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Article

Nuciferine's dual pathway regulation of lipid metabolism: A biomechanical perspective based on HepG2 cells and *Caenorhabditis elegans*

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Abstract: The lipid-lowering properties of lotus leaf are primarily attributed to the active alkaloid, nuciferine. In this study, the role and underlying mechanisms of nuciferine in lipid metabolism regulation from a biomechanical perspective were investigated using molecular docking and in vitro and in vivo studies, employing HepG2 cells and *Caenorhabditis elegans* (*C. elegans*) as experimental models. The results indicated that nuciferine exhibited significant binding affinity to the targets Sterol Regulatory Element-Binding Protein-1c (SREBP-1c) and Peroxisome Proliferator-Activated Receptor alpha (PPARα), suggesting that it may modulate lipid metabolism via the SREBP-1c/PPARα pathway. In vitro experiments demonstrated that nuciferine significantly inhibited the oleic acid-induced accumulation of triglycerides (TG) and total cholesterol (TC) in HepG2 cells. Furthermore, atomic force microscopy (AFM) was employed to detect the changes in cell elasticity and adhesion force before and after nuciferine treatment. We hypothesized that the lipid-lowering effect of nuciferine might be related to the alterations in the mechanical properties of cells, which could further influence lipid metabolism pathways. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis revealed that nuciferine could regulate the expression of SREBP-1c and PPARα, as well as their downstream target genes, which further affects the biomechanical properties of cells. In vivo experiments showed that nuciferine effectively attenuated hepatic steatosis and reduced TG levels in *C. elegans*, while modulating the expression of Sterol regulatory element-binding protein-1 (sbp-1), Nuclear hormone receptor 49 (nhr-49), and their downstream target genes. These changes may affect lipid metabolism through intercellular mechanical signaling. Furthermore, using mutant strains defective in sbp-1, nhr-49, fatty acid and triglyceride synthase 5 (fat-5); fat mass and obesity associated-like 6 (fat-6), and fat-6; fat mass and obesity associated-like 7 (fat-7) genes, the study provided further evidence that nuciferine's lipid-lowering effects are mediated through the sbp-1/nhr-49 pathway. This study is the first to employ molecular docking to identify SREBP-1c and PPARα as the lipid-lowering targets of nuciferine and to confirm through in vitro and in vivo experiments that its efficacy depends on the SREBP-1c/sbp-1 and PPARα/nhr-49 pathways, thereby offering a new perspective for the treatment of lipid metabolic disorders from a biomechanical perspective.

Keywords: nuciferine; obesity; biomechanics; lipid accumulation; *Caenorhabditis elegans*; molecular docking techniques

1. Introduction

Lipid metabolism dysfunction is a widespread global health issue, defined by elevated concentrations of cholesterol, triglycerides, and other lipids in the bloodstream. This metabolic dysfunction not only disrupts blood circulation but also poses a risk for a spectrum of cardiometabolic disorders, including those linked to

central obesity, impaired glucose levels [1], lipid imbalances, and elevated blood pressure [2]. Currently, while statins and fibrates are effective therapeutic agents [3], prolonged use may result in adverse reactions [4,5]. Therefore, it is essential to investigate alternative therapies that offer both safety and efficacy. Nuciferine, a significant bioactive compound found in lotus leaves, has garnered considerable attention in recent years for its ability to regulate lipid metabolism and facilitate weight loss [6,7]. Nuciferine has exhibited anti-inflammatory properties, as well as anti-HIV [8], antitumor, and weight lose effects [9], along with the ability to reduce lipase activity [7]. In studies on lipid metabolism, nuciferine has demonstrated its regulatory effects, particularly by reducing the liver index (an important biomarker of liver health and lipid accumulation), epididymal fat pad weight, and kidney fat mass, as well as improving hepatic steatosis [7,10,11]. Nuciferine has been demonstrated to lower serum and hepatic biomarkers such as Aminotransferase (AST), TC, TG, low-density lipoprotein cholesterol (LDL-C), and Free fatty acids (FFA) [10,11]. Nuciferine exerts regulatory effects on lipid metabolism through multiple pathways, such as its anti-inflammatory properties, antioxidant activities, and the reconstitution of the gut microbiota composition [7,8,10–14]. However, the precise mechanisms mediating these mechanisms are yet to be fully clarified.

SREBP-1c is a transcription factor that controls the synthesis of TC, fatty acids, and TG, a key role in the regulation of liver lipid metabolism [15]. PPARα exerts potential therapeutic effects on obesity, diabetes, and cardiovascular diseases by modulating fatty acid oxidation and energy metabolism [16]. SREBP-1c serves as a pivotal regulator of lipid synthesis, and its activation drives the upregulation of Acetyl-CoA Carboxylase (ACC) and Fatty Acid Synthase (FAS), consequently augmenting fat synthesis [17]. As a pivotal therapeutic target for lipid metabolism-associated disorders, PPARα is a master regulator, that oversees hepatic lipid homeostasis, and involves the production of cholesterol, fatty acids, and TG [18]. PPARα enhances fatty acid oxidation and mitigates fat accumulation through the regulation of Carnitine palmitoyltransferase-1 (CPT-1) [19].

The purpose of this research is to elucidate the role and underlying mechanism of nuciferine in modulating lipid metabolic processes within HepG2 cells and *C. elegans* through molecular docking techniques and in vitro and in vivo experiments. The research results indicate that nuciferine attenuates oleic acid-triggered lipid aggregation in HepG2 cells through the SREBP-1c/sbp-1 pathway. Additionally, the research utilized the C. elegans model to delve deeper into the lipid-modulating effects of nuciferine. This study provided the first evidence that nuciferine regulated lipid metabolism through the SREBP-1c/sbp-1 and PPARα/nhr-49 pathways, offering innovative avenues for addressing lipid metabolic disorders.

2. Materials and methods

Human hepatoma HepG2 cells were procured from Harbin Medical University. The *C. elegans* strains utilized in this investigation were furnished by the Caenorhabditis Genetics Center (CGC) at the University of Minnesota. The strains included *C. elegans* wild-type strain N2, mutant *C. elegans* sbp-1 (ep79) III, fat-6 (tm331) IV; fat-5 (tm420), fat-6 (tm331) IV; fat-7 (wa36) V, nhr-49 (ok2165) I.

Standards of nuciferine and simvastatin (the article is then represented by Sim) were obtained from Chengdu Sodium Potassium Lithium Biotechnology Co., Ltd.

