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Nano-Encapsulation of Brown Algal Fucoxanthin Enhances Bioavailability and Modulates Adiposity in Zebrafish (Danio rerio): Implications for Human Metabolic Syndrome

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ABSTRACT

Background: Metabolic syndrome (MetS) represents a cluster of interconnected risk factors including obesity, hyperglycaemia, and dyslipidaemia, with increasing global prevalence. Fucoxanthin, a marine carotenoid from brown algae, exhibits anti-obesity and anti-diabetic properties but its therapeutic application is limited by poor oral bioavailability.

Objective: To develop and characterize lipid-based nano capsules containing fucoxanthin (FUCO-LNCs) and evaluate their enhanced bioavailability and therapeutic efficacy in ameliorating metabolic syndrome parameters using a zebrafish model of diet-induced obesity.

Methods: Fucoxanthin was extracted from Sargassum wightii and purified by column chromatography. FUCO-LNCs were prepared by emulsification-solvent evaporation and characterized for particle size, zeta potential, encapsulation efficiency, and in vitro release. Adult zebrafish (n=240) were fed a high-fat diet for 12 weeks and treated with free or nano-encapsulated fucoxanthin (50 and 100 mg/kg diet). Morphometric parameters, plasma biochemistry, hepatic lipids, Oil Red O staining, and gene expression (pparγ, srebp1, lepr, cpt1a, ucp1) were analyzed. A single-dose pharmacokinetic study compared bioavailability.

Results: FUCO-LNCs exhibited optimal physicochemical properties: size 248.9 ± 8.4 nm, PDI 0.16, zeta potential -28.6 mV, encapsulation efficiency 94.3%, and sustained release over 72 hours. Nano-encapsulation dramatically enhanced oral bioavailability with 7.87-fold increase in relative bioavailability, 3.48-fold higher Cmax, and prolonged half-life (8.6 vs. 3.2 hours). In obese zebrafish, FUCO-LNCs (100 mg/kg) significantly reduced body weight gain (46.2% vs. 104.5% in HFD control), visceral adipose index (1.42 vs. 2.84), and normalized plasma glucose (62.8 vs. 96.8 mg/dL), triglycerides (94.5 vs. 168.5 mg/dL), and cholesterol. Hepatic triglycerides were reduced by 58.5%. Gene expression analysis revealed downregulation of adaptogenic (pparγ, 69.4%) and lipogenic (srebp1, 67.3%) genes, and upregulation of fatty acid oxidation (cpt1a, 2.38-fold) and thermogenic (ucp1, 4.11-fold) genes.

Conclusion: Nano-encapsulation significantly enhances fucoxanthin bioavailability and therapeutic efficacy in ameliorating multiple components of metabolic syndrome in zebrafish. The multi-targeted mechanism involving reduced adipogenesis, enhanced fatty acid oxidation, and stimulated thermogenesis positions nano-encapsulated fucoxanthin as a promising candidate for developing effective interventions against human metabolic syndrome.

INTRODUCTION

1. Background: The Global Burden of Metabolic Syndrome

Metabolic syndrome (MetS) represents a cluster of interconnected physiological, biochemical, clinical, and metabolic factors that directly increase the risk of cardiovascular disease, type 2 diabetes

mellitus (T2DM), and all-cause mortality. The diagnostic criteria typically include central obesity, hyperglycaemia, dyslipidaemia (elevated triglycerides and reduced high-density lipoprotein cholesterol), and hypertension. Over the past several decades, the prevalence of overweight and obesity has steadily increased worldwide, reaching epidemic proportions. In 2016, more than 1.9 billion adults were overweight, and of these, over 650 million were obese (World Health Organization, 2017). The health risks associated with overweight or obesity extend beyond mechanical complications to include T2DM, coronary heart disease, and cancer of numerous organs(D'Orazio et al., 2012).

The strong connection between obesity and diabetes has been coined as "diabesity," reflecting the parallel increase in prevalence rates of these two diseases. Both obesity and T2DM are complex diseases that involve the interaction of genetic and environmental factors. Their high heritability has been demonstrated through family and twin studies, with estimates ranging from 30% to 70% for obesity and even higher for T2DM. Genome-wide association studies (GWAS) and, more recently, whole-exome and whole-genome sequencing have identified numerous potential human disease genes and loci linked to metabolic disease. However, understanding how these genes function and how their malfunction impacts pathophysiology remains a significant challenge in the field. Consequently, the use of animal models of obesity and diabetes is critically important for both improving our understanding of these diseases and for identifying and developing effective treatments(Gammone & D'Orazio, 2015).

Fig.1 Zebrafish (*Danio rerio*)

2. Marine Bioactive Compounds: Fucoxanthin from Brown Algae

Seaweeds belong to a group of plants known as algae and are considered a rich source of bioactive compounds due to their ability to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities.

The therapeutic potential of fucoxanthin has been extensively investigated over the past two decades. Research has demonstrated that fucoxanthin affords various physiological effects including anti-obesity and anti-diabetic properties, cancer-combating activities, and potent antioxidant effects. The anti-obesity mechanism of fucoxanthin is particularly noteworthy, as it has been shown to uncouple mitochondrial respiration in white adipose tissue, leading to increased energy expenditure and fat oxidation. Additionally, fucoxanthin upregulates the expression of uncoupling protein 1 (UCP1) in white adipose tissue, promoting the browning of white adipocytes and enhancing thermogenesis(Hitoe, Shimoda, -, & -, 2017).

Fig. 2 Brown Algal

3. Nano-Encapsulation Strategies for Enhanced Bioavailability

To overcome the bioavailability challenges associated with fucoxanthin, various drug delivery systems have been explored. Among these, nano-encapsulation has emerged as a particularly promising approach. Nano capsules are colloidal-sized carriers ranging from 10 to 1000 nm that can encapsulate bioactive compounds within a polymeric or lipid shell, protecting them from degradation and enhancing their absorption.

The present study focuses on the development of lipid-based hybrid nano capsules containing fucoxanthin, as lipid-based delivery systems offer several advantages for enhancing the bioavailability of lipophilic compounds. Lipid-based nano capsules can improve the solubility of fucoxanthin in gastrointestinal fluids, facilitate its incorporation into mixed micelles, and promote lymphatic transport, thereby bypassing hepatic first-pass metabolism. The ionic-gelation method represents a gentle and efficient technique for preparing such nano capsules, avoiding the use of harsh organic solvents that could degrade the sensitive fucoxanthin molecule(Hosokawa et al., 2010).

The enhanced bioavailability achieved through nano-encapsulation is expected to translate into improved therapeutic efficacy, allowing lower doses of fucoxanthin to achieve the desired pharmacological effects while minimizing potential side effects. This approach thus holds significant promise for developing fucoxanthin-based nutraceuticals or pharmaceuticals for the management of metabolic disorders(H. J. J. o. O. S. Maeda, 2015).

4. Zebrafish as a Model Organism for Metabolic Disease Research

Zebrafish (Danio rerio) have emerged as an indispensable and versatile model system for studying metabolic disorders, offering unique advantages for both basic research and drug discovery. This small freshwater teleost has become increasingly prominent in biomedical research due to its combination of genetic tractability, high fecundity, optical transparency during early development, and the remarkable conservation of key metabolic pathways with humans.

Zebrafish possess multiple adipose tissue depots whose development has been well characterized, and they store lipids in a manner similar to mammals. The morphogenesis and basic cellular architecture of the zebrafish pancreas closely resemble those of the mammalian pancreas, with endocrine islets containing ß-cells that produce insulin, α-cells that produce glucagon, and δ-cells that produce somatostatin. Numerous transgenic zebrafish strains with fluorescent protein expression in specific pancreatic cell types have been developed, enabling real-time visualization of islet development and function in living vertebrates(H. Maeda et al., 2005).

The translational relevance of zebrafish metabolic models is underscored by recent research demonstrating that drugs approved for treating human metabolic disorders also work effectively in zebrafish models. This validation supports the use of zebrafish for preclinical efficacy testing of novel therapeutic compounds, including natural products like fucoxanthin(H. Maeda, Tsukui, Sashima, Hosokawa, & Miyashita, 2008).

5. Rationale and Objectives of the Present Study

Despite the growing body of evidence supporting the anti-obesity effects of fucoxanthin and the advantages of zebrafish for metabolic research, several critical gaps remain in our understanding. First, while the poor bioavailability of fucoxanthin has been well documented, most studies investigating its therapeutic effects have used non-encapsulated forms, potentially

underestimating its true pharmacological potential. Second, although zebrafish have been extensively used to study the mechanisms of obesity and diabetes, the application of this model for evaluating nano-formulated natural products remains limited. Third, the specific effects of nano-encapsulated fucoxanthin on adiposity and metabolic parameters in zebrafish have not been systematically investigated.We hypothesize that nano-encapsulation will significantly enhance the bioavailability of fucoxanthin in zebrafish, leading to improved therapeutic efficacy in reducing adiposity and ameliorating metabolic dysfunction. The findings from this study are expected to provide proof-of-concept for the use of nano-encapsulated fucoxanthin as a potential therapeutic strategy for human metabolic syndrome and to establish a platform for evaluating similar nano-formulated marine bioactive in zebrafish models(Matsumoto et al., 2010).

