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Enhanced antidiabetic efficacy and bioavailability of curcumin-loaded sporopollenin microcapsules in a type 2 diabetes rat model

Noha M. Meligi^{1*} , Noura A. Saad¹, Bahaa K. A. Abdel-Salam¹, Amro K. F. Dyab² and Diao B. Al-Azhary¹

Abstract

Background The rising global prevalence of type 2 diabetes mellitus (T2DM) necessitates the identification of efficient medications for its management. Curcumin (Cur), the principal curcuminoid found in turmeric, exhibits numerous beneficial effects on T2DM. The application of Cur for the treatment of T2DM presents several limitations, including inadequate penetration, decreased stability, poor solubility, and low bioavailability. Consequently, a versatile protective Cur encapsulation system is needed to address its natural instability. This study presents the application of *Lycopodium clavatum* sporopollenin (LCS) microcapsules, derived from natural micrometer-sized raw pollens, for Cur microencapsulation to enhance curcumin efficacy in an experimental rat model of T2DM.

Results Over a 4-week treatment, the high-fat diet/Streptozotocin (HFD/STZ)-induced diabetic rats administered Cur-loaded LCS reduced FBG by 60%, decreased serum TNF- α by 20%, and increased IL-10 by 45% compared to curcumin diabetic rats. Furthermore, histological investigations of the kidney, pancreas, and liver revealed that Cur-loaded LCS ameliorated the organs' degenerative alterations in diabetic rats.

Conclusion The current study demonstrates that microencapsulation of Cur within pollen-derived biomaterials facilitates Cur delivery and improves bioavailability, establishing it as a promising method for addressing complications related to T2DM.

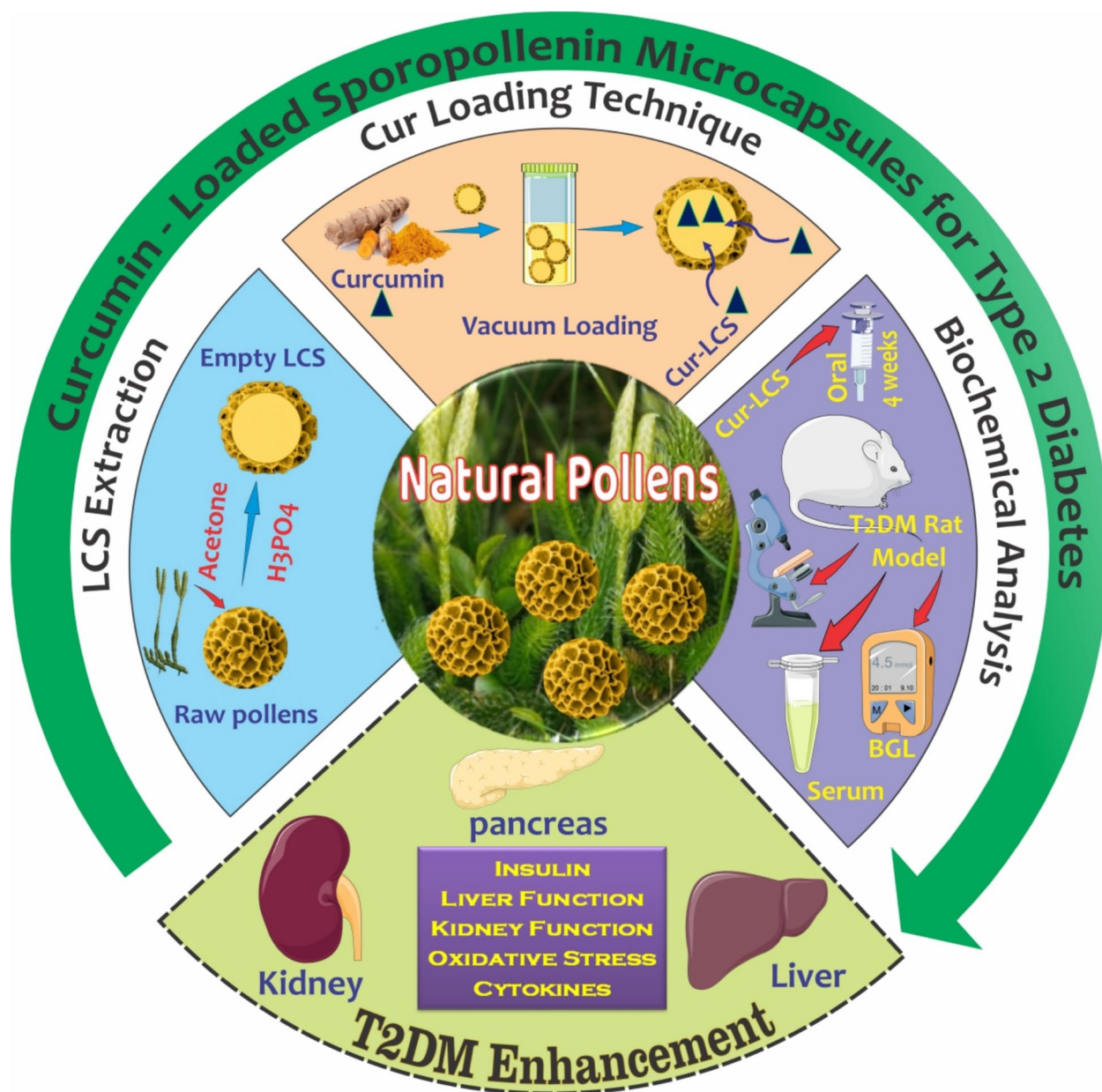
Keywords Type 2 diabetes mellitus (T2DM), Curcumin, Antidiabetic, Sporopollenin, Encapsulation, Biochemical parameters, Histopathology, Albino rats, Bioavailability enhancement, Drug delivery system

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Graphical abstract

**Introduction**

The International Diabetes Federation (IDF) projects that the global diabetic population will increase to 578 million by 2030 and is expected to hit 700 million by 2045, with T2DM accounting for approximately 90% of these cases. The prevalence is significantly greater

in urban (10.8%) than rural (7.2%) regions, as well as in high-income (10.4%) than low-income countries (4.0%). One in every two (50.1%) patients with diabetes are unaware of their condition. The worldwide rate of impaired glucose tolerance is expected to be 8.0% (454 million) by 2030 and rising to 8.6% (548 million)

by 2045 [1]. Pancreatic beta-cell dysfunction along with insulin resistance defines the metabolic condition known as T2DM [2, 3]. STZ is a pharmacological agent frequently utilized to induce DM in experimental animals by destroying pancreatic beta cells, resulting in hyperglycemia. It may be administered alongside an HFD to establish a T2DM animal model [4, 5]. Natural products exhibiting specific bioactivity have garnered considerable attention for their potential in managing hyperglycemia and other diabetes-related issues [6–8]. Cur, a polyphenol derived from the rhizomes of *Curcuma longa* Linn, has been utilized in India for millennia to enhance food color and flavor, as well as to address various medical conditions. This plant is acknowledged in folklore for its various pharmacological benefits, which encompass antibacterial, anti-inflammatory, antioxidant, antiviral, antiangiogenic, and antidiabetic properties [9].

Despite promoting a paradigm shift in the pharmaceutical and functional food areas, Cur is regarded as prime example of a “biopharmaceutical hurdle” owing to its rapid metabolism, poor solubility in aqueous media (0.4 µg/mL), and restricted absorption in the gastrointestinal (GI) tract, all of which lead to diminished bioavailability [10–16]. It was reported that the half-life of Cur in phosphate buffer (pH 7.4) is 10 min that was attributed to its instability in alkaline media, which further notoriously compromises its clinical translation [10]. Oral consumption of Cur may be affected by the dietary matrix, including proteins as well as lipids, which may alter its bioavailability. The majority is excreted in feces, with only a minor fraction absorbed in the GI tract, subsequently undergoing rapid metabolism in the plasma and liver. A significant conjugation rate through sulfation and glucuronidation leads to the formation of water-soluble metabolites (glucuronides and sulfates) that are excreted in the urine, which accounts for the low concentration of Cur in the blood [17–19]. Low bioavailability results in diminished serum concentrations, thereby limiting the potential for positive health outcomes. Therefore, it is imperative to develop a promising delivery platform to overcome such limitations of Cur biomedical applications by utilizing the two distinct technological trajectories, namely nanotechnology and microencapsulation.

Numerous nanotechnology techniques [11, 20–23] have been employed to enhance Cur's bioavailability, durability, and solubility. For instance, Cur bioavailability can be enhanced by constructing Cur complexes or combining piperine and other bioavailability enhancers [24, 25]. The advancement of nanobiotechnology, including nanogels, polymer micelles, liposomes, solid nanoparticles, hydrophilic prodrugs, and microemulsions, has generated numerous opportunities for the research and

development of Cur therapeutic uses [26–28]. Nanocurcumin exhibited better bioavailability, absorption capacity, half-life, and drug delivery efficacy than regular free Cur [29]. Notwithstanding their superior advantages in terms of high surface area and cellular/tissue uptake, nano-formulations present considerable challenges, for instance, their high-cost fabrication and nanotoxicity/biodistribution restrictions [30]. The widely used microencapsulation technologies may offer a feasible platform to overcome some of the limitations of nano-formulations via enhancing the stability and bioavailability of encapsulated bioactives [22]. This could be achieved by engineering the core and/or the shell of microcapsules to protect the bioactives from potential chemical degradation, harsh GI environment, and control its release. While microcarriers derived from plant or animal-based polymers show great interest and promise, their fabrication involves sophisticated equipment with lengthy and intricate processes. Inspired by nature, a novel, greener microencapsulation platform based on pollen grains has gained considerable attention recently, offering a uniquely efficient alternative as one of the most resilient, naturally pre-formed biopolymer microcarriers [31].

In selecting an encapsulation platform, the balance between therapeutic benefit, economic feasibility, and safety is paramount. Motivated by this alignment along with the unique properties of the natural microcarriers, we propose in the current study a novel technique for microencapsulating Cur into plant-based pollen microcapsules to enhance the clinical benefits of Cur in regulating T2DM. As natural biomaterials, sporopollenin microcapsules derived from the outer raw pollen shells like *Lycopodium clavatum* have recently been recognized as a viable encapsulating platform for pharmaceuticals [32–40], live cells [41], probiotics [42], pollen microgels [43], proteins [44], vitamin [45], and vaccines [46]. Unlike synthetic micro- and nanocarriers, the low-cost sporopollenin microcapsules offer unique advantages: (i) natural origin with inherent biocompatibility and size homogeneity, (ii) hollow core architecture enabling high loading capacity (27% in this study vs. typical 5–10% for liposomal formulations), (iii) extraordinary UV, chemical and mechanical resilience allowing protection through the GI tract, and (iv) surface ornamentation facilitating mucoadhesion and prolonged intestinal residence time. These characteristics entail sporopollenin as a superior alternative to conventional delivery systems for sensitive phytochemicals. Therefore, they can be utilized for diverse potential applications in several areas [47–49].

This study proposes a protocol for extraction of LCS microcapsules from raw pollens, followed by Cur loading utilizing a vacuum technique to enhance the clinical

benefits of Cur in regulating T2DM (Fig. 1). Cur-loaded LCS efficacy is evaluated in a T2DM rat model induced by an HFD and low-dose STZ injection, utilizing biochemical methods as well as correlated histological investigations. The microencapsulation of Cur into the versatile LCS natural biomaterials represents a promising strategy for enhancing Cur bioactivity and stability, establishing it as a promising platform for biomedical applications.

Materials and methods

Materials

Cur, with a total curcuminoid content of 95%, was obtained from the turmeric rhizome sourced from (Alfa Aesar-Germany-CAS Number: 458-37-7). LCS (~27 μm type in powder form) and STZ were obtained from Sigma-Aldrich, USA. Sodium hydroxide (NaOH) was acquired from (Al-Gomhoria, Egypt). Liquid anhydrous di-sodium hydrogen orthophosphate (Na_2HPO_4), orthophosphoric acid (H_3PO_4), and HCl acid were sourced from ADWIC, Egypt. Tween 80 extra pure and potassium di-hydrogen orthophosphate (KH_2PO_4) were obtained from Alpha Chemika, Mumbai, India. Acetone and ethanol were procured from PIOCHEM, Egypt. Distilled water was utilized in all experiments.

Cur loading into unloaded LCS sporopollenin microcapsules

LCS sporopollenin isolation from raw pollens

The suspension of raw *Lycopodium clavatum* pollens was carried out in acetone, followed by refluxing at 50 °C with gentle stirring for a duration of 3 h. Defatted pollens

were subsequently filtered and air-dried overnight. The suspension of dried pollens was done in 85% orthophosphoric acid, followed by refluxing at 70 °C with continuous stirring for a duration of 5 h [50]. Sample dilution was carried out utilizing water and then filtered and washed with pure ethanol, hot acetone, 2 M sodium hydroxide, and 2 M hydrochloric acid prior to drying at 60 °C until achieving a constant weight. The yield of extracted sporopollenin was $35 \pm 3.1\%$ w/w as revealed from the difference between the mass of raw pollens and extracted sporopollenin. The obtained sporopollenin was subsequently stored at room temperature (for further analysis).