2.1. Cell culture and handling

Adjustments were made in accordance with the methodologies described in the referenced literature [20]. HepG2 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS). The cell suspension was adjusted to a density of 1.5×10^5 cells/mL and seeded into a six-well plate, with 1 mL of the suspension added to each well. The cells were then cultured under conditions of 37 ℃, 5% CO_2, and saturated humidity. Following cell adherence, treatments were administered to HepG2 cells using oleic acid (OA) (soluble in methanol) and nuciferine (soluble in DMSO), either concurrently or sequentially. A preliminary incubation with 0.5 mM OA for 24 h at 37 ℃ was performed, succeeded by exposure to 10, 20, 30, or 40 μM of nuciferine for an additional 24 h at the same temperature. For the control group, cells were initially subjected to treatment with 0.3% methanol for 24 h at 37 ℃, followed by treatment with 0.1% DMSO for another 24 h at 37 ℃. In the OA control group, cells underwent a 24-hour preincubation with 0.5 mM OA at 37 ℃, succeeded by a 24-hour preincubation with 0.1% DMSO at 37 ℃. The positive control group received a 24-hour preincubation with 0.5 mM OA at 37 ℃, then each group of cells was treated with Sim (10 μM) for 24 h at 37 ℃ for replicate analysis, with the entire procedure being conducted in triplicate.

2.2. Cell viability assay

The cells were treated under 2.1 culture conditions. Refer to the method in the article [21], cells were seeded into 12-well plates at a concentration of 5×10^4 cells per well after digestion and cultured overnight. Subsequently, two separate groups of cells were exposed to varying concentrations of nuciferine and oleic acid, respectively, for 24 h. Afterward, 0.5 mg/mL MTT solution was introduced into each group and incubated at 37 ℃ for 4 h. Finally, the formazan crystals were solubilized in 150 μL of DMSO, followed by absorbance measurement at 570 nm using a microplate spectrophotometer to assess cell viability.

2.3. Oil red O staining as a method to determine intracellular triglyceride levels

The adjustment is made on the basis of experimental operation [22], under the culture conditions described in Section 2.1, cells treated with OA, Sim and nuciferine were washed thrice with PBS. The cells were fixed in 4% paraformaldehyde for a duration of 15 min and subsequently stained with a 0.5% solution. Oil Red O for 30 min, followed by observation under an optical microscope. Subsequently, 150 μL of isopropanol was added, and the cells were agitated for 15 min before absorbance was determined at 510 nm using a microplate spectrophotometer.

2.4. TG and TC contents assays of HepG2 cell

The adjustment is made on the basis of experimental operation [23]. After treating the HepG2 cells, they were rinsed twice with PBS and then lysed using 1% Triton X-

100 lysis buffer. The intracellular levels of TG, TC, and total protein levels were assessed using TG assay kits, TC assay kits, and BCA protein assay kits, respectively. The relative contents of TG and TC were calculated by dividing their absolute amounts by the total protein content.

2.5. Strains and maintenance conditions

At a temperature of 20 °C, *C. elegans* were maintained on agar plates formulated with nematode growth medium (NGM). The NGM was composed of 1.7% agar, 2.5 g/L peptone, 51 mM NaCl, and 25 mM KPO_4 buffer (pH 6.0), 5 μg/L cholesterol, 1 mM $CaCl_2$, and 1 mM $MgSO_4$. Escherichia coli OP50 was supplied as a fresh food source. To achieve a synchronized worm population, eggs were harvested using a bleach solution. Subsequently, the eggs were rinsed with M9 buffer. This study involved several *C. elegans* strains, including *C. elegans* wild-type strain N2, sbp-1 (ep79) III, fat-6 (tm331) IV; fat-5 (tm420), fat-6 (tm331) IV; fat-7 (wa36) V and nhr-49 (ok2165) I. Sbp-1 (ep79) III was cultured at 15 °C. Glucose-supplemented plates were formulated by incorporating glucose (2%, sterile-filtered) into the NGM, to simulate an obesity-induced *C. elegans* model in a high glucose environment. A conventional hypochlorite bleaching technique was employed to bleach gravid adults, thereby obtaining a cohort of nematodes with synchronized age. The nematodes were cultured on NGM agar plates, with approximately 2000–4000 nematodes per group. The plates were either supplemented or not supplemented with 2% glucose, and *E. coli* OP50 was provided as a nutritional substrate until the nematodes reached the L2 stage. Subsequently, the nematodes were transferred to freshly prepared NGM plates that had been pre-treated with 20 μM Sim or 20, 30, 40 μM nuciferine, with or without glucose.

2.6. The quantification of lipofuscin and reactive oxygen species (ROS)

Under the culture conditions described in Section 2.6 for a duration of 3 days, 2000 *C. elegans* wild-type strain N2 were selected. The experimental setup included a control group, a glucose control group, glucose plus varying concentrations of nuciferine groups (20, 30, 40 μM), and a positive control group treated with Sim. The levels of the age pigment lipofuscin were then measured. The worms were irradiated to stimulate lipofuscin fluorescence and observed by a fluorescence microscope. With an excitation at 350 nm and emission at 577 nm via a DAPI filter. The fluorescence intensity of *C. elegans* was assessed utilizing the ImageJ software.

In accordance with the methodology documented by Lee et al., measurements were conducted using 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) [24]. Briefly, L4-stage synchronized *C. elegans* from each group were selected, the *C. elegans* samples were washed thrice using M9 buffer., and then incubated with DCFH-DA solution at 37 °C for 30 min. Subsequently, the nematodes were subjected to washing three more times with M9 buffer and observed employing a fluorescence microscope configured with an excitation wavelength set at 485 nm and an emission wavelength at 530 nm. The fluorescence intensity of the nematodes was quantified using ImageJ software.

2.7. Quantitative real-time PCR analysis of cells and *C. elegans*

Total RNA was extracted from HepG2 cells and *C. elegans* wild-type strain N2 samples using TRIzol reagent. Subsequently, Complementary DNA (cDNA) synthesis was initiated using a reverse transcription kit according to the established protocol. Following cDNA synthesis, RT-qPCR was performed. Primer sequences, which are critical for the amplification process, are listed in **Table S1**. The relative changes in gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method.

2.8. Statistical analysis

In each group of the *C. elegans* experiments, we used a sample size of 2000 worms. Additionally, to ensure the robustness and reproducibility of our results, each experiment was independently repeated three times. One-way ANOVA was then applied to statistically analyze and identify significant disparities across the groups. Presented as mean ± SD, the outcomes were examined using Tukey's test for multiple comparisons and Student's *t*-tests within SPSS, defining statistical significance at a $p < 0.05$.