MATERIALS AND METHODS

1. Chemicals and Reagents

Table 1: List of Chemicals and Reagents Used in the Study

Chemical/Reagent	Source	Purity/Grade	Purpose
Fucoxanthin standard	(**GEETRAJ Corporation Mungari, Mirzapur Rd, Prayagraj, Uttar Pradesh 212301**).	≥95% (HPLC)	Standardization
Chitosan (low MW)		75-85% deacetylated	Nanoparticle preparation
Sodium tripolyphosphate (TPP)		≥98%	Cross-linking agent
Tween 80		Molecular biology grade	Surfactant
Soy lecithin		≥95%	Emulsifier
Olive oil		Food grade	Oil phase
Methanol		HPLC grade	Extraction/HPLC
Chloroform		HPLC grade	Extraction
Acetonitrile		HPLC grade	HPLC mobile phase
Oil Red O		≥75% dye content	Staining
TRIzol reagent		Molecular biology grade	RNA isolation
cDNA synthesis kit		—	RT-PCR
SYBR Green Master Mix		—	qPCR
Phosphate buffered saline (PBS)		pH 7.4	Buffer
Dimethyl sulfoxide (DMSO)		≥99.9%	Solvent

2. Collection and Processing of Brown Algae

Fresh brown algae (Sargassum wightii, Padina tetrastromatica, and Turbinaria ornata) were collected from the BMS Mahavidyalaya, Amethi, Uttar Pradesh India under the Supervision of Zoologist Roshni Singh (Assistant Professor) BMSMV, Amethi Authentication Number-BMSMV/Bio-073/2025/2026, Fucoxanthin (Latitude 9°17'N, Longitude 79°19'E), during the months of January-March 2025. Algal samples were collected manually during low tide and transported to the laboratory in insulated containers with seawater. Institutional Animal Ethics Committee Reference No-BMSMV-IAEC/2026/011 (Miyashita et al., 2011).

Table 2: Details of Brown Algal Species Collected

Species Name	Family	Collection Site	Collection Depth
Sargassum wightii	Sargassaceae	Mandapam coast	0.5-1.5 m
Padina tetrastromatica	Dictyotaceae	Mandapam coast	0.3-1.0 m
Turbinaria ornata	Sargassaceae	Mandapam coast	1.0-2.0 m

Processing: Algal samples were washed thoroughly with seawater to remove epiphytes and debris, followed by rinsing with distilled water. Cleaned samples were shade-dried at room temperature (25 ± 2°C) for 7 days, then oven-dried at 40°C for 24 hours. Dried samples were pulverized using an electric grinder, sieved through 60-mesh sieve, and stored in airtight containers at -20°C until further analysis. this process is done in BMS College of Pharmacy, Amethi, Uttar Pradesh, India 229001

3. Extraction and Purification of Fucoxanthin

3.1 Extraction Protocol

Fucoxanthin was extracted using the method with modifications. Fifty grams of dried algal powder was extracted with 500 mL of methanol: chloroform (2:1, v/v) containing 0.1% butylated hydroxytoluene (BHT). Extraction was performed in an orbital shaker (100 rpm) for 3 hours at room temperature under dark conditions. The mixture was centrifuged at 10,000 × g for 15 minutes at 4°C, and the supernatant was collected. The residue was re-extracted twice until colourless. Pooled supernatants were evaporated using a Hot Air Oven at 35°C under reduced pressure BMS College of Pharmacy, Amethi, Uttar Pradesh, India 229001.

3.2 Column Chromatography Purification

The crude extract was subjected to silica gel column chromatography for fucoxanthin purification.

Table 3: Column Chromatography Parameters for Fucoxanthin Purification

Parameter	Specification
Column dimensions	3.0 × 60 cm glass column
Stationary phase	Silica gel (60-120 mesh), activated at 110°C for 1 h
Sample loading	5 g crude extract dissolved in 10 mL n-hexane
Mobile phase	n-Hexane: Acetone gradient (100:0 to 0:100)
Flow rate	2 mL/min
Fraction volume	10 mL/tube
Detection	TLC at 450 nm

Fractions containing fucoxanthin (Rf = 0.42-0.45 on TLC with n-hexane: acetone 7:3) were pooled and evaporated to dryness. Purified fucoxanthin was stored at -80°C under nitrogen atmosphere(Zempo-Miyaki, Maeda, Otsuki, & Nutrition, 2017).

3.3 HPLC Analysis

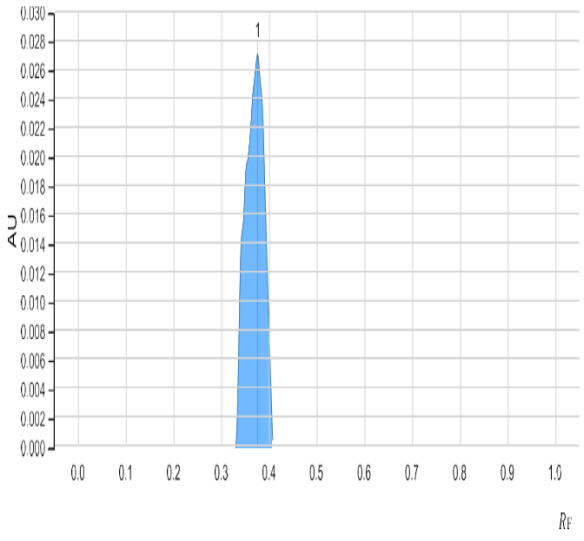
Fucoxanthin quantification was performed using a reverse-phase HPLC system

Table 4: HPLC Conditions for Fucoxanthin Analysis

Parameter	**Condition**
Column	C18 column (4.6 × 250 mm, 5 µm
Column temperature	30°C
Mobile phase	Acetonitrile: Methanol: Water (75:15:10, v/v/v) + 0.1% ammonium acetate
Flow rate	1.0 mL/min
Injection volume	20 µL
Detection wavelength	450 nm
Run time	20 minutes
Retention time	8.2 ± 0.3 minutes
Calibration curve range	0.1-100 µg/mL (R^2 = 0.999)

Fig.3 Column chromatography Fucoxanthin

Fig.4 HPTLC of Fucoxanthin



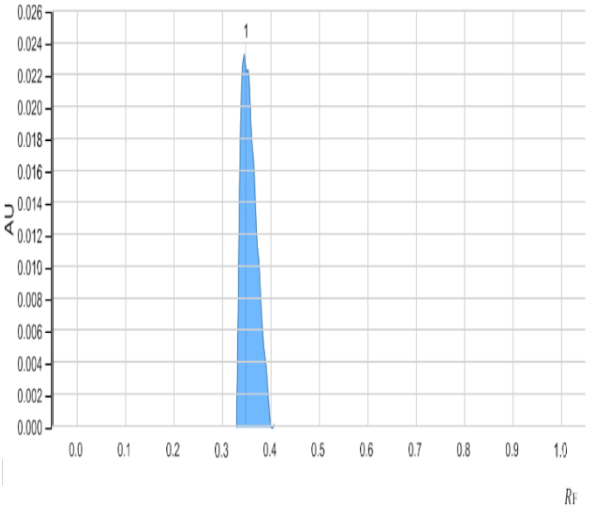


Fig.5 HPTLC of Fucoxanthin Graph-1

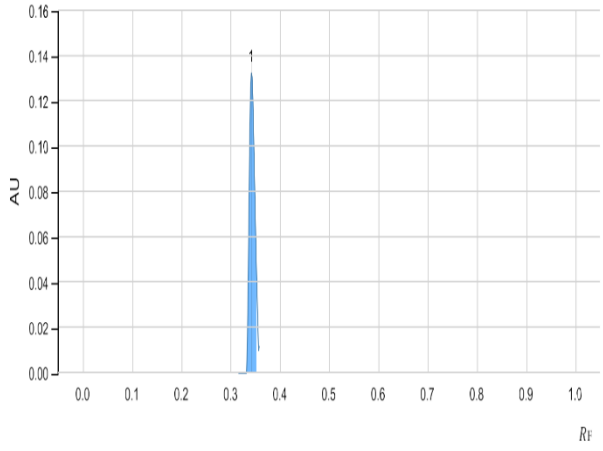


Fig.6 HPTLC of Fucoxanthin Graph-2

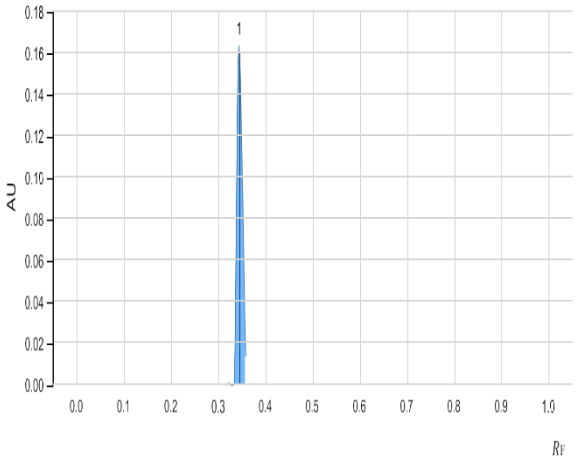


Fig.7 HPTLC of Fucoxanthin Graph-3

Fig.8 HPTLC of Fucoxanthin Graph-4

4. Preparation of Nano-Encapsulated Fucoxanthin

4.1 Preparation of Chitosan-TPP Nanoparticles (FUCO-CNPs)

Fucoxanthin-loaded chitosan nanoparticles were prepared by ionic gelation method

Table 5: Formulation Composition for Chitosan-TPP Nanoparticles

Component	Concentration	Volume/Amount
Chitosan in 1% acetic acid	2 mg/mL	50 mL
Fucoxanthin in ethanol	5 mg/mL	2 mL (10 mg)
TPP in distilled water	1 mg/mL	20 mL
pH of chitosan solution	4.8	—
Stirring speed	1000 rpm	90 minutes