Cur loading into LCS sporopollenin

Following a vacuum-assisted loading protocol [33], in brief, we first prepared a curcumin solution by dissolving 0.1 g of curcumin in 10 mL of PBS (pH 6.8) supplemented with 2% (v/v) Tween 80. This specific medium was selected for its ability to enhance curcumin solubility, reaching a maximum of 80 mg/mL as reported previously without compromising microcapsule integrity [11, 51], followed by stirring for 10 min (for complete dissolution). Subsequently, we added dry unloaded LCS (0.07 g) suspended in the Cur solution, followed by stirring at 500 rpm for 10 min, resulting in a theoretical drug loading (TDL) of 58.82% w/w. The tube was subsequently placed in a vacuum desiccator at approximately 20 mbar for a duration of 2 h. The Cur-loaded LCS microparticles were collected via filtration and subsequently washed with an 8 ml mixture of PBS and water containing Tween 80 (1:1 v/v) to eliminate any excess Cur that adhered to the surface. Eventually, the cur-loaded LCS suspension dried at 78–80 °C until a consistent weight was obtained; then, it was kept in brown vessels in dark environment at room temperature for the next study. This drying temperature of 78–80 °C was selected to ensure rapid and complete moisture removal, preventing microbial growth. It was reported that curcumin is thermally stable at this range, given its high melting point of around 183 °C [52, 53], and exhibits negligible degradation when exposed to 80 °C for short durations of few hours. Importantly, the robust, highly cross-linked biopolymer shell of the sporopollenin exine serves as a thermal safeguard, providing a protective microenvironment that shields the encapsulated curcumin from direct thermal stress and oxidative degradation during processing [54].

Cur-loading capacity and encapsulation efficiency

Cur-loaded LCS (10 mg) was suspended in PBS (5 mL, pH 6.8) containing 2% (v/v) Tween 80. The mixture was agitated for 15 min before being sonicated at room temperature for 2 min with Ivymen® ultrasonic homogenizer, Spain, using three cycles of 30% amplitude. Following

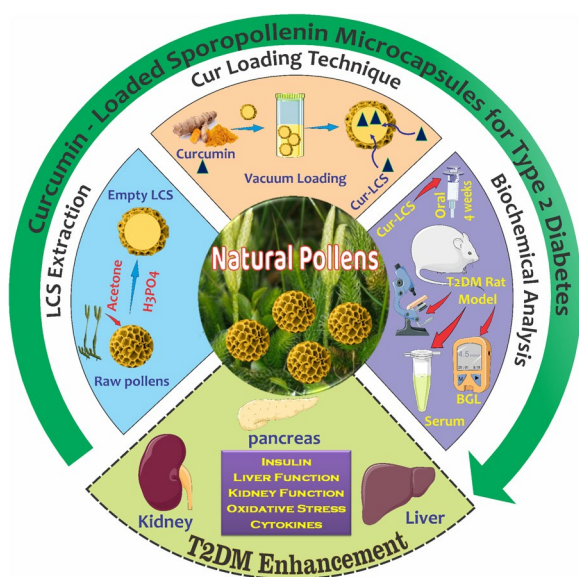


Fig. 1 Schematic representation of the protocol used

sonication, the suspension went through filtration, and the filtrate (1 mL) was diluted to a final volume of 10 mL. To determine the amount of Cur encapsulated in the microcapsules, the clear solution absorbance (diluted filtrate) was calculated using a UV–Vis spectrophotometer (Unicam Helios Alpha) at $\lambda_{\text{max}}=420$ nm, with the appropriate blank as a reference.

The determination of Cur content was done utilizing the relevant calibration curve, accounting for the dilution factor. The solid microcapsules that remained following filtration were resuspended in fresh PBS containing Tween 80, went through more sonication cycles as already noted, and were filtered again to recover any residual Cur. The final step was reiterated twice until no Cur was observed in the filtrate. For calculating the loading capacity (LC%) and encapsulation efficiency (EE%), Eqs. 1 and 2 are employed [36]:

$$\text{Loading capacity (\%)} = \frac{(\text{amount of drug entrapped})}{(\text{weight of drug loaded sporopollenin})} \times 100 \quad (1)$$

$$\text{Encapsulation efficiency (\%)} = \frac{(\text{practical drug loading})}{(\text{theoretical drug loading})} \times 100 \quad (2)$$

All measurements were taken in triplicate ($n=3$ independent experiments), and results are expressed as mean $\pm$ SD. The loading capacity (LC) was $27.0 \pm 2.3\%$, and the encapsulation efficiency (EE) was $45.90 \pm 3.8\%$.

LCS and cur-loaded LCS characterization

Confocal laser scanning microscopy (CLSM) analysis

CLSM analysis was performed on Cur-loaded LCS microcapsules and unloaded LCS using a Carl Zeiss LSM780 (Germany) confocal microscope with an EC Plan-Neofluar 63 $\times$ /1.40 Oil DIC (differential interference contrast) M27 objective lens. Fluorescence from both the Cur-loaded and unloaded LCS was measured using a photomultiplier with emission filters 561–640, 488–535, and 405–453. Scanning was done using a plane mode (pixel dwell durations of 6.3 and 12.6 μ s) with a beam splitter (MBS-InVis-405, MBS 405/488/561).

For each sample, three images were captured at a designated slice position or via Z-stack collection (within the LCS microcapsules). Videos and images were recorded and processed with ZEISS ZEN 3.9 software (ZEISS-Germany).

Scanning electron microscopy (SEM)

A ZEISS Crossbeam 540 (Germany) was used for SEM evaluation over several magnitudes at 15 kV acceleration

voltage. Samples were covered in a 10-nm gold layer using a (JEOL JFC-1100E) gold sputter device.

Elemental analysis

CHNS elemental analysis was performed utilizing a UNI-CUBE[®] trace ORGANIC ELEMENTAL ANALYZER (Elementar-Germany) with infrared (IR) detector as well as thermal conductivity Detector (TCD). Sulfanilamide and acetanilide were used as certified reference materials for the calibration and triplicate measurements [51]. Before performing elemental analysis, all samples have been dried at 60 °C for at least 1 h to guarantee combustion efficiency. They were then combusted in excess oxygen (at high temperatures) to produce compositional carbon (%C), nitrogen (%N), hydrogen (%H), and sulfur (%S). Protein percentages in samples were calculated using the nitrogen percentage and the total Kjeldahl nitrogen (TKN) conversion factor of 6.25 as shown in Eq. 3 [34]:

$$\% \text{Protein} = \% \text{Nitrogen} \times 6.25 \quad (3)$$

Attenuated total reflectance fourier transform infrared (ATR-FTIR) analysis

The ATR-FTIR spectra of powdered dry samples on ATR diamond crystal were recorded in the range 4000–400 cm^{-1} , adjusting the resolution to 4 cm^{-1} , using Nicolet iS12 ATR-FTIR spectrometer (Thermo Scientific, USA).

In vitro cytotoxicity assay

The human skin fibroblast (HSF) cell line was obtained from Nawah Scientific Inc. (Egypt) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with streptomycin (100 μ g/mL), penicillin (100 U/mL), and 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere containing 5% CO₂. The Sulforhodamine B (SRB) assay was applied for cytotoxicity examination [55]. Following adequate growth, a suspension of cells (density of 5×10^3 cells/well) was inoculated onto 96-well plates and cultured in full medium for 24 h. The cells received an additional 100 μ L of medium with varying amounts (0.01, 0.1, 1, 10, 100 μ g/mL) of the analyzed materials. After 72 h of samples exposure, the cells were fixed by replacing the media with 150 μ L of 10% trichloroacetic acid (TCA) and then incubated at 4 °C for 1 h. The TCA was aspirated and cells were washed five times with distilled water and aliquots of 70 μ L SRB solution (0.4% w/v) were added and incubated in a dark place at room temperature for 10 min. The plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Then, 150 μ L of 10 mM tris(hydroxymethyl)aminomethane)

base solution (TRIS) was added to dissolve protein-bound SRB, and the absorbance was measured at 540 nm using an Infinite F50 microplate reader (TECAN, Switzerland). The percentage of cell viability was determined by comparing the mean optical density of the treated cells to that of the untreated control cells, following blank adjustment. The experiment was conducted in triplicate, and the results were shown as mean $\pm$ SE.

In Vivo investigation

Animals

The current investigation utilized adult male Sprague–Dawley rats weighing 150–200 g. The Laboratory Animal Centre in Dokki, Giza, Egypt, provided the animals. Rats were maintained under standard laboratory conditions of 25 ± 3 °C with a 12/12-h natural daylight cycle during the experiment. Throughout the trial, the animals were provided with unrestricted access to standard food and water and were housed for a duration of two weeks to acclimate to laboratory conditions. All ethical considerations regarding animal treatment and methodologies were thoroughly addressed and approved.

Induction of T2DM

As described in a previous work, a rat model of T2DM was created from the fat-fed/STZ rat model [56]. The experimental animals were administered a HFD for three weeks prior to receiving a low-dose STZ injection. The HFD comprised 60% fat, 20% protein, and 20% carbohydrate (No. D12492diet; Research Diets Inc, USA) [4, 5, 57]. The rats' weight was recorded weekly during the feeding period to ensure weight gain. T2DM was induced in rats through a single intraperitoneal injection

of a freshly prepared STZ solution (35 mg/kg) [58–60] diluted in citrate buffer (0.01 M, pH=4.5) following a 14-h fasting period. To prevent mortality and hypoglycemia resulting from STZ rapid action, the rats were provided with a 5% glucose solution ad libitum overnight following the injection (on the first day). Fasting blood glucose (FBG) levels (in the rats) were monitored three days post-STZ injection utilizing a handy Accu-Chek-Active glucometer (Roche Diagnostic, Germany), with samples taken from the tip of their tail. The research involved animals exhibiting FBG levels exceeding 270 mg/dL [56]. A typical diet was given to normal control rats. Following blood glucose determination, treatment with Cur or Cur-loaded LCS began on the third day after the STZ injection.

Design of experiments

Thirty-six rats were randomized into six groups administered daily doses via oral gavage for a duration of four weeks, following the specified scheme (Fig. 2): Group 1 (served as controls) was administered 0.9% NaCl, commonly referred to as physiological saline; Group 2 (Cur group) received a 100 mg kg^{-1} dose [61–63] (suspended in normal saline); Group 3: (vehicle group) was administered unloaded LCS (270 mg kg^{-1}) suspended in normal saline; Group 4: untreated diabetic group (Dia group); Group 5: the diabetic group (Dia + Cur) received Cur at a dosage of 100 mg kg^{-1} (suspended in saline); and Group 6: The diabetic cohort received Cur-loaded LCS at a dosage of 370 mg kg^{-1} , corresponding to 100 mg kg^{-1} Cur, suspended in saline according to Cur-loading capacity in LCS, which is 27%.

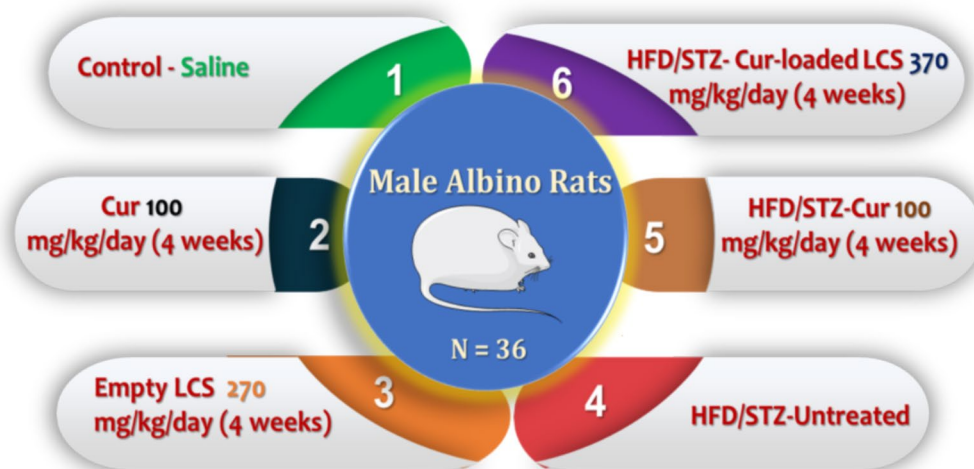


Fig. 2 Experimental design and group arrangement

The 100 mg/kg dose was selected based on previous studies demonstrating its efficacy in diabetic rat models without observed toxicity [61–63]. This dose has been shown to significantly reduce oxidative stress and inflammation in streptozotocin-induced diabetic rats, while higher doses (200–300 mg/kg) did not show proportional increases in therapeutic benefit in preliminary studies. This dose is also within the range commonly reported in the literature for evaluating curcumin's antidiabetic effects.

During the study, all animals received the same diet: a basal diet for vehicle, control, and Cur groups, and HFD for the other experimental animals. All doses and treatments were adjusted weekly based on body weight alterations, ensuring a consistent dose per kilogram of body weight (for each group) throughout the treatment period.

Body weight change assessment

The experimental groups' body weight alterations were assessed at week 0 (post-diabetes induction and prior to treatment initiation) and week 4 (conclusion of the treatment period) utilizing an automated scale (AND GX-600, Japan).

Blood glucose level assessment

FBG levels were determined from animals' tail tips in the various examined groups at week 0 (prior to treatment initiation), week 2, and week 4 (conclusion of the experiment) by using a handy Accu-Chek-Active glucometer (Roche Diagnostic, Germany).

Samples collection

Post-experiment, all animals were fasted overnight (12 h) with free access to water; the fasting duration was identical for all groups. Animals were then sedated using diethyl ether and decapitated and blood samples were collected from their trunks. The blood samples were allowed to clot at room temperature for 15–30 min before being centrifuged at 3000 rpm (4 °C) for serum separation. Subsequently, sera were collected and stored at –80 °C for further determination of biochemical and inflammatory biomarkers. Following blood sampling, liver, kidney, and pancreas tissues were immersed in 10% formaldehyde for histopathology.

