



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Core-shell nanofiber-mediated delivery of fucoxanthin enhances its bioavailability and alleviates non-alcoholic fatty liver disease

Jiaxuan Li¹, Xueying Tian¹, Yu Li¹, Yujuan Zhu^{2,*}, Yukun Song^{1,*}, Mingqian Tan¹, Jialin Miao³, Yihua Bai³ and Wentao Su^{1,*}

¹ State Key Laboratory of Marine Food Processing and Safety Control, National Engineering Research Center of Seafood, Dalian Key Laboratory for Precision Nutrition, Academy of Food Interdisciplinary Science, School of Food Science and Technology, Dalian 116034, Liaoning, China

² Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, Nantong 226001, China

³ Weihai Yuwang Group Co., Ltd, Weihai 264200, Shandong, China

ABSTRACT: Non-alcoholic fatty liver disease (NAFLD) remains a major health challenge, and although fucoxanthin (Fx) shows therapeutic potential, its poor stability and low bioavailability limit clinical application. In this study, a novel delivery system for Fx, a marine-derived xanthophyll carotenoid with anti-obesity, anti-diabetic, and hepatoprotective properties, was developed by encapsulating it within coaxial electrospun nanofibers. GZ12/ZFx exhibited uniform morphology with a distinct core-shell structure and superior encapsulation efficiency, coaxial electrospinning increased the Fx encapsulation by 1.17 times. In a high-fat diet-induced obese mouse model, GZ12/ZFx significantly outperformed free fucoxanthin and uniaxial nanofibers in improving metabolic parameters, reducing hepatic lipid accumulation, and modulating gut microbiota. The GZ12/Fx group limited weight increase to 3.64 g, while GZ12/ZFx performed only 3.02 g gain. The measurement results of lipid-related indicators in the serum indicated that the intervention of GZ12/ZFx was much more effective than that of GZ12/Fx. The reduction rates of TC and TG were 1.17 times and 1.75 times higher, respectively. The advanced delivery systems significantly enhanced the bioactivity of Fx, demonstrating improved metabolic outcomes and favorable safety profiles. These findings provide insights into designing effective food-grade delivery systems for hydrophobic bioactives and their potential in the intervention of NAFLD.

Keywords: Fucoxanthin, coaxial electrospinning, bioactive delivery, NAFLD, gut microbiota

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) has emerged as one of the most prevalent chronic liver disorders globally, affecting approximately 25% of the population and representing a major public health challenge[1]. Strongly linked with obesity and metabolic syndrome, NAFLD is characterized by excessive hepatic fat accumulation, chronic low-grade inflammation, oxidative stress, and disrupted lipid metabolism[2]. The pathogenesis of NAFLD is multifactorial, involving insulin resistance, hepatic lipid dysregulation, inflammatory responses, and alterations in gut microbiota composition[3]. Currently, lifestyle

*Corresponding author

yujuanzhu@ntu.edu.cn(Y.J. Zhu); 593186469@qq.com(Y. K. Song); suwentao2020@yeah.net (W.T. Su)

Received 29 September 2025

Received in revised form 6 January 2026

Accepted 20 March 2026

modification remains the cornerstone of NAFLD management, whereas pharmacological candidates often show limited efficacy and potential adverse effects, which restrict their clinical application. These limitations underscore the urgent need for safe and effective alternative strategies. Therefore, food bioactive compounds with antioxidant, anti-inflammatory, and lipid-regulating properties have attracted considerable attention as promising nutritional interventions for the prevention of NAFLD.

Fucoxanthin (Fx), a marine-derived xanthophyll carotenoid predominantly found in brown seaweeds, has demonstrated considerable potential in metabolic disease management[4,5]. Notably, Fx has been shown to enhance lipid metabolism by upregulating uncoupling protein 1 (UCP1) in white adipose tissue, improve insulin sensitivity via activation of the AMP-activated protein kinase (AMPK) pathway, and attenuate hepatic steatosis by downregulating sterol regulatory element-binding protein 1c (SREBP-1c)[6]. Furthermore, recent studies indicate that Fx may beneficially modulate the gut microbiota, suggesting an additional pathway through which it exerts metabolic effects.

Despite its therapeutic potential, the clinical application of Fx is limited by its poor aqueous solubility, low oral bioavailability, and instability under gastrointestinal conditions[7,8]. To address these limitations, advanced encapsulation strategies are essential to enhance its stability and absorption[9–11]. Electrospinning technology has emerged as a versatile and scalable platform for encapsulating hydrophobic bioactives, offering advantages such as high encapsulation efficiency, tunable release profiles, and improved bioavailability[12]. Uniaxial electrospinning has also been used to embed vanillin into zein nanofibers for active food packaging, with encapsulation efficiencies reaching 74%[13]. Juliani et al. encapsulated curcumin in starch-based capsules and fibers, achieving high loading capacities (79% and 97%, respectively) and enhanced thermal stability, alongside preserved antioxidant activity[14]. In particular, coaxial electrospinning enables the fabrication of core–shell nanofibers, where the core protects the active compound from harsh gastric conditions, while the shell modulates release kinetics[15]. For instance, a coaxial electrospun nanocomposite scaffold comprising poly (3-hydroxybutyrate)/poly (glycerol sebacate) delivering curcumin and ciprofloxacin exhibited distinct core–shell morphology, sustained dual-agent release over five days for regenerative applications[16]. Additionally, the high surface-area-to-volume ratio of electrospun nanofibers facilitates enhanced intestinal permeability, thereby improving the oral delivery of poorly soluble compounds[17]. Overall, coaxial electrospinning represents a promising strategy to address the intrinsic limitations of Fx and broaden its potential applications in functional food delivery systems.

In this study, a coaxial electrospun nanofiber delivery system (GZ12/ZFx) was developed using food-grade zein, gelatin, and Fx, and compared with a uniaxial electrospun system (GZ12/Fx) fabricated under optimized parameters according to previous study (voltage: 18 kV; core-to-shell flow rate ratio: 0.26:1)[18]. Structural and morphological characteristics of both systems were evaluated, and encapsulation efficiency was quantified. The *in vivo* efficacy of GZ12/ZFx was evaluated using a high-fat diet (HFD)-induced obese mouse model (Scheme 1). Comprehensive analyses including body composition, serum lipid profiling, glucose tolerance testing, insulin sensitivity, liver histopathology, and gut microbiota profiling

was conducted. This work provides novel insights into the development of effective Fx delivery systems and highlights their potential applications in functional foods for NAFLD prevention.

2. Materials and methods

2.1 Materials

Fucoxanthin was obtained from Jiejing Group Co., Ltd in Shandong, China. Zein was sourced from Macklin Reagent Co., Ltd. (Shanghai, China), and gelatin was purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Isoflurane was acquired from RWD Life Science Co., Ltd. (Shenzhen, China). All the commercial kit including TC, TG, HDL-C, LDL-C, AST, ALT, CAT, GSH, SOD and MDA were obtained from Nanjing jiancheng bioengineering institute Co., Ltd. (Nanjing, China). All other chemicals and reagents were analytical grade and without further treatment.

2.2 Preparation of uniaxial GZ12/Fx and coaxial GZ12/ZFx nanofiber

The electrospinning solutions for GZ12/Fx were formulated as follows, a homogeneous gelatin-zein blend solution was prepared by dissolving 0.8 g gelatin and 1.6 g zein in a solvent mixture consisting of acetic acid, ethanol, and deionized water (3:3:2, v/v/v). The GZ12 mixture was continuously stirred under water bath at 24 °C for 4 h, and then 0.04 g of Fx was dissolved in 4 mL of the mixture in a dark environment to obtain GZ12/Fx solution. Subsequently, GZ12/Fx nanofibers were fabricated using a uniaxial electrospinning system with a 30 G stainless steel needle employed, and the solution was squeezed at voltage of 18 kV under the flow rate of 0.2 mL/h.

To fabricate the coaxial GZ12/ZFx nanofibers, shell solution and core solution were GZ12 and ZFx respectively. The core ZFx solution was prepared by dissolving 0.02 g Fx and 0.3 g zein in 2 mL of 90% ethanol aqueous solution under light-protected conditions to prevent bioactive degradation. Both GZ12 and ZFx solutions were loaded into separate syringes connected to a coaxial spinneret, with an inner stainless nozzle (ϕ 350 μ m, ϕ 650 μ m) and an outer stainless nozzle (ϕ 1050 μ m, ϕ 1200 μ m). The core flow rate was 0.052 mL/h with a core-shell flow rate ratio of 0.26:1, which was optimized in our previous study[18]. The collector covered with aluminum foil was positioned at a fixed distance of 15 cm from the needle and the whole electrospinning process was conducted in a controlled environment maintained at 24 ± 2 °C with a relative humidity of 20-25% at the voltage of 18 kV. The resulting GZ12/Fx and GZ12/ZFx nanofibers were carefully peeled off and stored in a desiccator at room temperature for further characterization and application.