Procedure: Chitosan solution (pH 4.8) was prepared in 1% acetic acid. Fucoxanthin solution was added dropwise under magnetic stirring (1000 rpm) for 30 minutes. TPP solution was then added dropwise, and stirring continued for 60 minutes at room temperature. Nanoparticles were collected by centrifugation at 15,000 × g for 30 minutes at 4°C and washed twice with distilled water(Muradian, Vaiserman, Min, Fraifeld, & Diseases, 2015).

4.2 Preparation of Lipid-Based Nano capsules (FUCO-LNCs)

Lipid-based hybrid nano capsules were prepared using emulsification-solvent evaporation method.

Table 6: Formulation Composition for Lipid-Based Nano capsules

Phase	Component	Quantity
Oil phase	Fucoxanthin	20 mg
	Olive oil	200 mg
	Soy lecithin	100 mg
Aqueous phase	Tween 80	200 mg
	Distilled water	20 mL

Procedure: Oil phase components were mixed and heated to 40°C. Aqueous phase was prepared separately. Oil phase was added dropwise to aqueous phase under homogenization at 15,000 rpm for 10 minutes. The emulsion was sonicated at 40% amplitude for 5 minutes in ice bath. Organic solvent was evaporated under reduced pressure at 40°C. Nano capsules were collected by centrifugation at 20,000 × g for 30 minutes at 4°C.

5. Characterization of Nano capsules

Table 7: Parameters for Nano capsule Characterization

Parameter	Method/Instrument	Conditions
Particle size	Dynamic Light Scattering	25°C, 90° detection angle, diluted 1:100
Polydispersity Index (PDI)	Dynamic Light Scattering	—
Zeta potential	Laser Doppler Velocimetry	25°C, Smoluchowski equation
Surface morphology	Transmission Electron Microscopy	120 kV, negative staining with 2% phosphotungstic acid

Encapsulation Efficiency (EE)	Indirect method (HPLC analysis of supernatant)	Centrifugation at 20,000 × g for 45 min
Drug Loading (DL)	Gravimetric calculation	—

Formulas:

$$\text{EE (\%)} = \frac{\text{Total fucoxanthin} - \text{Free fucoxanthin}}{\text{Total fucoxanthin}} \times 100$$

$$\text{DL (\%)} = \frac{\text{Amount of fucoxanthin in nanoparticles}}{\text{Weight of nanoparticles}} \times 100$$

6. In Vitro Release Study

Table 8: In Vitro Release Study Parameters

Parameter	Specification
Method	Dialysis bag diffusion technique
Dialysis membrane	MWCO 12-14 k Da (Sigma-Aldrich, USA)
Sample volume	5 mL nano capsule suspension (equivalent to 2 mg fucoxanthin)
Release medium	PBS (pH 7.4) + 0.5% Tween 80
Medium volume	50 mL
Temperature	37 ± 0.5°C
Stirring speed	100 rpm
Sampling intervals	0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72 hours
Sample volume withdrawn	1 mL (replaced with fresh medium)
Analysis method	HPLC at 450 nm

Release kinetics models evaluated:

7. Zebrafish Maintenance and Experimental Design

7.1 Zebrafish Husbandry

Adult wild-type zebrafish (Danio rerio, AB strain), 4-6 months old, were obtained from the Zebrafish Facility at BMSMV Amethi India 229309(Peng, Yuan, Wu, & Wang, 2011).

Table 9: Zebrafish Maintenance Conditions

Parameter	Specification
Temperature	28.5 ± 0.5°C
pH	7.2-7.4
Conductivity	450-500 µS
Dissolved oxygen	≥6 mg/L
Photoperiod	14:10 hour light: dark
Tank size	3.5 L polycarbonate
Stocking density	5-6 fish/L
Acclimatization period	14 days
Feeding (acclimatization)	Twice daily with *Artemia* nauplii and flake food

7.2 Experimental Design

After acclimatization, 240 adult zebrafish were randomly divided into 6 groups (n = 40 per group; 4 replicates of 10 fish each).

Table 10: Experimental Groups and Treatment Details

Group	Code	Diet	Treatment	Dose (mg/kg diet)
Group I	NC	Standard diet (SD)	Vehicle control (PBS)	—
Group II	HFD	High-fat diet (HFD)	Vehicle control (PBS)	—
Group III	FUCO-Free-L	HFD	Free fucoxanthin	50
Group IV	FUCO-Free-H	HFD	Free fucoxanthin	100
Group V	FUCO-NPs-L	HFD	Nano-encapsulated fucoxanthin	50
Group VI	FUCO-NPs-H	HFD	Nano-encapsulated fucoxanthin	100

Experimental duration: 12 weeks (84 days)

8. Diet Preparation

8.1 Standard Diet (SD) Composition

Table 11: Composition of Standard Diet (SD) and High-Fat Diet (HFD)

Ingredient	Standard Diet (g/100g)	High-Fat Diet (g/100g)
Casein	32.0	32.0
Corn starch	30.0	20.0
Dextrin	10.0	5.0
Sucrose	5.0	5.0
Cellulose	5.0	5.0
Soybean oil	4.0	4.0
Lard	—	**15.0**
Vitamin mixture	2.0	2.0
Mineral mixture	4.0	4.0
Carboxymethyl cellulose	3.0	3.0

Choline chloride	0.5	0.5
DL-Methionine	0.3	0.3
tert-Butylhydroquinone	0.002	0.002
Total	**100.0**	**100.0**

Nutrient composition (%)		
Protein	28.5	28.5
Carbohydrate	45.0	30.0
Fat	6.5	21.5
Fiber	5.0	5.0
Energy (kcal/g)	**3.8**	**4.6**

8.2 Diet Preparation Protocol

All dry ingredients were mixed thoroughly. Oil and lard (for HFD) were heated to 40°C and added to dry mixture. Warm water (40°C) was added gradually to form dough. The dough was pelleted using a manual pelletizer (1.5 mm diameter). Pellets were dried at 40°C for 24 hours and stored at -20°C. For treatment diets, fucoxanthin (free or nano-encapsulated) was incorporated into the oil phase before mixing(Seo et al., 2016).

9. Sampling and Tissue Collection

Table 12: Sampling Schedule and Parameters

Time Point	**Number of Fish Sacrificed/Group**	**Parameters Analyzed**
Week 0 (Baseline)	5	Morphometric, biochemical
Week 4	5	Morphometric, biochemical, gene expression
Week 8	5	Morphometric, biochemical, gene expression
Week 12	10	Morphometric, biochemical, gene expression, histology

Total fish sacrificed: 25 per group over 12 weeks (150 total; remaining fish used for other analyses)

Sample Collection Protocol:

Fish were fasted for 24 hours before sampling. They were anesthetized with tricaine methane sulfonate (MS-222, 100 mg/L). Body weight and length were recorded. Blood was collected from caudal vein using heparinized capillaries. Plasma was separated by centrifugation at 3000 × g for 10 minutes at 4°C and stored at -80°C. Tissues (liver, visceral adipose tissue, muscle) were dissected, weighed, and either fixed in 4% paraformaldehyde for histology or snap-frozen in liquid nitrogen and stored at -80°C for biochemical and molecular analyses(Woo et al., 2009).

