatively consistent with the study by Aldhanhani *et al.*,[10] which identified 13 phenolic compounds in ZSC fruits. Quantitatively, their findings partially align with ours. Specifically, Aldhanhani *et al.*[10] reported that fruit maturation is associated with an increase in catechin and quercetin levels, while rutin levels also rise. Additionally, their study highlighted an overall increase in most phenolic acids during maturation.

In obese rats, our results showed that the oral administration of ZFU, ZFHR, ZFR, and ZL decreased intestinal lipase activity and consequently led to a reduction in body weight. The most powerful and remarkable activity was observed with ZFHR, while the weakest effect was noted with ZL. The potent anti-obesity effect of ZFHR is probably due to the presence of polyphenolic compounds that are abundant at this stage of fruit maturation, such as catechin, quercetin, as well as acids as gallic acid and 1,2-dihydroxybenzene. Our results align with

subsequent studies[56–58] that have demonstrated the effectiveness of catechin and quercetin in obesity prevention and body weight reduction. Moreover, gallic acid and 1,2-dihydroxybenzene have been characterized by anti-obesity activity,[59,60] which may be attributed to, on one hand, the inhibition of lipase activity and, on the other hand, the activation of lipid metabolism. In addition, gallic acid has been associated with a 6% reduction in body weight in obese rats, according to previous studies.[61,62] This therapeutic effect may occur either directly by inhibiting lipid accumulation in adipose tissue and the liver or indirectly by reducing intestinal lipase activity. According to the study by Hsu *et al.*,[63] the administration of rutin and coumaric acid to HFD-fed rats for eight weeks increased organ weights and suppressed epididymal adipose tissue weight.

On the other hand, our study is the first to highlight the effects of oral administration of ZFU, ZFHR, ZFR, and ZL on obesity-induced lymphocyte infiltration and pancreatic inflammation, also known as OB/T2D. The administration of these phenolic fractions from ZSC fruits at different maturation stages, as well as from its leaves, provides remarkable protection of pancreatic function in obese rats. This is evidenced by a significant reduction in lymphocyte infiltration and a protective effect against the loss of pancreatic β-cell biomass. All four extracts ZFU, ZFHR, ZFR, and ZL exert a protective effect on pancreatic architecture by suppressing lymphocyte infiltration, preserving the number and biomass of β-cells in the islets of Langerhans, and significantly reducing pancreatic MPO activity, an indicator of lymphocyte infiltration. The strongest protective activity was observed with ZFHR, followed by ZFU, ZFR, and ZL. These protective effects on pancreatic function and inflammation are also attributed to the ability of these fractions to inhibit key pancreatic inflammatory enzymes such as PLA2, ELA, and PGS, thereby safeguarding β-cells from damage and death. These protective effects of the pancreas against lymphocytic infiltration, inflammation, and the preservation of β-cell biomass in the islets of Langerhans are likely due to the diversity of phenolic compounds and acids present in the four fractions, particularly in the immature fruits of ZFU and the ZFHR. These two fractions exhibit the most potent protective effects, probably due to their high content of bioactive compounds with anti-inflammatory properties. Indeed, the major polyphenols found in the half-ripe fruits of ZFHR, such as catechin and quercetin, as well as the high rutin content in the immature fruits ZFU, are well known for their anti-inflammatory activities. These findings are consistent with previous studies, including that of Shen *et al.*,[64] who reported that quercetin exerts powerful anti-inflammatory effects, mainly by inhibiting cytokine production and reducing the expression of cyclooxygenase and lipoxygenase. Furthermore, Okoko *et al.*[65] demonstrated that quercetin treatment at a dose of 25 mg kg^{-1} in diabetic rats reduced the production of prostaglandin E-2, interleukin-1β, and leukotriene B-4, thereby promoting wound healing. Another study by Li *et al.*[66] also highlighted the anti-lymphocytic infiltration activity of quercetin and its protective effects against inflammatory and oxidative damage in cells and tissues. Additionally, the polyphenolic compounds catechin and rutin, which are present in high concentrations in ZFHR and ZFU, are characterized by their strong anti-inflammatory activity as well as their potent anti-lymphocyte infiltration effects.[67,68] Also, the phenolic acids, which are present in high concentrations in the four fractions, are compounds known for their anti-inflammatory activities, such as gallic acid,[69] coumaric acid,[70] salicylic acid,[71] cinnamic acid,[72] and vanillic acid.[73] This powerful anti-lymphocyte infiltration and, consequently, anti-inflammatory activity at the pancreatic level in obese rats, induced by the different fractions of ZSC fruits at different maturation stages as well as the leaves, with an advantage for ZFHR and ZFU, results in a reduction of the recruitment and accumulation of immune cells, such as neutrophils and lymphocytes, in the pancreas. These immune cells, which are ROS producers, lead to a reduction in ROS in this organ, as evidenced by a significant increase in net TAS, alongside a parallel reduction in H_2O_2, TOS, and TBARS levels in the pancreas. This corresponds to significant protection of the integrity and functionality of the pancreas.