3. Results and discussion

3.1. Nuciferine's protective role against oxidative stress in OA-treated HepG2 cells

High concentrations of nuciferine exhibited significant inhibitory effects on the viability of various cancer cells [25]. Nevertheless, as the exposure concentration increases, nuciferine may exert an impact on the viability of HepG2 cells. Hence, establishing the non-toxic concentration range of nuciferine for HepG2 cells is essential. Based on the literature [26,27], the concentration range of 10 to 100 mM was investigated. According to the MTT data (**Figure 1A**), nuciferine showed no obvious toxicity to HepG2 cells at concentrations between 10 μM and 40 μM. At 50 μM, the cells appeared to be considerably damaged. Consequently, the concentration range of 10 μM to 40 μM is deemed safe for use, and this range was chosen for the subsequent experimental procedures. OA is frequently utilized to trigger lipid accumulation in HepG2 cells, an action that typically results in increased oxidative stress [28]. In MTT and TG assays, 0.5 mM OA did not show suppression of HepG2 cells, making it a suitable choice for modeling (**Figure 1B,C**).

ROS that are produced due to disruptions in lipid metabolism contribute to the process of adipogenesis [29]. This indicates that oxidative stress not only initiates obesity but also amplifies it by mediating the overall process. To evaluate the oxidative stress within cells, H2DCF-DA probes are utilized to measure the levels of oxidative factors. The fluorescent staining data in **Figure 1D** and E illustrated that exposure to OA treatment led to a marked increase in intracellular ROS levels, which were approximately twofold higher than the levels observed in the control group. This elevation in ROS was effectively counteracted by the subsequent administration of nuciferine, which induced a rapid and significant reduction in ROS content, especially at a 30 μM concentration, where the levels were reduced to a point inferior to the corresponding values in the positive control Sim group. The ROS generation

escalation following OA treatment indicates the oxidative stress that can arise from hepatic lipid metabolism dysregulation. The notable attenuation of ROS production upon nuciferine supplementation underscored its potential to mitigate the oxidative stress induced by OA induction lipid peroxidation in HepG2 cells.

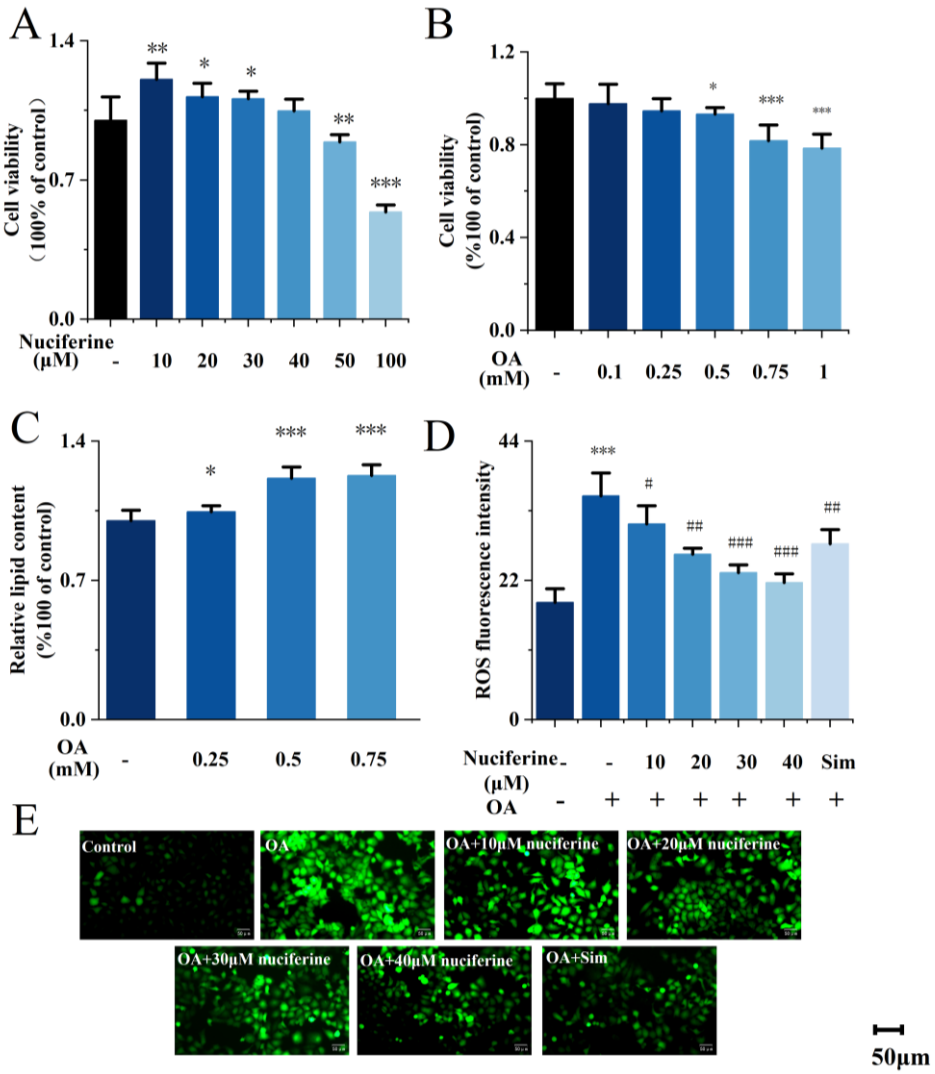

Figure 1. The effects of nuciferine and OA on HepG2 cell viability, OA-mediated lipid degeneration in HepG2 cells, while nuciferine can alleviate oleic acid-mediated oxidative stress in HepG2 cells. **(A)** Effect of nuciferine on HepG2 cell viability; **(B)** effect of OA on HepG2 cell viability; **(C)** OA-induced lipid degeneration of HepG2 cells; **(D)** intracellular ROS content; **(E)** ROS fluorescence images of cells taken under 200×.

Figures represented the significance of three independent experiments: *$p < 0.05$, **$p < 0.01$, OA and control groups, ##$p < 0.01$, ###$p < 0.001$, the nuciferine group and OA control group.

3.2. Nuciferine ameliorated lipid deposition in HepG2 cells induced by OA

TG and TC are vital components of lipid metabolism, playing essential roles in the regulation of energy storage and transport within the body [28]. As depicted in **Figure 2A,B**, OA treatment resulted in elevated TG levels in HepG2 cells. The

significant reduction in TG levels following nuciferine supplementation indicates its potential for lipid-lowering effects. A dose of 40 μM nuciferine was particularly effective, reducing TC levels by 46%, a decrease that was significantly greater than the 31% reduction observed in the positive control Sim group (as illustrated in **Figure 2B**). Lipid droplets are central to lipid metabolism and serve as the primary cellular sites for the storage and digestion of lipids [30], and the accumulation and size of these organelles can indeed serve as direct biological indicators of lipid accumulation within cells [31]. The study conducted quantitative analysis using the Oil Red O staining method. Referencing the data in **Figure 2C,D**, consistent with the results of TC and TG, nuciferine effectively alleviates lipid metabolic abnormalities with increasing doses, achieving the most pronounced effect at a concentration of 40 μM, surpassing that of the Sim group. Based on the experimental data, nuciferine significantly impacts lipid accumulation in HepG2 cells, suggesting its potential as an intervention for lipid metabolic disorders.