2.3 Morphological characterization of nanofibers

The morphologies of nanofibers were observed by SEM and TEM. For the SEM analysis, the samples were coated with palladium (Pt) and then checked by SU8010 microscope (Hitachi, Tokyo, Japan). To confirm the core-shell structure of coaxial electrospun nanofibers, high-resolution TEM imaging was conducted. A thin layer of nanofibers was carefully collected on carbon-coated copper grids, and then then examined using a JEM-2100 TEM (JEOL, Tokyo, Japan) operated at an accelerating voltage of 100 kV. The

average diameter of fibers was quantified using ImageJ software based on measurements from at least 100 randomly selected fibers per sample.

2.4 Evaluation of nanofiber efficacy in HFD-induced mice

Fifty SPF-grade male C57BL/6J mice (6 weeks old, 18–20 g) were purchased from Liaoning Changsheng Biotechnology Co., Ltd (Liaoning, China) and acclimatized for 7 days under controlled conditions ($23 \pm 2^\circ\text{C}$, 40–60% humidity, 12 h light/dark cycle). All procedures complied with the Dalian Polytechnic University Guide for the Care and Use of Laboratory Animals (DLPU2023045). After acclimation, mice were randomly divided into five groups ($n=10/\text{group}$): Control (normal diet + distilled water), Model (60% HFD + distilled water), Fx (HFD + free Fx), GZ12/Fx (HFD + GZ12/Fx nanofibers), and GZ12/ZFx (HFD + GZ12/ZFx nanofibers). After 6 weeks, obesity induction was confirmed via MRI, the therapeutic intervention was initiated. Control group mice received daily oral gavage of 0.2 mL sterile distilled water, while all other groups maintained on 60% HFD were administered with 250 μM Fx in 0.2 mL distilled water. Fx group was received free Fx, GZ12/Fx and GZ12/ZFx group were used uniaxial nanofibers and coaxial nanofibers containing equivalent Fx. Body weights were recorded weekly before gavage.

2.5 Body composition monitoring of mice

Throughout the 10-week experimental timeline encompassing 6 weeks of HFD induction and 4 weeks of therapeutic intervention, body fat distribution was quantitatively tracked using Nuclear Magnetic Resonance Imaging (MRI) (MesoQMR23-060H scanner, Niumag, Suzhou, China). Because repeated anesthesia and handling may affect body weight and metabolism, MRI-based body composition monitoring was performed on a randomly selected subset of three mice per group. The three mice were randomly chosen at the first imaging session and then marked/identified and followed longitudinally at subsequent time points to minimize disturbance to the remaining animals. Prior to each imaging session, mice were anesthetized with 2% isoflurane and maintained at 1.5% isoflurane in oxygen (1 L/min)[19]. Three-dimensional reconstruction of adipose tissue volumes was performed using manufacturer software.

2.6 Assessment of glucose and insulin tolerance

To evaluate metabolic function, fasting blood glucose and insulin sensitivity were measured through standardized glucose tolerance tests (GTT) and insulin tolerance tests (ITT). For GTT, following a 12–14 h fasting period (with free access to water), glucose was oral administrated to mice and the blood glucose levels were monitored at 0, 30, 60, 90, and 120-min intervals using a calibrated glucometer via tail vein sampling. The ITT was conducted after a 2-hour fast by administering intraperitoneal insulin (0.75 U/kg body weight), followed by measurement protocols[20]. The insulin level was tested by the same time period with glucose test, and data were analyzed with results normalized to baseline (0 min) values.

2.7 Serum biochemical and liver function analysis

After 4-week intervention, blood was collected via orbital puncture under isoflurane anesthesia and centrifuged at 4000 rpm for 10 min to isolate serum and then stored at -80°C . The serum samples were

analyzed for lipid profiles and liver function markers according to manufacturer protocols. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were quantified using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), with absorbance measured on a microplate reader. All samples were run in duplicate with internal quality controls, and values were normalized to pooled reference serum.

Liver tissues were immediately excised, weighed, and divided for preservation, and a portion of each liver sample was snap-frozen in liquid nitrogen and stored at -80 °C for subsequent biochemical analysis. Frozen liver tissues were homogenized in iced PBS using a tissue grinder (1:9, w/v), followed by centrifugation at $12,000 \times g$ for 15 min at 4°C to obtain clear supernatants. TC, TG, SOD, CAT, GSH and MDA levels were measured by commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.8 Histopathological examination of main tissues

Multiple organs (heart, spleen, lung, kidney, stomach, intestine, and testis) samples fixed in 4% paraformaldehyde were processed for histological analysis to evaluate morphological changes and lipid accumulation. Following fixation, tissues were dehydrated through a graded ethanol series (70% to 100%), embedded in paraffin, and sectioned at 5 µm thickness using a microtome. For hematoxylin and eosin (H&E) staining, sections were dewaxed, rehydrated, and stained with hematoxylin for 5 min and eosin for 3 min. For Oil Red O staining, frozen liver sections were prepared using a cryostat, stained with 0.5% Oil Red O solution for 15 min at 37 °C to visualize neutral lipid droplets, and counterstained with hematoxylin for nuclear identification.

2.9 Gut microbiota profiling

The fecal samples of each group were collected three days before sacrifice, DNA from different samples was extracted using the CTAB according to manufacturer's instructions and the total DNA was eluted in 50 µL of elution buffer and stored at -80 °C until measurement in the PCR. The composition of the samples was sequenced on an Illumina NovaSeq platform and analyzed in the V3-V4 regions with the primers of 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R(5'-GACTACHVGGGTATCTAATCC-3').

2.10 Statistics analysis

All experimental results were expressed as mean $\pm$ standard derivation to accurately represent data variability. Statistical analyses were performed using SPSS Statistics 20 (IBM Corporation, USA), with one-way analysis of variance (ANOVA) for multiple comparisons between treatment groups. Graphical representations were generated using Origin 2021 (OriginLab Corporation, USA).

3. Results and discussions

3.1 Structural properties of uniaxial and coaxial electrospun nanofibers

The nanofibers were composed of gelatin, zein and Fx in Fig.1A, and the morphological analysis revealed that fucoxanthin-loaded uniaxial gelatin-zein nanofibers (GZ12/Fx) exhibited smooth and bead-free structures as confirmed by SEM in Fig. 1B. The uniaxial fibers displayed uniform diameters of 663 ± 113 nm, which was reported in our previous report[18]. With a core flow rate of 0.052 mL/h and core-to-shell flow rate ratio of 0.26:1, Fig. 1C demonstrated the linear and bead-free coaxial fibers with uniform diameters of 663 ± 116 nm. It is noteworthy that although the coaxial spinning nanofibers with core-shell structure have one more layer than the uniaxial spinning nanofibers, their average diameters remain unchanged. This is due to the interaction between zein and Fx, which increases the viscosity of the electrospinning solution and is more conducive to releasing finer nanofibers[21]. As depicted in Fig. 1D, with the addition of zein in core layer, the encapsulation efficiency of Fx increased from 84.3% to 98.6% due to the interaction between Fx and zein. The excellent encapsulation efficiency in coaxial GZ12/ZFx nanofibers was attributed to the strong hydrophobic interaction between the non-polar domain of zein and the conjugated polyene skeleton of Fx, which was similar to the previous study[22]. High-resolution TEM clearly demonstrated the core-shell architecture of coaxial fibers in Fig. 1E, with a distinct interface between the Fx-loaded zein core in black and the gelatin-zein shell in grey, matching the structural characteristics reported by Craig et al. for bioactive compound encapsulation[23].