This preservation of pancreatic function in OB/T2D rats by ZFU, ZFHR, ZFR, and ZL is metabolically manifested through the regulation of hepatic GP activity, induction of GS, and modulation of hepatic glycogen levels, providing protection against elevated blood glucose levels. No subsequent studies have linked this effect to our plant, *Ziziphus spina-christi*, but this effect could be attributed to the protective actions of these fractions on the pancreas against inflammation and oxidative stress, as well as the preservation of βcells in the pancreas against damage and dysfunction. Consequently, this leads to better regulation of circulating metabolic hormones such as insulin, which helps regulate blood glucose levels.

Even more interestingly, this study shows, for the first time, that the oral administration of ZFU, ZFHR, ZFR, and ZL induces and promotes glucose catabolism while inhibiting glucose anabolism in the liver of obese rats. These metabolic effects are observed through the inhibition of hepatic glucose biosynthesis enzymes such as G6PDH, G6P, FBP, and PEPCK, while inducing the activity of glucose catabolism enzymes like HK, PK, LDH, and PFK. Additionally, these extracts stimulate the activity of glucose catabolism enzymes in the Krebs cycle, such as IDH, SDH, and MDH. No further study has explored the effects of our plant on these metabolic enzymes. These interesting and therapeutic metabolic effects of ZFU, ZFHR, ZFR, and ZL may be attributed to several mechanisms, including the prevention of obesity by inhibiting lipid absorption and accumulation in the body through inhibition of intestinal lipase and induction of lipid catabolism, protection of the pancreas against inflammation and dysfunction, and increased circulating catabolic hormones such as insulin. Furthermore, the induction of insulin signaling is observed through the suppression of DPP-4 and PTP1B.

These preventive and beneficial effects of ZFU, ZFHR, ZFR, and ZL on OB/T2D are also highlighted by the ability of these fractions to inhibit key digestive enzymes responsible for hyperglycemia and T2D, such as α-amylase, as well as the significant reduction in blood glucose levels and HbA1c. The HbA1c level, a key biomarker of long-term glycemic control, is

an indicator of diabetes management and associated complications. In this study, the reduction in HbA1c levels in rats treated with these fractions approaches the levels observed in normal rats, emphasizing the effectiveness of ZFU, ZFHR, ZFR, and ZL in managing type 2 diabetes and obesity.

Although this study provides promising results regarding the therapeutic potential of ZSC fruits and leaves at different stages of maturation linked to changes in phenolic compound profiles for the management of OB/T2D, with particularly notable effects observed for the semi-ripe fruits, several limitations must be acknowledged. First, the findings are based on an animal model, which limits the direct extrapolation of results to humans. Second, the mechanisms of action were primarily evaluated using biochemical and enzymatic markers, without in-depth validation at the molecular or cellular levels. Further studies, including clinical trials and mechanistic investigations, are necessary to confirm and expand upon these findings.