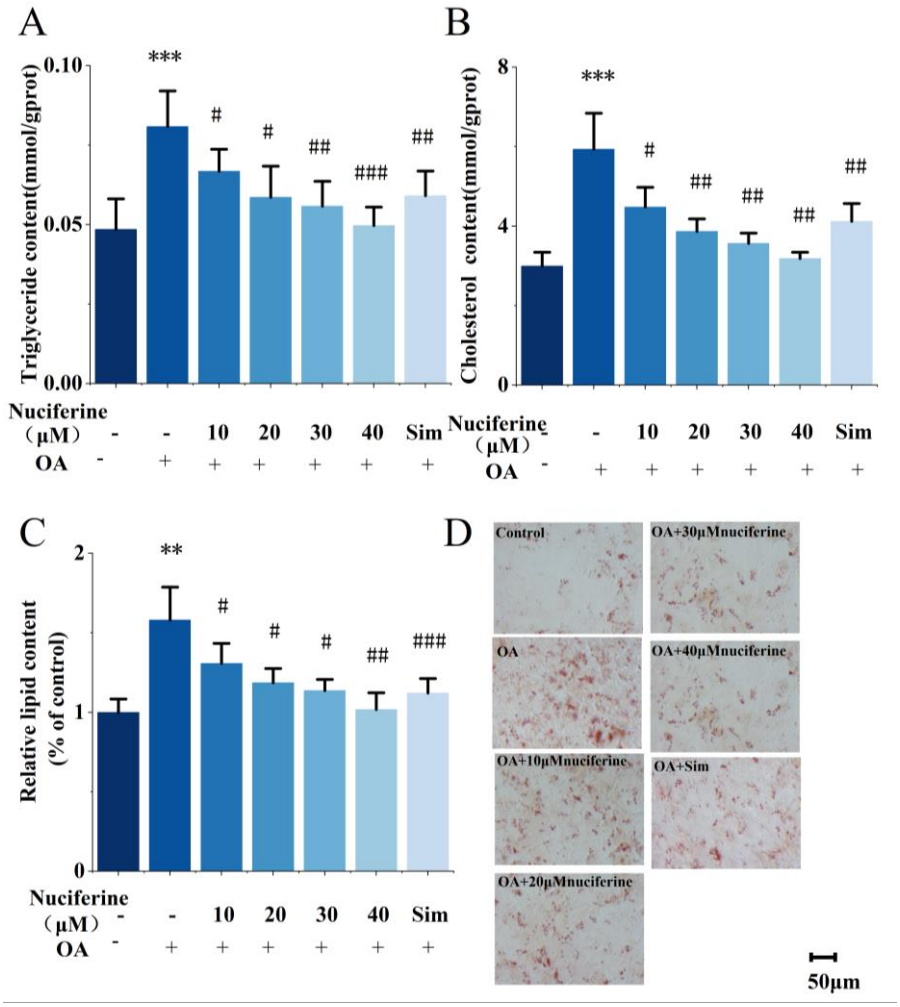

Figure 2. Effects of nuciferine on lipid accumulation in HepG2 cells induced by OA. **(A)** Intracellular TG content; **(B)** intracellular TC content; **(C)** quantitative staining of oil red; **(D)** Oil Red O-stained cell images were captured at 200× magnification, Scale bar, 50 μm.

The figures represented the significance of three independent experiments: *$p < 0.05$, **$p < 0.01$, OA group and control group, ##$p < 0.01$, ###$p < 0.001$, nuciferine group and OA control group.

3.3. Effect of nuciferine on gene expression of HepG2 fine lipid metabolism

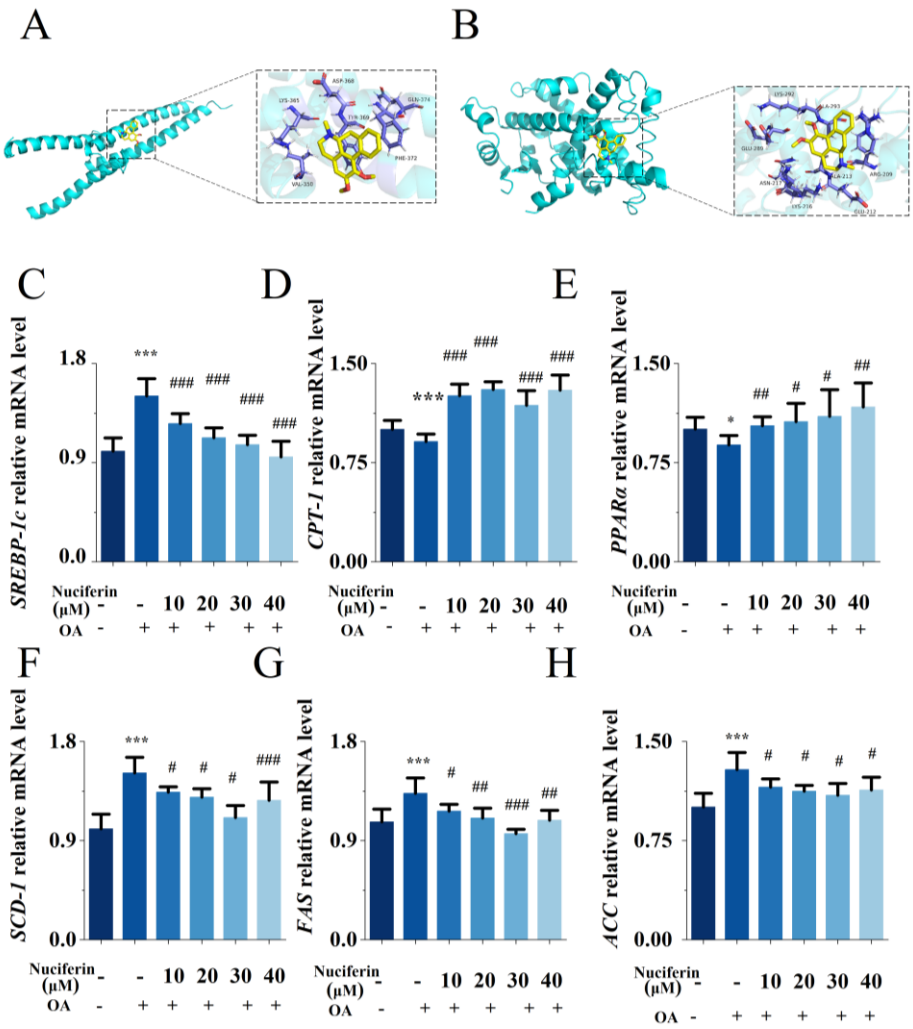

Figure 3. Molecular docking diagram and influence of nuciferine on lipogenesis and lipolysis gene expression in HepG2 cells. The impact of nuciferine on the expression levels of: **(A)** SREBP-1c- nuciferine; **(B)** PPARα-nuciferine; **(C)** SREBP-1c; **(D)** CPT-1; **(E)** PPARα; **(F)** SCD-1; **(G)** FAS; **(H)** ACC.