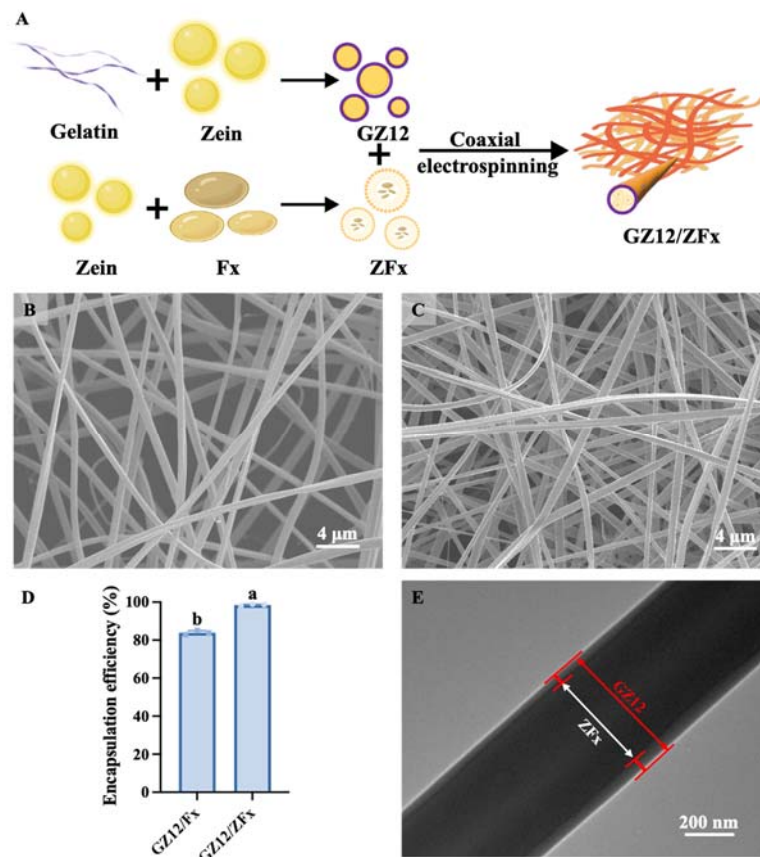
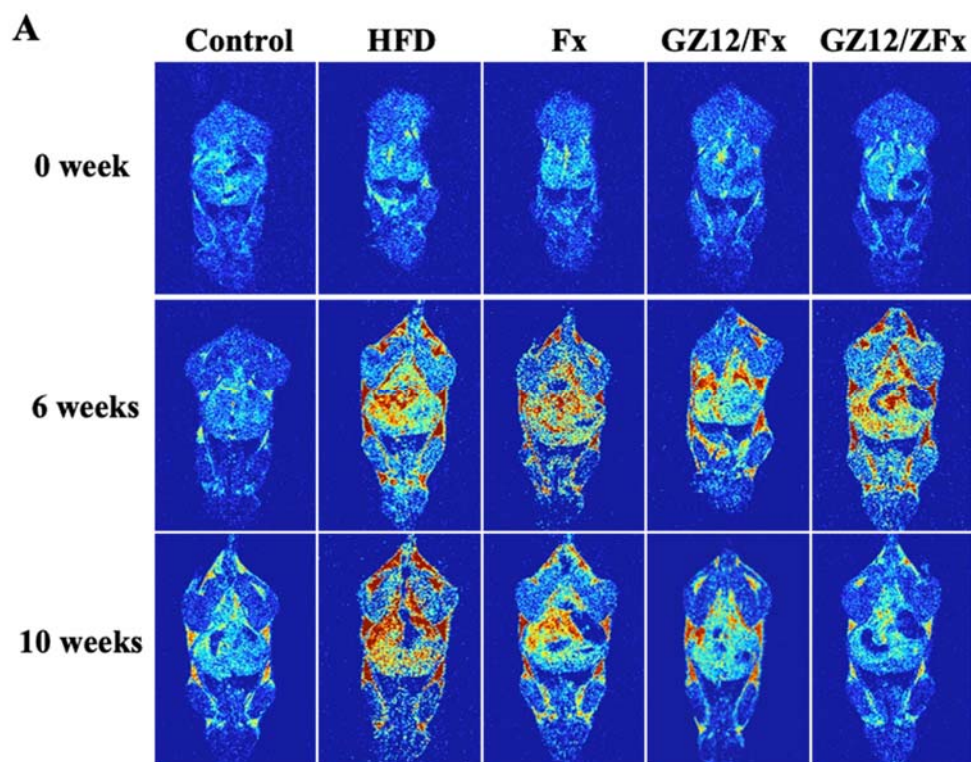


Figure 1. Morphological characterization of nanofibers. (A) Schematic graph of coaxial nanofiber fabrication, SEM results of (B) uniaxial electrospun nanofibers GZ12/Fx and (C) coaxial electrospun nanofibers GZ12/ZFx. (D) Encapsulation efficiency of Fx in both nanofibers. Different lowercase letters above bars indicate statistically significant differences among groups at $P < 0.05$, $n = 3$. (E) TEM images of core-shell GZ12/ZFx nanofibers, white arrow stands for the core while red lines stand for the shell.

3.2 Analysis of body fat distribution in experimental mice

Body composition was monitored in three randomly selected mice per group due to the potential confounding effect of repeated anesthesia/handling on body weight. To evaluate changes in adipose tissue accumulation during the study, MRI was performed at three critical time points, including baseline (week 0), post-modeling (week 6), and post-intervention (week 10) in Fig. 2A. The control group fed with a standard diet maintained relatively uniform fat distribution throughout the study, with only minimal lipid deposition observed around the neck and visceral regions. In contrast, mice subjected to a HFD for 6 weeks exhibited significant increases in body size, accompanied by blurred liver margins and pronounced abdominal fat accumulation, particularly around the liver. Following the 4-week intervention period, distinct patterns of fat modulation emerged among treatment groups. The free Fx group showed slight additional fat deposition, whereas both nanofiber formulations demonstrated measurable reductions in lipid accumulation (Fig. 2B). Notably, the GZ12/ZFx group exhibited the most substantial improvement, with visibly decreased visceral and subcutaneous fat deposition compared to the model group. To improve data transparency, representative MRI images of the rest mice per group at different time points are provided in the Fig. S1. MRI analysis revealed that GZ12/ZFx treatment not only attenuated liver steatosis but also reduced overall adiposity, as evidenced by clearer organ boundaries and diminished fat signals across abdominal regions. The progressive fat reduction observed from free Fx to GZ12/Fx and GZ12/ZFx formulations established a clear structure-activity relationship, with the coaxial delivery system providing optimal therapeutic outcomes against diet-induced adiposity.



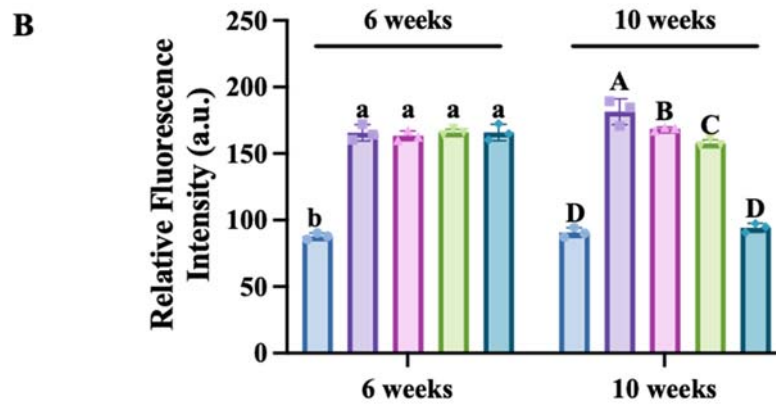
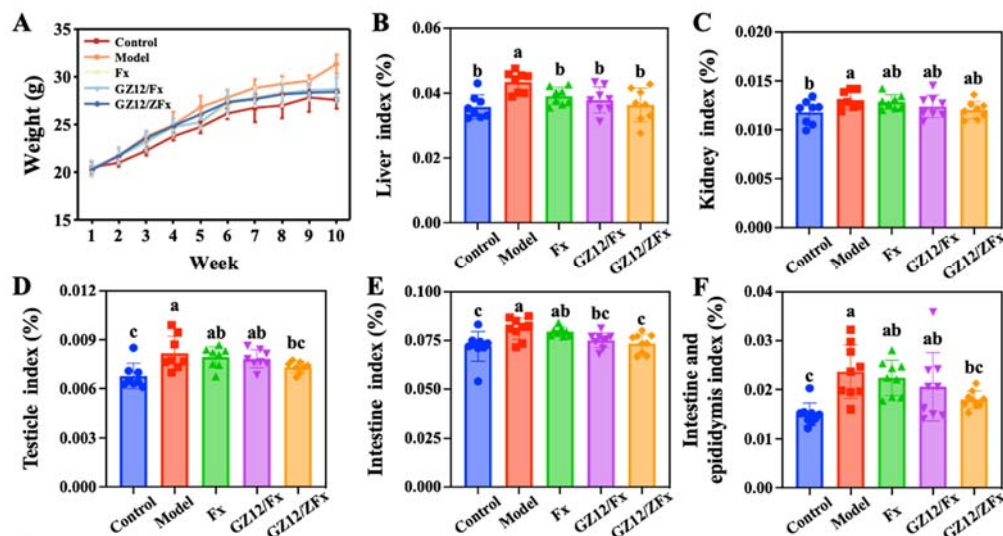


Figure 2. Body composition of mice in different groups. (A) MRI at 0, 6, and 10 weeks to observe body fat distribution in NAFLD mice after intervention by Fx, GZ12/Fx, and GZ12/ZFx. (B) Relative fluorescence intensity of body fat in mice from different groups after 6 weeks and 10 weeks. Different lowercase letters (a, b) indicate statistically significant differences among groups at 6 weeks ($P < 0.05$), while different uppercase letters (A–D) indicate statistically significant differences among groups at 10 weeks ($P < 0.05$), $n = 3$. Statistical analyses were performed independently for each time point.

