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Protective effects and mechanism of Qiangli Dingxuan Tablets on glucose-induced obesity in caenorhabditis elegans

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Abstract. Objective: This study aimed to evaluate the anti-obesity efficacy of Qiangli Dingxuan Tablets using a glucose-induced obese model in *Caenorhabditis elegans (C. elegans)* and to clarify its molecular mechanisms associated with lipid metabolism regulation. Methods: Obesity was induced in C. elegans using 2% glucose, followed by treatment with Qiangli Dingxuan Tablets at concentrations of 0.125, 0.25, and 0.5 mg/mL. To assess lipid accumulation and triglyceride content, we performed Oil Red O (ORO) staining, Nile Red staining, and triglyceride assays. Locomotion and body size were evaluated. The mRNA expression levels of lipid metabolism-, IIS pathway-, and stress-related genes were determined by RT-qPCR. Results: Qiangli Dingxuan Tablets significantly reduced lipid accumulation and triglyceride content in obese nematodes and improved obesity-associated locomotor impairment and body enlargement. RT-qPCR analysis showed that treatment downregulated *daf-2, sbp-1, fat-5, and fat-6*, while upregulated *daf-16, sod-2, and lipl-4*. Conclusion: Qiangli Dingxuan Tablets ameliorated glucose-induced obesity in *C*. elegans by regulating lipid metabolism, inhibiting lipogenesis, and promoting lipolysis. These findings provide experimental evidence for its potential application in obesity-related metabolic disorders.

Keywords: Qiangli Dingxuan Tablets, *Caenorhabditis elegans*, obesity, triglyceride, lipid accumulation

1. Introduction

As a prevalent metabolic disorder, obesity is marked by abnormal lipid overaccumulation and correlates with an elevated risk of various chronic diseases, such as type 2 diabetes and cardiovascular pathologies [1, 2]. It has become a major global public health concern. Dysregulated lipid metabolism not only disrupts energy homeostasis but also induces oxidative stress and cellular dysfunction [3], thereby aggravating metabolic abnormalities. Hence, the modulation of lipid metabolism and the maintenance of lipid homeostasis are essential for addressing obesity and its associated metabolic pathologies. Although current anti-obesity drugs exhibit certain therapeutic effects, their clinical application is limited by adverse effects and concerns regarding long-term safety [4].

Traditional Chinese Medicine (TCM) formulas possess the characteristics of multi-component, multi-target synergistic effects and relatively high safety, showing unique advantages in regulating lipid metabolism and

improving metabolic disorders [5]. Qiangli Dingxuan Tablets are derived from the classical prescription "Xiongma Decoction" and consist of several herbal medicines, including *Gastrodia elata, Chrysanthemum indicum, Eucommia ulmoides, and Ligusticum chuanxiong*. Clinically, they are mainly used for the treatment of vertigo and hypertension [6]. Previous studies have demonstrated that their components exhibit lipid metabolism-regulating, antioxidant, and lipid-lowering activities [7]. However, the effects and molecular mechanisms of Qiangli Dingxuan Tablets in obesity-related lipid metabolic disorders remain unclear.

The nematode *C. elegans* is a well-established model organism widely used in studies of metabolic diseases and drug mechanisms due to its short lifespan, clear genetic background, ease of manipulation, and highly conserved lipid metabolism-related signaling pathways with mammals [8]. A stable obesity model can be established in *C. elegans* through exogenous glucose induction, providing an ideal system for evaluating the effects of drugs on lipid accumulation and metabolic regulation.

Therefore, in this study, a glucose-induced obesity model of *C. elegans* was established to systematically evaluate the effects of Qiangli Dingxuan Tablets on lipid accumulation, triglyceride levels, and locomotor behavior. Moreover, the underlying molecular mechanisms related to the regulation of lipid metabolism imbalance and oxidative stress were analyzed by examining the expression of relevant genes, which lays an experimental foundation for the clinical application of Qiangli Dingxuan Tablets in treating obesity and related metabolic disorders.

