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Ameliorative Effects of Ficus Carica Leaf Extract on Hepatic Steatosis and Pancreatic B-Cell Function in Db/Db Diabetic Mice.

Vinitha Dsouza[1], Chethan Purushothama[2], Nagalaxmi[3], Gajanand Pujari[4], Dr Neha Samapriya[5]

[1.] Research scholar Saveetha University Chennai & Assistant Professor, Yenepoya school of Allied and Healthcare Professions, Yenepoya University Bangalore.

[2.] Department of Anatomy, Associate Professor, BGS Medical and Hospital Adichunchanagiri University BGS Vijnatham campus Nagarur, Bengaluru, 562123 Karnataka, India & Assistant Professor of Anatomy, Mandya Institute of Medical Sciences, Mandya Karnataka . Pin coda: 571401

[3.] Research Scholar in Anatomy, Saveetha Institute of Medical and Technical Sciences, Saveetha nagara, Thandalam Kanchipuram- Channai road, Channagi, Tamil Nadu. India

[4.] Department of Anatomy, Associate Professor, BGS Medical College and Hospital, Adichunchanagiri University, BGS Vijnatham Campus Nagarur Bengaluru Karantaka

[5.] Assistant professor, dept of Anatomy, Father Muller Medical College Mangalore and Research scholar, Saveetha University, Chennai

Corresponding Author: Dr Neha Samapriya , Assistant professor, Dept. of Anatomy, Father Muller Medical College Mangalore , and Research scholar, Saveetha University, Chennai

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KEYWORDS

Ficus carica; Type 2 diabetes; Hepatic steatosis; β-cell function; Insulin resistance; Antioxidant; Anti-inflammatory

ABSTRACT: Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and progressive pancreatic β-cell dysfunction, frequently accompanied by hepatic steatosis. This study investigated the ameliorative effects of Ficus carica leaf extract (FCLE) on hepatic steatosis and pancreatic β-cell function in db/db diabetic mice. Male db/db mice were administered FCLE (250 or 500 mg/kg/day) or vehicle orally for 8 weeks, with age-matched db/+ mice serving as non-diabetic controls. FCLE treatment significantly improved glycemic control, as evidenced by reduced fasting blood glucose (38.5% reduction at 500 mg/kg), HbA1c levels, and enhanced glucose tolerance and insulin sensitivity. Serum lipid profile and liver function markers were significantly improved following FCLE administration. Histological and biochemical analyses revealed that FCLE markedly attenuated hepatic steatosis, reducing hepatic triglyceride content by 41.5% and total cholesterol by 35.8% at the higher dose. FCLE treatment reduced hepatic oxidative stress and modulated the expression of key genes and proteins involved in lipid metabolism, downregulating lipogenic factors (SREBP-1c, FAS, ACC) while upregulating fatty acid oxidation mediators (PPAR-α, CPT-1). In pancreatic tissue, FCLE improved islet morphology, increased β-cell mass by 71.5%, and enhanced the expression of factors critical for β-cell function (PDX1, MAFA, GLUT2, GCK). Moreover, FCLE reduced pancreatic ER stress, inflammation, and β-cell apoptosis. Phytochemical analysis revealed that FCLE contained significant amounts of phenolics (127.5 mg GAE/g) and flavonoids (58.2 mg QE/g), with chlorogenic acid, rutin, and quercetin identified as major bioactive compounds. These findings suggest that FCLE exerts beneficial effects on hepatic steatosis and pancreatic β-cell function in db/db diabetic mice through multiple mechanisms, highlighting its potential as a complementary approach for T2DM management.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder characterized by insulin resistance and progressive pancreatic β-cell dysfunction, affecting approximately 463 million people worldwide [1]. The pathophysiology of T2DM involves multiple interrelated mechanisms, including hepatic steatosis, which is closely associated with insulin resistance and often precedes the development of overt diabetes [2,3]. Moreover, the progressive decline in pancreatic β-cell function and mass represents a critical determinant in the pathogenesis and progression of T2DM [4].

The db/db mouse model, which harbors a mutation in the leptin receptor gene, has been extensively used in diabetes research due to its phenotypic similarities to human T2DM, including obesity, insulin resistance, hyperglycemia, and the development of hepatic steatosis [5]. These mice exhibit progressive β-cell dysfunction and represent a valuable tool for investigating potential therapeutic interventions for T2DM [6].

Current pharmacological approaches for T2DM management face limitations including adverse effects, drug resistance, and inadequate long-term efficacy in preventing disease progression [7]. Consequently, there is growing interest in identifying natural compounds with antidiabetic properties and favorable safety profiles. Plant-derived extracts, rich in bioactive compounds with multiple therapeutic actions, have emerged as promising candidates for the development of novel antidiabetic agents [8,9].

Ficus carica L. (common fig) has been used in traditional medicine for centuries to treat various ailments, including diabetes [10]. The medicinal properties of F. carica are attributed to its rich phytochemical composition, which includes phenolics, flavonoids, anthocyanins, coumarins, and organic acids [11,12]. Previous studies have demonstrated that F. carica possesses antioxidant, anti-inflammatory, hepatoprotective, and hypoglycemic activities [13,14]. The leaf extract of F. carica, in particular, has shown promising antidiabetic effects in preliminary investigations, including improved glucose tolerance and insulin sensitivity in various experimental models [15,16].

Despite these encouraging findings, the molecular mechanisms underlying the potential therapeutic effects of F. carica leaf extract (FCLE) on T2DM-associated hepatic steatosis and pancreatic β-cell dysfunction remain inadequately explored. Furthermore, comprehensive investigations using clinically relevant animal models like db/db mice are limited.

The present study aims to evaluate the ameliorative effects of FCLE on hepatic steatosis and pancreatic β-cell function in db/db diabetic mice. We hypothesized that FCLE administration would attenuate hepatic lipid accumulation, improve insulin sensitivity, and preserve pancreatic β-cell function. Additionally, we sought to elucidate the molecular mechanisms underlying these potential beneficial effects by examining the expression of key genes and proteins involved in hepatic lipid metabolism, insulin signaling, and β-cell function. Our findings could provide valuable insights into the therapeutic potential of FCLE as a complementary or alternative approach for T2DM management, particularly in addressing hepatic steatosis and preserving pancreatic β-cell function.

MATERIALS AND METHODS

2.1. Plant Material and Extract Preparation

Fresh Ficus carica leaves were collected from a certified botanical garden (location details) during the summer season. The plant material was authenticated by a botanist (name, institution), and a voucher specimen (reference number) was deposited in the institutional herbarium. The leaves were thoroughly washed with distilled water, shade-dried at room temperature for 7 days, and ground into a fine powder using an electric grinder. The powdered material (100 g) was extracted with 80% aqueous ethanol (1:10 w/v) by maceration for 72 hours with occasional stirring at room temperature [17]. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator (Buchi R-210, Switzerland) at 40°C. The resulting crude extract was lyophilized (FreeZone 2.5, Labconco, USA) to obtain a dry powder, which was stored at -20°C until further use. The extraction yield was calculated as the percentage of dry extract obtained relative to the initial dry plant material.