Figures represented the significance of three independent experiments: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, comparison between, OA group and control group, ##$p < 0.01$, ###$p < 0.001$, comparison of the nuciferine group and OA control group.

This study employed molecular docking techniques to preliminarily investigate the interactions between nuciferine and two target sites, SREBP-1c and PPARα. The results demonstrated that nuciferine can bind to the active pockets of both SREBP-1c and PPARα receptors. Calculations using Autodock 4.2.6 software revealed a binding energy of −7.6 kcal/mol between nuciferine and SREBP-1c (**Figure 3A**). The binding energy between nuciferine and PPARα was −7.8 kcal/mol (**Figure 3B**). These findings suggest that nuciferine has a strong affinity for these targets, implying that it may regulate lipid metabolism through the SREBP-1c and PPARα metabolic pathways.To further elucidate the regulatory effects of nuciferine on lipid metabolism, this study investigated its modulation of key transcription factors and their target genes,

including SREBP-1c, PPARα, ACC, FAS, Stearoyl-CoA Desaturase 1 (SCD-1), and CPT-1. Observations from the experiments revealed that the enhancement of SREBP-1c expression due to OA could correspond to a surge in lipid accumulation within HepG2 cells. In contrast to the HepG2 cells subjected to the OA-induced model, the treatment with 30 μM nuciferine led to a 30% reduction in the SREBP-1c expression levels (as shown in **Figure 3C**). Moreover, the treatment with nuciferine was determined to upregulate the expression of genes pivotal for the stimulation of lipolysis, including CPT-1 and PPARα (**Figure 3D,E**). The downregulation of SREBP-1c's target genes, such as ACC, FAS, and SCD-1, which are pivotal for fatty acid synthesis and de novo lipogenesis, has been associated with reduced lipid accumulation in hepatic cells (**Figure 3F–H**). This finding revealed that nuciferine regulated lipid metabolism through the SREBP-1c and PPARα pathways.

3.4. Nuciferine-induced enhancement of antioxidant defensey in *C. elegans*

As depicted in Supplementary **Figure S1**, within the tested concentration range of lotus leaf alkaloid (20–40 μM), no cytotoxic effects were observed in nematodes. Moreover, it was capable of extending the lifespan of *Caenorhabditis elegans*, enhancing its growth capacity, and improving locomotor ability.Stress can induce oxidative damage to biomacromolecules, leading to lipid metabolism disorders [32]. As shown in **Figure 4A**. administration of 20 μM nuciferine led to an 18% decrease in ROS levels in *C. elegans* under high-glucose conditions relative to the glucose control group, implying an enhancement of antioxidant capabilities by nuciferine in these nematodes. This ability may decrease lipid accumulation and extend lifespan. *C. elegans* exhibits lipofuscin, a blue autofluorescent substance, that is considered a promising biomarker for tracking stress and aging within the species [33,34]. An excessive buildup of lipofuscin in nematodes can accelerate aging through the induction of oxidative stress. **Figure 4B** illustrated that on day three of the study, an increase in lipofuscin deposition was observed in *C. elegans*. Treatment with nuciferine at a concentration of 20 μM in high glucose *C. elegans* resulted in a 12% reduction in lipofuscin accumulation, which is comparable to the levels observed in the control group. These findings suggested that nuciferine may possess the potential to mitigate lipofuscin accumulation. The findings indicated that nuciferine could enhance the antioxidant capacity of *C. elegans* by effectively lowering the levels of lipofuscin and ROS.

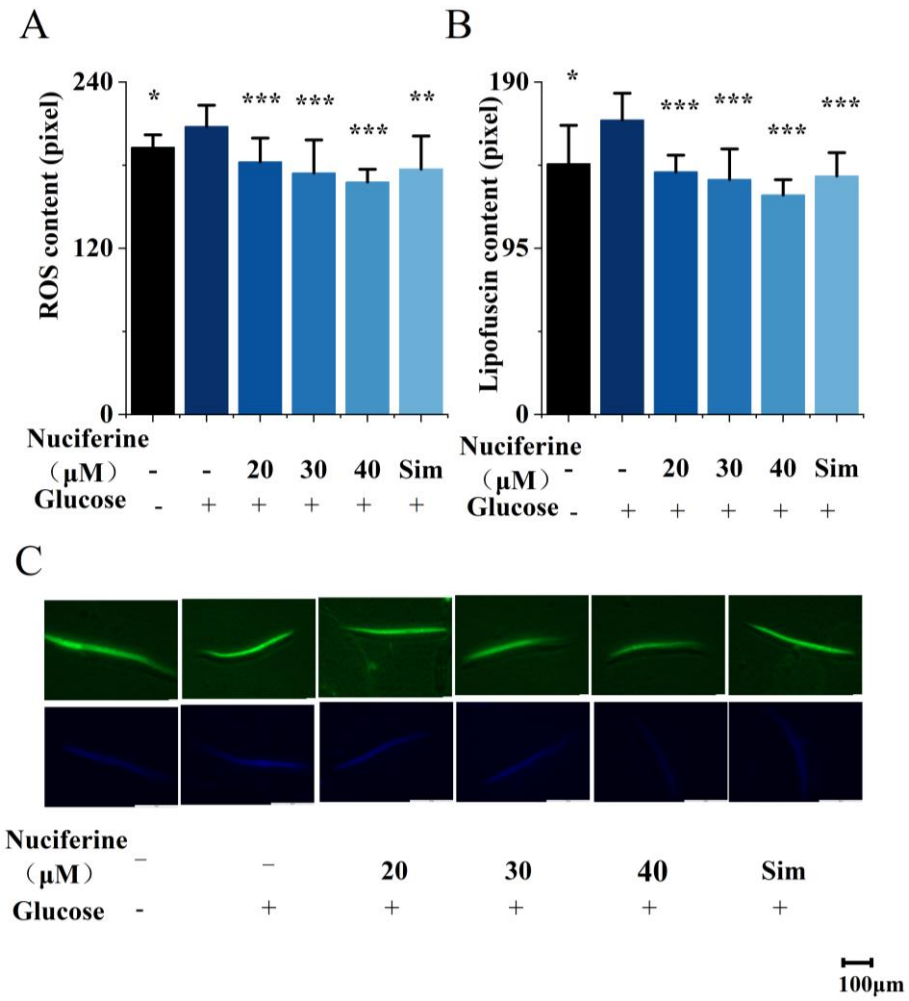

Figure 4. Effect of nuciferine on oxidative stress in high glucose *C. elegans* model. **(A)** Histogram of lipofuscin quantification; **(B)** ROS quantitative histogram; **(C)** ROS images and lipofuscin images of *C. elegans* on the third day after exposure to different conditions, Scale bar, 100 μm.