2. Materials

2.1. Nematodes and bacterial strains

Wild-type *C. elegans* (N2) was provided by the Caenorhabditis Genetics Center. All nematodes were raised on conventional NGM agar plates at 20 °C, using *Escherichia coli* OP50 as the food source. Worm culture, propagation, and synchronization were performed according to standard protocols.

2.2. Drugs and main reagents

Qiangli Dingxuan Tablets (Batch No. 015301) were sourced from Shaanxi Hanwang Pharmaceutical Co., Ltd. Oil Red O (ORO) staining solution was acquired from Beijing Solarbio Science & Technology Co., Ltd. Nile Red dye was procured from Shanghai Macklin Biochemical Co., Ltd. Triglyceride (TG) detection kits and protein quantitation kits were purchased from Beijing Solarbio Science & Technology Co., Ltd. TRIzol reagent was obtained from Shanghai Yuanye Bio-Technology Co., Ltd. Reverse transcription reagents and SYBR Green qPCR kits were provided by TIANGEN Biotech Co., Ltd. All other reagents applied in the present study were of analytical grade or better.

2.3. Main instruments

Stereomicroscope (ZOOM-7045, SHPQYQ, China); inverted microscope (CKX53, Olympus Corporation); fluorescence microscope (DMi8, Leica Microsystems); real-time quantitative PCR system (CFX96, Bio-Rad Laboratories); high-speed refrigerated centrifuge (5424R, Eppendorf); ultrasonic cell disruptor (Scientz-IID, Ningbo Scientz Biotechnology Co., Ltd., China); electronic analytical balance (BSA124S, Sartorius); constant-temperature incubator (SPX-150B, Shanghai Yiheng Scientific Instrument Co., Ltd., China).

3. Methods

3.1. Preparation of NGM medium

NGM medium was prepared according to standard procedures. Briefly, 3 g NaCl, 2.5 g peptone, and 17 g agar were dissolved in distilled water to a final volume of 1 L. After autoclaving, the medium was cooled to approximately 55 °C and transferred to a laminar flow hood. Sterile $CaCl_2$ (1 mmol/L), $MgSO_4$ (1 mmol/L), cholesterol (5 mg/mL in ethanol), and phosphate buffer (pH 6.0) were sequentially added. The medium was thoroughly mixed, poured into Petri dishes, and dried at room temperature before use.

3.2. Sample preparation and treatment

Prior to use, the sugar coating of Qiangli Dingxuan Tablets was stripped, and the tablets were ground into powder. The powder was dissolved in DMSO to prepare a stock solution, then diluted to the desired concentrations, with the final DMSO concentration kept at 0.01%. Drug solutions at 0.125, 0.25, and 0.5 mg/mL were mixed 1:1 with OP50 suspension ($OD_{600} \approx 0.6$), spread evenly on NGM plates, and air-dried at room temperature. An equal volume of 0.01% DMSO was used as the control, and orlistat served as the positive control.

3.3. Assessment of locomotor activity in C. elegans

The assay was performed according to the method described by Zhang et al. [9]. Adult nematodes from each group were transferred into drops of sterile M9 buffer, and locomotor activity was observed under a microscope. One body bend was defined as a complete movement from one side to the other and back to the original position. The number of body bends within 30 s was recorded for each worm. At least 30 worms were randomly selected per group, and the average value was calculated to evaluate the effects of different treatments on locomotor activity.

3.4. Measurement of body length and body width

Body size was measured according to the method described by Lee et al. [10]. Adult worms from each group were mounted on glass slides and imaged under a microscope. Body length and width were measured with ImageJ software. At least 30 nematodes were randomly picked from each group for measurement, and average values were calculated to assess morphological changes.

3.5. Determination of triglyceride content in C. elegans

Nematodes (≥1000 individuals) from each group were collected and rinsed three times with M9 buffer, then harvested via centrifugation. After adding lysis buffer, the worms were homogenized by ultrasonication. Following centrifugation at 8000 r/min for 10 min at 4 °C, the supernatant was collected. Protein concentration was quantified by the BCA method, and triglyceride (TG) levels were detected following the kit protocol. Data were presented as TG content relative to total protein content.