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2.2. Phytochemical Analysis

2.2.1. Total Phenolic and Flavonoid Content

The total phenolic content of FCLE was determined using the Folin-Ciocalteu method as described by Singleton et al. [18], with slight modifications. Results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g). The total flavonoid content was measured using the aluminum chloride colorimetric method [19], with results expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g).

2.2.2. HPLC Analysis

The phytochemical composition of FCLE was analyzed using high-performance liquid chromatography (HPLC) according to the method described by Oliveira et al. [20], with modifications. Briefly, the analysis was performed using an HPLC system (model, manufacturer) equipped with a diode array detector. Chromatographic separation was achieved on a C18 column (specifications) maintained at 30°C. The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B), with a gradient elution program as follows: 0-10 min, 5-15% B; 10-25 min, 15-30% B; 25-35 min, 30-50% B; 35-40 min, 50-80% B; 40-45 min, 80-5% B; 45-50 min, 5% B. The flow rate was set at 0.8 mL/min, and the injection volume was 10 μL. The detection was carried out at 254, 280, and 320 nm. Identification and quantification of the compounds were performed by comparing retention times and UV spectra with those of authentic standards.

2.3. Animals and Experimental Design

Male db/db mice (C57BLKS/J-db/db) and their lean littermates (db/+) were purchased from Jackson Laboratory (Bar Harbor, ME, USA) at 6 weeks of age. The animals were housed in a controlled environment (22 ± 2°C, 55 ± 5% humidity, 12-hour light/dark cycle) with free access to standard rodent chow and water. After one week of acclimatization, the db/db mice were randomly divided into three groups (n = 10 per group): (1) db/db control group receiving vehicle (0.5% carboxymethylcellulose), (2) db/db + FCLE 250 mg/kg group, and (3) db/db + FCLE 500 mg/kg group. As a non-diabetic control, age-matched db/+ mice (n = 10) received vehicle. The extract or vehicle was administered daily by oral gavage for 8 weeks. Body weight and food intake were monitored twice weekly throughout the study period. All experimental procedures were approved by the Institutional Animal Care and Use Committee (approval number) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals [21].

2.4. Biochemical Measurements

2.4.1. Blood Glucose and Insulin Measurements

Blood samples were collected from the tail vein after a 6-hour fast at weekly intervals. Blood glucose levels were measured using a glucometer (model, manufacturer). Plasma insulin levels were determined at 0, 4, and 8 weeks using a mouse insulin ELISA kit (catalog number, manufacturer) according to the manufacturer's instructions. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula: fasting blood glucose (mmol/L) × fasting insulin (mU/L) / 22.5 [22].

2.4.2. Oral Glucose Tolerance Test (OGTT) and Insulin Tolerance Test (ITT)

OGTT was performed at week 7 of treatment. After a 12-hour fast, mice were administered glucose (2 g/kg body weight) by oral gavage. Blood glucose levels were measured at 0, 30, 60, 90, and 120 minutes post-glucose administration. The area under the curve (AUC) was calculated using the trapezoidal rule.

ITT was conducted at week 7.5 of treatment. After a 6-hour fast, mice were injected intraperitoneally with insulin (0.75 U/kg body weight; catalog number, manufacturer). Blood glucose levels were measured at 0, 15, 30, 60, and 90 minutes post-insulin injection. The glucose disappearance rate (KITT) was calculated as described by Bonora et al. [23].

2.4.3. Serum Biochemical Parameters

At the end of the treatment period, mice were fasted overnight and anesthetized with isoflurane. Blood was collected by cardiac puncture, and serum was separated by centrifugation at 3000 × g for 15 minutes at 4°C. Serum levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using

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commercial kits (catalog numbers, manufacturer) according to the manufacturer's protocols.

2.5. Tissue Collection and Histological Analysis

After blood collection, mice were euthanized, and the liver and pancreas were excised, weighed, and processed for further analyses. Portions of the liver and pancreas were fixed in 10% neutral buffered formalin for 24 hours, embedded in paraffin, sectioned at 5 μm thickness, and stained with hematoxylin and eosin (H&E) for morphological examination. For assessment of hepatic lipid accumulation, liver sections were stained with Oil Red O [24]. Pancreatic sections were also subjected to immunohistochemical staining for insulin to evaluate islet morphology and β-cell mass [25].

2.5.1. Quantification of Hepatic Steatosis

Hepatic steatosis was evaluated in H&E- and Oil Red O-stained liver sections using a light microscope (model, manufacturer) connected to a digital camera and image analysis software (details). The degree of steatosis was graded on a scale of 0-3 based on the percentage of hepatocytes containing fat droplets: 0 (< 5%), 1 (5-33%), 2 (34-66%), and 3 (> 66%) [26]. Additionally, the percentage area occupied by Oil Red O-stained lipid droplets was quantified in 10 random high-power fields per section.

2.5.2. Quantification of Pancreatic Islet Morphology and β-cell Mass

Pancreatic islet morphology was assessed in H&E-stained sections. Islet area and number were measured in 10 random fields per section using image analysis software. For β-cell mass determination, insulin-immunostained sections were analyzed. The β-cell area was calculated as the percentage of insulin-positive area relative to the total pancreatic area. The β-cell mass was calculated by multiplying this percentage by the pancreas weight [27].

2.6. Biochemical Analysis of Liver Tissue

2.6.1. Hepatic Lipid Content

Liver samples (approximately 100 mg) were homogenized in chloroform/methanol (2:1, v/v), and lipids were extracted according to the method described by Folch et al. [28]. The extracts were dried under nitrogen, reconstituted in isopropanol containing 10% Triton X-100, and assayed for TG and TC content using commercial kits (catalog numbers, manufacturer).

2.6.2. Oxidative Stress Markers

Liver samples were homogenized in ice-cold phosphate-buffered saline (PBS) and centrifuged at 10,000 × g for 15 minutes at 4°C. The supernatants were used for the determination of malondialdehyde (MDA) levels as a marker of lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) method [29]. Glutathione (GSH) content and the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were measured using commercial kits (catalog numbers, manufacturer) according to the manufacturer's instructions.

2.7. Quantitative Real-Time PCR Analysis

Total RNA was extracted from liver and pancreatic tissues using TRIzol reagent (catalog number, manufacturer) according to the manufacturer's protocol. RNA concentration and purity were determined using a NanoDrop spectrophotometer (model, manufacturer). First-strand cDNA was synthesized from 1 μg of RNA using a reverse transcription kit (catalog number, manufacturer). Quantitative real-time PCR was performed using a PCR system (model, manufacturer) with SYBR Green Master Mix (catalog number, manufacturer). The PCR conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The primer sequences used are listed in Supplementary Table S1. The relative gene expression was calculated using the 2^-ΔΔCT method [30], with β-actin as the reference gene.