3.3 Anti-obesity effects on mice body and organ weights

Body weight serves as a crucial health indicator during the whole experiments, and the changes of body weight were monitored during the experimental period in Fig. 3A. Mice fed a normal diet exhibited gradual weight gain from 20.5 ± 0.763 g to 27.4 ± 0.82 g over 10 weeks, whereas HFD induced rapid obesity development, with body weight surging to 31.24 ± 0.98 g. After 4 weeks of treatment, all intervention of different Fx formations showed significantly better control of weight gain compared to model group. The GZ12/Fx group limited weight increase to 3.64 g, while GZ12/ZFx performed only 3.02 g gain. The increase of body weight gain of both nanofiber groups substantially lower than the increase seen in the free Fx group with 3.85 g. Overall, the GZ12/ZFx group showed a more favorable trend in controlling weight gain and improving NAFLD-related readouts compared with free Fx and the uniaxial nanofiber group. The liver weight of GZ12/ZFx group was 24.04% lower than that of HFD group in Table S1, while free Fx and GZ12/Fx nanofibers only had a decrease of 17.4% and 20.2% respectively, suggesting that the core-shell architecture may contribute to improved delivery of Fx and associated metabolic benefits.



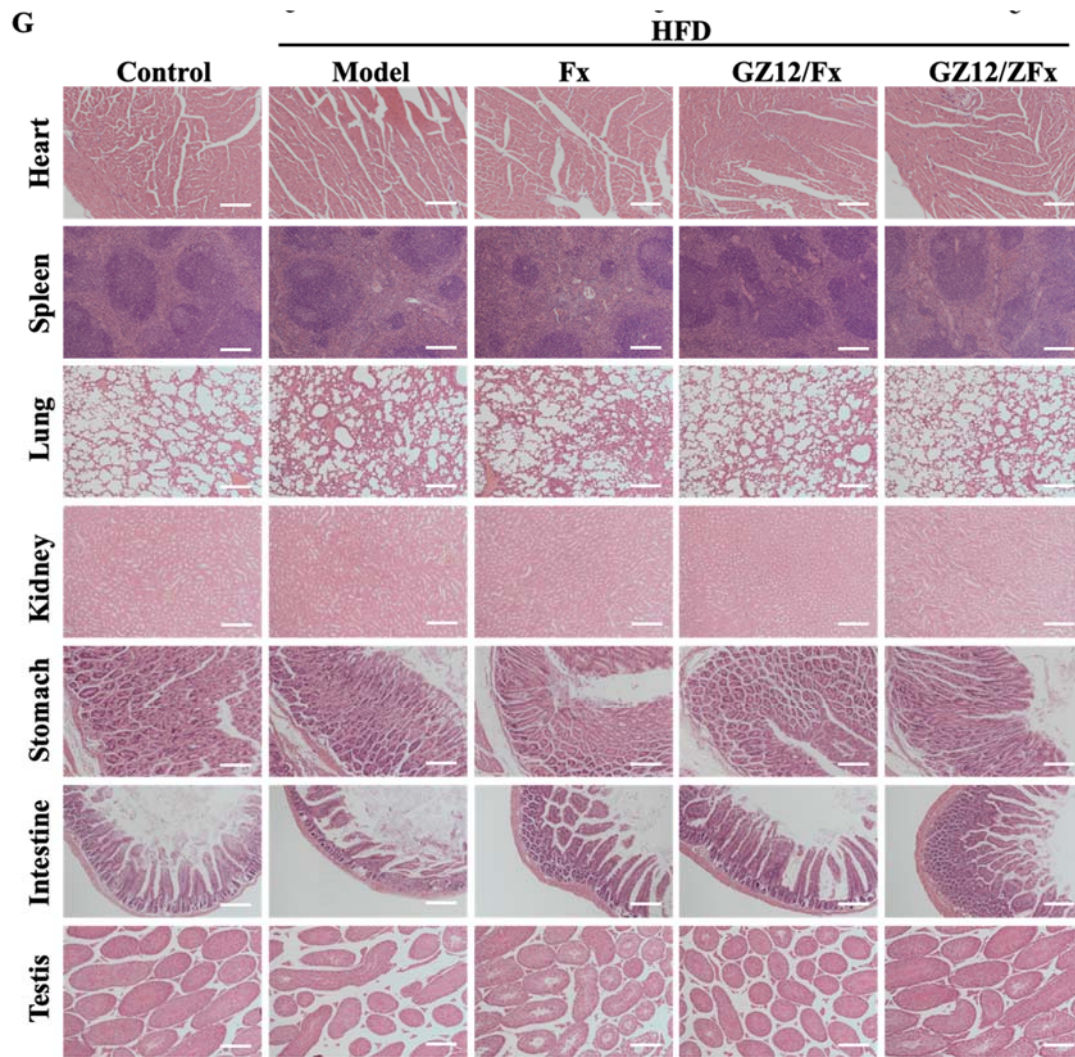


Figure 3. Body weight and organ indices variation in high fat-diet mice. (A) Body weight changes of mice fed a HFD for six weeks and nutritional intervention with Fx, GZ12/Fx, and GZ12/ZFx for four weeks. Changes of (B) Liver, (C) kidney, (D) testis, (E) intestinal and (F) intestine and epididymis index after intervention of Fx, GZ12/Fx and GZ12/ZFx. Different lowercase letters above bars indicate statistically significant differences among groups at $P < 0.05$, $n = 9$. (G) H&E staining section of the organs (heart, spleen, lung, kidney, stomach, intestine and testis). All histological images were acquired at the same magnification, and scale bars stood for 200 μm .

The organ index analysis revealed significant effects of free Fx, GZ12/Fx and GZ12/ZFx on tissue weights in mice ($p = 0.0392$, < 0.001 , < 0.001 , compared to model group respectively). Calculated as the ratio of organ mass to body weight, the index for liver, intestine, kidney, testis and epididymis showed significant increases in model group compared to control, while treatment with Fx, GZ12/Fx and GZ12/ZFx modulated the changes of these indices. The liver index showed clear reductions across all treatment groups in Fig. 3B, with the free Fx group achieving a 10.1% decrease, GZ12/Fx and GZ12/ZFx group showing a 12.5% and 15.8% reduction ($p = 0.048$, 0.0081 , 0.006 , compared to model group). As shown in Fig. 3C, changes in kidney indices were more modest, with all groups demonstrating less than 10% reduction, potentially attributable to the relatively small size of murine kidneys. Testicular index, as an important marker of metabolic disturbance, displayed progressive improvements with the testis indices of the free Fx group and the GZ12/Fx group were 97.1% and 95.8% of the model group, respectively, while the GZ12/ZFx group was 89.6% of the model group (Fig. 3D, $p = 0.868$, 0.649 , 0.035 , compared to model group). Besides, combined

with H&E staining section analysis, Fx formulations were able to effectively protect the testicular damage caused by high fat-diet with the appearance of decrease in the number of spermatogenic cells and structural blurring [24]. The final morphology of the GZ12/ZFx intervention group was similar to that of the control group, with a complete and neatly arranged structure, suggesting that the coaxial delivery system may help attenuate HFD-associated testicular alterations. In addition, the small intestine index of the GZ12/ZFx group was 0.073, approaching 0.072 of the control group, while the indices of the free Fx and uniaxial spinning groups were 0.079 and 0.075, respectively (Fig. 3E, $p = 0.933, 0.011, 0.509$ compared to control group). Adipose tissue measurements followed this same pattern of graduated efficacy, with the free Fx group showing 1.5% reduction, the GZ12/Fx group achieving 5.1% decrease, and the GZ12/ZFx group demonstrating the most significant 7.9% reduction compared to the model group in Fig. 3F. Among other organs, histological assessment demonstrated favorable biocompatibility, as evidenced by the absence of observable pathological alterations or inflammatory infiltration in major organs compared with the control group (Fig. 3G). These consistent findings across multiple organ systems clearly demonstrated the enhanced effectiveness of the core-shell nanofiber formulation, indicating that the coaxial encapsulation method improved Fx delivery and bioavailability, which may partially explain the observed improvements in metabolic outcomes. Although the anti-obesity effects of coaxial electrospun encapsulated Fx were statistically significant, the magnitude of changes in body weight and several metabolic parameters was modest. This is consistent with food-based nutritional interventions rather than pharmacological treatments; therefore, the findings should be interpreted with appropriate caution. In addition, one mouse in the Fx group was humanely euthanized during the final week due to gavage-related complications, and endpoint analyses were standardized to $n = 9$ per group, which may have reduced statistical power for certain comparisons.