3.6. ORO and Nile Red staining

The staining procedures were performed according to Wang et al. [11]. Adult nematodes from each group were washed three times with PBS and fixed before staining.

For ORO staining, worms were fixed in 60% isopropanol for 3 min, then stained with ORO working solution in the dark at room temperature for 4 h. After being washed three times with PBS, the worms were

observed and imaged under a light microscope.

For Nile Red staining, worms were collected into 1.5 mL centrifuge tubes, rinsed three times with PBS, and kept on ice for 15 min. The samples were then incubated with Nile Red dye in the dark for 30 min. Following three PBS washes, fluorescence images were captured using a fluorescence microscope, and fluorescence intensity was analyzed with ImageJ software to reflect lipid accumulation in nematodes.

3.7. Detection of lipid metabolism-related gene expression in *C. elegans*

Total RNA was extracted from nematodes of each group using TRIzol reagent, and cDNA was synthesized according to the manufacturer's instructions. The expression levels of lipid metabolism-related genes, including *daf-2, sbp-1, mdt-15, fat-5, lips-17, sod-3, hsp-12.6,* and *lipl-4*, were determined by real-time quantitative PCR (RT-qPCR), with act-1 used as the internal reference gene. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are listed in Table 1.

Table 1. Primer sequences of target gene

Gene name	Primer sequences(5'→3')
daf-2	F: CTCGTCGACGACTTCAACAA
	R: CGTCTCATTGTGATGTGCTCG
daf-16	F: CCGGAATGAGTTTTTCCACTGATTT
	R: CATTGCTCATTGCTCCCGTAT
sod-2	F: GCGGTCTCCAAAGGAAACGT
	R: CCAGCGCTGAAATTCAATGGT
fat-5	F: AGTCGCGCTTAATAGTGTCCA
	R: TCCTCCCAAAAGAGATTGAAGTCA
hsp-12.6	F: GTAACAACGAGTAATGTTACGAGAG
	R: GTAACAACGAGTAATGTTACGAGAG
hsp-16.1	F: T TGGCTCAGATGGAACGTCAA
	R: TGGCTTGAACTGCGAGACAT
daf-12	F: CAACAAACGTGCGGCATACA
	R: ATCTCTTCGGCAGCATCACC
nhr-80	F: TGAAACCGGAAGAGCATTTTTC
	R: TTTTCCGAGGTGTAGGGGTT
fat-6	F: AATAACCGCCGGAGCTCATC
	R: GCCATCCCATATGAGCGAAGA
sbp-1	F: ATTCTATGCGGAGTCGGCAG
	R: TAGAGATCACCGCCAATGGC
lipl-4	F: TTGCATTGGCTCCAATTGGC
	R: TTGGCTGGCTGCATTTGTTC
nhr-17	F: CTGAATGCGGGATGGAGTGA
	R: CAAATTCGCCGTTTGCTCCA
acs-2	F: TGCGCTCAATCCTCGTATCC
	R: TGATGTGTCCAACTCCTGGC

Table 1. Continued

mdt-15	F: GGGTAATGGTCAGGTGCCAA R: CTGAAGTGCATCAATCGGCG
act-1	F: CTCTACGCTTCCGGACGTAC R: AGCGGTGATTTCCTTCTGCA

3.8. Statistical analysis

All experiments were performed in triplicate independently, and data are shown as the mean ± standard deviation (mean ± SD). ImageJ software was used for quantifying ORO staining intensity, Nile Red fluorescence, as well as body length and width of nematodes. Statistical analysis and figure plotting were carried out using GraphPad Prism 9.0. Differences among multiple groups were analyzed by Dunnett's multiple comparisons test. A p-value < 0.05 was regarded as statistically significant.