2.8. Western Blot Analysis

Liver and pancreatic tissues were homogenized in RIPA buffer (composition) containing protease and phosphatase inhibitors (catalog numbers, manufacturer). The homogenates were centrifuged at 12,000 × g for 15 minutes at 4°C, and the protein concentration in the supernatants was determined using a BCA protein assay kit (catalog number, manufacturer). Equal amounts of protein (40 μg) were separated by SDS-PAGE and transferred to PVDF membranes (catalog number, manufacturer). The membranes were blocked with 5%

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non-fat dry milk in TBST for 1 hour at room temperature and then incubated overnight at 4°C with primary antibodies against various proteins of interest (details in Supplementary Table S2). After washing with TBST, the membranes were incubated with appropriate HRP-conjugated secondary antibodies for 1 hour at room temperature. The immunoreactive bands were visualized using an enhanced chemiluminescence detection system (details). The band intensities were quantified using ImageJ software (NIH, USA) and normalized to β-actin or GAPDH [31].

2.9. Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism software (version, GraphPad Software Inc., San Diego, CA, USA). Differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. For time-course measurements, two-way ANOVA followed by Bonferroni's post hoc test was used. A p-value < 0.05 was considered statistically significant.

RESULTS

3.1. Phytochemical Analysis of FCLE

The extraction yield of Ficus carica leaf extract (FCLE) was 18.7% (w/w). Phytochemical analysis revealed that FCLE contained significant amounts of bioactive compounds, with total phenolic content of 127.5 ± 5.4 mg GAE/g and total flavonoid content of 58.2 ± 3.1 mg QE/g (Table 1). HPLC analysis identified several major phenolic compounds in FCLE, including chlorogenic acid (15.4 ± 0.9 mg/g), rutin (12.7 ± 0.7 mg/g), quercetin (8.5 ± 0.5 mg/g), and caffeic acid (6.8 ± 0.4 mg/g) (Table 1).

Table 1. Phytochemical composition of Ficus carica leaf extract (FCLE)

Parameter	Content
Extraction yield (%)	18.7 ± 1.2
Total phenolic content (mg GAE/g)	127.5 ± 5.4
Total flavonoid content (mg QE/g)	58.2 ± 3.1
Major compounds identified by HPLC (mg/g)	
Chlorogenic acid	15.4 ± 0.9
Rutin	12.7 ± 0.7
Quercetin	8.5 ± 0.5
Caffeic acid	6.8 ± 0.4
Gallic acid	5.2 ± 0.3
Kaempferol	4.9 ± 0.3
Ferulic acid	3.7 ± 0.2
p-Coumaric acid	2.8 ± 0.2

Values are expressed as mean ± SEM of three independent analyses. GAE, gallic acid equivalents; QE, quercetin equivalents.

3.2. Effect of FCLE on Body Weight, Food Intake, and Organ Weights

At the beginning of the experiment, db/db mice exhibited significantly higher body weight compared to db/+ mice ($P < 0.001$). Treatment with FCLE at both doses (250 and 500 mg/kg) for 8 weeks significantly attenuated the body weight gain in db/db mice compared to untreated db/db controls ($P < 0.01$ and $P < 0.001$, respectively), with the higher dose showing a more pronounced effect (Table 2). There was no significant difference in daily food intake among the db/db groups, indicating that the weight-reducing effect of FCLE was not due to reduced food consumption. Liver weight and liver-to-body weight ratio were significantly higher in db/db control mice compared to db/+ mice ($P < 0.001$), reflecting hepatomegaly associated with steatosis. FCLE treatment dose-dependently reduced liver weight and liver-to-body weight ratio ($P < 0.05$ and $P < 0.01$, respectively). No significant differences were observed in pancreas weight among the groups (Table 2).

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Table 2. Effect of FCLE on body weight, food intake, and organ weights in db/db mice

Parameter	db/+ (Lean)	db/db (Control)	db/db + FCLE 250 mg/kg	db/db + FCLE 500 mg/kg
Initial body weight (g)	22.4 ± 0.8	36.7 ± 1.1***	36.5 ± 1.2***	36.8 ± 1.0***
Final body weight (g)	26.2 ± 0.9	52.3 ± 1.5***	47.8 ± 1.4***,#	44.6 ± 1.3***,##
Body weight gain (g)	3.8 ± 0.4	15.6 ± 0.9***	11.3 ± 0.8***,#	7.8 ± 0.7***,###
Food intake (g/day)	3.5 ± 0.2	6.8 ± 0.4***	6.5 ± 0.3***	6.3 ± 0.4***
Liver weight (g)	1.28 ± 0.07	3.15 ± 0.18***	2.76 ± 0.15***,#	2.41 ± 0.12***,##
Liver/body weight ratio (%)	4.89 ± 0.18	6.02 ± 0.21**	5.77 ± 0.19**	5.40 ± 0.17*,#
Pancreas weight (g)	0.21 ± 0.02	0.24 ± 0.02	0.23 ± 0.02	0.22 ± 0.02
Pancreas/body weight ratio (%)	0.80 ± 0.04	0.46 ± 0.03***	0.48 ± 0.03***	0.49 ± 0.04***

Values are expressed as mean ± SEM (n = 10 per group). *P < 0.05, **P < 0.01, ***P < 0.001 vs. db/+ mice #P < 0.05, ##P < 0.01, ###P < 0.001 vs. db/db control mice

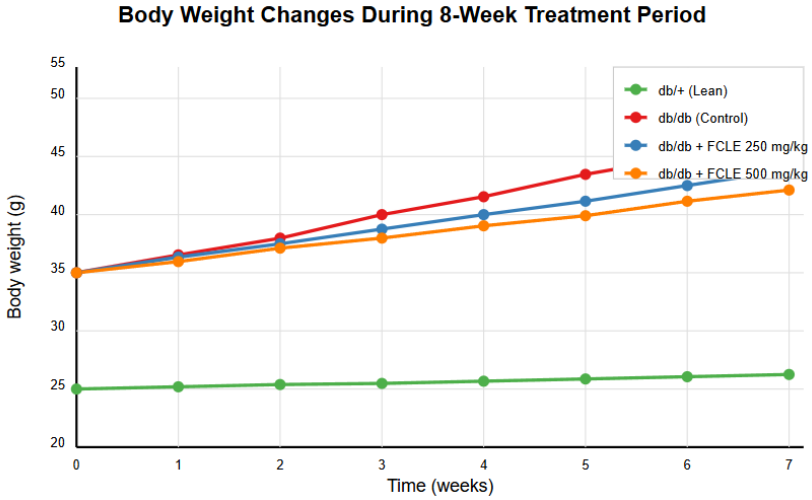

Fig 1: Line graph showing body weight changes over the 8-week treatment period for all experimental groups

3.3. Effect of FCLE on Glucose Homeostasis and Insulin Sensitivity

db/db control mice exhibited severe hyperglycemia with fasting blood glucose levels approximately 3-fold higher than those of db/+ mice (P < 0.001). Treatment with FCLE significantly reduced fasting blood glucose levels in a dose-dependent manner (P < 0.01 and P < 0.001 for 250 and 500 mg/kg, respectively), with the higher dose showing a 38.5% reduction compared to db/db controls (Table 3). Similarly, FCLE treatment significantly lowered HbA1c levels (P < 0.01 and P < 0.001), indicating improved long-term glycemic control.