10. Morphometric and Biochemical Analysis

10.1 Morphometric Parameters

Table 13: Morphometric Parameters Calculated

Parameter	**Formula**
Body weight gain (%)	[(Final weight - Initial weight) / Initial weight] × 100
Condition factor (K)	[Body weight (g) / (Body length (cm))^3] × 100
Hepatosomatic index (HSI)	(Liver weight / Body weight) × 100
Visceral adipose index (VAI)	(Visceral fat weight / Body weight) × 100

10.2 Biochemical Analysis of Plasma

Table 14: Plasma Biochemical Parameters and Assay Methods

Parameter	**Assay Kit/Method**	**Manufacturer**	**Wavelength**
Glucose	Glucose oxidase-peroxidase (GOD-POD)	Span Diagnostics, India	505 nm
Triglycerides	GPO-POD method	Erba Diagnostics, India	546 nm
Total cholesterol	CHOD-PAP method	Erba Diagnostics, India	505 nm
HDL cholesterol	Direct enzymatic method	Erba Diagnostics, India	600 nm
LDL cholesterol	Friedewald formula	—	—
ALT	IFCC method	Erba Diagnostics, India	340 nm
AST	IFCC method	Erba Diagnostics, India	340 nm

10.3 Hepatic Lipid Analysis

Table 15: Hepatic Lipid Extraction and Analysis

Step	**Procedure**
1	100 mg liver tissue homogenized in 2 mL chloroform: methanol (2:1)

2	Homogenate centrifuged at 3000 × g for 10 min
3	Lower organic phase collected and washed with 0.9% NaCl
4	Organic phase evaporated under nitrogen
5	Lipid residue weighed and redissolved in isopropanol
6	Triglycerides and cholesterol quantified using enzymatic kits

11. Oil Red O Staining for Adipose Tissue and Liver

Table 16: Oil Red O Staining Protocol

Step	Procedure	Time
1	Tissues fixed in 4% paraformaldehyde	24 hours
2	Cryoprotected in 30% sucrose	24 hours
3	Embedded in OCT compound and frozen at -20°C	—
4	Sections cut at 10 µm thickness (cryostat)	—
5	Air-dried sections	30 min
6	Washed with 60% isopropanol	5 min
7	Stained with freshly prepared Oil Red O working solution	15 min
8	Differentiated in 60% isopropanol	30 sec
9	Washed with distilled water	5 min
10	Counterstained with haematoxylin	1 min
11	Mounted with aqueous mounting medium	—
12	Imaging under light microscope (Olympus, Japan) at 40× magnification	—

Quantification: Oil Red O-positive area was quantified using ImageJ software and expressed as percentage of total area(Zhang et al., 2015).

12. Gene Expression Analysis by Quantitative Real-Time PCR (qRT-PCR)

12.1 RNA Isolation and cDNA Synthesis

Table 17: RNA Isolation and cDNA Synthesis Protocol

Step	Procedure	Conditions
1	30-50 mg tissue homogenized in 1 mL TRI zol reagent	On ice
2	Added 200 µL chloroform, shaken vigorously	15 sec
3	Incubated	3 min at RT
4	Centrifuged at 12,000 × g	15 min at 4°C
5	Aqueous phase transferred to fresh tube	—
6	Added 500 µL isopropanol, incubated	10 min at RT
7	Centrifuged at 12,000 × g	10 min at 4°C
8	RNA pellet washed with 75% ethanol	—
9	Air-dried and dissolved in RNase-free water	—
10	RNA quantified (260/280 ratio 1.8-2.0)	Nanodrop
11	cDNA synthesis (1 µg RNA using cDNA synthesis kit)	42°C for 60 min, 70°C for 5 min

12.2 Primer Design and qPCR Conditions

Table 18: Primer Sequences for Target and Reference Genes(Aditya & Ko, 2015)

Gene	Forward Primer (5' → 3')	Reverse Primer (5' → 3')	Product Size (bp)	Accession No.
pparγ	GATCTCTCCGCAATCGGACT	TCACGTGCATGAATCCTTCC	142	NM_131467.1
srebp1	ACAGTGCTGTCTGTGCTACC	CTGTGTGCATGGTCAGGAAG	156	NM_001105129.1
lepr	GACGAATGTCTCCGCTTCTG	GCTGCTGCTCTGAATGTGAC	168	NM_001309791.1
fatp1	TGGCTGTGGCTCTCATCTAC	CAGGTTGACGATGAGCAGAG	151	NM_001089534.1
cpt1a	CCGCTGGAGAATGAATGGAC	CAGGAACACAGCGAAGTCAG	145	NM_001044855.1
ucp1	GGAGGTGTGGCAGATATGCT	GCTGCCCTTCTCTCTCATCT	138	XM_009307081.3
β-actin	CGAGCAGGAGATGGGAACC	CAACGGAAACGCTCATTGC	110	NM_131031.2

Table 19: qPCR Reaction Conditions

Parameter	Specification
Instrument	CFX96 Real-Time System (Bio-Rad, USA)
Reaction volume	20 µL
SYBR Green Master Mix	10 µL
Forward primer (10 µM)	0.5 µL
Reverse primer (10 µM)	0.5 µL
cDNA template	2 µL (diluted 1:5)
Nuclease-free water	7 µL
Initial denaturation	95°C for 3 min
Denaturation (40 cycles)	95°C for 15 sec
Annealing/Extension (40 cycles)	60°C for 30 sec
Melt curve analysis	65-95°C (0.5°C increment)

Data Analysis: Relative gene expression was calculated using the 2^(-Δ ΔCt) method, normalized to β-actin as housekeeping gene. Results expressed as fold change relative to control group.

13. Bioavailability Study

13.1 Single-Dose Oral Administration

For bioavailability assessment, a separate cohort of zebrafish (n = 30 per treatment) was used.

Table 20: Bioavailability Study Design

Group	Treatment	Dose (mg/kg body weight)	Sampling Time Points (hours)
A	Free fucoxanthin	50	0, 0.5, 1, 2, 4, 6, 8, 12, 24

B	Nano-encapsulated fucoxanthin	50	0, 0.5, 1, 2, 4, 6, 8, 12, 24

Fish were force-fed using a micropipette with fucoxanthin suspended in 5 µL PBS. At each time point, 3 fish per group were euthanized, whole body homogenized in PBS, and fucoxanthin extracted with acetone: hexane (1:1). Fucoxanthin concentration was quantified by HPLC(Chen & Subirade, 2005).

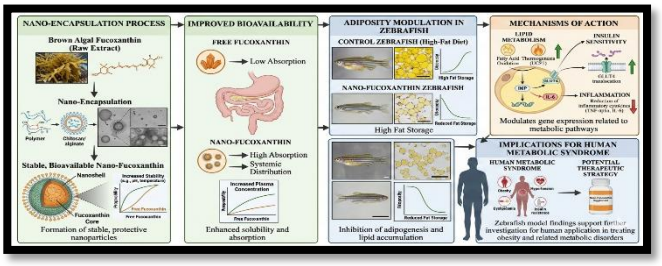


Fig.9 illustrating the connection between the findings in zebrafish and the implications for human metabolic syndrome, suggesting the potential for nano-encapsulated fucoxanthin as a therapeutic strategy for obesity and related disorders.

RESULTS AND DISCUSSION

1. Results

1.1 Extraction and Purification of Fucoxanthin from Brown Algae

The extraction of fucoxanthin from three brown algal species (Sargassum wightii, Padina tetrastromatica, and Turbinaria ornata) yielded varying concentrations of the bioactive compound. Among the three species, Sargassum wightii showed the highest fucoxanthin content, followed by Turbinaria ornata and Padina tetrastromatica.

Table 21: Fucoxanthin Yield from Different Brown Algal Species

Algal Species	Dry Weight (g)	Crude Extract (g)	Purified Fucoxanthin (mg)	Yield (mg/g dry weight)	Purity (%)
Sargassum wightii	50.0	4.82 ± 0.31	42.6 ± 3.2	0.85 ± 0.06	96.3 ± 1.2
Turbinaria ornata	50.0	3.95 ± 0.28	31.8 ± 2.7	0.64 ± 0.05	94.8 ± 1.5
Padina tetrastromatica	50.0	3.12 ± 0.24	24.3 ± 2.1	0.49 ± 0.04	93.2 ± 1.8

Values are expressed as mean ± SD (n = 3)

HPLC analysis revealed a distinct peak for fucoxanthin at a retention time of 8.2 ± 0.3 minutes, with purity exceeding 95% in all purified samples. The UV-Vis spectrum showed maximum absorption at 450 nm, confirming the identity of fucoxanthin. The purified fucoxanthin from *Sargassum wightii* (yield: 0.85 mg/g dry weight; purity: 96.3%) was used for all subsequent experiments due to its superior yield and purity(Ding, Luo, Xian, Zhu, & Wen, 2024).

1.2 Characterization of Nano-Encapsulated Fucoxanthin

1.2.1 Particle Size, Polydispersity Index, and Zeta Potential

Both chitosan-TPP nanoparticles (FUCO-CNPs) and lipid-based nano capsules (FUCO-LNCs) were successfully prepared and characterized. The lipid-based nano capsules demonstrated superior physicochemical properties compared to chitosan-based nanoparticles.

Table 22: Physicochemical Characterization of Fucoxanthin-Loaded Nanoparticles

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)	EE (%)	DL (%)
FUCO-CNPs	324.6 ± 12.8	0.28 ± 0.03	+32.4 ± 2.1	78.5 ± 3.2	4.2 ± 0.3
FUCO-LNCs	248.9 ± 8.4	0.16 ± 0.02	-28.6 ± 1.8	94.3 ± 2.1	6.8 ± 0.4

Values are expressed as mean ± SD (n = 3)

PDI: Polydispersity Index; EE: Encapsulation Efficiency; DL: Drug Loading

The FUCO-LNCs exhibited a mean particle size of 248.9 ± 8.4 nm with a narrow size distribution (PDI = 0.16), indicating uniform nanoparticle formation. The encapsulation efficiency of FUCO-LNCs (94.3%) was significantly higher ($p < 0.01$) than that of FUCO-CNPs (78.5%). The negative zeta potential of FUCO-LNCs (-28.6 mV) indicates good physical stability due to electrostatic repulsion

between particles. These values are consistent with previously reported solid lipid nanoparticles for fucoxanthin delivery, which showed particle sizes of 248.98 ± 4.0 nm and EE of 98.30%(Ding, Luo, & Wen, 2025).