3.4 Assessment of blood lipid profiles and serum enzyme levels

To evaluate the effects of Fx formulations on metabolic dysfunction in non-NAFLD, the serum levels of TC, TG, HDL-C, and LDL-C were measured. The model group exhibited significant dyslipidemia compared to the control with markedly elevated TC, TG, and LDL-C levels in Fig. 4A-C, and Fx interventions showed improvements in lipid profiles relative to the model group. Both the GZ12/Fx and GZ12/ZFx nanofiber groups demonstrated significant reductions in TC (23.9% and 28%, $p = 0.011, 0.0003$, respectively) compared to the model group in Fig. 4B. Specifically, LDL-C decreased from 1.05 ± 0.13 mmol/L in model mice to 0.90 ± 0.2 , 0.69 ± 0.17 , and 0.258 ± 0.04 mmol/L in the Fx, GZ12/Fx and GZ12/ZFx groups, respectively (Fig. 4C). Notably, the GZ12/ZFx group restored LDL-C to near-normal levels with 0.236 ± 0.07 mmol/L in control ($p = 0.999$ compared to control group). Concurrently, HDL-C levels increased from 0.102 ± 0.03 mmol/L in model mice to 0.151 ± 0.02 , 0.226 ± 0.07 , and 0.374 ± 0.11 mmol/L in the Fx, GZ12/Fx and GZ12/ZFx groups, respectively, indicating improved cholesterol clearance (Fig. 4D).

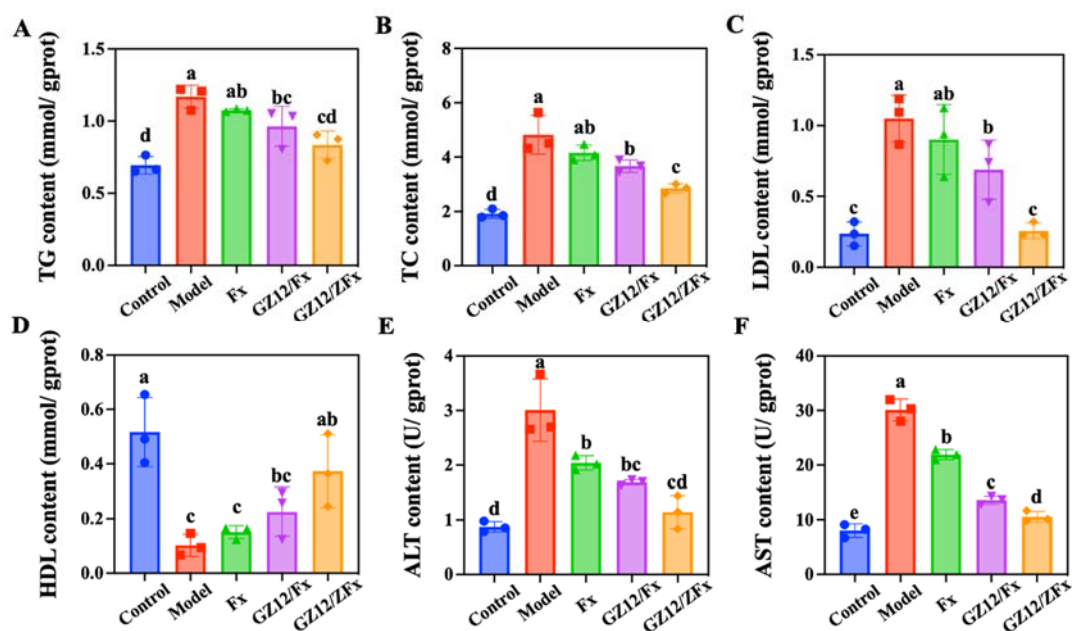


Figure 4. Determination of lipid levels in mice. (A) TC, (B) TG, (C) MDA, (D) SOD, (E) ALT, (F) AST level in serum. Different lowercase letters above bars indicate statistically significant differences among groups at $P < 0.05$, $n = 3$.

Serum ALT and AST levels, as the key markers of hepatic injury, were significantly elevated in model group compared to controls (Fig. 4E, F). All treatment groups showed reduced ALT and AST levels, with GZ12/ZFx showing a more favorable trend in reducing ALT and AST levels. These results suggest that the core-shell nanofiber system (GZ12/ZFx) may help alleviate HFD-associated metabolic dysregulation by partially restoring lipid homeostasis and reducing hepatocellular injury. Notably, Serum lipid and enzyme assays were conducted using samples from a subset of three mice per group due to resource constraints. Samples were selected by blinded randomization, and the same animals were used consistently across kit-based measurements; however, the smaller n may reduce power to detect subtle differences.

3.5 Evaluation of blood sugar and insulin resistance

The evaluation of glucose homeostasis through oral glucose tolerance tests (OGTT) and insulin tolerance tests (ITT) revealed significant metabolic dysfunction in HFD-fed mice, with area under the curve (AUC) analysis providing quantitative assessment of glucose uptake capacity and insulin sensitivity[25]. The results of intraperitoneal glucose tolerance in mice were shown in Fig. 5A, the blood glucose tolerance curve of the model group significantly increased after oral administration of glucose for 30 min. The blood glucose values of the Fx, GZ12/Fx and GZ12/ZFx intervention groups were consistently lower than those of the model group, with the GZ12/ZFx group exhibited a quickest blood glucose recovery and closet to the normal level. The model group exhibited markedly impaired glucose tolerance evidenced by AUC of OGTT increased to approximately 1.68-fold of that in control group (Fig. 5B), while GZ12/Fx and GZ12/ZFx interventions increased the glucose uptake capacity with OGTT AUC of 1328.4 and 1221.1 mM/min, respectively. As depicted in Fig. 5C, the blood glucose of the model group after insulin intraperitoneal injection showed an upward trend during 60-90 min. At the time point of 120 min, the blood glucose of the model group reached to 6.92 ± 0.72 , which was 1.5 times higher than that of the control, indicating the reduced insulin sensitivity.

Therapeutic intervention with Fx formulations demonstrated that GZ12/ZFx had a superior efficacy by reducing ITT AUC by 25.4% versus model values, while the GZ12/Fx and free Fx groups showed intermediate effects (Fig. 5D, $p = 0.0001, 0.0506, 0.0003$, compared to model group respectively). The results indicated that the GZ12/Fx and GZ12/ZFx intervention groups can improve insulin resistance induced by NAFLD in mice, which GZ12/ZFx showed better effect with higher bioavailability of Fx.

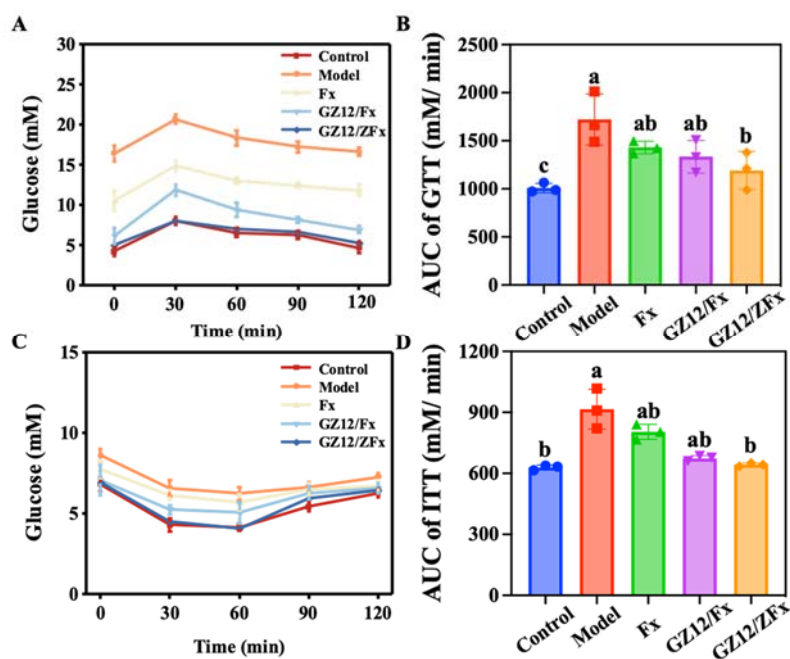


Figure 5. Assessment of oral glucose tolerance (OGTT) and insulin tolerance (ITT). NAFLD model mice (A) OGTT and (B) area under the curve and (C) ITT curve and (D) area under the curve. Different lowercase letters above bars indicate statistically significant differences among groups at $P < 0.05$, $n = 3$.

3.6 Histopathological evaluation and measurement of metabolic parameters in liver

Macroscopic and microscopic examinations were performed to evaluate hepatic alterations induced by a high-fat diet (HFD) and the effects of fucoxanthin formulations in Fig. 6A. The mice in control group exhibited normal reddish-brown liver morphology with well-defined margins, while model group displayed marked hepatomegaly, yellowish discoloration, indicative of severe lipid accumulation and tissue damage. Oil red and H&E staining revealed that hepatocytes in model group contained numerous large lipid droplets, while both nanofiber-encapsulated Fx formulations (GZ12/Fx and GZ12/ZFx) substantially ameliorated these pathological changes, with the GZ12/ZFx showing superior efficacy by reducing hepatic lipid deposition and restoring normal hepatocyte morphology. Low-magnification ($4\times$) histological images and the complete set of histological sections for all mice are provided in the Fig. S2. Due to budget limitations, liver histological staining was performed in three randomly selected mice per group, therefore, the histological conclusions should be interpreted with this subset size in mind. The GZ12/ZFx group showed an improvement in gross appearance and histological features, with reduced lipid deposition and inflammatory signs compared with the model group.