Biochemical analysis

Serum liver, kidney, and lipid profile assays Following prior study methods, aspartate transaminase (AST) and alanine transaminase (ALT) were determined calorimetrically by spectrophotometer utilizing commercial kits (Biosystems, S.A, Barcelona, Spain) [64]. Assessment of serum alkaline phosphatase (ALP) level was done calorimetrically according to Rosalki et al. [65] utilizing

commercial diagnostic kits (Biosystems-S.A-Barcelona-Spain). Total protein (TP) and albumin (ALB) levels (CAT. No. TP116250 and CAT. No. ALB100250) were estimated with commercial kits (BioMed, Cairo, Egypt). Triglycerides (TGs), low-density lipoprotein-cholesterol (LDL-C), total cholesterol (TC), and high-density lipoprotein-cholesterol (HDL-C) in serum were quantified utilizing commercial test kits (Biosystems, S.A, Barcelona, Spain) following the corresponding standard procedures outlined in prior research [66–68]. According to prior research [69], creatinine and urea serum levels were estimated utilizing commercial kits (BioSystems, S.A, Barcelona, Spain).

Assessment of insulin and HOMA-IR The assessment of serum fasting insulin was done utilizing a Rat Insulin Sandwich ELISA Kit (CAT. No. KE20008, Proteintech, USA). Estimation of insulin resistance index was done utilizing the homeostasis model of assessment of insulin resistance (HOMA-IR) using Eq. 4 as follows [60]:

$$\text{HOMA} - \text{IR} = \frac{\text{fasting insulin (ng/mL)} \times \text{FBG (mg/dL)}}{405} \quad (4)$$

Serum oxidative stress Malondialdehyde (MDA) levels [70] (CAT. No. MD 25 29), reduced form of glutathione (GSH) [71] (CAT. No. GR 25 11), and superoxide dismutase (SOD) [72] (CAT. No. SD 25 21) were determined utilizing commercial test kits (Bio-Diagnostic Giza, Egypt).

Serum pro-inflammatory factors We used ELISA kits made by Elabscience Biotechnology Inc. in the USA to find out the levels of interleukin-6 (IL-6, CAT. No. E-EL-R0015) and tumor necrosis factor-alpha (TNF- α , CAT. No. E-EL-R0019) in serum samples. Additionally, serum interleukin-10 levels (IL-10, CAT. No. E0108Ra) were estimated utilizing ELISA ready-made commercial kits (Bioassay Technology Lab). The manufacturer's directions guided all of the biochemical tests.

Histological procedures

The rats were dissected for histopathological examination, and the pancreas, liver, and kidney were promptly excised. The organs were preserved in 10% formal saline (for 24 h). Standard histological techniques were employed, including immersion in a series of ascending ethanol dilutions. The tissue specimens were subsequently embedded in paraffin. Paraffin blocks were sectioned to a 5- μ m thickness, followed by staining H&E. Light microscopy facilitated the examination of stained sections for histological changes (Olympus

CH20BIMF200, Japan), with images obtained with a PHD-5MP Micro camera (Lainsy, Egypt).

Statistical analysis

The findings of the current study were analyzed utilizing version 22 of the SPSS software. The normality of data distribution was first confirmed using the Shapiro–Wilk test. The one-way analysis of variance (ANOVA) was employed to assess significance, subsequently followed by Tukey’s multiple comparison procedure. Results were presented as mean $\pm$ SE, with significance defined at $P < 0.05$.

Results

Cur encapsulation into LCS microcapsules

Prior to utilizing pollens as microcapsules, it is essential to extract the sporoplasm core materials from the raw pollens to form unloaded cavities and mitigate potential allergic reactions associated with certain proteins [73]. The extraction protocol employed in this study was analogous to previously reported methods [50] but with a partial modification. We performed a two-step extraction using acetone and phosphoric acid for pollen defatting and acidolysis. Defatting removes oils from the pollen surface, whereas acidolysis removes proteins mainly from the pollen’s inner part (intine), leaving the outer part (exine) as a hollow sporopollenin shell. A schematic

diagram of the extraction procedure is depicted in Fig. S1 (in ESI).

The morphological structure of the obtained sporopollenin was analyzed using SEM, as illustrated in Fig. 3A–C. The distal face of the microcapsule displays a reticular architecture characterized by perforated muri, resembling a honeycomb structure, on the exine shell. The proximal face of sporopollenin is characterized by the trilete scar (Y-shaped) structure, as indicated in the inset of Fig. 3C. It was found that the obtained LCS microcapsules retained the original morphology of the raw pollens, indicating the resilience of these LCS to chemical treatments. A vacuum encapsulation protocol was used to load Cur into the obtained LCS shells where no significant residual Cur agglomerates were found on the Cur-loaded LCS surface, as shown in Fig. 3D–F. The inset of Fig. 3F depicts the morphology of the large inner cavity of a cracked-open loaded microcapsule, where it shows a smooth surface, a thick shell wall, and some entrapped materials.

To examine the extent of the extraction process, we performed a combustion elemental analysis (for Cur-loaded LCS, the raw pollens, and unloaded LCS), as depicted in Table 1. The raw pollens’ protein content ($N\% \times 6.25$) [49] was found to be 8.75%, while for the obtained LCS, it was 2.25%. Therefore, a reduction of 74.3% in protein content was achieved, indicative of the

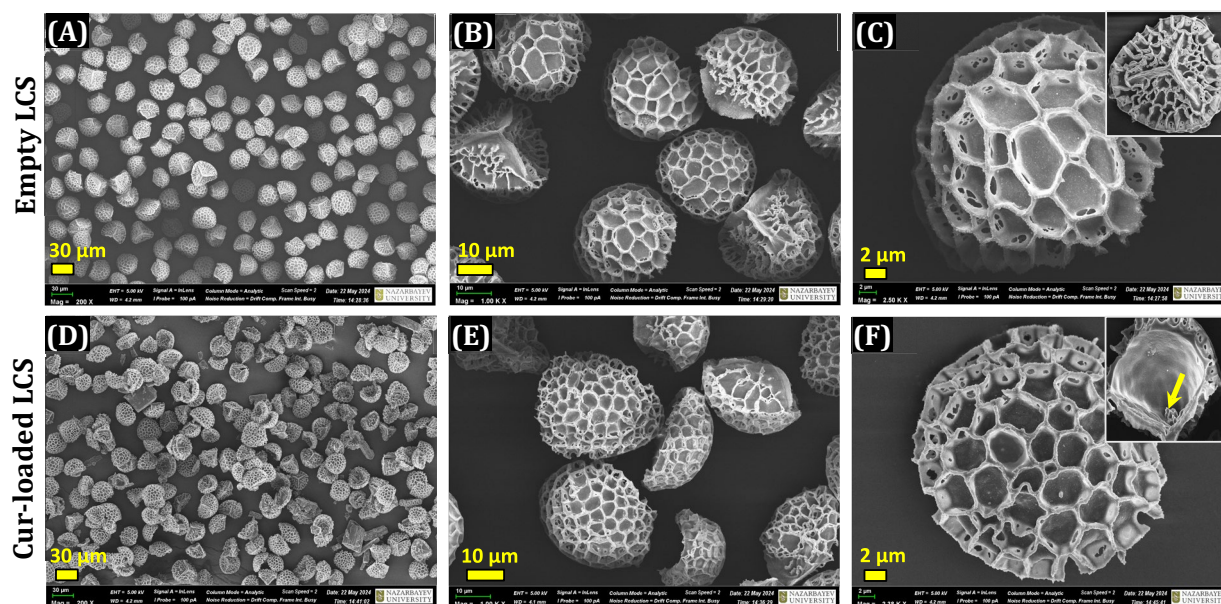


Fig. 3 SEM photos of LCS microcapsules illustrating the surface architecture and morphology. **A–C** Empty LCS before loading at different magnifications showing the characteristic reticular structure of the distal face of the sporopollenin. The inset in **C** showing the trilete scar of the proximal face. **D–F** represent the Cur-loaded LCS at different magnification after washing and drying. The inset in **F** shows a cracked opened microcapsule inner cavity smooth structure with remaining of entrapped material (indicated by a yellow arrow). The empty and Cur-loaded LCSs exhibited robust and intact structure after the extraction and loading steps

successful extraction process. In addition, the change in elemental composition, particularly the reduction in carbon and hydrogen percentages upon Cur loading, confirms that Cur is successfully encapsulated into microcapsules and constitutes a significant fraction of the total mass analyzed. This is because of the "dilution effect" where curcumin ($C_{21}H_{20}O_6$) has zero nitrogen and its presence within the LCS cavities increases the total mass of the microcapsules.

The SEM and elemental CHNS analyses do not demonstrate a successful Cur encapsulation within LCS cores. Consequently, it was essential to validate the loading process of the desired material through the CLSM technique. We obtained 2D (XY) and Z-stack CLSM images along with videos utilizing blue, red, and overlay

channels at random and middle focal positions for both unloaded and Cur-loaded LCS microcapsules. Figure 4A depicts CLSM images at the middle of (XY) depth position of unloaded LCS microcapsules where the autofluorescence of the unloaded LCS shells is evident with black cores (yellow arrow). It is known that the characteristic autofluorescence of the exine shells of pollens is due to the phenolic components of the shell's chemical structure [32–38]. A Z-stack images were recorded as a movie at different focal positions of unloaded LCS, as shown in Video S1 (in ESI), and a split image is constructed and presented in Figure S2 (in ESI). This further confirms the removal of the nitrogenous components from inside the raw pollens, as previously reported elsewhere [32, 74, 75]. Interestingly, after loading the LCS with Cur, a significant gain of enhanced fluorescence was seen at a middle slice position inside the core of the Cur-loaded LCS upon excitation at 561–645 nm, as shown in Fig. 4B, indicating a successful encapsulation of the material. A video demonstrating the enhanced fluorescence cores of overlay Z-stack slices of Cur-loaded LCS sporopollenin at various focal depths has been recorded and is available as Video S2 (in ESI). Additional CLSM images of Cur-loaded LCS microcapsules are presented in Figs. S3, S4, and S5 (in ESI).

Table 1 Elemental CHNS analysis (wt.%) of raw pollens, unloaded LCS, and Cur-loaded LCS

	N [%]	C [%]	H [%]	S [%]
Raw pollens	1.4	58.47	8.371	0.414
Unloaded LCS	0.36	52.2	6.776	0
Cur-loaded LCS	0.32	44.31	6.013	0

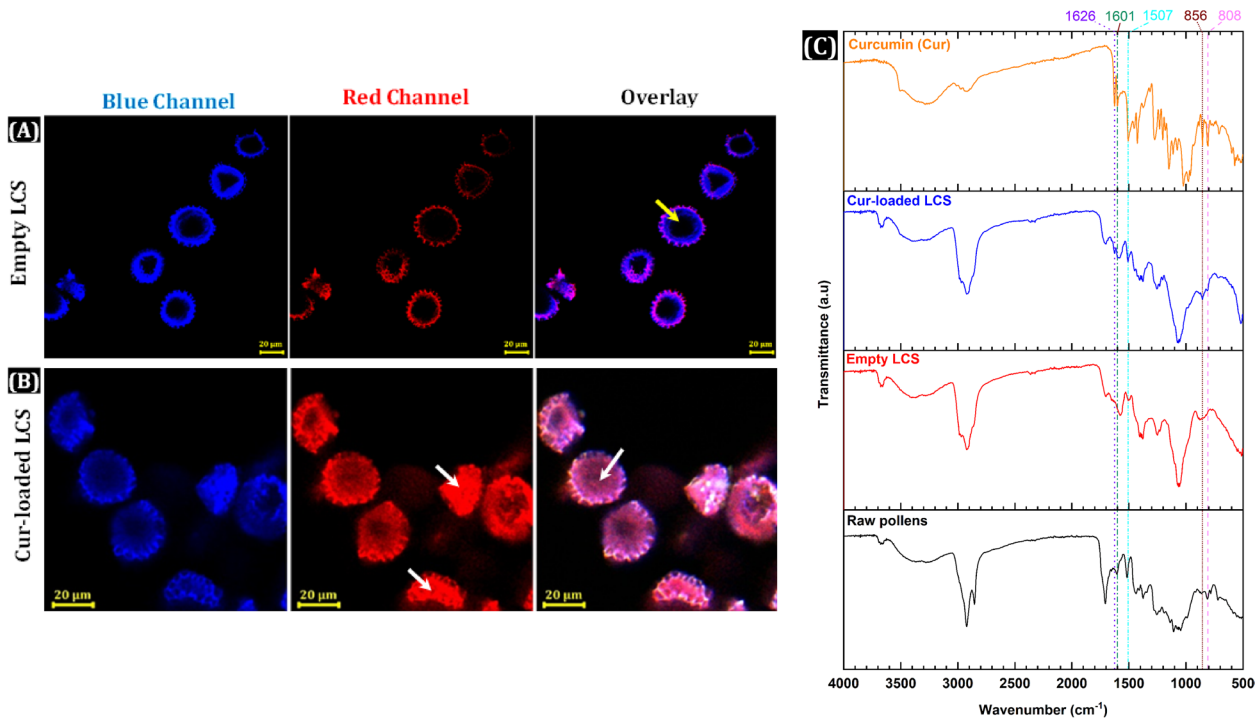


Fig. 4 Confocal laser scanning microscopy (CLSM) images of unloaded (A) and Cur-loaded LCS *Lycopodium clavatum* sporopollenin (B) at blue, red, and overlay channels. The images in A show the autofluorescence of the unloaded LCS with black cavities (yellow arrow). Images in B represent the gain of enhanced fluorescence cores of the LCS as a result of the curcumin payload (white arrows). The photographs were captured at the central slice of the microcapsules in the XY position. C FTIR spectra of raw pollens, unloaded LCS, Cur-loaded LCS, and pure curcumin

FTIR spectra were carried out to further evaluate the encapsulation of Cur into unloaded LCS and to test whether there is any interaction between different samples. Figure 4C shows the FTIR spectra for of raw pollens, unloaded LCS, Cur-loaded LCS, and pure curcumin. The spectra of raw LCS and unloaded LCS are mainly similar but with notable decrease of intensities of some bands of unloaded LCS at 2922.8, 2854.16 and 1707.91 cm^{-1} that was attributed to protein and/or lipid removal from raw pollens after chemical treatment for LCS extraction [34, 76]. In addition, the band at 1065.9 cm^{-1} for unloaded LCS was intensified and can be assigned to stretching vibration of (C–O–C) in cellulose [34]. As seen in Fig. 4C, the spectrum of pure curcumin exhibited characteristics bands at: 3510–3284.21 cm^{-1} (O–H stretching vibration), 2923.53 cm^{-1} (C–H asymmetric stretching), 1626.68 and 1601.46 cm^{-1} (aromatic C=C stretching), 1507.51 cm^{-1} (C=O stretching), 1456.27 cm^{-1} (CH_3 in-plane methoxy group bending vibration), 1427.46 cm^{-1} (olefinic C–H bending vibration), 1275.5 cm^{-1} (aromatic C–O stretching), 1232.19 cm^{-1} (C–O–H in plane methoxy group bending vibration), and 855.92 and 808.05 cm^{-1} , which was attributed to aromatic ring C–C–H out-of-plane bending vibration [77]. Meanwhile, the spectrum of cur-loaded LCS retained characteristic bands of pure curcumin at 1626.68 cm^{-1} , 1601.46 cm^{-1} , 1507.51 cm^{-1} , 1456.27 cm^{-1} , 855.92 cm^{-1} , and 808.05 cm^{-1} , indicative of the presence of curcumin in the loaded LCS, and no significant interaction was observed. However, these retained bands were less intense compared to the pure curcumin, which could be attributed to the encapsulation of most of curcumin inside the cavities of the unloaded LCS microcapsules.