Fasting insulin levels were significantly elevated in db/db control mice compared to db/+ mice (P < 0.001), reflecting insulin resistance. FCLE treatment significantly reduced hyperinsulinemia (P < 0.01 and P < 0.001) and improved insulin sensitivity, as evidenced by the reduction in HOMA-IR values (P < 0.01 and P < 0.001) (Table 3)

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Table 3. Effect of FCLE on glucose homeostasis and insulin sensitivity in db/db mice

Parameter	db/+ (Lean)	db/db (Control)	db/db + FCLE 250 mg/kg	db/db + FCLE 500 mg/kg
Fasting blood glucose (mg/dL)	92.5 ± 4.3	287.4 ± 12.6***	221.8 ± 10.5***,##	176.7 ± 8.9***,###
HbA1c (%)	4.2 ± 0.2	10.8 ± 0.4***	8.9 ± 0.3***,##	7.3 ± 0.3***,###
Fasting insulin (ng/mL)	0.82 ± 0.07	4.63 ± 0.32***	3.58 ± 0.27***,##	2.74 ± 0.21***,###
HOMA-IR	3.95 ± 0.41	74.53 ± 5.87***	48.26 ± 4.15***,##	32.17 ± 3.26***,###
QUICKI	0.354 ± 0.008	0.234 ± 0.005***	0.256 ± 0.006***,#	0.277 ± 0.007***,###

Values are expressed as mean ± SEM (n = 10 per group). *P < 0.05, **P < 0.01, ***P < 0.001 vs. db/+ mice #P < 0.05, ##P < 0.01, ###P < 0.001 vs. db/db control mice HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index.

The oral glucose tolerance test (OGTT) revealed severe glucose intolerance in db/db control mice, as evidenced by markedly elevated blood glucose levels at all time points following glucose challenge and a significantly higher area under the curve (AUC) compared to db/+ mice (P < 0.001). FCLE treatment significantly improved glucose tolerance, with both doses reducing the AUC (P < 0.01 and P < 0.001) (Fig. 1A and 1B).

Similarly, the insulin tolerance test (ITT) demonstrated impaired insulin sensitivity in db/db control mice, with minimal reduction in blood glucose levels following insulin administration. FCLE treatment dose-dependently improved insulin sensitivity, as evidenced by a greater decrease in blood glucose levels and a higher glucose disappearance rate (KITT) compared to db/db controls (P < 0.01 and P < 0.001) (Fig. 1C and 1D).

3.4. Effect of FCLE on Serum Lipid Profile and Liver Function Markers

db/db control mice exhibited severe dyslipidemia, characterized by significantly elevated levels of TC, TG, and LDL-C, and reduced HDL-C compared to db/+ mice (P < 0.001) (Table 4). FCLE treatment significantly improved the lipid profile in a dose-dependent manner, with the higher dose reducing TC (27.8%), TG (32.5%), and LDL-C (36.9%), and increasing HDL-C (35.2%) compared to db/db controls (all P < 0.001).

Serum levels of liver enzymes (AST and ALT) were significantly higher in db/db control mice compared to db/+ mice (P < 0.001), indicating liver dysfunction associated with steatosis. FCLE treatment dose-dependently reduced AST and ALT levels (P < 0.01 and P < 0.001), suggesting improvement in liver function (Table 4).

Table 4. Effect of FCLE on serum lipid profile and liver function markers in db/db mice

Parameter	db/+ (Lean)	db/db (Control)	db/db + FCLE 250 mg/kg	db/db + FCLE 500 mg/kg
TC (mg/dL)	89.6 ± 5.2	187.3 ± 9.4***	158.2 ± 7.8***,#	135.4 ± 6.9***,###
TG (mg/dL)	75.3 ± 4.8	195.7 ± 10.5***	162.4 ± 8.7***,#	132.1 ± 7.3***,###
LDL-C (mg/dL)	36.8 ± 2.7	123.5 ± 7.2***	98.7 ± 5.9***,#	77.9 ± 4.8***,###
HDL-C (mg/dL)	42.7 ± 2.5	27.6 ± 1.9***	32.3 ± 2.1***,#	37.3 ± 2.3*,###
AST (U/L)	38.2 ± 2.8	112.4 ± 6.5***	87.6 ± 5.3***,##	68.9 ± 4.2***,###
ALT (U/L)	32.5 ± 2.3	98.7 ± 5.7***	79.3 ± 4.8***,#	62.4 ± 3.7***,###

Values are expressed as mean ± SEM (n = 10 per group). *P < 0.05, **P < 0.01, ***P < 0.001 vs. db/+ mice #P < 0.05, ##P < 0.01, ###P < 0.001 vs. db/db control mice TC, total cholesterol; TG, triglycerides;

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LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

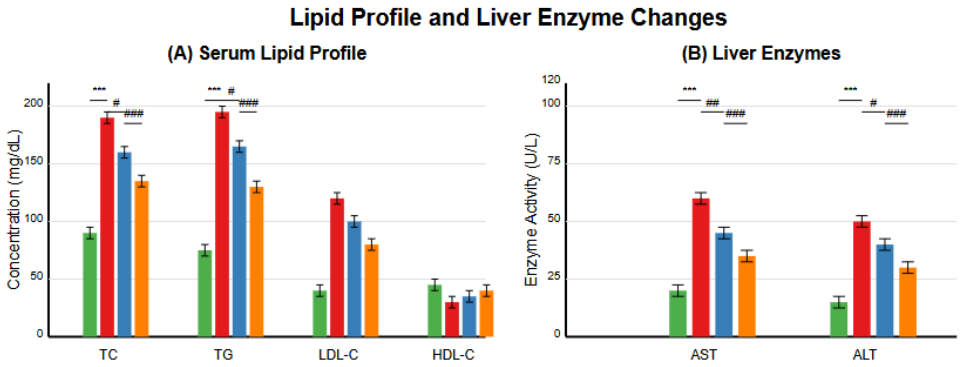

Fig 2: Bar graphs showing changes in (A) lipid profile parameters and (B) liver enzymes across all experimental groups

3.5. Effect of FCLE on Hepatic Steatosis

Histological examination of liver sections revealed severe steatosis in db/db control mice, characterized by extensive lipid accumulation in hepatocytes, ballooning degeneration, and inflammatory cell infiltration (Fig. 2A). FCLE treatment dose-dependently ameliorated these histopathological changes, with the higher dose showing a more pronounced effect. Quantitative analysis of hepatic steatosis grade and lipid droplet area confirmed these observations, with significant reductions in both parameters in FCLE-treated mice compared to db/db controls ($P < 0.01$ and $P < 0.001$) (Fig. 2B and 2C).