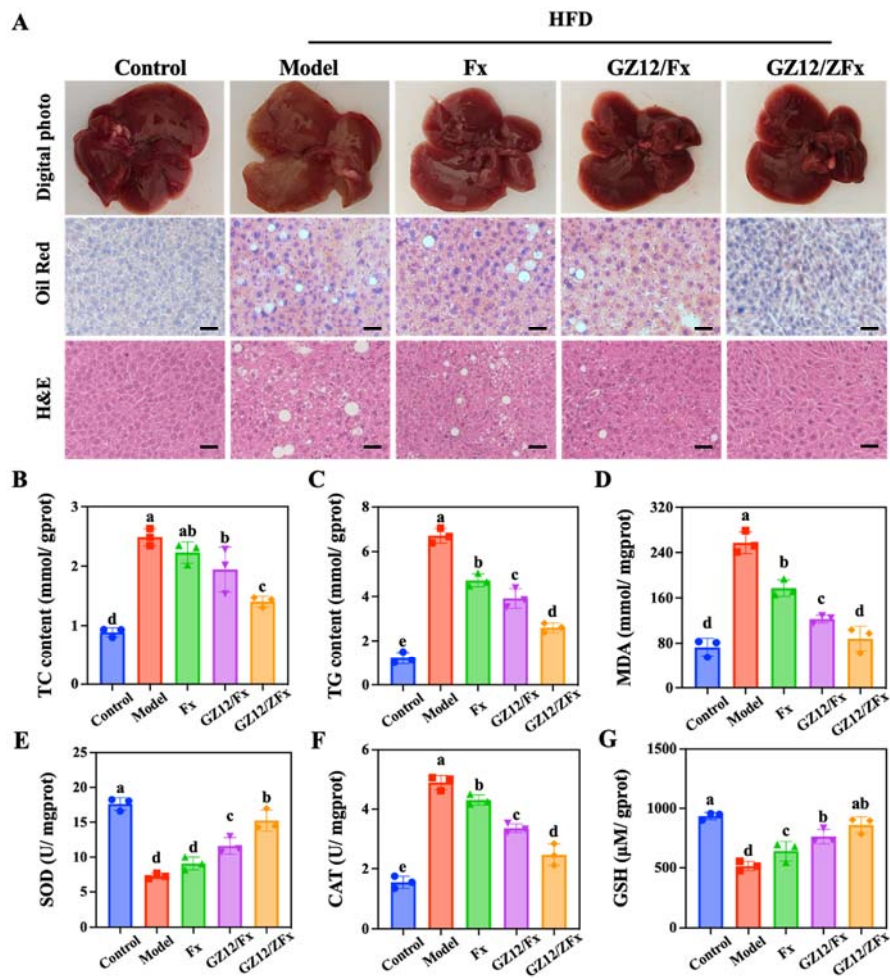


Figure 6. Liver histological analysis and determination of liver tissues levels in mice. (A) The digital photos of photographs, oil red O-stained sections, and H&E-stained sections of mice after 4 weeks of Fx, GZ12/Fx, and GZ12/ZFx interventions, with a scale bar of 200 μ m. (B) TC, (C) TG, (D) MDA, (E) SOD, (F) CAT and (G) GSH level in liver tissue. Different lowercase letters above bars indicate statistically significant differences among groups at $P < 0.05$, $n = 3$.

Disruption of lipid homeostasis leads to increased uptake of non-esterified fatty acids and enhanced endogenous synthesis of fatty acid esters, thereby promoting the production and transport of TC and TG. As demonstrated in Fig. 6B and 6C, mice fed a HFD exhibited nearly 3-fold and 5.5-fold increases in TC and TG levels compared to the control group, which may induce hepatic steatosis. Both GZ12/Fx and GZ12/ZFx nanofibers intervention groups significantly reduced hepatic TC and TG content in NAFLD model mice, though levels remained elevated relative to the control. Excessive TC and TG accumulation may ultimately induce oxidative stress, therefore, changes of MDA, SOD, CAT and GSH levels were also measured[26]. As a marker of lipid peroxidation, MDA level significantly increased in model group ($P < 0.0001$), indicating a severe oxidative stress. After the intervention, GZ12/Fx and GZ12/ZFx group exhibited 30.7% and 50.3% reductions in MDA content, respectively, higher than that in free Fx group (Fig. 6D). In Fig. 6E, SOD activity was significantly enhanced in the GZ12/ZFx group, showing a 31.3% increase over GZ12/Fx-treated mice ($P < 0.0001$). The regulation of CAT levels showed a differential trend in Fig. 6F, with GZ12/ZFx reduced activity of 22% and 42.7% relative to Fx and GZ12/Fx groups, respectively. Conversely, GSH concentrations rose by 19.2% and 34.5% in GZ12/Fx and GZ12/ZFx groups compared to free Fx in Fig. 6G. Collectively,

these findings suggested that the Fx-loaded core-shell nanofiber system may attenuate hepatic lipid accumulation and oxidative stress, thereby improving liver injury markers in this model. The relatively better performance of GZ12/ZFx supported the potential value of advanced delivery systems for enhancing the efficacy of bioactive compounds. However, some biochemical endpoints were assessed in a blinded, randomly selected subset of mice due to resource constraints, which may reduce statistical power for certain comparisons. Future studies should aim to include larger animal cohorts with longitudinal monitoring to strengthen statistical robustness.

3.7 Impact of therapeutic interventions on gut microbiota regulation

Based on the intervention effect on obesity, fecal samples from control group, model group, free Fx group, and GZ12/ZFx group were selected for 16S rRNA sequencing. As shown in Fig. 7A, initial assessment of microbial communities revealed 118 shared operational taxonomic units (OTUs) across all groups, with control, model, free Fx, and GZ12/ZFx groups exhibiting 78, 103, 104, and 157 unique OTUs respectively. Alpha diversity analysis demonstrated that HFD significantly reduced microbial richness and diversity compared to control, as evidenced by decreased Simpson and Shannon indices (Fig. 7B and 7C). Both Fx interventions reversed this trend, with GZ12/ZFx showing efficacy in restoring microbial[27]. Consistent results were observed in Chao1 index (Fig. 7D), confirming the ability of GZ12/ZFx to ameliorate HFD-induced microbiota depletion. Beta diversity analysis using Bray-Curtis metrics revealed distinct clustering patterns in Fig. 7E, PCoA showed clear separation with Fx and GZ12/ZFx groups clustering separately from model along PC2 while maintaining distance from control along PC1[28]. NMDS analysis (stress = 0.0904, Fig. 7F) confirmed significant HFD-induced dysbiosis, with GZ12/ZFx treatment driving microbiota composition closer to control than free Fx.