The UV analysis of the encapsulated Cur demonstrated a loading capacity (LC) of $27.0 \pm 2.3\%$ and an entrapping efficiency (EE) of $45.90 \pm 3.8\%$. The values were utilized to modify the dosage of the formulations in the in vivo study. In addition, the LC of Cur obtained in the current study is higher when compared to Cur nano-delivery systems; for instance, the LC of 5% was reported for Cur-loaded liposomal nanoparticles [78]. As a profound departure from the typical “bottom-up” nano-formulations techniques, the “top-down” sporopollenin microcapsules platform exhibited several key advantages, among others: (i) the large cores of the sporopollenin allow high payload compared to nano-delivery systems [40]; (ii) it is now established that sporopollenin exhibited decent chemical, thermal, GI, and photochemical stabilities, entailing them to offer superior protection levels for the encapsulated labile bioactives [79]; (iii) sporopollenin delivery platform is regarded as eco-friendly, non-toxic, scalable, and sustainable, whereas some of nano-formulations are costly and raise nanotoxicity concerns [30]; (iv)

the unique surface ornamental morphology and chemistry of sporopollenin offer a desired mucoadhesion ability that can enhance the bioaccessibility by prolonging the residence time during the localized GI delivery [37, 40, 79].

Cytotoxicity of LCS Microcapsules formulations

It was imperative to explore the noteworthy and safe usage of Cur/LCS formulations for biomedical and drug delivery platforms. Therefore, before improving their therapeutic qualities, we demonstrate the first cytotoxicity evaluation of LCS microparticles loaded with Cur toward normal HSF cell line to ensure the selection of optimal concentration of Cur and its encapsulant. It was reported that human dermal fibroblasts model has been used for the diagnosis and examination of different diseases, such as delayed wound healing in T2DM and Parkinsons and Alzheimers [80]. The effect of different concentrations of pure Cur, unloaded LCS, and Cur-loaded LCS on the viability of HSF after 72 h is depicted in Fig. 5. The selected concentration range (up to 100 $\mu\text{g}/\text{mL}$) for the cytotoxicity assay is in accordance with ISO 10993-5 guidelines for establishing a comprehensive safety margin for biomaterials [81]. Although systemic plasma levels of curcumin are typically lower, such concentrations are physiologically relevant for assessing localized exposure within the gastrointestinal lumen and for determining the maximum tolerated dose of the delivery system [82, 83]. No cytotoxic effect was observed for unloaded LCS and Cur-loaded LCS samples at all concentrations. However, Cur sample exhibited concentration-dependent trend where the cell viability decreased to ~60% at higher concentration of 100 $\mu\text{g}/\text{mL}$ with respect to the control. This is consistent with few studies suggesting decreased cell viability of human dermal fibroblasts cells, based on its origin, at high Cur concentration which can be regulated by coadministration of antioxidants [84, 85]. Our findings suggested that encapsulation of Cur into robust natural LCS microcapsules enhanced cell viability when compared to pure non-encapsulated Cur at concentrations > 100 $\mu\text{g}/\text{mL}$. This could be attributed primarily to the controlled and sustained release pattern provided by the sporopollenin shell. While free curcumin exposes the cells to a sudden, high-concentration “bolus” that can trigger localized oxidative stress or membrane disruption, the LCS microcapsules release the curcumin steadily. This prevents the “burst effect,” keeping the concentration of active curcumin below the toxic threshold for the cells throughout the incubation period. Additionally, the sporopollenin exine itself is highly biocompatible and chemically inert, acting as a protective barrier that minimizes direct, high-density contact between the drug and the cellular membrane [48].

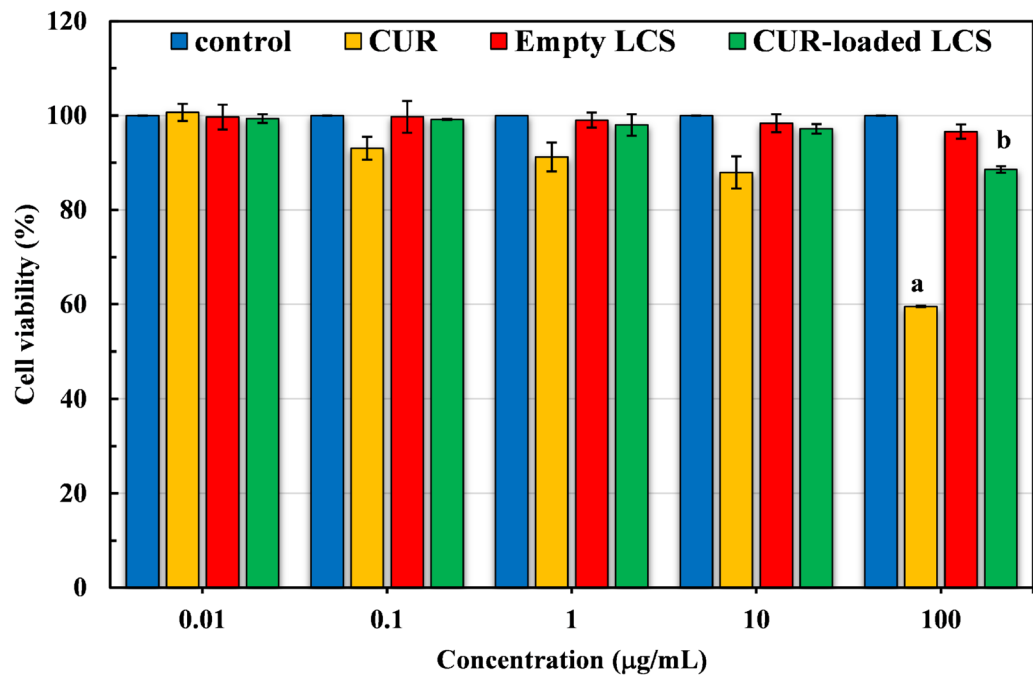


Fig. 5 Cell viability of pure Cur, LCS, and Cur-loaded LCS assessed by SRB protocol in HSF cell line for 72 h. Normal untreated HSF cells represents the control sample. The data shown as mean $\pm$ SE ($n=3$) with significance defined at $p<0.05$. Statistically significant differences were obtained relative to; ^athe control group. ^b $p<0.05$ indicates a significant difference between Cur-loaded LCS and free Cur at the same concentration

Changes in body weight

As indicated in Table 2, at week 0, following diabetes induction and prior to the commencement of treatments, all diabetic rat groups (4, 5, and 6) showed a statistically significant rise in body weight relative to LCS, the control, and Cur groups ($p<0.05$). At the conclusion of the treatment period (the fourth week), a substantial weight reduction was observed in the untreated diabetic animals (group 4) relative to all experimental groups, with the exception of group 5 ($p<0.05$). The Dia+Cur-loaded LCS group (group 6) maintained their body weight significantly ($p<0.05$) in comparison with the Dia+Cur and Dia groups (groups 4 and 5, respectively), indicating a better weight preservation. No substantial difference was observed in the body weight between LCS and Cur-treated rats (groups 2 and 3) and control rats.

Blood glucose level

Following diabetes induction and before the commencement of treatment (week 0), all diabetic rats exhibited a substantial elevation ($p<0.05$) in FBG compared to the control, LCS, and Cur groups (Fig. 6). All diabetic rats that received treatment exhibited a variable decrease in FBG throughout the treatment period. During the second week of the treatment period, treated diabetic rats receiving Cur-loaded LCS exhibited substantially decreased FBG levels compared to untreated diabetic rats (Dia group) ($p<0.05$). In contrast, the Dia+Cur-treated rats demonstrated no major differences.

At the conclusion of the treatment period (week 4), both the Dia+Cur-loaded LCS and Dia+Cur groups exhibited considerably decreased FBG levels compared to the untreated diabetic group ($p<0.05$). Furthermore, Dia rats receiving Cur-loaded LCS demonstrated superior

Table 2 Mean values of initial, final body weights (g) of all studies groups over an experimental period

Parameters	Groups				Dia + Cur	Dia + Cur-loaded LCS
	Control	Cur	LCS	Dia		
Initial weight (g)	207.67 $\pm$ 12.74	206.33 $\pm$ 9.80 ^{def}	207.50 $\pm$ 8.31 ^{def}	259.83 $\pm$ 11.17 ^{a,b,c}	261.83 $\pm$ 17.13 ^{a,b,c}	259.50 $\pm$ 7.71 ^{a,b,c}
Final weight (g)	270.67 $\pm$ 4.66	273.33 $\pm$ 4.90 ^{de}	262.50 $\pm$ 3.69 ^{de}	187.67 $\pm$ 16.67 ^{a,b,c,f}	205.83 $\pm$ 6.85 ^{a,b,c}	249.50 $\pm$ 8.22 ^d

Values are given as (mean $\pm$ SE, $n=6$), with significance defined at $p<0.05$. Statistically significant differences were observed relative to; ^athe control group, ^bthe Cur group, ^cthe LCS group, ^dthe untreated Dia group, ^ethe Dia + Cur group, and ^fthe Cur-loaded LCS group

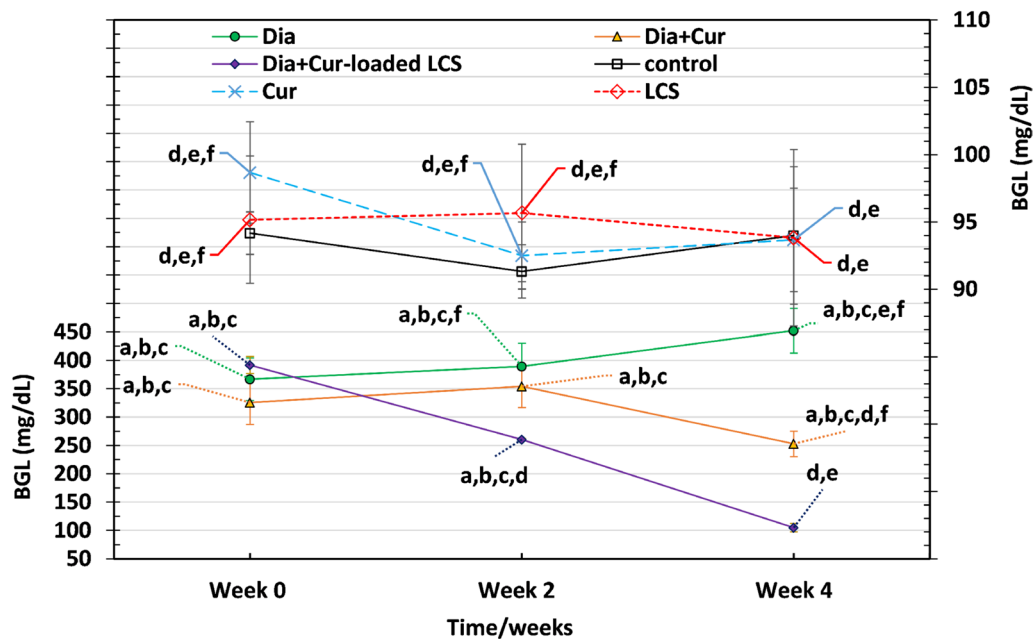


Fig. 6 Variation of blood glucose levels (BGL, mg/dL) against the treatment period for various examined groups. Values are given as (mean $\pm$ SE, $n=6$), with significance defined at $p<0.05$. Statistically significant differences were revealed relative to ^athe control group, ^bthe Cur group, ^cthe LCS group, ^dthe untreated Dia group, ^ethe Dia + Cur group, and ^fthe Cur-loaded LCS group

maintenance of glycemic values, indicating the beneficial impact of our formulation on glycemic control (Fig. 6).

Biochemical analysis

Insulin and HOMA-IR

At the conclusion of the treatment period, the results obtained (Table 3) indicated a substantial decline in serum insulin levels in untreated diabetic animals (group 4) relative to the LCS, Cur, and control groups ($p<0.05$). The Cur-loaded LCS group treatment in group 6 displayed markedly elevated ($p<0.05$) serum insulin levels, approaching normalization when compared to controls. At the conclusion of the treatment period, all diabetic animals in the treated groups (5 and 6) exhibited a substantial decline in HOMA-IR compared to the untreated diabetic group (4) ($p<0.05$), with nearly total correction of the insulin resistance index observed in the Cur-loaded LCS group (Table 3).