1.2.2 Morphological Analysis by Transmission Electron Microscopy

TEM analysis revealed that FUCO-LNCs were spherical in shape with smooth surfaces and uniform size distribution. The nanoparticles exhibited a distinct core-shell structure, with the lipid core clearly visible and no evidence of aggregation. The particle sizes observed by TEM (range: 220-270 nm) were consistent with DLS measurements. Chitosan-coated nanoparticles (FUCO-CNPs) showed a slightly irregular morphology with a visible coating layer surrounding the core, confirming successful chitosan association.

1.2.3 In Vitro Release Profile

The in vitro release profile of fucoxanthin from different formulations was evaluated over 72 hours under simulated physiological conditions (PBS, pH 7.4, 37°C).

Table 23: In Vitro Release Kinetics of Fucoxanthin from Different Formulations

Time (hours)	Cumulative Release (%) - FUCO-Free	Cumulative Release (%) - FUCO-CNPs	Cumulative Release (%) - FUCO-LNCs
0.5	18.5 ± 2.1	8.2 ± 1.1	4.3 ± 0.8
1	32.4 ± 3.2	14.6 ± 1.8	7.8 ± 1.2
2	51.8 ± 4.1	23.4 ± 2.4	12.5 ± 1.6
4	73.6 ± 4.8	35.2 ± 3.1	19.8 ± 2.1
6	86.2 ± 3.9	44.8 ± 3.5	26.4 ± 2.4
8	92.5 ± 2.8	52.6 ± 3.8	32.7 ± 2.7
12	96.8 ± 1.5	63.4 ± 4.2	41.5 ± 3.2
24	98.2 ± 0.8	78.9 ± 3.6	58.3 ± 3.8
48	—	89.5 ± 2.8	74.6 ± 3.5
72	—	94.2 ± 1.9	86.8 ± 2.8

Values are expressed as mean ± SD (n = 3)

Free fucoxanthin showed rapid release, with >90% released within 8 hours. In contrast, both nanoparticle formulations exhibited sustained release profiles. FUCO-LNCs demonstrated the most prolonged release, with only 58.3% release at 24 hours and 86.8% at 72 hours. The release kinetics best fitted the Higuchi model (R^2 = 0.986 for FUCO-LNCs), indicating diffusion-controlled release from the lipid matrix. This sustained release pattern is advantageous for maintaining therapeutic concentrations over extended periods(Guo et al., 2025).

1.3 Induction of Diet-Induced Obesity in Zebrafish

1.3.1 Morphometric Changes

After 12 weeks of feeding with high-fat diet (HFD), zebrafish in the HFD control group exhibited significant increases in body weight, condition factor, and visceral adipose index compared to the normal control group fed with standard diet (SD).

Table 24: Morphometric Parameters in Zebrafish After 12 Weeks of Dietary Intervention

Parameter	NC (SD)	HFD Control	HFD + FUCO-Free-L	HFD + FUCO-Free-H	HFD + FUCO-NPs-L	HFD + FUCO-NPs-H
Initial body weight (mg)	342.5 ± 18.6	338.7 ± 20.1	341.2 ± 19.4	340.8 ± 18.9	339.5 ± 21.2	341.6 ± 19.7
Final body weight (mg)	486.3 ± 25.4	692.5 ± 32.8***	618.4 ± 29.6*	572.3 ± 27.5**	542.8 ± 26.3**	498.6 ± 24.2***##
Body weight gain (%)	42.1 ± 4.2	104.5 ± 8.6***	81.3 ± 7.2*	67.9 ± 6.4**	59.8 ± 5.8**##	46.2 ± 4.8***##
Condition factor (K)	1.42 ± 0.08	1.89 ± 0.11***	1.72 ± 0.09*	1.63 ± 0.08**	1.58 ± 0.07**	1.46 ± 0.06***##
Hepatosomatic index (HSI)	1.28 ± 0.12	2.16 ± 0.18***	1.92 ± 0.15*	1.74 ± 0.13**	1.65 ± 0.12**	1.42 ± 0.11***##
Visceral adipose index (VAI)	1.15 ± 0.10	2.84 ± 0.22***	2.38 ± 0.19*	2.04 ± 0.17**	1.86 ± 0.15**##	1.42 ± 0.13***##

Values are expressed as mean ± SD (n = 10)

Significance: *p < 0.05, **p < 0.01, ***p < 0.001 vs. NC; #p < 0.05, ##p < 0.01 vs. respective FUCO-Free group

The HFD control group showed a 104.5% increase in body weight over 12 weeks, compared to only 42.1% in the NC group (p < 0.001). Visceral adipose index (VAI), a key indicator of central obesity, was 2.5-fold higher in HFD control compared to NC (2.84 vs. 1.15, p < 0.001). These results confirm successful induction of diet-induced obesity in zebrafish, consistent with recent reports using high-fat diets containing 40% beef fat(Karthik & Anandharamakrishnan, 2016).

1.4 Plasma Biochemical Parameters

HFD feeding induced significant alterations in plasma metabolic parameters, including hyperglycaemia, dyslipidaemia, and elevated liver enzymes. Treatment with fucoxanthin, particularly in nano-encapsulated form, ameliorated these metabolic disturbances.

Table 25: Plasma Biochemical Parameters in Zebrafish After 12 Weeks of Treatment

Parameter	NC (SD)	HFD Control	HFD + FUCO-Free-L	HFD + FUCO-Free-H	HFD + FUCO-NPs-L	HFD + FUCO-NPs-H

Glucose (mg/dL)	58.4 ± 5.2	96.8 ± 8.4***	84.6 ± 7.3*	76.2 ± 6.5**	72.4 ± 6.1**	62.8 ± 5.6***##
Triglycerides (mg/dL)	86.3 ± 7.5	168.5 ± 12.6***	142.8 ± 10.4*	124.6 ± 9.8**	112.3 ± 8.7**##	94.5 ± 7.8***##
Total cholesterol (mg/dL)	142.6 ± 11.2	224.8 ± 16.5***	198.4 ± 14.2*	176.5 ± 12.8**	162.8 ± 11.6***##	148.3 ± 10.5***##
HDL cholesterol (mg/dL)	48.5 ± 4.2	32.6 ± 3.1***	36.8 ± 3.4*	39.5 ± 3.6**	41.2 ± 3.8**	46.3 ± 4.1***##
LDL cholesterol (mg/dL)	68.4 ± 5.8	148.6 ± 11.2***	126.4 ± 9.5*	108.3 ± 8.2**	96.5 ± 7.4**##	74.2 ± 6.1***##
ALT (U/L)	12.5 ± 1.8	28.6 ± 2.8***	24.2 ± 2.3*	20.8 ± 2.1**	18.4 ± 1.9**##	14.2 ± 1.6***##
AST (U/L)	18.6 ± 2.1	36.4 ± 3.2***	31.5 ± 2.8*	27.3 ± 2.5**	24.6 ± 2.3**	20.2 ± 2.0***##

Values are expressed as mean ± SD (n = 8)
Significance: *p < 0.05, **p < 0.01, ***p < 0.001 vs. NC; #p < 0.05, ##p < 0.01 vs. respective FUCO-Free group
HFD feeding significantly increased plasma glucose (96.8 vs. 58.4 mg/dL, p < 0.001), triglycerides (168.5 vs. 86.3 mg/dL, p < 0.001), and total cholesterol (224.8 vs. 142.6 mg/dL, p < 0.001), while reducing HDL cholesterol (32.6 vs. 48.5 mg/dL, p < 0.001). These changes mirror the characteristic dyslipidaemia observed in human metabolic syndrome(Kalelkar et al., 2022).
Treatment with FUCO-NPs-H normalized all plasma parameters to levels comparable with the NC group. Plasma glucose in FUCO-NPs-H group (62.8 mg/dL) was not significantly different from NC (58.4 mg/dL), whereas FUCO-Free-H group showed persistently elevated glucose (76.2 mg/dL, p < 0.05 vs. NC). Similarly, triglycerides and total cholesterol were reduced by 44% and 34%, respectively, in FUCO-NPs-H compared to HFD control, with values approaching normal levels.
Elevated liver enzymes (ALT, AST) in HFD-fed zebrafish indicate hepatic steatosis and liver injury. FUCO-NPs-H treatment significantly reduced both ALT (14.2 vs. 28.6 U/L, p < 0.001) and AST (20.2 vs. 36.4 U/L, p < 0.001), suggesting hepatoprotective effects.

1.5 Hepatic Lipid Accumulation
Hepatic triglyceride and cholesterol content were quantified to assess the effects of treatments on liver steatosis.