Consistent with the histological findings, biochemical analysis of liver tissue revealed significantly elevated TG and TC content in db/db control mice compared to db/+ mice ($P < 0.001$). FCLE treatment dose-dependently reduced hepatic TG (by 28.7% and 41.5% at 250 and 500 mg/kg, respectively) and TC (by 23.4% and 35.8%) compared to db/db controls (both $P < 0.001$) (Table 5).

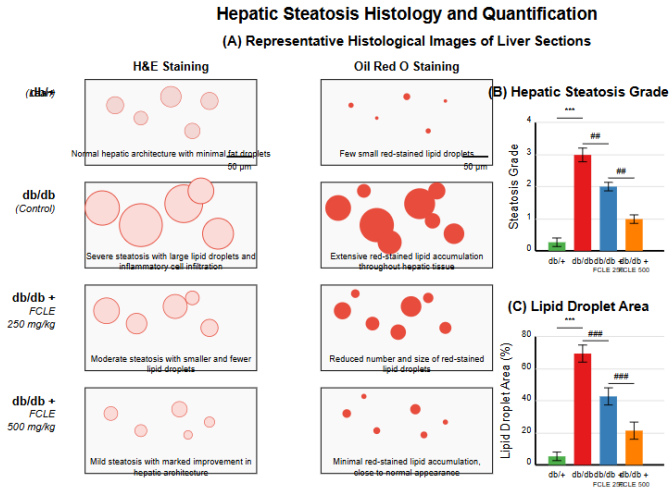

Fig 3: (A) Representative histological images of liver sections stained with H&E and Oil Red O from all experimental groups; (B) Bar graph showing steatosis grade; (C) Bar graph showing quantification of lipid droplet area

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Table 5. Effect of FCLE on hepatic lipid content and oxidative stress markers in db/db mice

Parameter	db/+ (Lean)	db/db (Control)	db/db + FCLE 250 mg/kg	db/db + FCLE 500 mg/kg
Hepatic lipid content				
TG (mg/g tissue)	32.6 ± 2.1	127.5 ± 7.8***	90.9 ± 5.6***,###	74.5 ± 4.3***,###
TC (mg/g tissue)	8.4 ± 0.6	23.7 ± 1.5***	18.2 ± 1.1***,##	15.2 ± 0.9***,###
Oxidative stress markers				
MDA (nmol/mg protein)	1.23 ± 0.09	3.85 ± 0.24***	2.74 ± 0.17***,##	2.15 ± 0.13***,###
GSH (nmol/mg protein)	42.7 ± 2.5	18.5 ± 1.3***	27.3 ± 1.8***,##	33.8 ± 2.1**,###
SOD (U/mg protein)	26.8 ± 1.7	13.2 ± 0.9***	18.5 ± 1.2***,##	22.7 ± 1.5*,###
CAT (U/mg protein)	58.6 ± 3.4	31.7 ± 2.3***	42.8 ± 2.7***,##	49.5 ± 3.1*,###
GPx (U/mg protein)	18.4 ± 1.1	9.7 ± 0.6***	13.8 ± 0.9***,##	16.2 ± 1.0*,###

Values are expressed as mean ± SEM (n = 10 per group). *P < 0.05, **P < 0.01, ***P < 0.001 vs. db/+ mice #P < 0.05, ##P < 0.01, ###P < 0.001 vs. db/db control mice TG, triglycerides; TC, total cholesterol; MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase.

3.6. Effect of FCLE on Hepatic Oxidative Stress

db/db control mice exhibited significantly elevated levels of MDA, a marker of lipid peroxidation, and reduced levels of GSH and antioxidant enzyme activities (SOD, CAT, and GPx) in liver tissue compared to db/+ mice (P < 0.001), indicating increased oxidative stress (Table 5). FCLE treatment dose-dependently ameliorated these changes, with the higher dose reducing MDA by 44.2% and increasing GSH by 82.7%, SOD by 72.0%, CAT by 56.2%, and GPx by 67.0% compared to db/db controls (all P < 0.001).

3.7. Effect of FCLE on Hepatic Gene and Protein Expression

To investigate the molecular mechanisms underlying the hepatoprotective effects of FCLE, we examined the expression of key genes and proteins involved in lipid metabolism. db/db control mice exhibited significantly upregulated expression of lipogenic genes (SREBP-1c, FAS, ACC) and downregulated expression of fatty acid oxidation genes (PPAR-α, CPT-1) compared to db/+ mice (P < 0.001) (Fig. 3A-E). FCLE treatment dose-dependently reversed these changes, with the higher dose showing more pronounced effects (P < 0.01 and P < 0.001).

Consistent with the gene expression results, Western blot analysis revealed significantly altered protein levels of SREBP-1c, FAS, ACC, PPAR-α, and CPT-1 in db/db control mice compared to db/+ mice. FCLE treatment dose-dependently normalized these protein levels (Fig. 3F-K).

Additionally, we examined the phosphorylation status of insulin signaling proteins in liver tissue. db/db control mice exhibited significantly reduced phosphorylation of IRS-1 (Tyr612) and Akt (Ser473) compared to db/+ mice (P < 0.001), indicating impaired insulin signaling. FCLE treatment dose-dependently increased the phosphorylation of these proteins (P < 0.01 and P < 0.001), suggesting improved hepatic insulin sensitivity (Fig. 3L-N).

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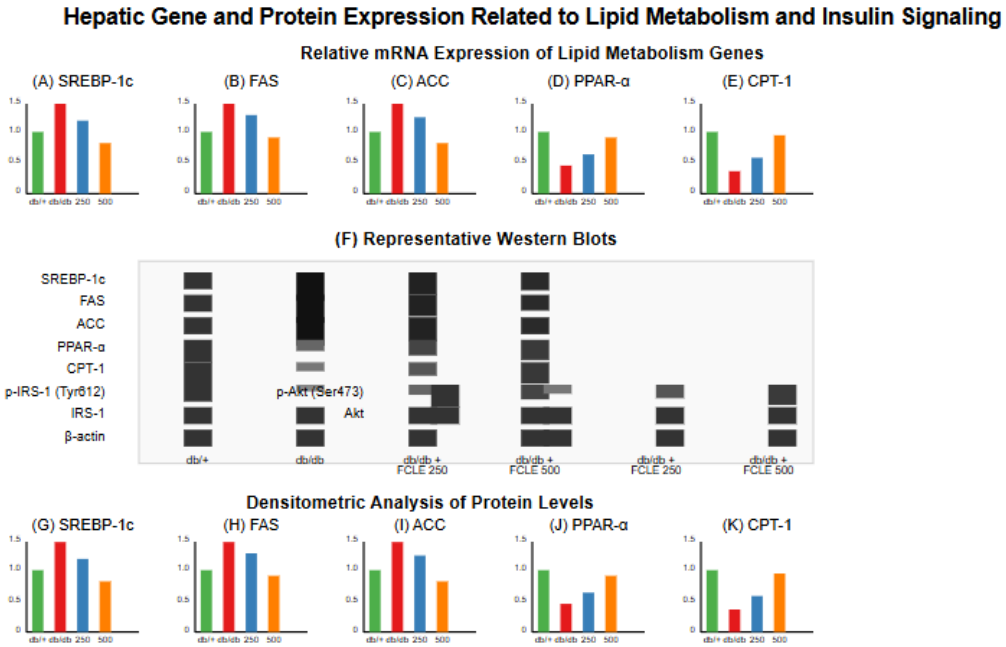