Lipid profile

Table 4 implies that all diabetic rats exhibited hyperlipidemia, characterized by markedly decreased HDL-C and substantially elevated TGs, LDL-C, and TC levels relative to the majority of other groups ($p<0.05$). The administration of Cur-loaded LCS and Cur to HFD/STZ-induced rats resulted in a significant reduction in TGs, LDL-C, and TC levels, accompanied by a substantial rise in HDL-C levels when compared to the untreated diabetic group (group 4). The Cur-loaded LCS markedly improved the serum lipid profile in group 6 relative to the Dia group ($p<0.05$). Cur-loaded LCS treatment demonstrated a substantial difference ($p<0.05$) in lipid profile relative to Cur-treated animals (group 5), achieving almost full normalization of TGs, LDL-C, and TC levels in group 6.

Table 3 Data for serum insulin levels and HOMA-IR across the various studied groups

Parameters	Groups				Dia + Cur	Dia + Cur-loaded LCS
	Control	Cur	LCS	Dia		
Insulin (ng/ml)	1.74 $\pm$ 0.06	1.81 $\pm$ 0.04 ^{d,e}	1.81 $\pm$ 0.05 ^{d,e}	0.83 $\pm$ 0.13 ^{a,b,c,f}	1.04 $\pm$ 0.09 ^{a,b,c,f}	1.81 $\pm$ 0.07 ^{d,e}
HOMA-IR	0.40 $\pm$ 0.03	0.42 $\pm$ 0.02 ^{d,e}	0.41 $\pm$ 0.03 ^{d,e}	0.87 $\pm$ 0.21 ^{a,b,c,e,f}	0.65 $\pm$ 0.06 ^{a,b,c,d}	0.48 $\pm$ 0.04 ^d

Significance is defined at $p<0.05$, and the results are presented as the mean $\pm$ SE, with a sample size of $n=6$. Statistically significant differences were observed relative to; ^athe control group, ^bthe Cur group, ^cthe LCS group, ^dthe untreated Dia group, ^ethe Dia + Cur group, and ^fthe Cur-loaded LCS group

Hepatic function biomarkers

Table 5 indicates that serum AST, ALP, and ALT levels were substantially higher in the Dia group compared to the LCS, control, and Cur groups ($p < 0.05$). The Cur treatment in the Dia + Cur group induced substantial decreases in all of the aforementioned parameters compared to the Dia group, although values remained higher than those observed in the LCS, Cur, and control groups ($p < 0.05$). Treatment of Dia rats with Cur-loaded LCS resulted in almost normalized ALP and ALT values. Dia rats exhibited considerably lower ALB and TP levels than the LCS, control, and Cur groups ($p < 0.05$). In contrast to Cur, Cur-loaded LCS treatment groups significantly mitigated hepatotoxicity effects and resulted in higher concentrations of ALB and TP compared to the Dia group ($p < 0.05$).

Kidney function

The Dia group exhibited elevated serum levels of creatinine and urea compared to the other groups ($p < 0.05$). The HFD/STZ-induced rats (treated with Cur-loaded LCS) exhibited a greater decrease in creatinine levels as well as serum urea, closer to control values, compared to the group treated solely with Cur (group 5) (Table 6).

Oxidative stress

MDA, SOD, and GSH serum values for all groups are depicted in Fig. 7A–C. There were increased MDA levels in HFD/STZ-induced rats significantly compared to other groups ($p < 0.05$). Cur-loaded LCS treatment alleviated the oxidative stress, leading to markedly declined MDA relative to Dia + Cur and Dia-treated groups ($p < 0.05$). Nevertheless, the GSH and SOD activities were substantially decreased in the untreated diabetic group than other animals ($p < 0.05$). Cur oral administration markedly elevated antioxidant activity, notably SOD

Table 4 Different investigated groups' serum values of the lipid profile parameters

Parameters	Groups				Dia + Cur	Dia + Cur-loaded LCS
	Control	Cur	LCS	Dia		
TC (mg/dl)	120.92 ± 4.27	117.05 ± 9.84 ^{d,e}	119.14 ± 7.45 ^{d,e}	300.43 ± 9.75 ^{a,b,c,e,f}	198.93 ± 20.38 ^{a,b,c,d,f}	148.39 ± 4.09 ^{d,e}
HDL-C (mg/dl)	47.77 ± 2.36	51.93 ± 2.88 ^{d,e}	48.84 ± 2.64 ^{d,e}	32.56 ± 1.28 ^{a,b,c,f}	33.96 ± 0.99 ^{a,b,c,f}	47.71 ± 2.44 ^{d,e}
LDL-C (mg/dl)	39.94 ± 1.66	42.64 ± 2.09 ^{d,e,f}	37.29 ± 2.83 ^{d,e,f}	213.72 ± 5.66 ^{a,b,c,e,f}	125.39 ± 11.71 ^{a,b,c,d,f}	80.06 ± 3.92 ^{a,b,c,d,e}
TGs (mg/dl)	100.39 ± 6.41	98.18 ± 2.63 ^{d,e}	98.81 ± 5.21 ^{d,e}	230.27 ± 14.77 ^{a,b,c,e,f}	167.33 ± 5.50 ^{a,b,c,d,f}	129.23 ± 2.21 ^{d,e}

Significance is defined at $p < 0.05$, and the results are presented as the mean ± SE, with a sample size of $n = 6$. Statistically significant differences were observed relative to; ^athe control group, ^bthe Cur group, ^cthe LCS group, ^dthe untreated Dia group, ^ethe Dia + Cur group, and ^fthe Cur-loaded LCS group

Table 5 Serum values of the hepatic function parameters in the different studied groups

Parameters	Groups				Dia + Cur	Dia + Cur-loaded LCS
	Control	Cur	LCS	Dia		
ALT (U/L)	45.76 ± 3.71	47.63 ± 2.25 ^{d,e}	42.54 ± 3.69 ^{d,e}	95.19 ± 4.68 ^{a,b,c,f}	92.25 ± 5.08 ^{a,b,c,f}	52.95 ± 2.47 ^{d,e}
AST (U/L)	67.84 ± 5.033	68.24 ± 3.62 ^{d,e,f}	66.97 ± 5.48 ^{d,e,f}	129.33 ± 8.71 ^{a,b,c,f}	108.74 ± 6.14 ^{a,b,c}	89.37 ± 2.73 ^{a,b,c,d}
ALP (U/L)	88.39 ± 4.89	95.24 ± 7.25 ^{d,e}	87.90 ± 6.30 ^{d,e}	181.06 ± 12.19 ^{a,b,c,e,f}	140.35 ± 11.50 ^{a,b,c,d}	112.47 ± 3.48 ^d
Total protein (g/dl)	8.93 ± 0.44	9.08 ± 0.53 ^{d,e}	9.17 ± 0.42 ^{d,e}	3.41 ± 0.17 ^{a,b,c,f}	4.77 ± 0.43 ^{a,b,c,f}	8.03 ± 0.32 ^{d,e}
Albumin (g/dl)	3.55 ± 0.15	4.04 ± 0.16 ^{d,e}	3.71 ± 0.16 ^{d,e}	2.05 ± 0.13 ^{a,b,c,f}	2.67 ± 0.20 ^{b,c,f}	4.35 ± 0.38 ^{d,e}

Significance is defined at $p < 0.05$, and the results are presented as the mean ± SE, with a sample size of $n = 6$. Statistically significant differences were observed relative to; ^athe control group, ^bthe Cur group, ^cthe LCS group, ^dthe untreated Dia group, ^ethe Dia + Cur group, and ^fthe Cur-loaded LCS group

Table 6 Serum levels of renal function biomarkers in the different studied groups

Parameters	Groups				Dia + Cur	Dia + Cur-loaded LCS
	Control	Cur	LCS	Dia		
Urea (mg/dl)	59.06 ± 2.37	48.39 ± 4.90 ^d	48.65 ± 5.35 ^d	107.53 ± 17.11 ^{a,b,c,f}	79.68 ± 4.00	45.98 ± 2.49 ^d
Creatinine (mg/dl)	0.82 ± 0.04	0.86 ± 0.03 ^{d,e}	0.85 ± 0.04 ^{d,e}	2.12 ± 0.11 ^{a,b,c,f}	1.79 ± 0.20 ^{a,b,c,f}	0.99 ± 0.05 ^{d,e}

Significance is defined at $p < 0.05$, and the results are presented as the mean ± SE, with a sample size of $n = 6$. Statistically significant differences were observed relative to; ^athe control group, ^bthe Cur group, ^cthe LCS group, ^dthe untreated Dia group, ^ethe Dia + Cur group, and ^fthe Cur-loaded LCS group

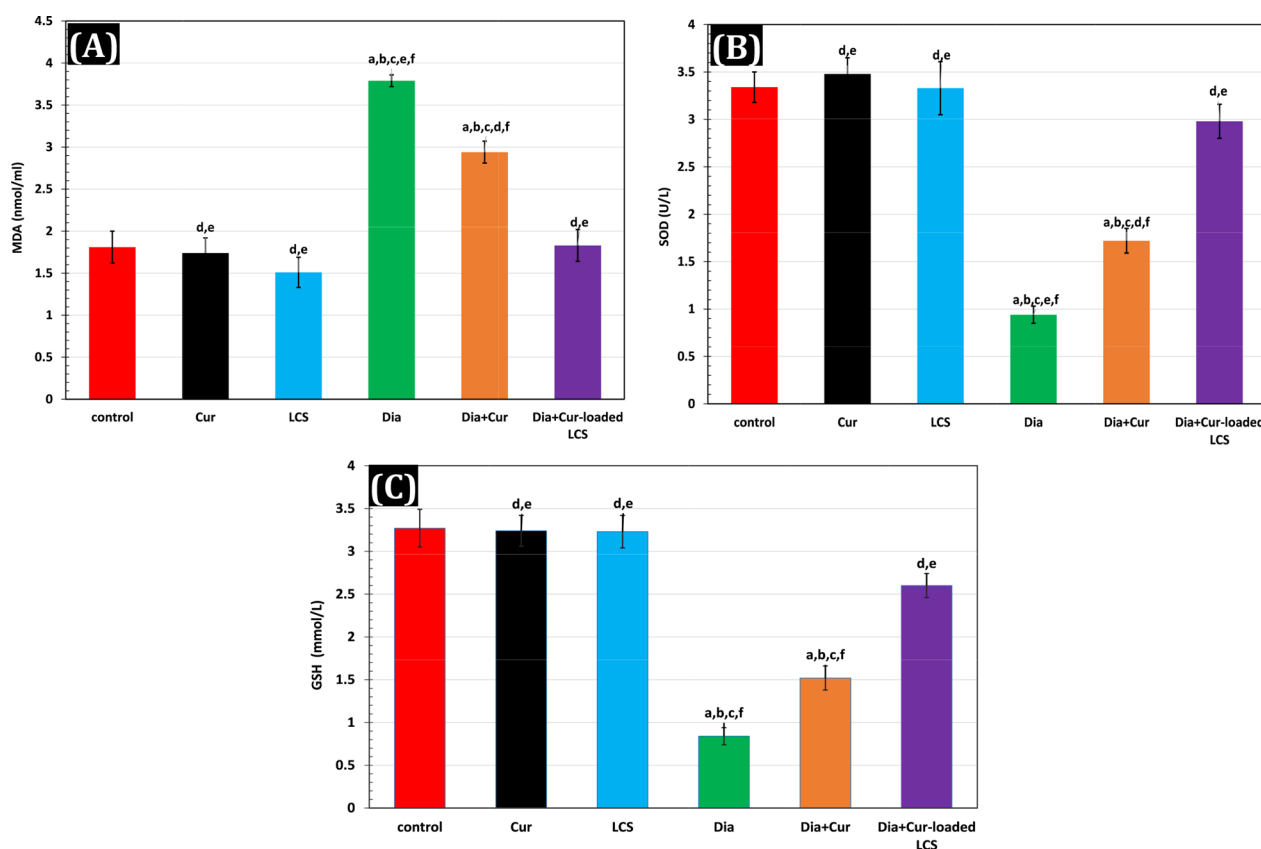


Fig. 7 **A** Levels of MDA, **B** SOD, and **C** GSH at the end of the treatment. Significance is determined at $p < 0.05$, and results are presented as the mean $\pm$ SE, with a sample size of $n = 6$. Statistically significant differences were obtained relative to; ^athe control group, ^bthe Cur group, ^cthe LCS group, ^dthe untreated Dia group, ^ethe Dia + Cur group, and ^fthe Cur-loaded LCS group

activity, than the Dia group ($p < 0.05$). However, the Cur-loaded LCS group demonstrated approximate normalization of GSH and SOD values in group 6.

Serum cytokines

At the conclusion of the treatment period, the Dia group exhibited significantly elevated serum levels of TNF- α and IL-6 in comparison with other groups (Fig. 8A, B). However, Cur and Cur-loaded LCS significantly reduced TNF- α and IL-6 levels than the Dia group. The serum levels of IL-10 were significantly reduced in the Dia group than in controls (Fig. 8C) ($p < 0.05$). Animals receiving Cur and Cur-loaded LCS showed considerably elevated IL-10 levels ($p < 0.05$) compared to Dia rats (Fig. 8C).