The results were replicated independently three times, expressed as mean ± SD, *$p < 0.05$, **$p < 0.01$, and the nuciferine and glucose model groups were compared.

3.5. Modulation of lipid homeostasis by nuciferine in *C. elegans*

TG were key components in lipid metabolism [35]. TG levels can be considered a definitive metric for the accumulation of body fat, highlighting the net result of lipid storage processes within the body [36]. To gain insights into the impact of nuciferine on lipid metabolism in *C. elegans*, this research quantified the levels of TG within the *C. elegans*. **Figure 5A** illustrates the levels of TG in *C. elegans* under various treatments. Excess glucose led to lipid accumulation, with a 26% increase in lipid content compared to the control group. The normalization of TG levels by nuciferine was evident in a dose-dependent trend, the 40 μM dose showed the most significant reduction of 51%. To delve deeper into the effects of nuciferine on the distribution of lipids within *C. elegans*, this study employed the Oil Red O staining technique. Oil Red O staining as depicted in **Figure 5B,C** demonstrated that the glucose model group exhibited a 12% increase in lipid accumulation relative to the control. Nuciferine treatments at concentrations of 20 μM, 30 μM, and 40 μM progressively reduced lipid

accumulation, with the highest dose of 40 μM achieving a 31% reduction. This reduction was more pronounced than the 25% decrease observed with the positive control Sim treatment. These aforementioned results suggest that nuciferine may substantially reduce lipid elevation triggered by glucose in *C. elegans*, underscoring its therapeutic potential for lipid accumulation disorders.

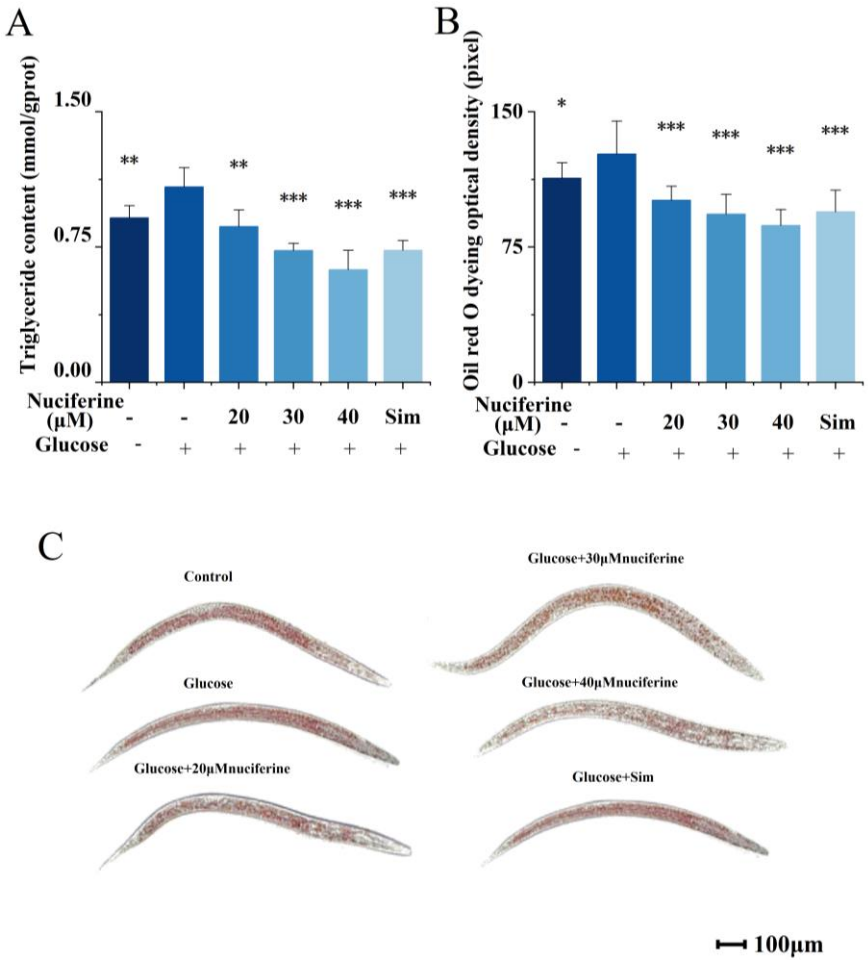

Figure 5. Lipid contents in a high glucose *C. elegans* model after 0–40 μM nuciferine treatment for 3 days. **(A)** Triglyceride content in *C. elegans* after nuciferine treatment; **(B)** quantitative histogram of oil red; **(C)** On the third day of exposure to different conditions of the worm, images of Oil Red O-stained lipids of *C. elegans* were taken, Scale bar, 100 μm.

The results were replicated independently three times, expressed as mean ± SD, *$p < 0.05$, **$p < 0.01$, and the nuciferine and glucose models were compared.

3.6. Nuciferine targeted sbp-1 and nhr-49 pathways reduced lipid accumulation in *C. elegans*

To investigate whether nuciferine regulates lipid metabolism in *C. elegans* via the SREBP-1c/PPARα pathway, this study focused on the homologous genes of human SREBP-1c, namely sbp-1, and the PPARα homolog nhr-49 in *C. elegans*. Yang et al. identified that the expression of genes fat-5, fat-6, and fat-7 is controlled by sbp-1 [36]. Nhr-49 is known to activate the transcription of genes fat-5, fat-6, acs-2 and fat-7, which are integral to lipid metabolic processes [37]. The genes sbp-1 and nhr-

49 are under the joint regulatory control of the co-activator known as Mediator 15 (mdt-15) [38].