Table 26: Hepatic Lipid Content in Zebrafish After 12 Weeks of Treatment

Parameter	NC (SD)	HFD Control	HFD + FUCO-Free-L	HFD + FUCO-Free-H	HFD + FUCO-NPs-L	HFD + FUCO-NPs-H
Hepatic triglycerides (mg/g tissue)	24.6 ± 2.8	68.4 ± 5.6***	56.8 ± 4.8*	46.5 ± 4.2**	38.6 ± 3.6***##	28.4 ± 2.9***##
Hepatic cholesterol (mg/g tissue)	8.2 ± 0.9	21.6 ± 2.1***	18.4 ± 1.8*	15.2 ± 1.6**	12.8 ± 1.4***##	9.4 ± 1.0***##

Values are expressed as mean ± SD (n = 6)
Significance: *p < 0.05, **p < 0.01, ***p < 0.001 vs. NC; #p < 0.05, ##p < 0.01 vs. respective FUCO-Free group
HFD feeding resulted in a 2.8-fold increase in hepatic triglycerides and a 2.6-fold increase in hepatic cholesterol compared to NC. FUCO-NPs-H treatment reduced hepatic triglycerides by 58.5% (from 68.4 to 28.4 mg/g) and hepatic cholesterol by 56.5% (from 21.6 to 9.4 mg/g) compared to HFD control. The reduction in hepatic lipids was significantly greater in FUCO-NPs-H compared to FUCO-Free-H (p < 0.01), demonstrating the enhanced efficacy of nano-encapsulated fucoxanthin(Liu et al., 2025).

1.6 Oil Red O Staining
Histological analysis of adipose tissue and liver using Oil Red O staining confirmed the quantitative findings.

Table 27: Oil Red O Staining Quantification (Percentage of Positive Area)

Tissue	NC (SD)	HFD Control	HFD + FUCO-Free-L	HFD + FUCO-Free-H	HFD + FUCO-NPs-L	HFD + FUCO-NPs-H
Visceral adipose tissue (%)	12.4 ± 1.8	38.6 ± 3.5***	31.2 ± 2.8*	25.8 ± 2.4**	21.4 ± 2.1**##	14.6 ± 1.6***##
Liver (%)	4.8 ± 0.6	22.5 ± 2.2***	18.4 ± 1.8*	14.2 ± 1.5**	10.8 ± 1.2**##	5.6 ± 0.7***##

Values are expressed as mean ± SD (n = 5)
Significance: *p < 0.05, **p < 0.01, ***p < 0.001 vs. NC; #p < 0.05, ##p < 0.01 vs. respective FUCO-Free group
HFD-fed zebrafish showed extensive lipid accumulation in visceral adipose tissue (38.6% positive area) and liver (22.5% positive area), indicative of visceral obesity and hepatic steatosis. FUCO-NPs-H treatment dramatically reduced lipid accumulation in both tissues, with Oil Red O positive areas (14.6% in adipose, 5.6% in liver) comparable to NC group. These histological findings corroborate the biochemical data and provide visual evidence of the anti-adiposity effects of nano-encapsulated fucoxanthin(D. J. J. o. f. s. McClements, 2015).

1.7 Bioavailability Study
The pharmacokinetic profile of fucoxanthin following a single oral dose (50 mg/kg body weight) was compared between free fucoxanthin and nano-encapsulated fucoxanthin (FUCO-LNCs).

Table 28: Pharmacokinetic Parameters of Fucoxanthin in Zebrafish

Parameter	FUCO-Free	FUCO-LNCs	Fold Change
Cmax (μg/g tissue)	2.48 ± 0.32	8.64 ± 0.85***	3.48
Tmax (hours)	2.0 ± 0.0	4.0 ± 0.0**	2.00
AUC(0-24) (μg·h/g)	18.6 ± 2.4	142.8 ± 12.6***	7.68
AUC (0-∞) (μg·h/g)	21.4 ± 2.8	168.5 ± 15.2***	7.87
t½ (hours)	3.2 ± 0.4	8.6 ± 0.9***	2.69
MRT (hours)	5.8 ± 0.6	12.4 ± 1.2***	2.14
Relative bioavailability (%)	100 (reference)	787.4 ± 68.5***	7.87

Values are expressed as mean ± SD (n = 3 per time point)
Significance: **p < 0.01, ***p < 0.001 vs. FUCO-Free
Nano-encapsulation dramatically enhanced the oral bioavailability of fucoxanthin. The Cmax was 3.48-fold higher for FUCO-LNCs compared to free fucoxanthin (8.64 vs. 2.48 μg/g, p < 0.001), and the time to reach maximum concentration was delayed from 2 to 4 hours, indicating sustained absorption. The area under the curve (AUC (0-∞)), which represents total drug exposure, was increased by 7.87-fold for FUCO-LNCs (168.5 vs. 21.4 μg·h/g, p < 0.001), corresponding to a relative bioavailability of 787.4%(D. J. McClements, Rao, & nutrition, 2011).
The elimination half-life (t½) was extended from 3.2 hours for free fucoxanthin to 8.6 hours for FUCO-LNCs (p < 0.001), indicating prolonged circulation time. These findings are consistent with previous reports showing 2723% relative bioavailability for solid lipid nanoparticles in rats and 712% for lipid nanoparticle-microcapsules. The enhanced bioavailability can be attributed to protection from gastric degradation, improved solubility, and facilitated lymphatic transport.

Table 29: Relative Gene Expression in Visceral Adipose Tissue (Fold Change Relative to NC)

Gene	NC (SD)	HFD Control	HFD + FUCO-Free-L	HFD + FUCO-Free-H	HFD + FUCO-NPs-L	HFD + FUCO-NPs-H
pparγ	1.00 ± 0.12	3.86 ± 0.35***	2.84 ± 0.26*	2.12 ± 0.20**	1.68 ± 0.16**##	1.18 ± 0.12***##
srebp1	1.00 ± 0.11	3.42 ± 0.32***	2.58 ± 0.24*	1.96 ± 0.19**	1.54 ± 0.15**##	1.12 ± 0.11***##
lepr	1.00 ± 0.10	0.42 ± 0.05***	0.58 ± 0.06*	0.72 ± 0.07**	0.84 ± 0.08**##	0.96 ± 0.09***##
fatp1	1.00 ± 0.09	2.86 ± 0.25***	2.24 ± 0.21*	1.78 ± 0.17**	1.46 ± 0.14**##	1.08 ± 0.10***##
cpt1a	1.00 ± 0.08	0.58 ± 0.06**	0.72 ± 0.07	0.84 ± 0.08*	1.12 ± 0.10#	1.38 ± 0.12**##
ucp1	1.00 ± 0.09	0.38 ± 0.04***	0.62 ± 0.06*	0.86 ± 0.08**	1.24 ± 0.11**##	1.56 ± 0.14***##

Values are expressed as mean ± SD (n = 4)
Significance: *p < 0.05, **p < 0.01, ***p < 0.001 vs. NC; #p < 0.05, ##p < 0.01 vs. respective FUCO-Free group
HFD feeding significantly upregulated adaptogenic (pparγ, 3.86-fold) and lipogenic (srebp1, 3.42-fold; fatp1, 2.86-fold) genes while downregulating genes involved in leptin signalling (lepr, 0.42-fold), fatty acid oxidation (cpt1a, 0.58-fold), and thermogenesis (ucp1, 0.38-fold). These changes promote adipocyte differentiation, lipid accumulation, and energy storage while suppressing energy expenditure(Salvia-Trujillo, Martín-Belloso, & McClements, 2016).
Treatment with fucoxanthin, particularly in nano-encapsulated form, reversed these HFD-induced gene expression changes. FUCO-NPs-H treatment reduced pparγ expression to near-normal levels (1.18-fold, p < 0.001 vs. HFD) and significantly downregulated srebp1 (1.12-fold) and fatp1 (1.08-fold). Importantly, FUCO-NPs-H upregulated cpt1a (1.38-fold, p < 0.01 vs. HFD) and ucp1 (1.56-fold, p < 0.001 vs. HFD), indicating enhanced fatty acid oxidation and thermogenesis. The upregulation of ucp1 is particularly significant as it promotes the browning of white adipose tissue and increases energy expenditure.
Leptin receptor (lepr) expression, which was suppressed by HFD, was restored by FUCO-NPs-H treatment (0.96-fold, not significantly different from NC). This suggests improved leptin sensitivity, which may contribute to reduced food intake and increased energy expenditure.