Histopathological assessments

Pancreas histopathology

Microscopic examination of the pancreas from the control, Cur, and LCS groups revealed normal cells of islets of Langerhans, regular blood capillaries, and

standard pancreatic acini with basal basophilia and apical acidophilia (Fig. 9a–c). In group 4, the combination of low-dose STZ injection and HFD resulted in severe degenerative changes, including islet of Langerhans atrophy with apoptotic appearance of most cells and vacuolations, degenerated acini, apoptotic acini cells, congested blood vessels, and interstitial hemorrhage (Fig. 9d). Cur treatment (group 5) resulted in a moderate improvement, with an increase in cells homing within the islets of Langerhans with a few vacuolations, inflammatory cell infiltration, and congested blood capillaries (Fig. 9e). Concretely, the Dia+Cur-loaded LCS group showed improvement with a rise in the number of cells homing islets of Langerhans, which were detected in their normal shape and scarce vacuolations. Intact blood capillaries and a few interstitial hemorrhages were observed (Fig. 9f). Furthermore, the pancreatic acini exhibited a normal microstructure in all specimens from the Cur-loaded LCS and Cur treatment groups.

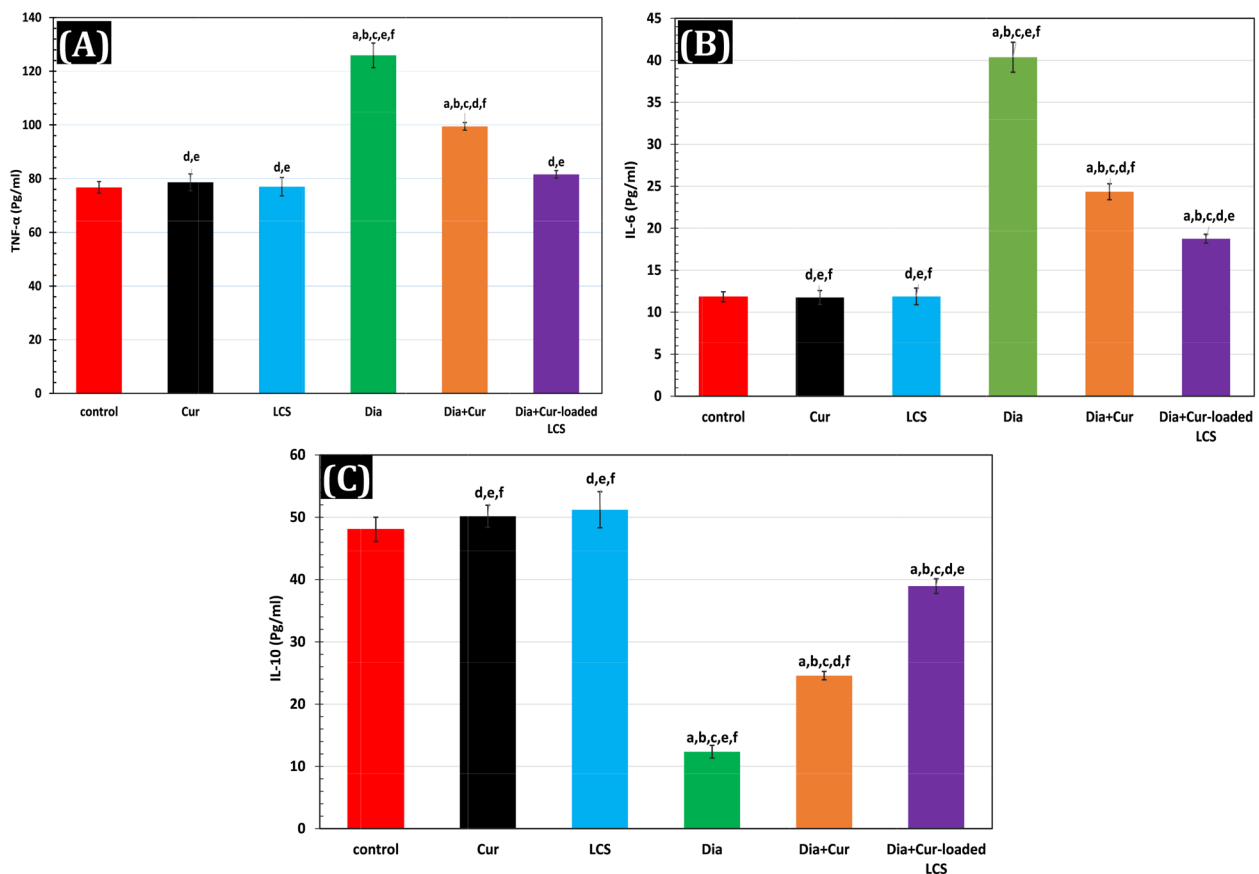


Fig. 8 **A** Levels of TNF- α , **B** IL-6, and **C** IL-10 at the end of the treatment. Significance is determined at $p < 0.05$, and results are presented as the mean $\pm$ SE, with a sample size of $n = 6$. Statistically significant differences were observed relative to; ^athe control group, ^bthe Cur group, ^cthe LCS group, ^dthe untreated Dia group, ^ethe Dia + Cur group, and ^fthe Cur-loaded LCS group

(See figure on next page.)

Fig. 9 Photomicrographs illustrate the histological variations in pancreatic tissue slices of investigated groups (Hematoxylin & Eosin Stain, Magnification Power = $\times 400$ & Scale Bar = $50 \mu\text{m}$) as follows: **a** Pancreas Sections from Control Group displaying normal cells of islets of Langerhans (rectangle), regular blood capillaries (arrowhead), and standard pancreatic acini with its basal basophilia and apical acidophilia (arrow). **b** Pancreas Sections from Cur Group exhibiting typical structure islets of Langerhans (rectangle), integral blood capillaries (arrowhead), and standard pancreatic acini with their basal basophilia and apical acidophilia (arrow). **c** Pancreas Sections from LCS Group signifying intact islets of Langerhans (rectangle), normal blood capillaries (arrowhead), and typical structure of pancreatic acini with its basal basophilia and apical acidophilia (arrow). **d** Pancreas Section from Dia Group highlighting severe degenerative changes assembled as atrophy of islets of Langerhans with apoptotic appearance of most cells and vacuolations (rectangle), degenerated acini (curvy arrow), apoptotic cells of acini (arrow), congested blood vessels (arrowhead), and interstitial hemorrhage (wave arrow). **e** Pancreas Section from Dia + Cur Group marking moderate improvement with obvious increase in number of cells homing in islets of Langerhans and detected in their normal shape, and few vacuolations (rectangle), inflammatory cells infiltration (arrow with tail), congested blood capillaries (arrowhead), along with limited areas suffering from interstitial hemorrhage (wave arrow). Observe pancreatic acini existed in their regular structure (arrow). **f** Pancreas Section from Dia + Cur-loaded LCS Group exhibiting the best noticeable improvement with rise in number of cells homing islets of Langerhans and detected in their normal shape and scarce vacuolations (rectangle), intact blood capillaries (arrowhead), few interstitial hemorrhages (wave arrow) along with regular existence of pancreatic acini (arrow)

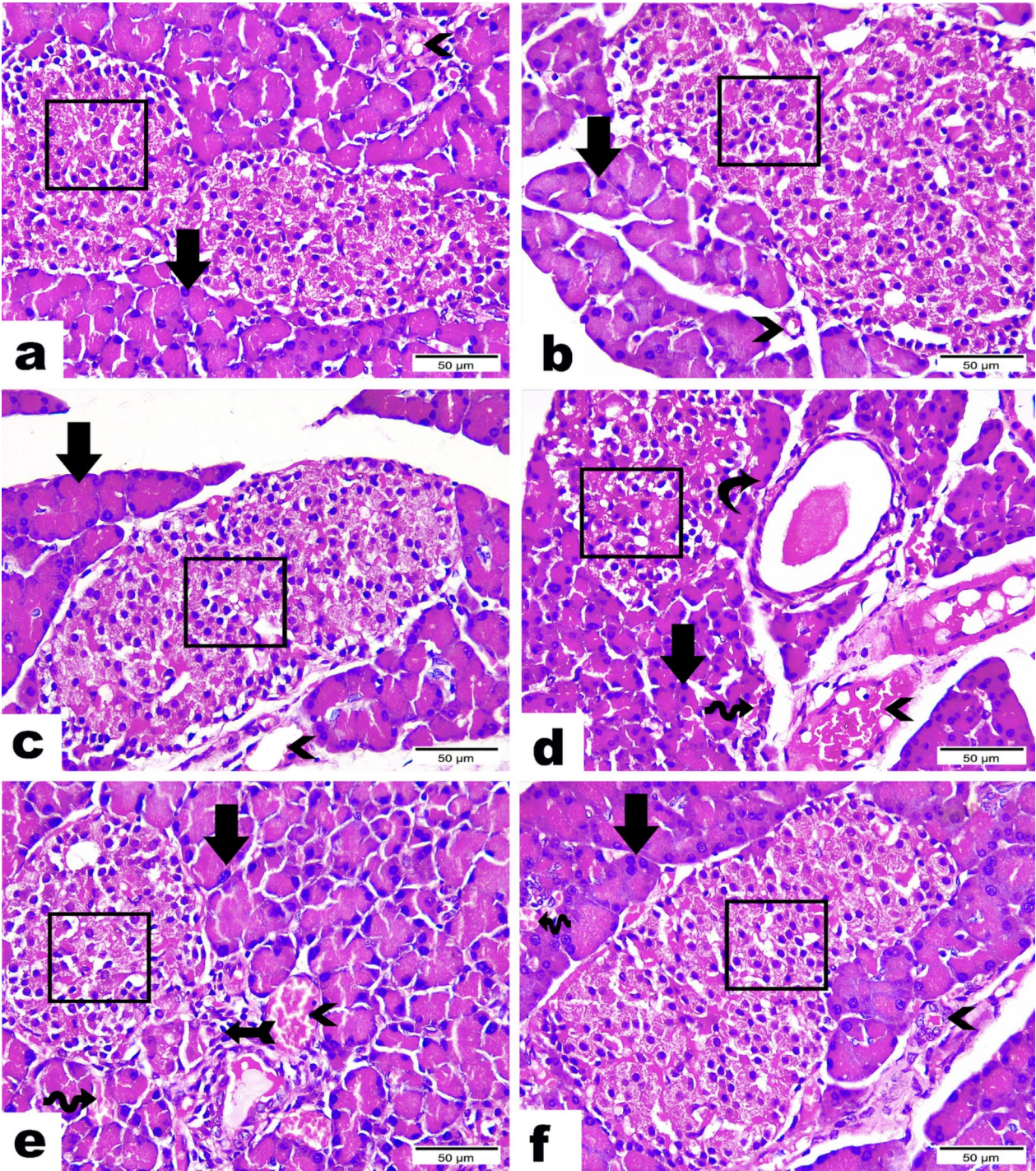


Fig. 9 (See legend on previous page.)

Liver histopathology

The sections of hepatic tissues from control, LCS, and Cur-treated rats revealed normal central vein architecture

characterized by intact endothelial lining, regular hepatic cords containing large hepatocytes with vesicular, central, and spherical nuclei, as well as normal blood sinusoids (Fig. 10a–c). Conversely, liver sections from the Dia rats showed severe hepatic damage assembling in

degraded endothelium of the central vein, hepatocellular degeneration with significant deep basophilic apoptotic hepatocytes with cytoplasmic vacuolations. Furthermore, elevated inflammatory cell infiltration and blood sinusoid dilation were detected (Fig. 10d). The administration of Cur in group 5 demonstrated a reduction in hepatic damage relative to the Dia group, evidenced by an intact central vein and minimal dilation of blood sinusoids. In addition to some cells exhibiting cytoplasmic vacuolations, there is a significant reduction in hepatocellular degeneration. A limited number of interstitial inflammatory cells were also detected (Fig. 10e). The Cur-loaded LCS (group 6) demonstrated significant enhancement in tissue architecture, featuring a normal central vein, intact blood sinusoids, and regular hepatic cords, with hepatocytes appearing nearly normal, aside from a few exhibiting cytoplasmic vacuolations. Nevertheless, fewer inflammatory cells were observed (Fig. 10f).

Kidney histopathology

The renal tissue sections from control, Cur, and LCS-treated rats displayed normal renal glomeruli and renal tubules (Fig. 11a–c). Dia rats exhibited significant renal pathology characterized by atrophy of the renal corpuscle, dilation of the glomerular space, and degeneration of Bowman's capsule. Renal tubules exhibited hydropic degeneration or pronounced basophilic apoptosis. Additionally, congested blood vessels, interstitial congestion, and infiltration of inflammatory cells were observed (Fig. 11d). Histological analysis of the Dia+Cur-treated group indicates moderate tissue recovery, characterized by the presence of recovered renal corpuscles, mild congestion, vacuolations, and degradation of Bowman's capsule. Renal tubules exhibited either minimal hydropic

degeneration of epithelial lining cells or a predominance of normal cells characterized by bright, vesicular nuclei. A notable reduction in aggregated inflammatory cells is observed, accompanied by minimal interstitial edema (Fig. 11e). Renal sections from the Cur-loaded LCS demonstrated restoration of the histological structure of the majority of renal corpuscles. Renal tubules exhibited predominantly normal architecture, with the exception of a few cells displaying cytoplasmic vacuolations. Mild interstitial hemorrhage and infiltrating inflammatory cells were observed (Fig. 11f).

Discussion

Diabetes, a prevalent endocrine disorder, is defined by lipid metabolic disorder and hyperglycemia [86]. Efforts are made to identify an ecologically sustainable, relatively safe, and economically viable solution for diabetes complications. Nutraceuticals provide advantages across various therapeutic domains, including diabetes; however, numerous nutraceuticals face challenges related to biodegradability, solubility, and bioavailability [87–89]. In the current study, the antidiabetic efficacies of Cur and Cur-loaded LCS were investigated using a classical T2DM model induced by HFD- and STZ-treated rats. The accepted model was employed to examine T2DM pathophysiology. HFD induces insulin resistance, while a low dose of STZ results in mildly impaired β -cell function [90, 91].