4. Results

4.1. Effects of Qiangli Dingxuan Tablets on locomotor activity in *C. elegans*

As shown in Figure 1, compared with the normal group, the number of body bends in the model group was significantly decreased ($p < 0.0001$), indicating impaired locomotor activity. Treatment with a low concentration of Qiangli Dingxuan Tablets significantly increased the number of body bends per unit time compared with the model group ($p < 0.0001$), suggesting that Qiangli Dingxuan Tablets ameliorated obesity-induced locomotor dysfunction.

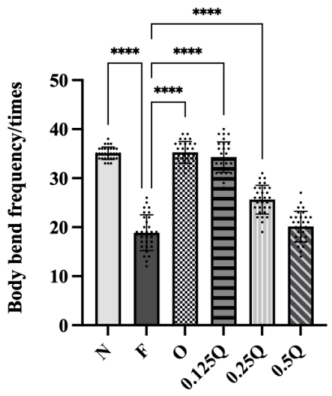

Figure 1. Effects of Qiangli Dingxuan Tablets on body bending frequency of *C. elegans* with glucose-induced obesity

Note: N, normal control group; F, glucose-induced obesity model group; O, orlistat positive control group; 0.125Q, 0.25Q, and 0.5Q, Qiangli Dingxuan Tablet treatment groups at different concentrations. Data are presented as $\bar{x}$ ± s. 3 independent replicates were performed for each group (n = 30). ****$p < 0.0001$ vs. the model group.

4.2. Effects of Qiangli Dingxuan Tablets on body length and body width in *C. elegans*

As depicted in Figure 2, nematodes in the model group exhibited markedly greater body length and width relative to the normal group ($p < 0.0001$), confirming that the obesity model was successfully established.

Treatment with low- and medium-dose Qiangli Dingxuan Tablets significantly alleviated glucose-induced body enlargement and reduced both body length and width to different degrees when compared with the model group ($p < 0.01$, $p < 0.0001$).

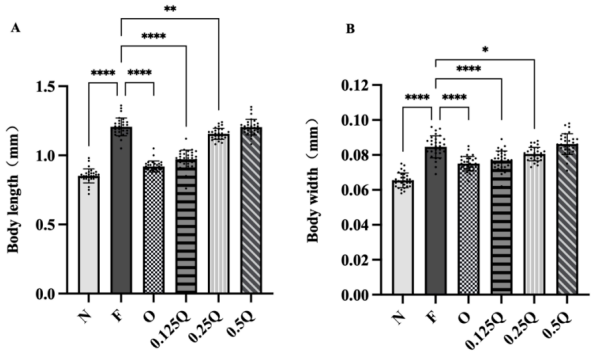

Figure 2. Effects of Qiangli Dingxuan Tablets on body length and width of *C. elegans* with glucose-induced obesity

Note: A, body length of nematodes in each group; B, body width of nematodes in each group. N, normal control group; F, glucose-induced obesity model group; O, orlistat positive control group; 0.125Q, 0.25Q, and 0.5Q, Qiangli Dingxuan Tablet treatment groups at different concentrations. Data are presented as $\bar{x} \pm s$. 3 independent replicates were performed for each group (n = 30). *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$ vs. the model group.

4.3. Effects of Qiangli Dingxuan Tablets on triglyceride content in *C. elegans*

As shown in Figure 3, compared with the normal group, triglyceride (TG) content in the model group was significantly increased ($p < 0.05$). Compared with the model group, treatment with a low concentration of Qiangli Dingxuan Tablets significantly reduced TG levels in nematodes, showing a dose-dependent trend ($p < 0.05$ *or* $p < 0.01$). These results suggest that Qiangli Dingxuan Tablets significantly decrease triglyceride accumulation in *C. elegans.*