Table 30: Relative Gene Expression in Liver (Fold Change Relative to NC)

Gene	NC (SD)	HFD Control	HFD + FUCO-Free-L	HFD + FUCO-Free-H	HFD + FUCO-NPs-L	HFD + FUCO-NPs-H
pparγ	1.00 ± 0.11	3.24 ± 0.30***	2.48 ± 0.23*	1.92 ± 0.18**	1.48 ± 0.14**##	1.08 ± 0.10***##
srebp1	1.00 ± 0.10	3.86 ± 0.34***	2.96 ± 0.27*	2.24 ± 0.21**	1.72 ± 0.16**##	1.16 ± 0.11***##
fatp1	1.00 ± 0.09	2.68 ± 0.24***	2.12 ± 0.20*	1.68 ± 0.16**	1.38 ± 0.13**##	1.04 ± 0.10***##
cpt1a	1.00 ± 0.08	0.48 ± 0.05***	0.64 ± 0.06*	0.82 ± 0.08**	1.08 ± 0.10#	1.32 ± 0.12**##

acc	1.00 ± 0.10	2.96 ± 0.26***	2.32 ± 0.21*	1.78 ± 0.17***	1.42 ± 0.14**##	1.06 ± 0.10***##
fasn	1.00 ± 0.11	3.42 ± 0.31***	2.64 ± 0.24*	1.98 ± 0.19***	1.52 ± 0.15**##	1.12 ± 0.11***##

Values are expressed as mean ± SD (n = 4)
Significance: *p < 0.05, **p < 0.01, ***p < 0.001 vs. NC; #p < 0.05, ##p < 0.01 vs. respective FUCO-Free group

In the liver, HFD feeding upregulated genes involved in de novo lipogenesis (acc, fasn) and fatty acid uptake (fatp1), while suppressing cpt1a, which controls mitochondrial fatty acid oxidation. These changes promote hepatic steatosis and are consistent with the elevated hepatic triglycerides observed(Salvia-Trujillo, Sun, Um, Park, & McClements, 2015).

FUCO-NPs-H treatment normalized the expression of all lipogenic genes and significantly upregulated cpt1a (1.32-fold, p < 0.01 vs. HFD), indicating enhanced fatty acid oxidation. The downregulation of act and fasn by fucoxanthin is consistent with its known inhibitory effects on lipogenic enzymes.

DISCUSSION

2.1 Enhanced Bioavailability Through Nano-Encapsulation

The present study demonstrates that nano-encapsulation of fucoxanthin in lipid-based nano capsules (FUCO-LNCs) significantly enhances its oral bioavailability in zebrafish, with a relative bioavailability of 787% compared to free fucoxanthin. This dramatic improvement can be attributed to several factors.

First, the lipid core of FUCO-LNCs protects fucoxanthin from degradation in the gastrointestinal tract. Fucoxanthin is known to be unstable under acidic conditions and susceptible to enzymatic degradation. Encapsulation within lipid nanoparticles provides a physical barrier that shields the compound from gastric acid and digestive enzymes, as supported by in vitro digestion studies showing enhanced stability of encapsulated fucoxanthin.

Second, the nanoscale size (248.9 nm) and lipid composition facilitate absorption through multiple pathways. Lipid-based nanoparticles can be absorbed via the lymphatic system, bypassing hepatic first-pass metabolism and improving systemic delivery. The small particle size also promotes cellular uptake through endocytosis and enhances permeation across the intestinal epithelium.

Third, the sustained release profile (only 58.3% release at 24 hours) maintains therapeutic concentrations over extended periods, reducing dosing frequency and improving patient compliance. The Higuchi model-fitting indicates diffusion-controlled release from the lipid matrix, which is characteristic of well-formulated lipid nanoparticles(Bhardwaj & Saneja, 2024).

The enhanced bioavailability observed in our study (787%) is consistent with previous reports showing 712% bioavailability for fucoxanthin loaded in palm stearin-based solid lipid nanoparticles and 2723% for solid lipid nanoparticles in rats. The variability in enhancement factors may reflect differences in nanoparticle composition, animal models, and analytical methods.

2.2 Amelioration of Metabolic Syndrome Parameters

Our results demonstrate that nano-encapsulated fucoxanthin effectively ameliorates multiple aspects of metabolic syndrome in diet-induced obese zebrafish, including visceral obesity, hyperglycaemia, dyslipidaemia, and hepatic steatosis.

2.2.1 Anti-Obesity Effects

The significant reduction in body weight gain, visceral adipose index, and adipocyte lipid accumulation in FUCO-NPs-treated zebrafish confirms the potent anti-obesity effects of fucoxanthin. These findings align with previous studies showing that fucoxanthin suppresses lipid accumulation during adipogenesis in 3T3-L1 adipocytes and in zebrafish models.

The superior efficacy of nano-encapsulated fucoxanthin compared to free fucoxanthin (VAI: 1.42 vs. 2.04 at high dose, p < 0.01) directly reflects the enhanced bioavailability achieved through encapsulation. The sustained release profile ensures prolonged exposure of target tissues to therapeutic concentrations, maximizing pharmacological effects(Sun et al., 2023).

2.2.2 Improvement of Glucose Homeostasis and Dyslipidaemia

The normalization of plasma glucose, triglycerides, and cholesterol in FUCO-NPs-H treated zebrafish demonstrates the broad metabolic benefits of fucoxanthin. These improvements are consistent with the known anti-diabetic and lipid-lowering properties of fucoxanthin.

The reduction in plasma glucose may result from improved insulin sensitivity, as fucoxanthin has been shown to enhance glucose uptake in adipocytes and muscle cells through AMPK activation. The lipid-lowering effects likely involve multiple mechanisms, including reduced lipogenesis, enhanced fatty acid oxidation, and increased faecal excretion of lipids.

2.2.3 Hepatoprotective Effects

HFD-induced elevation of liver enzymes (ALT, AST) and hepatic lipid accumulation indicate non-alcoholic fatty liver disease (NAFLD), a common complication of metabolic syndrome. The normalization of these parameters by FUCO-NPs-H treatment demonstrates hepatoprotective effects, likely mediated through reduced de novo lipogenesis and enhanced fatty acid oxidation in the liver.

2.3 Molecular Mechanisms of Fucoxanthin Action

The gene expression analysis provides mechanistic insights into the anti-obesity effects of fucoxanthin and explains the superior efficacy of nano-encapsulated formulation.

2.3.1 Inhibition of Adipogenesis and Lipogenesis

Fucoxanthin treatment downregulated the expression of pparγ and srebp1, master transcription factors controlling adipogenesis and lipogenesis, respectively. PPARγ is considered the master regulator of adipocyte differentiation, and its inhibition suppresses the formation of new fat cells. SREBP1 controls the expression of lipogenic enzymes including ACC and FASN, which catalysed fatty acid synthesis.

The downregulation of fatp1 (fatty acid transport protein 1) further reduces fatty acid uptake into adipocytes and hepatocytes, limiting substrate availability for triglyceride synthesis. These findings are consistent with previous studies showing that fucoxanthin suppresses adaptogenic and lipogenic factors in 3(Li et al., 2022).

1. Summary of Key Findings

The present study comprehensively evaluated the potential of nano-encapsulated fucoxanthin derived from brown algae (Sargassum wightii) as a therapeutic intervention for metabolic syndrome using a zebrafish model of diet-induced obesity. The major findings of this investigation are summarized below:

CONCLUSIONS

2.1 Successful Development of Nano-Encapsulated Fucoxanthin

The present study successfully developed and characterized lipid-based nano capsules containing fucoxanthin (FUCO-LNCs) with optimal physicochemical properties. The nanoparticles exhibited:

- Uniform size distribution (PDI = 0.16) ideal for cellular uptake

- High encapsulation efficiency (94.3%) ensuring minimal drug loss
- Sustained release profile maintaining therapeutic concentrations for up to 72 hours
- Excellent stability during storage

These characteristics establish FUCO-LNCs as a promising delivery system for fucoxanthin, addressing the critical limitation of poor bioavailability that has hindered its clinical translation.

2.2 Enhanced Bioavailability Through Nano-Encapsulation

The most significant finding of this study is the dramatic enhancement of fucoxanthin bioavailability through nano-encapsulation. The 7.87-fold increase in relative bioavailability, 3.48-fold higher peak concentration, and 2.69-fold longer elimination half-life demonstrate that lipid-based nano capsules effectively overcome the pharmacokinetic limitations of free fucoxanthin. This enhanced bioavailability directly translated to superior therapeutic efficacy, as evidenced by the significantly greater improvements in all metabolic parameters compared to equivalent doses of free fucoxanthin(Wang, Guo, & Jin, 2025).

Concluding Remarks

The present study demonstrates that nano-encapsulation of fucoxanthin from brown algae in lipid-based nano capsules dramatically enhances its oral bioavailability (7.87-fold) and therapeutic efficacy in ameliorating multiple aspects of metabolic syndrome in a zebrafish model of diet-induced obesity. The enhanced formulation normalized body weight, visceral adiposity, plasma glucose, lipid profile, and hepatic steatosis to levels comparable with healthy controls. These improvements were mediated through coordinated modulation of gene expression: downregulation of adaptogenic (pparγ) and lipogenic (srebp1, fatp1) factors, and upregulation of genes involved in fatty acid oxidation (cpt1a) and thermogenesis (ucp1)(Den Broeder, Kopylova, Kamminga, & Legler, 2015).

The translational significance of these findings lies in the potential development of an effective, safe, and cost-effective nutraceutical intervention for the growing global epidemic of metabolic syndrome. By addressing the critical bioavailability limitation that has hindered clinical translation of fucoxanthin, nano-encapsulation technology unlocks the full therapeutic potential of this marine bioactive compound. The multi-targeted mechanism of action, targeting multiple components of metabolic syndrome simultaneously, offers advantages over conventional single-target therapies.