The current data reveal that the Dia group is badly impacted by a number of variables, including body weight loss, hyperglycemia, insulin resistance, inflammation, oxidative stress, lipid metabolic abnormalities, and liver and kidney issues. These findings align with previous

(See figure on next page.)

Fig. 10 Photomicrographs showed histological changes in liver tissue slices (Central Vein Area) among groups (Hematoxylin & Eosin Stain, Magnification Power = $\times 400$, Scale Bar = 50 μm): **a** Liver section from Control Group showing normal central vein architecture with intact endothelium (rectangle), regular hepatic cords containing large hepatocytes with central, spherical, and vesicular nuclei (arrow), and blood sinusoids with von Kupffer cell lining (arrowhead). **b** Liver section from the Cur Group shows a structure comparable to the negative control group, with intact lining endothelium of the central vein (rectangle), regular hepatic cords, hepatocytes with central, spherical, and vesicular nuclei (arrow), along with standard blood sinusoids (arrowhead). **c** Liver section from LCS Group exhibiting a structure identical to negative control group that presented as integral lining endothelium of central vein (rectangle), consistent hepatic cords, hepatocytes with central, spherical, and vesicular nuclei (arrow), along with standard blood sinusoids (arrowhead). **d** Liver Section from Dia Group displaying serious hepatic injury assembling in deteriorated endothelium of central vein (rectangle), micro vesicular steatosis (circle) hepatocellular degeneration with marked deep basophilic apoptotic hepatocytes (arrow with tail), vacuolations (curvy arrow) except few hepatocytes still existed in normal structure (arrow). Moreover, dilatation along blood sinusoids (arrowhead) as well as infiltrations of inflammatory cells (wave arrow) were also noticed. **e** Liver Section from Dia + Cur-treated group revealing a lesser hepatic damage than the DIA group evidenced by intact central vein (rectangle) and mild dilatation of blood sinusoids (arrowhead). There is a notable decline in hepatocellular degeneration, characterized by the regular appearance of hepatic cords and intact hepatocytes (arrow), although a few cells still exhibit cytoplasmic vacuolations (curvy arrow). Minimal infiltration of interstitial inflammatory cells was observed between the hepatic cords (wave arrow). **f** Liver section from the Dia + Cur-loaded LCS-treated group exhibits significant improvement in tissue architecture, characterized by a normal central vein (rectangle), intact blood sinusoids (arrowhead), and regular hepatic cords with nearly normal hepatocytes (arrow) except scarce ones with cytoplasmic vacuolations (curvy arrow). Though, mild inflammatory cells (wave arrow) are still observed

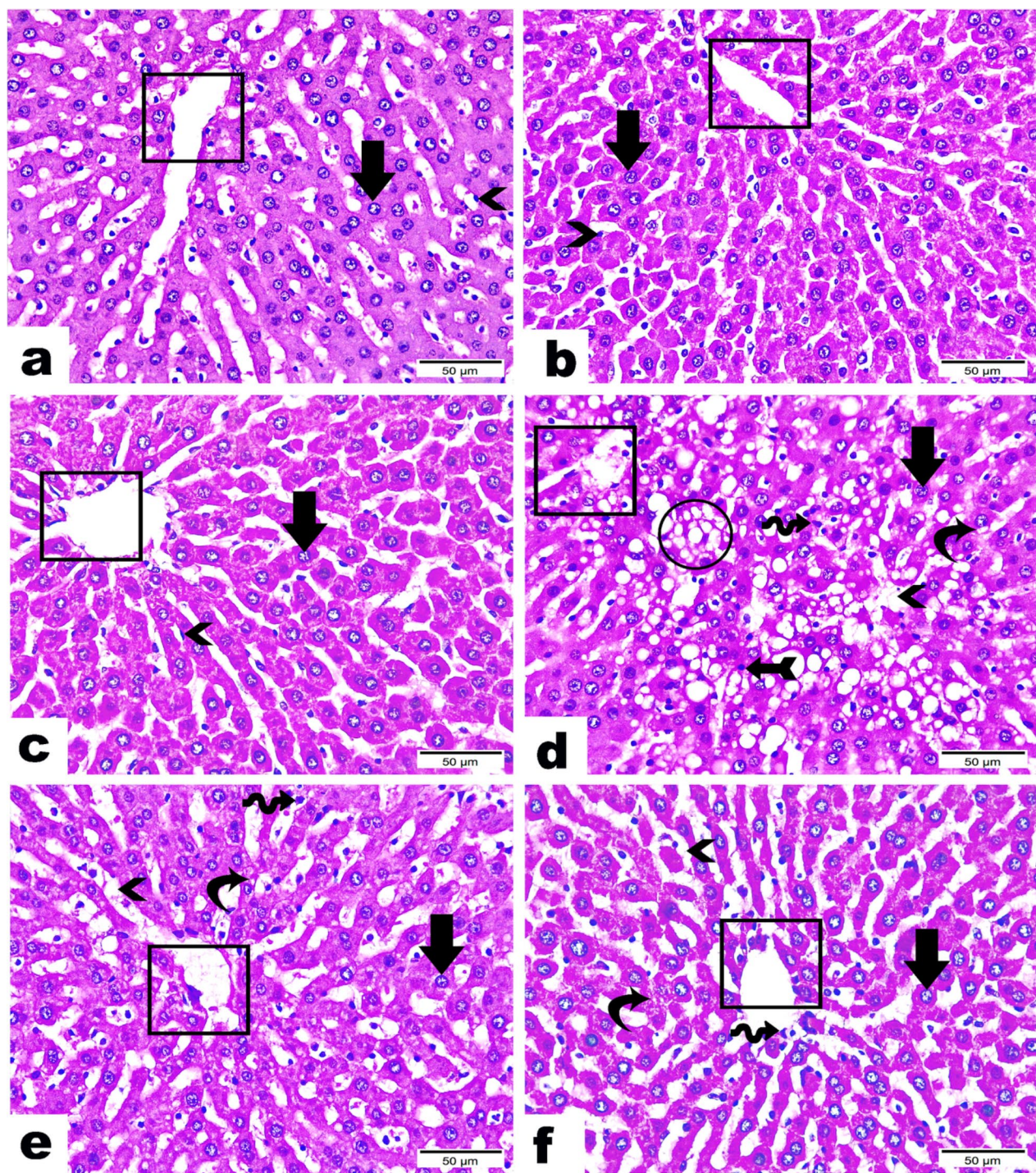


Fig. 10 (See legend on previous page.)

studies [36, 60, 92, 93]. Diabetic rats administered Cur and Cur-loaded LCS exhibited a greater rise in body weight when compared with diabetic rats, likely attributable to their protective effects in mitigating muscle loss. Prior research [94, 95] indicated that oral administration

of Cur (100 mg/kg) over 7 and 8 weeks prevents weight loss in various diabetic rat models. Furthermore, another study [96] reported that an 8-week treatment of Cur at a dosage of 80 mg/kg/day significantly enhances the body weight of diabetic rats.

The current study indicates that Dia rats administered Cur-loaded LCS exhibited the maintenance of the glycemia values after the treatment. This finding demonstrates the beneficial impacts of the optimized formula on glycemic control and its efficacy in maintaining Cur, as well as stabilizing blood glucose levels over an extended duration. The Cur-loaded LCS-treated group exhibited a substantial rise in serum insulin, whereas the Cur-treated group indicated no substantial alteration compared to untreated diabetic animals. Additionally, Cur-loaded LCS treatment displayed a notable improvement relative to Cur treatment regarding insulin resistance. Cur-loaded LCS treatment in group 6 displayed a substantial rise in serum insulin levels and a reduction in insulin resistance, approaching normalization of values compared to controls.

Multiple studies indicate that Cur is an effective anti-hyperglycemic agent. An experimental study found that Cur therapy can change insulin resistance and lower the levels of Glycogen Synthase Kinase-3 Beta (GSK-3 β) and islet amyloid polypeptide (IAPP) in the blood [97]. Another study determined that Cur treatment substantially decreased FBG, glycated hemoglobin (HbA1c), and estimated average glucose (eAG) levels [98]. Recent studies [99, 100] reported that Cur improved glucose intolerance and insulin sensitivity, along with reducing FBG and upregulating glucose transporter 4 (GLUT4) gene expression. Furthermore, Cur activates peroxisome proliferator-activated receptor gamma (PPAR γ), which modulates glucose metabolism, inhibiting glucose transporter 2 (GLUT2) surface expression and advanced glycation end products (AGEs) accumulation [101–103].

Compared to the individual treatment of Cur, the Cur-loaded LCS group demonstrated a substantial

enhancement in lipid profile. The results indicate that Cur-loaded LCS may normalize TGs, HDL-C, and TC values, suggesting a distinct functionality and characteristic of Cur in the formulation studied. A randomized, double-blind clinical trial [104] indicated that Cur treatment could downregulate TG levels in T2DM, potentially decreasing the risk of diabetic complications. Prior studies [105–107] concluded that Cur enhances insulin resistance and glucose homeostasis in mice model of T2DM via the modulation of lipid metabolism. Also, the results indicated that Cur significantly decreased plasma levels of free fatty acids (FFA), TC, and TGs in mice with T2DM. Another study [108] concluded that the effect of Cur on insulin resistance may be associated with reductions in FFA and LDL levels in T2DM rats. Previous studies [109, 110] have shown that Cur and nanocurcumin treatment significantly reduced serum levels of very low-density lipoproteins (VLDL), TC, LDL, and TGs while elevating HDL levels in diabetic rats. Furthermore, nanocurcumin demonstrated greater efficacy in improving the lipid profile than Cur in STZ-induced diabetic rats. Cur effectively enhanced insulin sensitivity, fat accumulation, and blood lipid levels in HFD-induced obese mice with T2DM by modulating the target genes of modulating sterol regulatory element-binding proteins (SREBPs) and metabolism-associated genes in adipose tissues and the liver [10, 111]. Prior research [112] has demonstrated comparable lipid-lowering effects of Cur, indicating that oral administration may reduce TC, TGs, and LDL-C in rats with sodium arsenite-induced liver disease.

The results indicated substantially elevated liver enzymes and reduced TP and ALB levels in the

(See figure on next page.)

Fig. 11 Photographs underlined the histological differences in kidney tissue sections (Renal Cortex Area) between investigated groups (Hematoxylin & Eosin Stain, Magnification Power = $\times 400$ & Scale Bar = 50 μm): **a** Renal Cortex Section from Control Group indicating the typical histological structure of renal cortex comprising renal corpuscle (rectangle), proximal (arrow) and distal (arrowhead) convoluted tubules. **b** Renal Cortex Section from Cur Group signifying a structure similar to negative control group that assembled with regular histological structure of renal cortex comprising renal corpuscle (rectangle), proximal (arrow) and distal (arrowhead) convoluted tubules. **c** Renal Cortex Section from LCS Group disclosing a structure identical to negative control group that presented with intact renal cortex including renal corpuscle (rectangle), proximal (arrow) and distal (arrowhead) convoluted tubules. **d** Renal Cortex Section from Dia Group highlighting severe renal damage including atrophy of renal corpuscle with dilated glomerular space and deteriorated Bowman's capsule (rectangle). Renal tubules existed either with hydropic degeneration (curvy arrow), deep basophilic apoptotic nucleus (arrow with tail) except few cells still with vesicular nucleus (arrow). Additionally, congested blood vessels (circle), interstitial congestion (arrowhead), and infiltrations of inflammatory cells (wave arrow) were pictured. **e** Renal Cortex Section from the Dia + Cur-treated group demonstrates moderate tissue enhancement, characterized by the restoration of renal corpuscles, accompanied by minor congestion, vacuolations and degeneration of Bowman's capsule (rectangle). Renal tubules exhibited either a limited number of cells with hydropic degeneration of the epithelial lining (curvy arrow) or predominantly normal cells characterized by light and vesicular nucleus (arrow). Noticeable reduction in aggregated inflammatory cells (wave arrow) and minimal interstitial edema (star). **f** Renal Cortex Section from Dia + Cur-loaded LCS-treated group exhibits significant improvement with a marked reduction in renal cortex damage. The section demonstrates a restoration of the majority of the histological structure of the renal corpuscle, with the exception of a few vacuolations (rectangle). Renal tubules had predominantly normal architecture (arrow), however a few cells displayed cytoplasmic vacuolations (curvy arrow). Mild interstitial hemorrhage (arrowhead) as well as infiltrated inflammatory cells (wave arrow) were detected