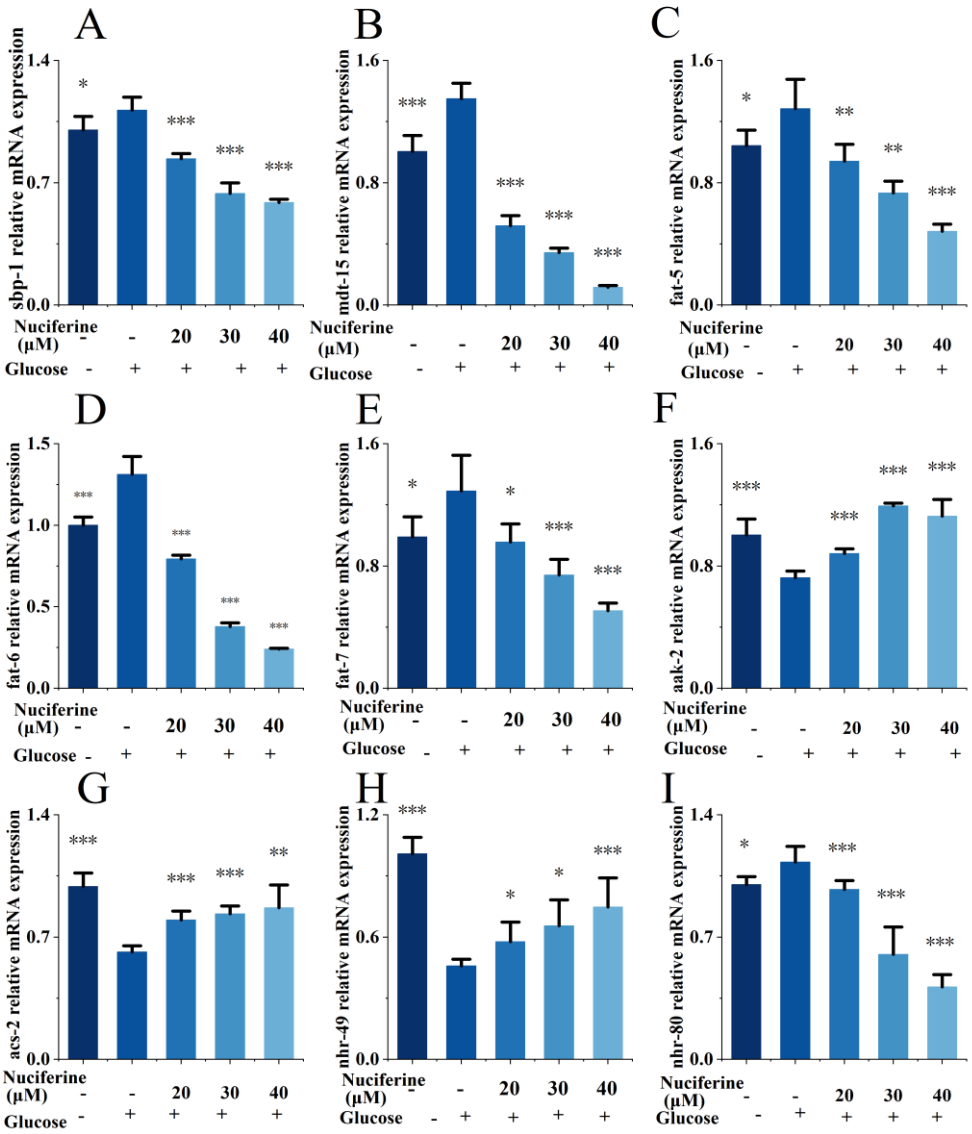

Figure 6. Effect of nuciferine on the expression of lipid metabolism-regulating genes in *C. elegans*. The impact of nuciferine on the expression levels of: **(A)** sbp-1; **(B)** mdt-15; **(C)** fat-5; **(D)** fat-6; **(E)** fat-7; **(F)** aak-2; **(G)** acs-2; **(H)** nhr-49; **(I)** nhr-80. Figures represent three independent experiments' significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and the nuciferine and glucose models were compared.

This study revealed that under high-glucose induction, the transcriptional levels of sbp-1 and mdt-15 were significantly upregulated, a phenomenon that was attenuated by the supplementation of nuciferine. Additionally, the genes sbp-1 and mdt-15 have a significant impact on the regulation of fat production, primarily by inhibiting the expression levels of key fatty acid desaturase enzymes, namely fat-5, fat-6, and fat-7 (**Figure 6A–E**). Conversely, even the minimal concentration of nuciferine introduced, which is 20 μM, efficiently enhanced the expression levels of AMP-activated kinase 2 (aak-2) and acs-2, as depicted in **Figure 6F,G**. After the supplementation of nuciferine, the gene expression of the nhr-49 in *C. elegans* was upregulated, while the

expression of nuclear hormone receptor 80 (nhr-80) was downregulated (**Figure 6H,I**). This study found that nuciferine regulates lipid accumulation in *C. elegans* through two distinct mechanisms involving the sbp-1 and nhr-49 signaling pathways. Specifically, nuciferine significantly reduced lipid deposition in the worms by inhibiting the expression of key lipid synthesis genes (such as fat-5, fat-6, and fat-7) and lipid degradation genes (such as Acetyl-CoA synthetase 2 (acs-2), aak-2, mdt-15, and nhr-80).

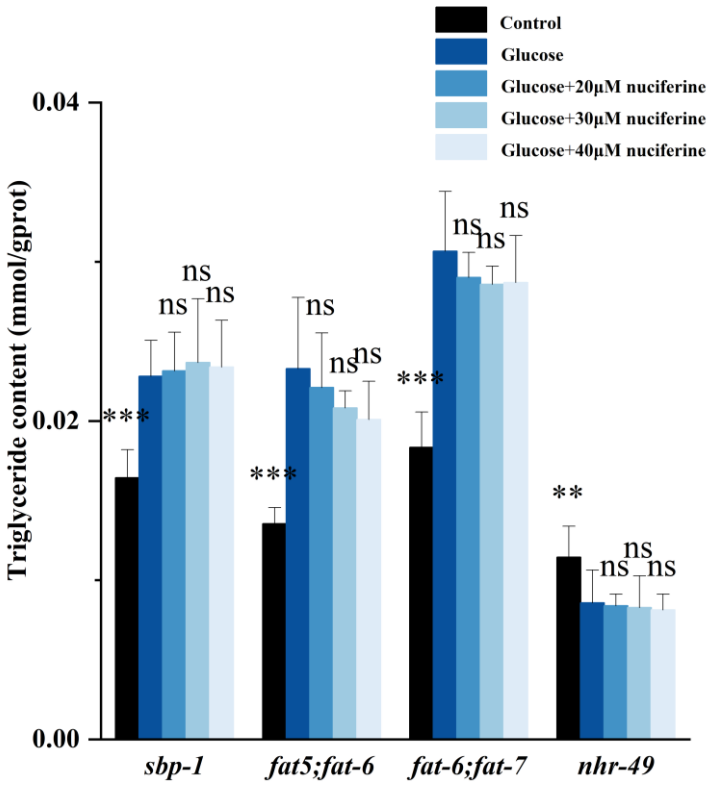

Figure 7. Influence of various concentrations of nuciferine on lipid accumulation in mutant nematodes.