5. Conclusion

This study demonstrates the phenolic profile changes of ZSC fruits at different stages of maturation, analyzed through DAD-HPLC, revealing distinct bioactive compounds. The results showed an increase in polyphenolic compounds and a decrease in total phenolic acids as the fruit matured. The consumption of fruits at different maturation stages and the leaves of ZSC exhibited significant anti-lipase and anti-obesity effects, along with protective actions against pancreatic inflammation, preventing lymphocytic infiltration in obese rats. Moreover, these extracts displayed notable antioxidant and anti-inflammatory activities, with the most pronounced effects observed in semi-mature fruits. Additionally, administration of the fruit and leaf fractions enhanced glucose metabolism in obese rats with type 2 diabetes (T2D). The extracts were shown to modulate key enzymes involved in glucose metabolism and insulin signaling, while lowering blood glucose and HbA1c levels. These results suggest that ZSC semi-ripe fruit extract may serve as a promising candidate for as a natural therapeutic agent for managing obesity, T2D, and related metabolic disorders. Additional clinical studies are needed to clarify its underlying mechanisms and evaluate its therapeutic applicability in humans.

Abbreviations

ZSC	*Ziziphus spina-christi*
ZFU	*Ziziphus* fruit unripe
ZFHR	*Ziziphus* fruit half-ripe
ZFR	*Ziziphus* fruit ripe
ZL	*Ziziphus* leaves
OB/T2D	Obesity and type 2 diabetes
MPO	Myeloperoxidase
PLA2	Phospholipase A2
ELA	Elastase
PGS	Prostaglandin synthase
TBARS	Thiobarbituric acid reactive substances
H_2O_2	Hydrogen peroxide
TOS	Total oxidant status
TAS	Total antioxidant status
HK	Hexokinase
PK	Pyruvate kinase
LDH	Lactate dehydrogenase
PFK	Phosphofructokinase
HbA1c	Hemoglobin A1c
DAD-HPLC	Diode array detector-high performance liquid chromatography

Ethical statement

All procedures complied with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) and were approved under ethical approval numbers CEEA-EMMV 24/25 and CCR-SMS 0013/20222020-0205 (01/2024).

Data availability

The corresponding author can provide the data used to support the study's conclusions upon request.

Author contributions

Conceptualization, A. A. A., N. A. A., S. A.; methodology, A. A. A., N. A. A., S. A.; analysis, A. A. A., N. A. A.; investigation A. A. A., N. A. A., S. A.; data analysis, A. A. A., N. A. A., S. A.; resources, A. A. A., N. A. A., S. A.; writing – original draft preparation, A. A. A.; writing – review and editing, S.A.; funding acquisition, A. A. A. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts of interest.

Acknowledgements

This research has been funded by Scientific Research Deanship at University of Ha'il – Saudi Arabia through project number "BA-24 021".

References

1 M. Tiss, Z. Souiy, N. ben Abdeljelil, M. Njima, L. Achour and K. Hamden, *J. Funct. Foods*, 2020, **67**, 103869.
2 T. Mohamed, Z. Souiy, L. Achour and K. Hamden, *Arch. Physiol. Biochem.*, 2022, **128**, 1453–1460.
3 F. Saad, T. M. Al-Shaikh, F. Zouidi, M. A. Taher, S. A. Saidi and K. Hamden, *J. Food Process. Preserv.*, 2023, **2023**, 9522830.
4 S. Abdelkader Saidi, T. M. Al-Shaikh and K. Hamden, *J. Food Qual*, 2023, 1–9.
5 W. I. El-Sofany, A. F. Alshammari, M. Z. Alshammari, H. K. Alshammari, N. S. Alshammari and K. Hamden, *J. Agric. Food Res.*, 2025, 101955.