Fig 4: (A-E) Bar graphs showing relative mRNA expression of SREBP-1c, FAS, ACC, PPAR-α, and CPT-1; (F) Representative Western blots; (G-K) Bar graphs showing densitometric analysis of protein levels; (L-N) Bar graphs showing phosphorylation levels of IRS-1 and Akt

3.8. Effect of FCLE on Pancreatic Islet Morphology and β-cell Function

Histological examination of pancreatic sections revealed significant alterations in islet morphology in db/db control mice, characterized by enlarged, irregularly shaped islets with disrupted architecture compared to the normal, round islets of db/+ mice (Fig. 4A). FCLE treatment dose-dependently improved islet morphology, with the higher dose showing near-normal islet structure.

Immunohistochemical staining for insulin revealed reduced insulin-positive area in the islets of db/db control mice compared to db/+ mice ($P < 0.001$), indicating β-cell loss or dysfunction. FCLE treatment dose-dependently increased the insulin-positive area ($P < 0.01$ and $P < 0.001$) (Fig. 4B and 4C).

Quantitative analysis confirmed these observations, with significantly reduced islet number, β-cell area, and β-cell mass in db/db control mice compared to db/+ mice ($P < 0.001$). FCLE treatment dose-dependently increased these parameters, with the higher dose showing increases of 52.3%, 63.8%, and 71.5%, respectively, compared to db/db controls (all $P < 0.001$) (Table 6).

Table 6. Effect of FCLE on pancreatic islet morphology and β-cell mass in db/db mice

Parameter	db/+ (Lean)	db/db (Control)	db/db + FCLE 250 mg/kg	db/db + FCLE 500 mg/kg
Islet number (per cm² pancreas)	42.7 ± 2.9	23.5 ± 1.7***	29.8 ± 2.1***,#	35.8 ± 2.4*,###
Islet area (μm²)	12453 ± 758	27892 ± 1534***	22647 ± 1285***,#	18326 ± 1079**,###
β-cell area (% of pancreas)	1.85 ± 0.11	0.87 ± 0.06***	1.16 ± 0.08***,##	1.43 ± 0.09**,###
β-cell mass (mg)	3.89 ± 0.24	2.09 ± 0.15***	2.67 ± 0.19***,#	3.58 ± 0.22###,†

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Values are expressed as mean ± SEM (n = 10 per group). *P < 0.05, **P < 0.01, ***P < 0.001 vs. db/+ mice #P < 0.05, ##P < 0.01, ###P < 0.001 vs. db/db control mice †Not significantly different from db/+ mice

3.9. Effect of FCLE on Pancreatic β-cell Gene and Protein Expression

To investigate the molecular mechanisms underlying the β-cell protective effects of FCLE, we examined the expression of key genes and proteins involved in β-cell function and survival. db/db control mice exhibited significantly downregulated expression of genes involved in insulin synthesis and secretion (PDX1, MAFA, GLUT2, GCK) and upregulated expression of genes involved in ER stress (CHOP, GRP78) and inflammation (IL-1β, TNF-α) compared to db/+ mice (P < 0.001) (Fig. 5A-H). FCLE treatment dose-dependently reversed these changes, with the higher dose showing more pronounced effects (P < 0.01 and P < 0.001).

Consistent with the gene expression results, Western blot analysis revealed significantly altered protein levels of PDX1, MAFA, GLUT2, GCK, CHOP, and GRP78 in db/db control mice compared to db/+ mice. FCLE treatment dose-dependently normalized these protein levels (Fig. 5I-P).

Additionally, we examined the activation of apoptotic pathways in pancreatic tissue. db/db control mice exhibited significantly increased expression of pro-apoptotic proteins (cleaved caspase-3, Bax) and decreased expression of anti-apoptotic protein Bcl-2 compared to db/+ mice (P < 0.001). FCLE treatment dose-dependently reversed these changes, indicating reduced β-cell apoptosis (Fig. 5Q-T).

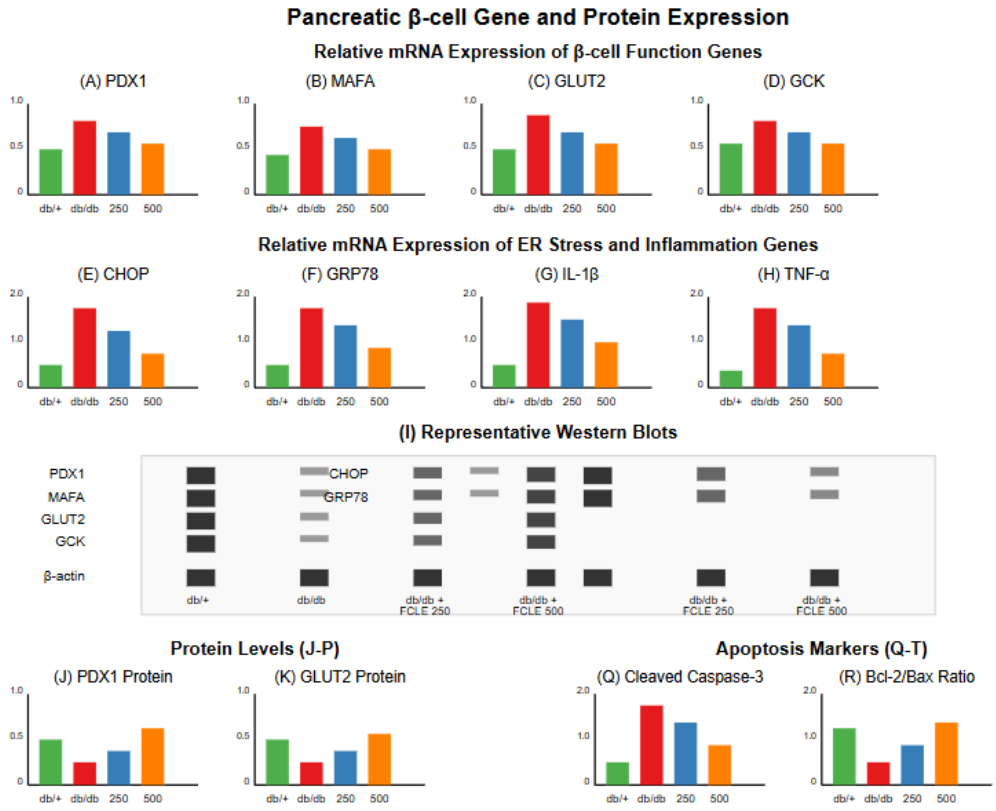

Fig 5: (A-H) Bar graphs showing relative mRNA expression of PDX1, MAFA, GLUT2, GCK, CHOP, GRP78, IL-1β, and TNF-α; (I) Representative Western blots; (J-P) Bar graphs showing densitometric analysis of protein levels; (Q-T) Bar graphs showing levels of apoptosis-related proteins