Figures represent three independent experiments' significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and the nuciferine and glucose models were compared.

To further elucidate whether nuciferine regulates lipid metabolism via the sbp-1 and nhr-49 signaling pathways in *C. elegans*, the study employed mutant strains sbp-1 (ep79) III, fat-6 (tm331) IV; fat-5 (tm420), fat-6 (tm331) IV; fat-7 (wa36) V and nhr-49 (ok2165) I to investigate the modulatory influence of nuciferine on lipid metabolism. **Figure 8** illustrates after glucose treatment, the triglyceride content in the glucose model group was 28% greater than that observed in the control group, indicating that excess glucose promotes fat accumulation in nematodes. However, after intervention with 20 μM–40 μM nuciferine, there was No substantial alteration in the triglyceride content in the sbp-1, nhr-49, fat-5, fat-6, fat-6, fat-7 mutant nematodes compared to the glucose model group (**Figure 7**). The results indicate depend on the sbp-1 and nhr-49 pathways to regulate energy homeostasis (**Figure 8**).

The findings of this study hold significant translational potential, especially considering the increasing prevalence of lipid metabolic disorders and the limitations of current treatments. Nuciferine, a natural compound derived from lotus leaves, has

demonstrated the ability to regulate lipid metabolism through multiple pathways, including modulation of both SREBP-1c and PPARα pathways. This dual mechanism represents a broader regulatory effect compared to conventional lipid-lowering agents like statins, which primarily target cholesterol synthesis via the HMG-CoA reductase pathway. However, transitioning from the *C. elegans* model to mammalian systems and clinical settings presents several challenges. These include the complexity of metabolic pathways in mammals, the need for rigorous evaluation of nuciferine's efficacy and safety in higher organisms, and the determination of its pharmacokinetics and bioavailability in humans. Additionally, potential off-target effects and long-term safety concerns must be thoroughly investigated in preclinical and clinical trials.

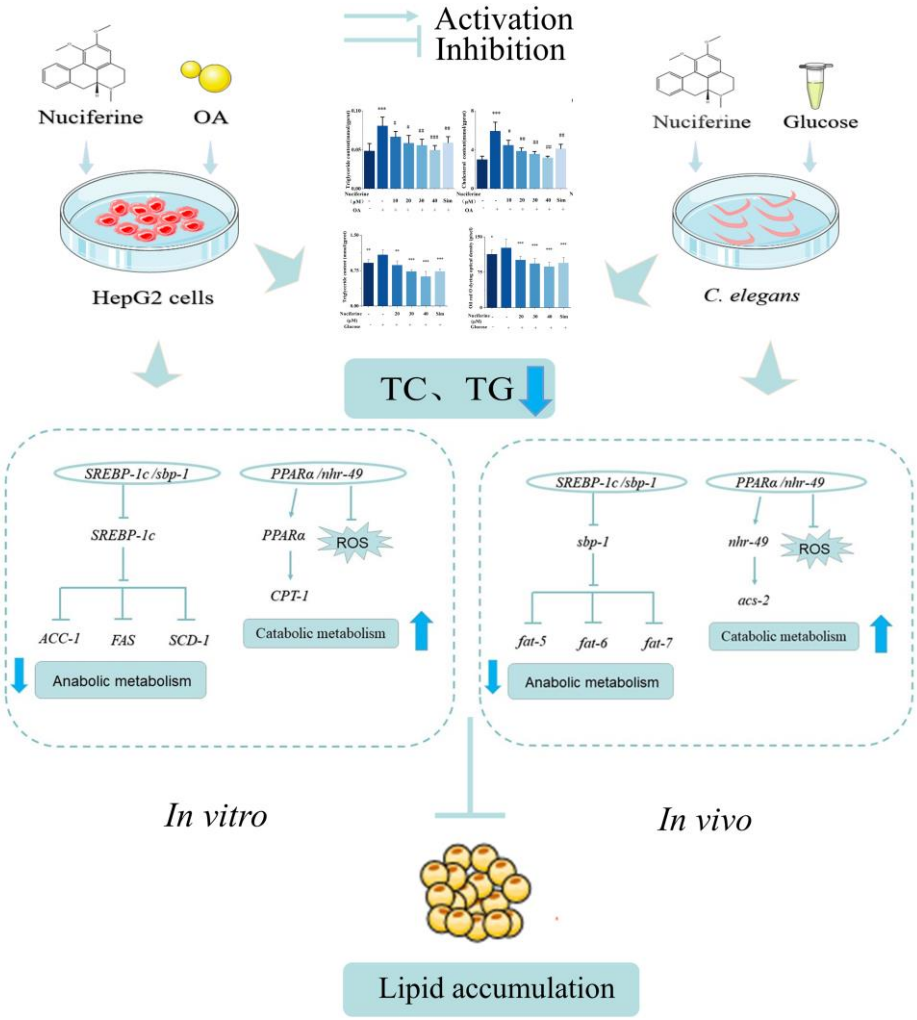

Figure 8. Mechanism of nuciferine regulating lipid metabolism.

4. Conclusion

Recent studies have focused on nuciferine, a key bioactive compound in lotus leaves, to explore its potential in regulating lipid metabolism and promoting weight loss. Building on this foundation, the present study delves into the positive effects and underlying mechanisms of nuciferine in modulating lipid metabolic pathways in HepG2 cells and *C. elegans*. Using molecular docking techniques and in vitro and in vivo experiments, we investigated the role and mechanisms of nuciferine in lipid

metabolism regulation. Molecular docking results showed that nuciferine has a strong binding affinity to the targets SREBP-1c and PPARα, suggesting that it may regulate lipid metabolism via the SREBP-1c/PPARα pathway. In vitro experiments demonstrated that nuciferine significantly inhibits the formation of ROS and lipids by suppressing the activity of several metabolic transcription factors (including SREBP-1c, ACC, FAS, CPT-1, PPARα, and SCD-1) and activating the SREBP-1c/sbp-1 and PPARα/nhr-49 signaling pathways, thereby reducing lipid accumulation and protecting cells from oxidative stress. In vivo experiments revealed that nuciferine significantly inhibits lipid accumulation in *C. elegans*, extends lifespan, enhances locomotor activity, reduces lipofuscin and ROS levels, and boosts antioxidant capacity. Oil Red O staining and TG level measurements further confirmed its lipid-reducing ability. Additionally, using mutant strains with defects in sbp-1, nhr-49, fat-5; fat-6, and fat-6; fat-7 genes, we suggest that nuciferine's lipid-lowering effects may depend on the SREBP-1c/sbp-1 and PPARα/nhr-49 pathways.

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