6 D. Dallali, J. Fakhfakh, C. Paris, K. Hamden, M. Varbanov and N. Allouche, *Bioorg. Chem.*, 2024, 107100.
7 F. I. A. Abdella, A. Toumi, S. Boudriga, T. Y. A. Alanazi, A. K. Alshamari, A. A. Alrashdi and K. Hamden, *Heliyon*, 2024, e27598.
8 W. I. El-Sofany, T. D. Alanezi, S. Latif, O. Abdelhedi and K. Hamden, *Enzyme Microb. Technol.*, 2025, 110639.
9 W. I. El-Sofany, A. F. Alshammari, M. Z. Alshammari, H. K. Alshammari, N. S. Alshammari and K. Hamden, *Food Biosci.*, 2025, 106569.
10 A. R. H. Aldhanhani, Z. F. R. Ahmed, N. Tzortzakis and Z. Singh, *Ann. Agric. Sci.*, 2022, **67**, 196–203.
11 S. Pareek and E. M. Yahia, in *Horticultural Reviews*, vol. 41, 2013, pp. 201–240.
12 J. Reche, M. S. Almansa, F. Hernández, A. Amorós and P. Legua, *Agronomy*, 2021, **11**, 132.
13 Y. Zhao, X. Zhu, Y. Hou, Y. Pan, L. Shi and X. Li, *Sci. Hortic.*, 2021, **277**, 109778.
14 Y. Yahia, M. A. Benabderrahim, N. Tlili, M. Bagues and K. Nagaz, *PLoS One*, 2020, **15**, e0232599.
15 N. S. El-Gazzar, R. S. Ibrahim, D. A. Ghareeb, A. S. Abdel-Hamid and A. A. El-Banna, *S. Afr. J. Bot.*, 2024, **174**, 963–977.
16 S. S. Elhady, M. S. Goda, E. T. Mehanna, N. M. El-Sayed, R. M. Hazem, M. A. Elfaky, A. J. Almalki, M. S. Mohamed and R. F. A. Abdelhameed, *Biomed. Pharmacother.*, 2024, **176**, 116823.
17 E. S. Saheed, M. A. Abdulkareem and F. C. Adeleke, *World J. Tradit. Chin. Med.*, 2025, **11**(2), 2019–2024.
18 G. A. Gregory, T. I. G. Robinson, S. E. Linklater, F. Wang, S. Colagiuri, C. de Beaufort, K. C. Donaghue, J. L. Harding, P. L. Wander and X. Zhang, *Lancet Diabetes Endocrinol.*, 2022, **10**, 741–760.
19 M. Tiss, Z. Souiy, N. ben Abdeljelil, M. Njima, L. Achour and K. Hamden, *J. Funct. Foods*, 2020, **67**, 103869.
20 M. Tiss and K. Hamden, *J. Evidence-Based Complement. Altern. Med.*, 2022, **2022**, 6816942.
21 M. Figorilli, F. Velluzzi and S. Redolfi, *Nutr. Metab. Cardiovasc. Dis.*, 2025, 104014.
22 S. M. Aghaei and S. M. Hosseini, *Cytokine*, 2024, **182**, 156724.
23 Y. Alimi and D. E. Azagury, *Clin. Gastroenterol.*, 2021, **50**, 859–870.
24 S. Kadri, M. El Ayed, F. Limam, E. Aouani and M. Mokni, *Microvasc. Res.*, 2020, **132**, 104054.
25 M. Mahmoudi, K. Charradi, F. Limam and E. Aouani, *Obes. Res. Clin. Pract.*, 2018, **12**, 115–126.
26 L. Cross, *Am. J. Heal. Pharm.*, 2025, **82**, 48–59.
27 O. Topaloglu and I. Sahin, *Med-Science*, 2021, **10**, 651–657.
28 I. Bahrini, M. Alamoudi, M. Alazami, J. Alrashdi and N. Alouche, *J. Agric. Food Res.*, 2025, 101951.
29 M. S. O. Alhar, W. I. El-Sofany, A. A. AlRashidi and K. Hamden, *J. Diabetes Res.*, 2025, **2025**, 8050374.
30 I. Bahrini, M. Alamoudi, J. Alrashdi, M. Alazami, K. Mnafgui, L. Achour and K. Hamden, *J. Food Sci. Technol.*, 2025, **21**, 1–13.
31 F. I. A. Abdella, T. Y. A. Alanazia, M. A. Alreshidi, H. D. A. Al-Enazy, H. K. Alshammari, N. Alouche and K. Hamden, *Food Biosci.*, 2025, **71**, 107122.