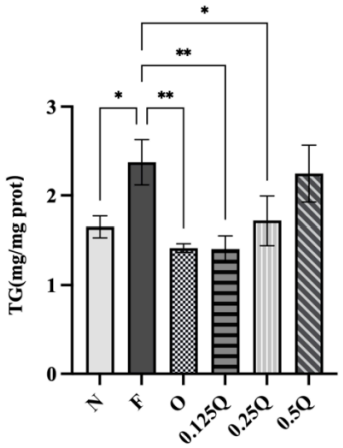

Figure 3. Effects of Qiangli Dingxuan Tablets on triglyceride content in *C. elegans* with glucose-induced obesity

Note: N, normal control group; F, glucose-induced obesity model group; O, orlistat positive control group; 0.125Q, 0.25Q, and 0.5Q, Qiangli Dingxuan Tablet treatment groups at different concentrations. Data are presented as $\bar{x} \pm s$. 3 independent replicates

were performed for each group. *$p < 0.05$, **$p < 0.01$ vs. the model group.

4.4. Effects of Qiangli Dingxuan Tablets on lipid accumulation in *C. elegans*

As presented in Figure 4A and 4B, ORO staining revealed that lipid droplet accumulation and staining intensity were both notably elevated in the model group relative to the normal control group. ($p < 0.0001$). Compared with the model group, the low-dose Qiangli Dingxuan Tablet group significantly reduced lipid droplet accumulation and attenuated staining intensity ($p < 0.0001$). Nile Red staining results are shown in Figure 4C and 4D. Compared with the normal group, fluorescence intensity in the model group was significantly increased ($p < 0.0001$). Both the low- and medium-dose Qiangli Dingxuan Tablet groups significantly reduced fluorescence intensity in nematodes ($p < 0.0001$). These findings indicate that Qiangli Dingxuan Tablets effectively inhibit lipid accumulation in *C. elegans*.