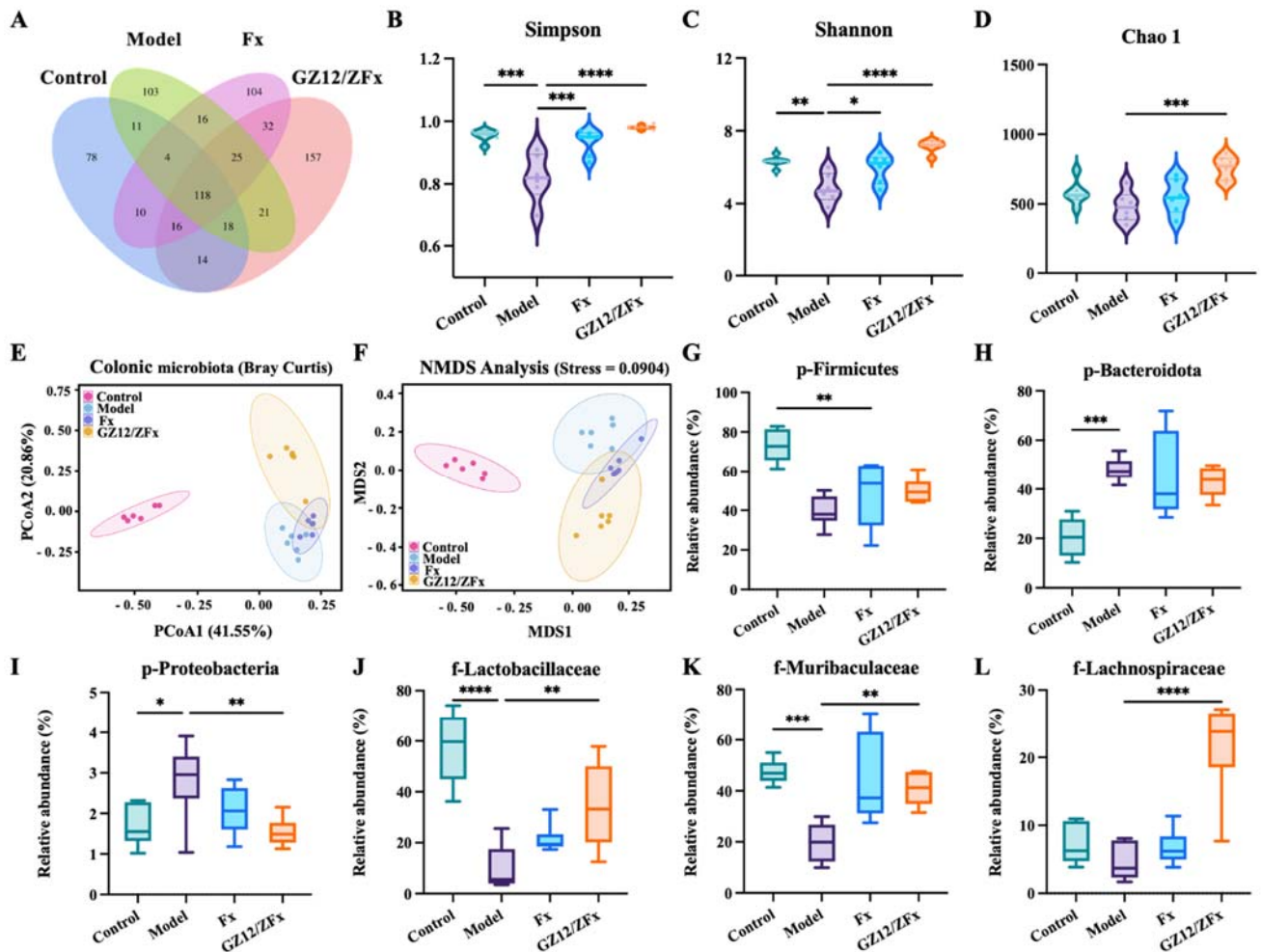


Figure 7. Gut microbiota regulation after intervention of Fx formulations. (A) Venn diagram of OTUs in each group.

Alpha diversity of microbiota with index of (B) Simpson, (C) Shannon, and (D) Chao 1. Beta diversity of colonic microbiota with (E) Bray Curtis and (F) NMDS analysis. Relative abundance of (G) Firmicutes, (H) Bacteroidota, (I) Proteobacteria, (J) Lactobacillaceae, (K) Muribaculaceae and (L) Lachnospiraceae. Statistical significance is indicated by asterisks (*, **, ***, ****) representing $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively, compared with the model group.

At the phylum level in Fig. 7G-I, HFD feeding decreased Firmicutes abundance while increased *Bacteroidetes* and *Proteobacteria*[29], whereas GZ12/ZFx intervention effectively normalized these ratios[30]. The intervention particularly reduced *Proteobacteria* abundance, potentially attenuating LPS-TLR4 mediated inflammatory pathways[31]. Importantly, family-level analysis demonstrated that GZ12/ZFx treatment significantly increased the relative abundance of beneficial bacterial families including *Lactobacillaceae* in Fig. 7J, which contributes to anti-inflammatory regulation, *Muribaculaceae* in Fig. 7K, which improves glycan metabolism, and *Lachnospiraceae* in Fig. 7L, which enhances gut barrier protection[32–34]. These comprehensive findings demonstrated that the core-shell delivery system (GZ12/ZFx) more effectively restored HFD-disrupted microbiota ecology than free Fx through multiple mechanisms including enhanced bacterial richness and diversity, normalization of obesogenic phylum ratios, selective enrichment of beneficial families, and suppression of pro-inflammatory *Proteobacteria*, with these superior modulatory effects likely attributable to the improved bioavailability, thereby positioning GZ12/ZFx as a promising strategy for microbiota-targeted obesity intervention.

4. Conclusion

In this study, GZ12/ZFx were developed using electrospinning technology and compared with GZ12/Fx in HFD-induced obese mice. GZ12/ZFx exhibited uniform morphology with a distinct core-shell structure and superior encapsulation efficiency. *In vivo*, all fucoxanthin formulations attenuated weight gain, organ enlargement, dyslipidemia, and insulin resistance, with GZ12/ZFx showing the most pronounced effects. Histopathological analyses confirmed reduced hepatic lipid accumulation and improved liver morphology, while gut microbiota profiling revealed restored diversity and increased beneficial bacteria. No structural abnormalities were observed in major organs, indicating biosafety. These findings demonstrate that GZ12/ZFx enhances FX bioavailability and provides multi-target protection against obesity-related metabolic disorders, highlighting its potential as a food-grade nutraceutical strategy for NAFLD intervention.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2024YFD2100404), Mount Tai Industrial Blue Talent Program of Shandong Province, China (Tsls20231209) and Liao Ning Revitalization Talents Program (XLYC2203091).

Data Availability Statement

Data will be made available on request.

References

- [1] Z.M. Younossi, P. Golabi, J.M. Paik, A. Henry, C. Van Dongen, L. Henry, The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review, *Hepatology* 77(4) (2023) 1335-1347. <https://doi.org/10.1097/HEP.0000000000000004>.
- [2] A.R. Saltiel, J.M. Olefsky, Inflammatory mechanisms linking obesity and metabolic disease, *J Clin. Invest.* 127(1) (2017) 1-4. <https://doi.org/10.1172/JCI92035>.
- [3] Z. Younossi, Q.M. Anstee, M. Marietti, T. Hardy, L. Henry, M. Eslam, J. George, E. Bugianesi, Global burden of NAFLD and NASH: Trends, predictions, risk factors and prevention, *Nat. Rev. Gastro. Hepat.* 15 (2018) 11-20. <https://doi.org/10.1038/nrgastro.2017.109>.
- [4] X. Zhong, Y. Wang, C. Song, X. Song, Y. Zhang, L. Cao, A.G. Skirtach, S. Song, M. Tan, W. Su, Engineered liver-targeted probiotic nanovesicles overcome oral absorption barriers to cure alcoholic liver disease, *Chem. Eng. J.* 522 (2025) 167270. <https://doi.org/10.1016/J.CEJ.2025.167270>.
- [5] K. Wang, X. Li, K. Huang, H. Wu, M. Tan, W. Su, Dietary probiotic-derived extracellular vesicles as delivery systems of bioactive compounds to maintain intestinal homeostasis, *Chem. Eng. J.* 505 (2025) 159546. <https://doi.org/10.1016/J.CEJ.2025.159546>.
- [6] H. Maeda, Nutraceutical effects of fucoxanthin for obesity and diabetes therapy: a review., *J. Oleo. Sci.* 64(2) (2015) 125-132. <https://doi.org/10.5650/jos.ess14226>.