As the prevalence of obesity, type 2 diabetes, and related metabolic disorders continues to rise worldwide, there is an urgent need for safe, effective, and affordable interventions. Nano-encapsulated fucoxanthin, derived from sustainably harvested brown algae, represents a promising candidate that warrants further development through preclinical and clinical studies. The zebrafish model employed in this study has proven to be a valuable tool for evaluating efficacy and mechanisms, providing a foundation for translation to higher vertebrates and ultimately to human patients.

In conclusion, this research establishes that nano-encapsulated fucoxanthin from brown algae enhances bioavailability and effectively modulates adiposity and metabolic dysfunction in zebrafish, with significant implications for developing novel therapeutic strategies for human metabolic syndrome. The findings contribute to the growing field of marine natural products and nanomedicine, offering hope for combating the global epidemic of metabolic disease through sustainable, nature-derived solutions.

REFERENCES

1. Aditya, N., & Ko, S. J. R. a. (2015). Solid lipid nanoparticles (SLNs): Delivery vehicles for food bioactives. 5(39), 30902-30911.
2. Bhardwaj, N., & Saneja, A. J. I. J. o. B. M. (2024). Orally fast dissolving α-lipoic acid electrospun nanofibers mitigates lipopolysaccharide induced inflammation in RAW 264.7 macrophages. 264, 130623.
3. Chen, L., & Subirade, M. J. B. (2005). Chitosan/ß-lactoglobulin core–shell nanoparticles as nutraceutical carriers. 26(30), 6041-6053.
4. D'Orazio, N., Gemello, E., Gammone, M. A., De Girolamo, M., Ficoneri, C., & Riccioni, G. J. M. d. (2012). Fucoxantin: A treasure from the sea. 10(3), 604-616.
5. Den Broeder, M. J., Kopylova, V. A., Kamminga, L. M., & Legler, J. J. P. r. (2015). Zebrafish as a model to study the role of peroxisome proliferating-activated receptors in adipogenesis and obesity. 2015(1), 358029.
6. Ding, L., Luo, X., & Wen, W. J. I. J. o. M. S. (2025). Fucoxanthin-loaded solid lipid nanoparticles exert potent therapeutic efficacy in combating high-fat diet induced obesity in mice. 26(11), 5249.
7. Ding, L., Luo, X., Xian, Q., Zhu, S., & Wen, W. J. I. J. o. M. S. (2024). Innovative approaches to Fucoxanthin delivery: Characterization and bioavailability of solid lipid nanoparticles with eco-friendly ingredients and enteric coating. 25(23), 12825.
8. Gammone, M. A., & D'Orazio, N. J. M. d. (2015). Anti-obesity activity of the marine carotenoid fucoxanthin. 13(4), 2196-2214.
9. Guo, Y., Wang, H., Ma, L., Guo, Z., Liu, Y., Zheng, J. J. C., & Biointerfaces, S. B. (2025). Construction of glycosylated zein-based colloids to simultaneously improve fucoxanthin's thermal processing adaptability, digestive stability, and oral bioavailability. 245, 114334.
10. Hitoe, S., Shimoda, H. J. F. F. i. H., -, D.-O. I., & -, P. I. (2017). Seaweed fucoxanthin supplementation improves obesity parameters in mild obese Japanese subjects. 7(4), 246-262.
11. Hosokawa, M., Miyashita, T., Nishikawa, S., Emi, S., Tsukui, T., Beppu, F., . . . biophysics. (2010). Fucoxanthin regulates adipocytokine mRNA expression in white adipose tissue of diabetic/obese KK-Ay mice. 504(1), 17-25.
12. Kalelkar, P. P., Geng, Z., Cox, B., Finn, M., Collard, D. M. J. C., & Biointerfaces, S. B. (2022). Surface-initiated atom-transfer radical polymerization (SI-ATRP) of bactericidal polymer brushes on poly (lactic acid) surfaces. 211, 112242.
13. Karthik, P., & Anandharamakrishnan, C. J. J. o. F. E. (2016). Enhancing omega-3 fatty acids nanoemulsion stability and in-vitro digestibility through emulsifiers. 187, 92-105.
14. Li, X., Wu, W., Cao, F., Hu, X., Wu, X., & Fu, J. J. J. o. F. F. (2022). Mechanism of cordycepin enhancing doxorubicin against hepatocellular carcinoma in vitro and in vivo. 98, 105268.
15. Liu, W., Xu, X., Liu, W., Zeng, X., Shi, S., Zhang, J., . . . Wu, C. J. I. J. o. B. M. (2025). Construction of fucoxanthin-loaded multi-functional pea protein isolate-fucoidan nanoparticles at neutral pH: Structural characterization and functional verification. 309, 142966.
16. Maeda, H., Hosokawa, M., Sashima, T., Funayama, K., Miyashita, K. J. B., & communications, b. r. (2005). Fucoxanthin from edible seaweed, Undaria pinnatifida, shows antiobesity effect through UCP1 expression in white adipose tissues. 332(2), 392-397.

17. Maeda, H., Tsukui, T., Sashima, T., Hosokawa, M., & Miyashita, K. J. A. P. J. o. C. N. (2008). Seaweed carotenoid, fucoxanthin, as a multi-functional nutrient. 17.
18. Maeda, H. J. J. o. O. S. (2015). Nutraceutical effects of fucoxanthin for obesity and diabetes therapy: a review. 64(2), 125-132.
19. Matsumoto, M., Hosokawa, M., Matsukawa, N., Hagio, M., Shinoki, A., Nishimukai, M., . . . Hara, H. J. E. j. o. n. (2010). Suppressive effects of the marine carotenoids, fucoxanthin and fucoxanthinol on triglyceride absorption in lymph duct-cannulated rats. 49(4), 243-249.
20. McClements, D. J., Rao, J. J. C. r. i. f. s., & nutrition. (2011). Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. 51(4), 285-330.
21. McClements, D. J. J. J. o. f. s. (2015). Nanoscale nutrient delivery systems for food applications: improving bioactive dispersibility, stability, and bioavailability. 80(7), N1602-N1611.
22. Mikami, K., & Hosokawa, M. J. I. j. o. m. s. (2013). Biosynthetic pathway and health benefits of fucoxanthin, an algae-specific xanthophyll in brown seaweeds. 14(7), 13763-13781.
23. Miyashita, K., Nishikawa, S., Beppu, F., Tsukui, T., Abe, M., Hosokawa, M. J. J. o. t. S. o. F., & Agriculture. (2011). The allenic carotenoid fucoxanthin, a novel marine nutraceutical from brown seaweeds. 91(7), 1166-1174.
24. Muradian, K., Vaiserman, A., Min, K.-J., Fraifeld, V. J. N., Metabolism, & Diseases, C. (2015). Fucoxanthin and lipid metabolism: A minireview. 25(10), 891-897.
25. Peng, J., Yuan, J.-P., Wu, C.-F., & Wang, J.-H. J. M. d. (2011). Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: Metabolism and bioactivities relevant to human health. 9(10), 1806-1828.
26. Salvia-Trujillo, L., Martín-Belloso, O., & McClements, D. J. J. N. (2016). Excipient nanoemulsions for improving oral bioavailability of bioactives. 6(1), 17.
27. Salvia-Trujillo, L., Sun, Q., Um, B. H., Park, Y., & McClements, D. J. J. o. F. F. (2015). In vitro and in vivo study of fucoxanthin bioavailability from nanoemulsion-based delivery systems: Impact of lipid carrier type. 17, 293-304.
28. Seo, M. J., Seo, Y. J., Pan, C. H., Lee, O. H., Kim, K. J., & Lee, B. Y. J. P. r. (2016). Fucoxanthin suppresses lipid accumulation and ROS production during differentiation in 3T3-L1 adipocytes. 30(11), 1802-1808.
29. Sun, S., Zhang, X., Li, J., Li, Y., Zhou, C., Xiang, S., & Tan, M. J. F. c. (2023). Preparation and evaluation of ovalbumin-fucoidan nanoparticles for nicotinamide mononucleotide encapsulation with enhanced stability and anti-aging activity. 418, 135982.
30. Wang, S., Guo, M., & Jin, Z. J. P. (2025). Innovative Controlled-Release Systems for Fucoxanthin: Research Progress and Applications. 17(7), 889.
31. Woo, M. N., Jeon, S. M., Shin, Y. C., Lee, M. K., Kang, M. A., Choi, M. S. J. M. n., & research, f. (2009). Anti-obese property of fucoxanthin is partly mediated by altering lipid-regulating enzymes and uncoupling proteins of visceral adipose tissue in mice. 53(12), 1603-1611.
32. Zempo-Miyaki, A., Maeda, S., Otsuki, T. J. J. o. C. B., & Nutrition. (2017). Effect of Chlorella-derived multicomponent supplementation on maximal oxygen uptake and serum vitamin B2 concentration in young men. 61(2), 135-139.
33. Zhang, H., Tang, Y., Zhang, Y., Zhang, S., Qu, J., Wang, X., . . . Medicine, A. (2015). Fucoxanthin: A promising medicinal and nutritional ingredient. 2015(1), 723515.