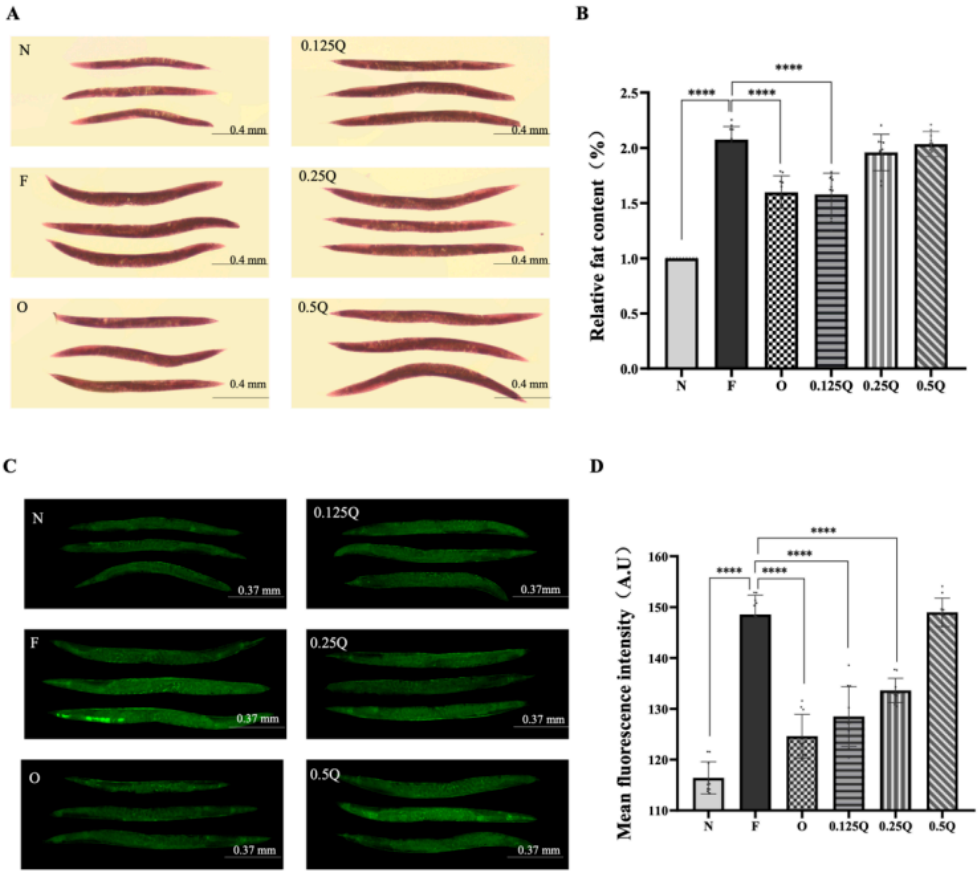

Figure 4. Effects of Qiangli Dingxuan Tablets on lipid accumulation in *C. elegans* with glucose-induced obesity

Note: A, ORO staining images; B, quantitative analysis of relative lipid content based on ORO staining; C, Nile Red staining images; D, quantitative analysis of mean fluorescence intensity from Nile Red staining. N, normal control group; F, glucose-induced obesity model group; O, orlistat positive control group; 0.125Q, 0.25Q, and 0.5Q, Qiangli Dingxuan Tablet treatment groups at different concentrations. Scale bars: 0.4 mm in A and 0.37 mm in C. Data are presented as $\bar{x} \pm s$. Three independent replicates were performed for each group (n = 10). ****$p < 0.0001$ vs. the model group.

4.5. Effects of Qiangli Dingxuan Tablets on lipid metabolism-related gene expression in *C. elegans*

As displayed in Figure 5, relative to the normal control group, the model group showed notably increased *daf-2* expression ($p < 0.0001$) but markedly reduced *daf-16* and *sod-2* levels ($p < 0.01$ and $p < 0.001$, respectively). Treatment with 0.125 mg/mL Qiangli Dingxuan Tablets significantly downregulated *daf-2* expression ($p < 0.0001$) and upregulated *daf-16* and *sod-2* expression ($p < 0.05$ and $p < 0.01$), indicating that the drug can inhibit abnormal activation of key genes in the IIS pathway.

Meanwhile, compared with the normal group, the model group showed significantly downregulated expression of lipid catabolism- and metabolism-related genes (*lipl-4, daf-12,* and *nhr-49*) ($p < 0.01$ or $p < 0.001$), whereas lipid synthesis-related genes (*sbp-1, fat-5,* and *fat-6*) were significantly upregulated ($p < 0.01$, $p < 0.001$, or $p < 0.0001$), indicating enhanced lipogenesis and suppressed lipolysis under obesity conditions.

Treatment with 0.125 mg/mL Qiangli Dingxuan Tablets significantly reversed these changes: expression levels of *sbp-1, fat-5,* and *fat-6* were markedly downregulated ($p < 0.05$ or $p < 0.001$), while *lipl-4* and *nhr-49* were significantly upregulated ($p < 0.05$, $p < 0.01$, or $p < 0.001$). In addition, stress-related genes *hsp-16.1* and *hsp-12.6* showed a slight increase compared with the model group, but the differences were not statistically significant.

In summary, Qiangli Dingxuan Tablets may improve glucose-induced lipid metabolic disorders in *C. elegans* by regulating the IIS signaling pathway, inhibiting lipid synthesis-related gene expression, and promoting lipid catabolism-related gene expression.