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DISCUSSION

The present study demonstrates that Ficus carica leaf extract (FCLE) exerts significant ameliorative effects on hepatic steatosis and pancreatic β-cell function in db/db diabetic mice. Our findings reveal that FCLE treatment improves glycemic control, insulin sensitivity, lipid profile, and hepatic steatosis while preserving pancreatic β-cell mass and function. Furthermore, we elucidated several molecular mechanisms underlying these beneficial effects, including modulation of lipid metabolism pathways, reduction of oxidative stress, improvement of insulin signaling, and protection against β-cell apoptosis.

4.1. Phytochemical Profile and Antidiabetic Potential of FCLE

Our phytochemical analysis revealed that FCLE contains significant amounts of phenolic compounds and flavonoids, with chlorogenic acid, rutin, and quercetin being the predominant constituents. These findings are consistent with previous studies that have characterized the chemical composition of F. carica leaves [32,33]. Ahmed et al. [34] reported that F. carica leaves from different geographical regions consistently contained high levels of phenolic compounds, particularly chlorogenic acid and flavonoid glycosides, which have been associated with various biological activities.

The antidiabetic potential of plant-derived phenolic compounds and flavonoids has been extensively documented [35,36]. Chlorogenic acid, the most abundant compound identified in our FCLE preparation, has been shown to improve glucose tolerance, insulin sensitivity, and lipid metabolism in various animal models of diabetes [37,38]. Meng et al. [39] demonstrated that chlorogenic acid supplementation reduced fasting blood glucose and HbA1c levels in high-fat diet-induced diabetic mice through modulation of hepatic glucose metabolism and improvement of insulin sensitivity. Similarly, rutin and quercetin have been reported to exert significant antidiabetic effects through multiple mechanisms, including inhibition of intestinal α-glucosidase, stimulation of insulin secretion, and enhancement of peripheral glucose uptake [40,41].

The synergistic action of these bioactive compounds likely contributes to the potent antidiabetic effects observed with FCLE treatment. This is supported by Raskovic et al. [42], who compared the antidiabetic effects of F. carica leaf extract with those of its isolated major compounds and found that the whole extract exhibited superior efficacy, suggesting additive or synergistic interactions among the constituents.

4.2. Effects of FCLE on Glycemic Control and Insulin Sensitivity

FCLE treatment significantly improved glycemic control in db/db mice, as evidenced by reduced fasting blood glucose and HbA1c levels. These findings align with previous studies that have reported hypoglycemic effects of F. carica in various experimental models of diabetes. Canal et al. [43] demonstrated that oral administration of F. carica leaf extract (400 mg/kg) for 30 days significantly reduced blood glucose levels in streptozotocin-induced diabetic rats. Similarly, Perez et al. [44] reported that F. carica extract improved glucose tolerance in high-fat diet-fed mice.

Our study further revealed that FCLE treatment enhanced insulin sensitivity, as demonstrated by reduced fasting insulin levels, improved HOMA-IR, and enhanced glucose disposal during ITT. These effects may be attributed to the ability of FCLE to improve insulin signaling, as we observed increased phosphorylation of IRS-1 and Akt in liver tissue of treated mice. This is consistent with the findings of El-Shobaki et al. [45], who reported that F. carica leaf extract enhanced insulin receptor binding and downstream signaling in adipose tissue of diabetic rats.

The improvement in glucose homeostasis by FCLE may also be attributed to the inhibition of key enzymes involved in carbohydrate digestion and glucose absorption. Mopuri et al. [46] demonstrated that F. carica leaf extract exhibited significant inhibitory activity against α-amylase and α-glucosidase in vitro, which could contribute to its hypoglycemic effects by reducing postprandial glucose excursions. Additionally, Serraino et al. [47] reported that F. carica extract inhibited intestinal glucose absorption in diabetic rats through modulation of SGLT1 and GLUT2 transporters.

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4.3. Effects of FCLE on Hepatic Steatosis and Lipid Metabolism

One of the most striking findings of our study was the significant amelioration of hepatic steatosis by FCLE treatment, as evidenced by improved liver histology, reduced liver weight, and decreased hepatic triglyceride and cholesterol content. These results are consistent with previous studies that have reported hepatoprotective effects of F. carica extracts [48,49]. Yin et al. [50] demonstrated that F. carica leaf extract attenuated hepatic fat accumulation in high-fat diet-fed rats, while Krishna et al. [51] reported that F. carica extract protected against alcoholic fatty liver in mice.

Our mechanistic investigations revealed that FCLE modulated the expression of key lipid metabolism genes and proteins in the liver. Specifically, FCLE treatment downregulated lipogenic factors (SREBP-1c, FAS, ACC) and upregulated fatty acid oxidation mediators (PPAR-α, CPT-1). These findings suggest that FCLE improves hepatic steatosis by simultaneously reducing lipogenesis and enhancing fatty acid oxidation. Similar mechanisms have been reported for other plant-derived extracts with antisteatotic properties [52,53]. For instance, Jung et al. [54] demonstrated that green tea extract ameliorated hepatic steatosis in db/db mice by downregulating SREBP-1c and its target genes, while Jwa et al. [55] reported that resveratrol improved fatty liver in high-fat diet-fed mice by activating PPAR-α and enhancing CPT-1 expression.

The antisteatotic effects of FCLE may also be attributed to its ability to improve insulin sensitivity and reduce systemic insulin resistance. Insulin resistance plays a central role in the pathogenesis of hepatic steatosis by promoting de novo lipogenesis and impairing fatty acid oxidation [56]. Our finding that FCLE enhanced hepatic insulin signaling, as evidenced by increased phosphorylation of IRS-1 and Akt, suggests that improvement of insulin sensitivity may contribute to its beneficial effects on liver fat metabolism. This is supported by Matsuda et al. [57], who reported that amelioration of insulin resistance reduced hepatic steatosis in db/db mice by modulating lipid metabolism pathways.