32 F. I. A. Abdella, T. Y. A. Alanazia, M. A. Alreshidi, H. D. A. Al-Enazy, H. K. Alshammari and K. Hamden, *Biomed. Pharmacother.*, 2025, **189**, 118277.
33 K. Hamden, N. Allouche, M. Damak and A. Elfeki, *Chem. Biol. Interact.*, 2009, **180**, 421–432.
34 S. M. Abubakar, S. A. Umar, I. Alexander, N. Abubakar, M. A. Abdulazeez and M. S. Sule, *J. Drug Deliv. Ther.*, 2018, **8**, 82–92.
35 C. G. Michel, D. I. Nesseem and M. F. Ismail, *J. Ethnopharmacol.*, 2011, **133**, 53–62.
36 Z. A. Othman, Z. Zakaria, J. B. Suleiman, K. M. F. Mustaffa, N. A. C. Jalil, W. S. Wan Ghazali, N. N. Zulkipli and M. Mohamed, *Int. J. Mol. Sci.*, 2022, **23**, 10266.
37 R. F. Queiroz, S. M. Vaz and O. Augusto, *Biochem. J.*, 2011, **439**, 423–434.
38 S. Chandra, P. Chatterjee, P. Dey and S. Bhattacharya, *Asian Pac. J. Trop. Biomed.*, 2012, **2**, S178–S180.
39 A. George, S. Chinnappan, M. Chintamaneni, V. Kotak C, Y. Choudhary, T. Kueper and A. K. Radhakrishnan, *BMC Complement. Altern. Med.*, 2014, **14**, 1–7.
40 J. P. N. Ribeiro, M. A. Segundo, S. Reis and J. L. F. C. Lima, *Talanta*, 2009, **79**, 1169–1176.
41 O. Erel, *Clin. Biochem.*, 2005, **38**, 1103–1111.
42 O. Erel, *Clin. Biochem.*, 2004, **37**, 112–119.
43 J. A. Buege and S. D. Aust, in *Methods in Enzymology*, Elsevier, 1978, vol. 52, pp. 302–310.
44 M. M. Bradford, *Anal. Biochem.*, 1976, **72**, 248–254.
45 N. Brandstrup, J. E. Kirk and C. Bruni, *J. Gerontol.*, 1957, **12**, 166–171.
46 C. I. Pogson and R. M. Denton, *Nature*, 1967, **216**, 156–157.
47 K. Hikaru and O. Toshitsugu, *Clin. Chim. Acta*, 1959, **4**, 554–561.
48 J. M. Gancedo and C. Gancedo, *Arch. Mikrobiol.*, 1971, **76**, 132–138.
49 L. F. Leloir and S. H. Goldemberg, in *Methods in Enzymology*, Elsevier, 1962, vol. 5, pp. 145–147.
50 J. G. Castano, A. Nieto and J. E. Feliu, *J. Biol. Chem.*, 1979, **254**, 5576–5579.
51 T. P. Mommsen, P. J. Walsh and T. W. Moon, *Mol. Physiol.*, 1985, **8**, 89–99.
52 M. J. Duncan and D. G. Fraenkel, *J. Bacteriol.*, 1979, **137**, 415–419.
53 C. Veeger, D. V DerVartanian and W. P. Zeylemaker, in *Methods in Enzymology*, Elsevier, 1969, vol. 13, pp. 81–90.
54 C. E. Shonk and G. E. Boxer, *Cancer Res.*, 1964, **24**, 709–721.
55 K. C. Ong and H.-E. Khoo, *Life Sci.*, 2000, **67**, 1695–1705.
56 V. Patial, S. Katoch, J. Chhimwal, G. Dadhich, V. Sharma, A. Rana, R. Joshi and Y. Padwad, *Life Sci.*, 2023, **316**, 121437.
57 I. Kazmi, S. Sarim Imam, F. A. Al-Abbasi, M. Afzal, M. Shahid Nadeem and S. Alshehri, *Int. J. Pharm.*, 2022, **628**, 122340.
58 H. Zeng, L. Peng, C. Liu, Y. Zhou, L. Wan, S. Wen, W. Fang, X. Yang, Q. Bai, J. Zeng, J. Huang, S. Zhang and Z. Liu, *Food Chem.*, 2025, **464**, 141785.
59 P. K. Behera, S. Devi and N. Mittal, *Obes. Med.*, 2023, **37**, 100473.
60 E. Kato, R. Nakagomi, M. D. P. T. Gunawan-Puteri and J. Kawabata, *Food Chem.*, 2013, **136**, 1239–1242.