- [7] H. Li, Y. Xu, X. Sun, S. Wang, J. Wang, J. Zhu, D. Wang, L. Zhao, Stability, bioactivity, and bioaccessibility of fucoxanthin in zein-caseinate composite nanoparticles fabricated at neutral pH by antisolvent precipitation, *Food Hydrocoll.* 84 (2018) 379–388. <https://doi.org/10.1016/j.foodhyd.2018.06.032>.
- [8] J. Li, Y. Li, W. Su, X. Zhang, D. Liang, M. Tan, In vivo anti-obesity efficacy of fucoxanthin/HP- β -CD nanofibers in high-fat diet induced obese mice, *Food Chem.* 429 (2023) 136790. <https://doi.org/10.1016/j.foodchem.2023.136790>.
- [9] K. Wang, X. Li, X. Xu, J. Chang, M. Sun, K. Huang, S. Huang, H. Jiang, H. Wang, M. Tan, W. Su, AI-driven target screening and microfluidic sonication-assembled oral delivery of fucoxanthin-loaded probiotic vesicles for targeted alleviation of diet-induced obesity, *Chem. Eng. J.* 518 (2025) 164598. <https://doi.org/10.1016/J.CEJ.2025.164598>.
- [10] K. Wang, K. Huang, X. Li, H. Wu, L. Wang, F. Bai, M. Tan, W. Su, Kelp nanocellulose combined with fucoxanthin achieves lipid-lowering function by reducing oxidative stress with activation of Nrf2/HO-1/NQO1 pathway, *Food Chem.* 464 (2025) 141588. <https://doi.org/10.1016/J.FOODCHEM.2024.141588>.
- [11] K. Huang, K. Wang, S. Wu, X. Li, X. Gao, Y. Xiong, F. Bai, X. Song, M. Tan, W. Su, One-step assembly of fucoxanthin delivery system based on kelp nanocellulose with improved environmental stability and bioavailability, *Food Biosci.* 66 (2025) 106295. <https://doi.org/10.1016/j.fbio.2025.106295>.
- [12] S.M.S. Shahriar, J. Mondal, M.N. Hasan, V. Revuri, D.Y. Lee, Y.K. Lee, Electrospinning nanofibers for therapeutics delivery, *Nanomaterials* 9(4) (2019) 532. <https://doi.org/10.3390/nano9040532>.
- [13] M. Zhang, A. Ahmed, L. Xu, Electrospun Nanofibers for Functional Food Packaging Application, *Materials* 16(17) (2023) 5937. <https://doi.org/10.3390/MA16175937>.
- [14] J.B. Pires, L.M. Fonseca, T.J. Siebeneichler, R.L. Crizel, F.N. dos Santos, H.C. dos S. Hackbart, D.H. Kringel, A.D. Meinhart, E. da R. Zavareze, A.R.G. Dias, Curcumin encapsulation in capsules and fibers of potato starch by electrospraying and electrospinning: Thermal resistance and antioxidant activity, *Food Res. Int.* 162 (2022) 112111. <https://doi.org/10.1016/J.FOODRES.2022.112111>.
- [15] X.X. Wang, G.F. Yu, J. Zhang, M. Yu, S. Ramakrishna, Y.Z. Long, Conductive polymer ultrafine fibers via electrospinning: Preparation, physical properties and applications, *Prog. Mater. Sci.* 115 (2021) 100704. <https://doi.org/10.1016/j.pmatsci.2020.100704>.
- [16] S.M.R. Hosseini, P. Heydari, R.N. Azadani, S. Irvani, A. Zarrabi, Sustained release investigation of curcumin and ciprofloxacin on coaxial electrospun nanocomposite scaffold of poly (3-hydroxybutyrate)-poly glycerol sebacate: an approach for skin regeneration, *Emergent. Mater.* 8(1) (2025) 505–516. <https://doi.org/10.1007/S42247-024-00874-Z/METRICS>.
- [17] J. Li, Y. Li, X. Zhang, S. Miao, M. Tan, W. Su, Microfluidic spinning of fucoxanthin-loaded nanofibers for enhancing antioxidation and clarification of fruit juice, *Food Funct.* 13(3) (2022) 1472–1481. <https://doi.org/10.1039/d1fo03766h>.
- [18] X. Tian, J. Li, K. Wang, S. Fei, X. Zhang, C. Wu, M. Tan, W. Su, Microfluidic fabrication of core-shell fucoxanthin nanofibers with improved environmental stability for reducing lipid accumulation in vitro, *Food Chem.* 442 (2024) 138474. <https://doi.org/10.1016/J.FOODCHEM.2024.138474>.
- [19] H. Li, W. Shang, S. Wu, M. Tan, H. Wang, Development of intestine-targeted microcapsules for enhanced delivery of fucoxanthin: A strategy to mitigate lipid accumulation in vitro and in vivo, *Food Biosci.* 59 (2024) 104167. <https://doi.org/10.1016/j.fbio.2024.104167>.
- [20] H. Li, Y. Hou, S. Jia, M. Tan, H. Wang, Oxidized paramylon self-assembled nanoparticles loaded with fucoxanthin attenuate insulin resistance in HpeG2 cells, *Carbohydr. Polym.* 345 (2024) 122597. <https://doi.org/10.1016/j.carbpol.2024.122597>.
- [21] T. İnanc Horuz, K.B. Belibağlı, Nanoencapsulation by electrospinning to improve stability and water solubility of carotenoids extracted from tomato peels, *Food Chem.* 268 (2018) 86–93. <https://doi.org/10.1016/J.FOODCHEM.2018.06.017>.
- [22] Z.H. Zhang, H. Peng, M.W. Woo, X.A. Zeng, M. Brennan, C.S. Brennan, Preparation and characterization of whey protein isolate-chlorophyll microcapsules by spray drying: Effect of WPI ratios on the physicochemical and antioxidant properties, *J. Food Eng.* 267 (2020) 109729. <https://doi.org/10.1016/J.JFOODENG.2019.109729>.
- [23] D. Craig, P. Ye, R. Yusufu, Z. Guan, T. Chen, S. Li, Y. Feng, X. Zeng, J. Lu, M. Luo, F. Wei, Multifunctional Bioactivity Electrospinning Nanofibers Encapsulating Emodin Provide a Potential Postoperative Management Strategy for Skin Cancer, *Pharmaceutics* 16(9) (2024) 1131. <https://doi.org/10.3390/PHARMACEUTICS16091131>.

- [24] M.M. Billah, S. Khatiwada, V. Lecomte, M.J. Morris, C.A. Maloney, Ameliorating high-fat diet-induced sperm and testicular oxidative damage by micronutrient-based antioxidant intervention in rats, *Eur. J. Nutr.* 61(7) (2022) 3741–3753. <https://doi.org/10.1007/s00394-022-02917-9>.
- [25] B. Merino, C.M. Fernández-Díaz, C. Parrado-Fernández, C.M. González-Casimiro, T. Postigo-Casado, C.D. Lobatón, M.A. Leissring, I. Cózar-Castellano, G. Perdomo, Hepatic insulin-degrading enzyme regulates glucose and insulin homeostasis in diet-induced obese mice., *Metabolism* 113 (2020) 154352. <https://doi.org/10.1016/j.metabol.2020.154352>.
- [26] D. Lou, Q. Fang, Y. He, R. Ma, X. Wang, H. Li, M. Qi, Oxymatrine alleviates high-fat diet/streptozotocin-induced non-alcoholic fatty liver disease in C57BL/6 J mice by modulating oxidative stress, inflammation and fibrosis, *Biomedicine* 174 (2024) 116491. <https://doi.org/10.1016/j.biopha.2024.116491>.
- [27] L. Xiao, S.B. Sonne, Q. Feng, N. Chen, Z. Xia, X. Li, Z. Fang, D. Zhang, E. Fjaere, L.K. Midtbø, M. Derrien, F. Hugenholtz, L. Tang, J. Li, J. Zhang, C. Liu, Q. Hao, U.B. Vogel, A. Mortensen, M. Kleerebezem, T.R. Licht, H. Yang, J. Wang, Y. Li, M. Arumugam, J. Wang, L. Madsen, K. Kristiansen, High-fat feeding rather than obesity drives taxonomical and functional changes in the gut microbiota in mice., *Microbiome* 5 (2017) 43. <https://doi.org/10.1186/s40168-017-0258-6>.
- [28] H. Jang, M.B. Kim, H. Kang, O. Corvino, J.Y. Lee, Y.K. Park, Dietary sugar kelp consumption inhibits adipose tissue inflammation and fibrosis in male mouse model of diet-induced obesity, *J. Agric. Food Res.* 17 (2024) 101226. <https://doi.org/10.1016/j.jafr.2024.101226>.
- [29] S. Sookoian, A. Salatino, G.O. Castaño, M.S. Landa, C. Fijalkowky, M. Garaycochea, C.J. Pirola, Intrahepatic bacterial metataxonomic signature in non-alcoholic fatty liver disease, *Gut* 69(8) (2020) 1483–1491. <https://doi.org/10.1136/gutjnl-2019-318811>.
- [30] I. Burakova, Y. Smirnova, M. Gryaznova, M. Syromyatnikov, P. Chizhkov, E. Popov, V. Popov, The Effect of Short-Term Consumption of Lactic Acid Bacteria on the Gut Microbiota in Obese People, *Nutrients* 14(16) (2022) 3384. <https://doi.org/10.3390/nu14163384>.
- [31] Zhong, Weibo, Wu, Kaihui, Long, Zining, Zhou, Xumin, Zhong, Chuanfan, Wang, Shuo, Lai, Houhua, Guo, Yufei, Lv, Daojun, Lu, Jianming, Mao, Xiangming, Gut dysbiosis promotes prostate cancer progression and docetaxel resistance via activating NF-κB-IL6-STAT3 axis, *Microbiome* 10(1) (2022) 94. <https://doi.org/10.1186/s40168-022-01289-w>.
- [32] G. Yuan, M. Tan, X. Chen, Punicic acid ameliorates obesity and liver steatosis by regulating gut microbiota composition in mice, *Food* 12(17) (2021) 7897–7908. <https://doi.org/10.1039/d1fo01152a>.
- [33] X. Li, Y. Qin, F. Yue, X. Lü, Comprehensive Analysis of Fecal Microbiome and Metabolomics Uncovered dl-Norvaline-Ameliorated Obesity-Associated Disorders in High-Fat Diet-Fed Obese Mice by Targeting the Gut Microbiota, *J Agric Food Chem* 73(4) (2025) 2381–2392. <https://doi.org/10.1021/acs.jafc.4c06638>.
- [34] T. Zou, F. Xie, P. Liang, J. Chen, Z. Wang, M. Du, J. You, Polysaccharide-rich fractions from *Enteromorpha prolifera* improve hepatic steatosis and gut barrier integrity in high-fat diet-induced obese mice linking to modulation of gut microbiota, *Biomedicine* 157 (2023) 114034. <https://doi.org/10.1016/j.biopha.2022.114034>.