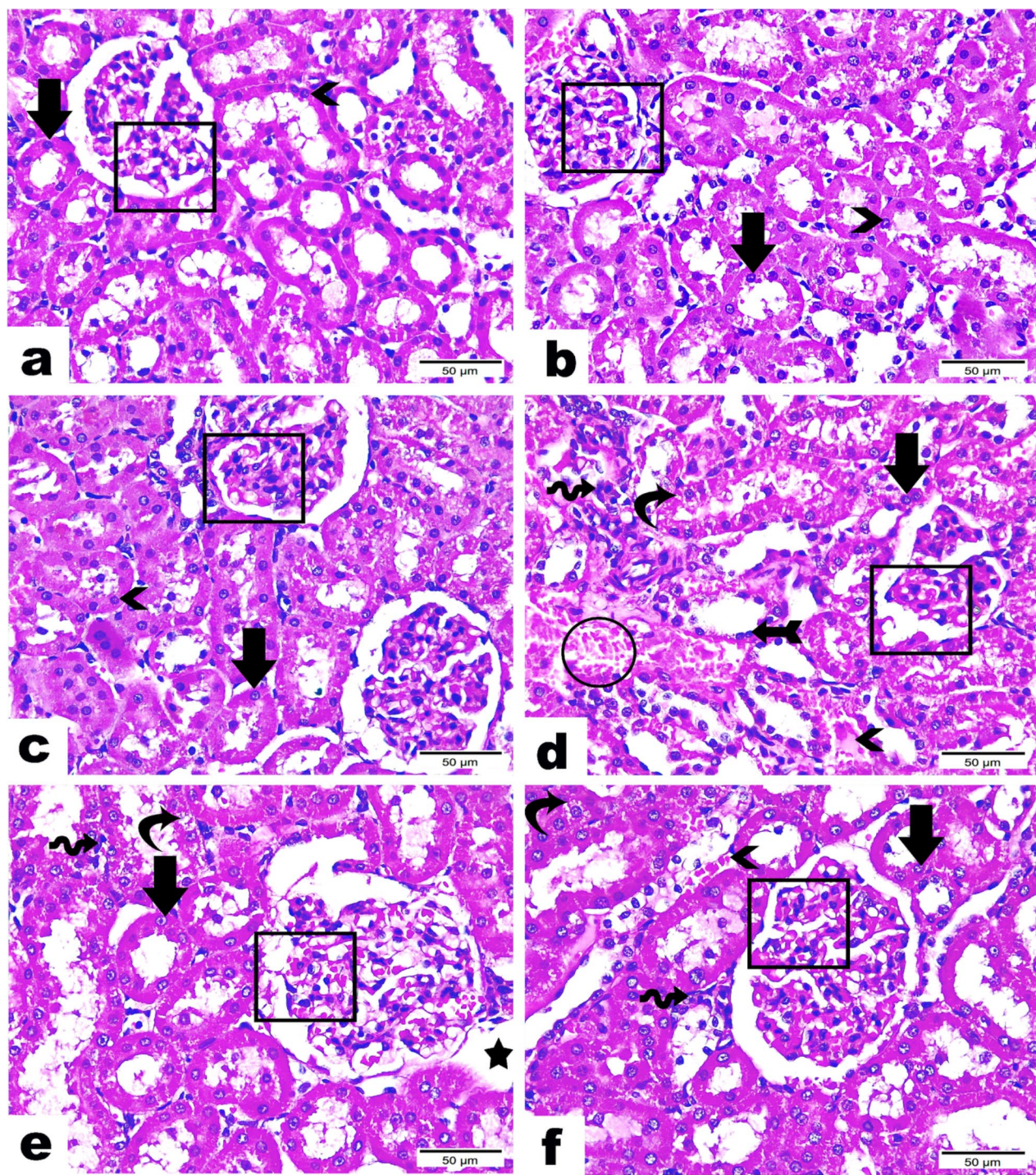


Fig. 11 (See legend on previous page.)

Dia + Cur group in comparison with control rats. Conversely, the Cur-loaded LCS group demonstrated a more significant improvement in these parameters compared to the diabetic rats treated solely with Cur.

Similarly, Ghosh et al. [113] found that oral post-treatment with 100 mg/kg/day of Cur for 56 days considerably restores elevated ALP, AST, and ALT levels in diabetic rats. Cur post-treatment also resulted in an

ALB level approaching normal levels. This supports the liver damage repair and hepatoprotective properties of Cur at the specified dose and duration. Other authors [114, 115] confirmed Cur's hepatoprotective effects by downregulating serum ALP and AST levels in propanil as well as nicotine-intoxicated rats.

Renal parameters indicated that creatinine and urea levels were reduced in diabetic rats receiving treatments with Cur and the Cur-loaded LCS group in comparison with untreated diabetic rats. Cur-loaded LCS values were normalized in comparison with the Dia group. Multiple studies [116, 117] have indicated that Cur may mitigate kidney damage by preventing AGEs accumulation in multiple tissues and reducing oxidative stress as well as inflammation associated with hyperglycemia. Soetikno et al. [95] demonstrated that oral pretreatment with 100 mg/kg/day of Cur for 8 weeks greatly reduced the activities of both protein kinase C isoforms (PKC- β 1 and PKC- α). Therefore, they concluded that Cur is considered a promising adjuvant therapy for managing and preventing diabetic nephropathy.

The current study demonstrated that diabetic groups receiving Cur-loaded LCS exhibited a greater reduction in oxidative stress compared to those treated with Cur alone. Previous literature indicated that Cur reduced MDA levels, increased SOD levels, and decreased apoptosis ratio in alloxan-treated pancreatic islet cells, suggesting that Cur may serve as a potential compound for pancreatic islet cell preservation and T2DM treatment [118]. Furthermore, in patients with T2DM, Shafabakhsh et al. [119] found that the oral pretreatment with 1000 mg/kg/day of Cur for 12 weeks improved PPAR- γ , MDA, GSH, and total antioxidant capacity (TAC) gene expression. Previous literatures [120, 121] suggested that Cur may serve as a more effective alternative for preventing and/or treating oxidative stress and mitochondrial dysfunction associated with obesity and diabetes. In diabetic gastroparesis rats, the administration of 150 mg/kg Cur intragastrically for 6 weeks markedly improved gastric emptying rates by increasing stem cell factor (SCF)/c-kit protein levels, disrupting nuclear factor kappa B (NF- κ B) signal transduction, and inhibiting oxidative stress production [122].

The data indicated that Cur enhanced the production of the anti-inflammatory factor IL-10 while diminishing the pro-inflammatory cytokines IL-6 and TNF- α levels in T2DM rats. The previous experiments [94] indicated that the Cur treatment (100 mg/kg/day) for 7 weeks downregulated the markers of oxidative stress as well as vascular inflammation levels in both the blood of diabetic rats and a cell-culture model. A prior study [123] demonstrated that Wistar rats' treatment with 200 mg/kg/day for

6 weeks of Cur induced a marked decline in TNF- α and IL-6 secretion in STZ-induced diabetic rats with heart injury. Gonzales et al. [124] suggested that Cur considerably inhibited adipocyte production of interleukin-1 β (IL-1 β), TNF- α , IL-6, and cyclooxygenase-2 (COX-2). A study [125] demonstrated that Cur and its analog mitigated diabetes-induced damage by modulating inflammation in the brains of diabetic rats. Multiple studies [104, 126] revealed that Cur administration downregulated TNF- α serum levels as well as high sensitivity C-reactive protein (hs-CRP) while upregulating adiponectin serum levels in T2DM patients. The previous data [127, 128] showed that applying topical Cur to STZ-induced acute diabetic rats increased the wound contraction by decreasing the inflammatory cytokine expression such as IL-1 β , matrix metalloproteinase-9 (MMP-9), and TNF- α , while upregulating anti-inflammatory cytokine levels like IL-10.

The superior cytokine modulation by Cur-LCS is attributed to the protective nature of the sporopollenin exine. By shielding curcumin from gastric acid, LCS facilitates higher systemic bioavailability, allowing for more potent inhibition of the NF- κ B signaling pathway—the primary regulator of TNF- α and IL-6 expression.

The histopathological results were consistent with biochemical ones. Previous studies [60, 129–131] found that the pancreas, liver, and kidney tissues architecture were severely damaged in diabetic rats. The current findings indicate improved outcomes in rats treated with Cur-loaded LCS, showing significant enhancement in tissue architecture and a reduction in degenerative changes in the pancreas, liver, and kidney compared to Dia rats, along with improvements in the measured biochemical indicators. This may be associated with the potential therapeutic benefits of Cur-loaded LCS, as the bioavailability of Cur can be enhanced, thereby improving its biological effects. These effects may include the inhibition of reactive oxygen species (ROS) production, which is significant in disorders linked to inflammation and oxidative stress, such as diabetes [53, 132].

The results demonstrated that compared to the Cur individually treated group, Cur-loaded LCS were far more effective in preserving the majority of the tested parameters. Additionally, this study found that the Cur-loaded LCS could largely alleviate pathological changes in the pancreas, liver, and kidney of the HFD/STZ rat model of T2DM. In contrast, previous investigations [62, 133] in the same HFD-administered model of T2DM indicated that a dose of 100 mg/kg of individually administered Cur provided evidence supporting Cur's significant role in T2DM prevention, attributed to its anti-inflammatory, antioxidant, antihyperglycemic, antihyperlipidemic, and other actions. This controversy could be attributed to the

length of Cur's administration in the above-mentioned studies. In these studies, treatment durations of 8 and 16 weeks, as opposed to 4 weeks in our current study, may more effectively enhance the antidiabetic activity of Cur.

Pollens and spores possess porous shells characterized by multidirectional, nano-sized channels, facilitating the transport of nutrients, water, and other agents across their walls. Also, these nano-channels might help make protein-free microcapsules and make it easier to trap active compounds that depend on physical absorption to the sporopollenin surface and/or the active component diffusing into its core. Additionally, they may serve as a mechanism for the controlled release of actives for drug delivery purposes [36, 38, 45, 134]. A recent nitrogen sorption/desorption investigation found that the mean pore width of both raw and extracted sporopollenin from *Lycopodium clavatum* is 3.2 nm. According to prior research, [37] after ingesting, some intact LCS were found in rats' blood samples after 1 h, indicating that such microparticles could penetrate the GI tract and enter the circulation. After two hours of administration, partially degraded microcapsules were detected by light microscopy. No observable deterioration of the LCS in stomach fluid was noted after 1 and 2 h of ingestion, indicating that the microcapsules can endure the harsh conditions of the GI tract. Additionally, whole LCS microcapsules were detected in feces two hours post-consumption, indicating the efficacy of these natural carriers within the GI tract, especially during gastric transit.

As a revolutionary microencapsulation platform, sporopollenin microcapsules can circumvent the known limitation of Cur, for instance, poor water solubility, low bioavailability (1%), rapid systemic metabolism, and fast hydrolytic degradation in physiological environment [78]. This can be attributed to the remarkable resilience of sporopollenin to harsh chemical and biological conditions where the encapsulated curcumin can withstand, for instance, the premature hydrolytic or enzymatic attack at the low pH of the stomach where Cur is usually compromised before reaching the absorption site, enhancing its bioavailability. In line with that, the mucoadhesion ability of sporopollenin allows extended contact with mucosa and the epithelial lining in the intestine where the prolonged residence time increases, resulting in enhanced bioavailability. In addition, based on the encapsulation protocol, Cur is loaded inside the core of the hollow porous sporopollenin microcapsules where a controlled and sustained release help increasing Cur bioaccessibility [40, 135].

Interestingly, the previous study's [37] preliminary findings suggested that LCS microcapsules attached to the mucosa of the rat stomach and duodenum. Furthermore,

LCS microcapsules preserved their decorative form in feces, stomach, and duodenum, showing their ability to withstand harsh GI tract conditions.

The toughness and mucoadhesion properties of LCS microcapsules may contribute to the sustained release of loaded Cur, enhancing drug bioavailability while reducing the duration and frequency of medication delivery. In the current HFD/STZ-induced type 2 diabetes animal model, oral glucose tolerance test (OGTT) and immunohistochemical techniques to further confirm apoptosis and inflammation should be taken in mind and considered as the limitations. In addition, pharmacokinetic studies to quantify plasma curcumin levels and confirm enhanced bioavailability are needed and are planned for future investigations.

Conclusions

Curcumin, the principal curcuminoid found in turmeric, exhibits numerous beneficial effects on T2DM, yet its therapeutic application is hindered by limited bioavailability. This study addressed this limitation by developing a curcumin-loaded *Lycopodium clavatum* sporopollenin (Cur-LCS) microcapsule based on a natural, "top-down" biomaterial platform. The successful extraction of sporopollenin microcapsules from raw pollen was confirmed by a 74.3% reduction in protein content and the efficient encapsulation of curcumin, as revealed by confocal microscopy. The formulation demonstrated a high curcumin loading capacity of 27%, and FTIR analysis indicated no significant chemical interactions post-encapsulation, preserving curcumin's native structure. As a crucial prerequisite for biomedical use, cytotoxicity assays confirmed the biocompatibility of the Cur-LCS formulations. The data obtained did not demonstrate that Cur, at the administered dose and duration, played a significant role in the treatment of T2DM in the current preclinical HFD/STZ rat model. However, the Cur-loaded LCS formulation effectively controlled lipid peroxidation and pro-inflammatory cytokines, maintaining lipid metabolism, and renal parameters, and enhanced histopathological changes in diabetic rats. Our data suggest that the LCS encapsulation platform could be utilized as a robust vehicle for sensitive phytochemicals where its systemic protective profile may contribute to its preclinical benefit. At the preclinical level, this approach could serve as a clinically relevant adjuvant therapy specifically targeted and mitigating the secondary complications of diabetes, such as oxidative stress-induced nephropathy and chronic systemic inflammation while reducing the required daily dosage and associated treatment costs. Dose-response optimization, long-term pharmacokinetic studies, and co-encapsulation should be

considered in future investigations. Clinical translation of this biocompatible, natural carrier could pave the way for more effective and affordable phytocompound-based therapies.

Supplementary Information

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Additional file 1.

Additional file 2.

Additional file 3.

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Author contributions

NMM (Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing-original draft, Writing-review & editing, Supervision), NAS (Investigation, Resources, Formal analysis, Visualization, Validation), BKAA-S (Methodology, Validation, review & editing, Supervision), AKFD (Conceptualization, Methodology, Writing-review & editing), DBA-A (Conceptualization, Methodology, Validation, Writing- original draft, Writing-review & editing, Supervision).

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Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate

All ethical considerations regarding animal treatment and methodologies were thoroughly addressed and approved. The Faculty of Pharmacy's Research Ethics Committee (Minia University) has approved the study protocol (protocol number: MPEC 2401202). To the best of our knowledge, animal distress and the quantity of animals used were reduced.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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