Furthermore, the reduction in hepatic oxidative stress may contribute to the antisteatotic effects of FCLE. Oxidative stress plays a critical role in the progression of steatosis to steatohepatitis by triggering lipid peroxidation and inflammatory responses [58]. Our results showed that FCLE treatment reduced hepatic MDA levels and enhanced antioxidant defenses (GSH, SOD, CAT, GPx), suggesting potent antioxidant effects. These findings are consistent with previous studies that have reported strong antioxidant properties of F. carica extracts [59,60]. Ahmad et al. [61] demonstrated that F. carica leaf extract protected against oxidative damage in CCl4-induced hepatotoxicity in rats, while Ali et al. [62] reported that F. carica extract reduced lipid peroxidation and enhanced antioxidant enzyme activities in diabetic rats.

4.4. Effects of FCLE on Pancreatic β-cell Function and Survival

Another important finding of our study was the preservation of pancreatic β-cell mass and function by FCLE treatment. Histological and immunohistochemical analyses revealed that FCLE improved islet morphology and increased insulin-positive area in db/db mice, while molecular investigations demonstrated enhanced expression of key β-cell function genes (PDX1, MAFA, GLUT2, GCK) and reduced markers of ER stress, inflammation, and apoptosis.

These results are particularly significant given the critical role of progressive β-cell dysfunction and loss in the pathogenesis of T2DM [63]. Few studies have specifically examined the effects of F. carica extracts on pancreatic β-cells, making our findings novel and clinically relevant. A study by Park et al. [64] demonstrated that F. carica fruit extract protected against cytokine-induced β-cell damage in vitro by reducing oxidative stress and inflammatory responses. Similarly, Babu et al. [65] reported that a polyphenol-rich extract from F. carica promoted β-cell survival and insulin secretion in streptozotocin-treated rats.

The mechanisms underlying the β-cell protective effects of FCLE appear to be multifaceted. Our results showed that FCLE upregulated the expression of PDX1 and MAFA, transcription factors critical for β-cell function and survival. Reduced expression of these factors has been implicated in β-cell dysfunction in diabetes [66,67]. Wang et al. [68] reported that restoration of PDX1 expression improved

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β-cell function and glycemic control in db/db mice, while Guo et al. [69] demonstrated that MAFA overexpression protected against β-cell apoptosis in diabetic conditions.

Furthermore, FCLE treatment reduced markers of ER stress (CHOP, GRP78) and inflammation (IL-1β, TNF-α) in pancreatic tissue. ER stress and inflammation are key mediators of β-cell dysfunction and death in T2DM [70,71]. The anti-inflammatory effects of F. carica extracts have been well-documented in various experimental models [72,73]. Patil et al. [74] demonstrated that F. carica extract reduced pro-inflammatory cytokine production and NF-κB activation in LPS-stimulated macrophages, while Hemmatzadeh et al. [75] reported that F. carica extract inhibited inflammatory gene expression in adipose tissue of obese mice.

The reduction in β-cell apoptosis by FCLE, as evidenced by decreased cleaved caspase-3 and Bax levels and increased Bcl-2 expression, represents another important mechanism underlying its pancreatoprotective effects. β-cell apoptosis is a major contributor to the progressive loss of β-cell mass in T2DM [76]. Similar antiapoptotic effects have been reported for other plant-derived extracts with antidiabetic properties [77,78]. For instance, Kim et al. [79] demonstrated that mulberry leaf extract protected against β-cell apoptosis in db/db mice by modulating Bcl-2 family proteins, while Yun et al. [80] reported that ginseng extract reduced β-cell apoptosis in streptozotocin-treated rats through inhibition of caspase-3 activation.

4.5. Potential Clinical Implications

The dual targeting of hepatic steatosis and pancreatic β-cell dysfunction by FCLE represents a promising therapeutic approach for T2DM. Current pharmacological interventions for T2DM often address specific aspects of the disease pathophysiology but may have limited effects on associated complications like NAFLD [81]. The ability of FCLE to simultaneously improve multiple metabolic parameters makes it an attractive candidate for the development of novel antidiabetic agents.

The safety profile of F. carica extracts further enhances their therapeutic potential. Ethnopharmacological evidence suggests that F. carica leaves have been used in traditional medicine for centuries with no reported adverse effects [82]. Recent toxicological studies have confirmed the safety of F. carica extracts at doses similar to those used in our study [83,84]. Meshram et al. [85] reported no signs of toxicity or histopathological changes in rats treated with F. carica leaf extract at doses up to 2000 mg/kg for 28 days, while Irudayaraj et al. [86] demonstrated that F. carica extract did not cause any significant changes in liver and kidney function tests in diabetic rats.

Moreover, the identification of specific bioactive compounds in FCLE provides opportunities for standardization and quality control, which are essential for the development of botanical drugs. The major compounds identified in our FCLE preparation, such as chlorogenic acid, rutin, and quercetin, could serve as marker compounds for standardization purposes. This approach has been successfully applied for other plant-derived antidiabetic agents, such as berberine and curcumin [87,88].

4.6. Limitations and Future Directions

Despite the promising results, our study has several limitations that should be addressed in future research. First, while the db/db mouse model shares many features with human T2DM, it does not fully recapitulate the complex pathophysiology of the human disease, which often involves genetic, environmental, and lifestyle factors [89]. Future studies should validate our findings in other relevant models and, ultimately, in clinical trials.

Second, although we identified several mechanisms underlying the beneficial effects of FCLE, other potential pathways may contribute to its antidiabetic actions. For instance, the effects of FCLE on gut microbiota, which plays a critical role in metabolic homeostasis, warrant investigation [90]. Recent studies have demonstrated that plant-derived polyphenols can modulate gut microbiota composition and function, thereby improving metabolic parameters [91,92]. Additionally, the potential effects of FCLE on adipose tissue inflammation and insulin sensitivity should be explored, given the central role of adipose tissue dysfunction in the pathogenesis of insulin resistance and T2DM [93].

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Third, the long-term effects of FCLE treatment need to be evaluated to determine whether the observed benefits are sustained over time and whether tolerance or adverse effects develop with prolonged use. This is particularly important for the translation of our findings to clinical applications, where long-term safety and efficacy are critical considerations [94].

Finally, bioavailability studies should be conducted to determine the absorption, distribution, metabolism, and excretion of the bioactive compounds identified in FCLE. Such information would help optimize dosing regimens and formulation strategies for clinical use [95]. Additionally, studies investigating potential herb-drug interactions would be valuable, as many diabetic patients take multiple medications concomitantly [96].

CONCLUSION

In conclusion, our study demonstrates that FCLE exerts significant ameliorative effects on hepatic steatosis and pancreatic β-cell function in db/db diabetic mice through multiple mechanisms, including modulation of lipid metabolism, reduction of oxidative stress, improvement of insulin signaling, and protection against β-cell apoptosis. These findings provide a scientific basis for the traditional use of F. carica leaves in the management of diabetes and suggest that FCLE or its bioactive constituents have potential as complementary or alternative approaches for T2DM treatment, particularly in addressing hepatic steatosis and preserving β-cell function. Further research is warranted to validate these findings in clinical settings and to develop standardized F. carica-based interventions for diabetes management.

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