61 J. N. Sousa, A. F. Paraíso, J. M. O. Andrade, D. F. Lelis, E. M. Santos, J. P. Lima, R. S. Monteiro-Junior, M. F. S. V. D'Angelo, A. M. B. de Paula and A. L. S. Guimarães, *Exp. Gerontol.*, 2020, **134**, 110881.
62 M. N. Muhd Rodhi, K. H. Ku Hamid, F. Hamzah, N. S. Kamarul Bahari and N. A. Abdul Rahman, *Malays. J. Chem. Eng. Technol.*, 2020, **3**, 1–10.
63 C.-L. Hsu, C.-H. Wu, S.-L. Huang and G.-C. Yen, *J. Agric. Food Chem.*, 2009, **57**, 425–431.
64 P. Shen, W. Lin, X. Deng, X. Ba, L. Han, Z. Chen, K. Qin, Y. Huang and S. Tu, *Front. Immunol.*, 2021, **12**, 689044.
65 T. Okoko and I. F. Oruambo, *Food Chem. Toxicol.*, 2009, **47**, 809–812.
66 J. Li, Z. Sun, G. Luo, S. Wang, H. Cui, Z. Yao, H. Xiong, Y. He, Y. Qian and C. Fan, *Front. Immunol.*, 2021, **12**, 649285.
67 R. Yousuf, P. K. Verma, P. Sharma, S. Sood and Z. F. Bhat, *Food Chem. Adv.*, 2023, **3**, 100380.
68 A. Sharma, N. V. Tirpude, M. Kumari and Y. Padwad, *Food Funct.*, 2021, **12**, 8492–8506.
69 S. G. Tsiogkas, K. Apostolopoulou, A. Mavropoulos, M. G. Grammatikopoulou, E. Dardiotis, E. Zafiriou and D. P. Bogdanos, *Immunol. Res.*, 2023, **71**, 475–487.
70 S. S. Bal, G. D. Leishangthem, R. S. Sethi and A. Singh, *Pestic. Biochem. Physiol.*, 2022, **180**, 104997.
71 J. K. N. Thuo, P. K. Towett, T. I. Kanui and K. S. P. Abelson, *Heliyon*, 2022, **8**(2), 1–7.
72 J.-G. Jiang, Q. Luo, S.-S. Li, T.-Y. Tan, K. Xiong, T. Yang and T.-B. Xiao, *World J. Gastrointest. Pharmacol. Ther.*, 2023, **14**, 4.
73 E. S. Alamri and H. A. El Rabey, *J. Diabetes Res.*, 2024, **2024**, 4873544.