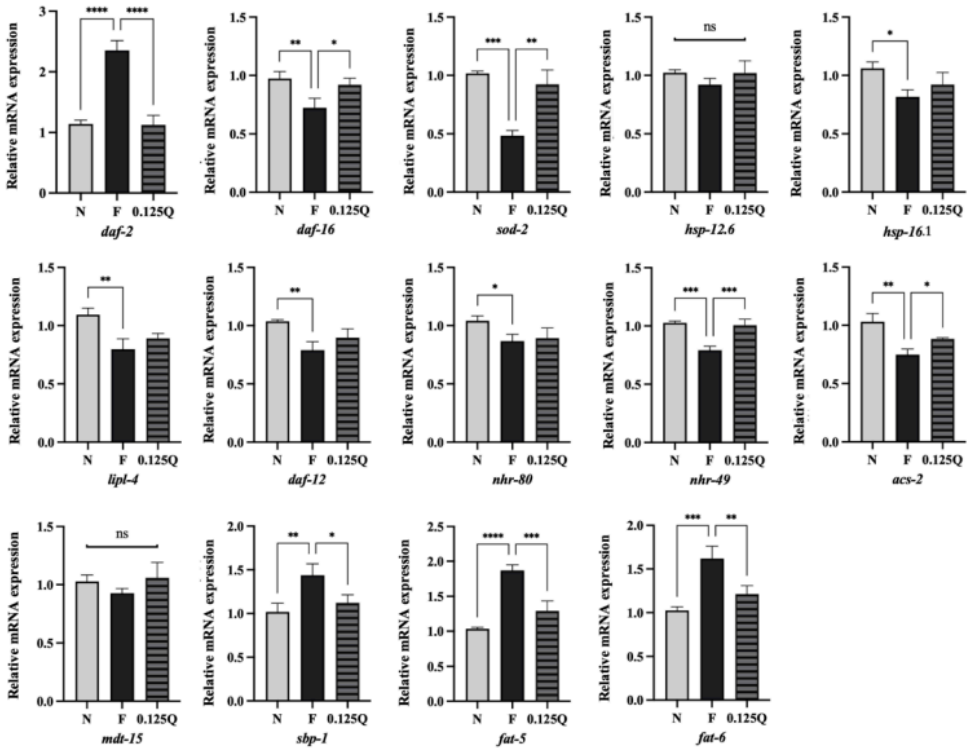

Figure 5. Effects of Qiangli Dingxuan Tablets on mRNA expression of lipid metabolism and related pathway genes in C. elegans with glucose-induced obesity

Note: N, normal control group; F, glucose-induced obesity model group; Q, Qiangli Dingxuan Tablet treatment group. *daf-2* and *daf-16* are key genes in the IIS pathway. *hsp-12.6* and *hsp-16.1* are heat shock protein-related genes. *lipl-4* is a lipolysis-related

gene. *acs-2, fat-5,* and *fat-6* are fatty acid metabolism-related genes. *mdt-15* and *sbp-1* are lipogenesis regulatory genes. *daf-12*, *nhr-49*, and *nhr-80* are nuclear receptor family-related genes. Data are presented as $\bar{x} \pm s$. 3 independent replicates were performed for each group. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 vs. the model group.

5. Discussion

Obesity exhibits excessive lipid deposition and disrupted lipid metabolism as its main pathological hallmarks, which may trigger insulin resistance, elevated oxidative stress, and damaged locomotor activity, thus severely endangering physical and mental health [12]. Although clinically used anti-obesity drugs show certain therapeutic effects, they are often associated with adverse effects and limited mechanisms of action. Therefore, identifying safe and effective lipid-lowering compounds from traditional Chinese medicine has important research value. In this study, a 2% glucose-induced *C. elegans* obesity model was established to investigate the effects and molecular mechanisms of Qiangli Dingxuan Tablets in improving obesity and lipid metabolic disorders. The results showed that Qiangli Dingxuan Tablets significantly reduced lipid accumulation and triglyceride levels in nematodes and improved obesity-induced locomotor impairment.

Obesity can lead to impaired locomotor function in nematodes. The model group exhibited a significant decrease in body bend frequency, along with increased body length and width, indicating obesity-related body enlargement and locomotor dysfunction. After treatment with Qiangli Dingxuan Tablets, body bend frequency was significantly increased, while body length and width were significantly reduced, suggesting that Qiangli Dingxuan Tablets ameliorated obesity-induced locomotor impairment and morphological abnormalities.

ORO and Nile Red staining results showed that glucose induction significantly increased lipid droplet accumulation and fluorescence intensity in nematodes, indicating successful establishment of the obesity model. After intervention with Qiangli Dingxuan Tablets, both lipid accumulation and fluorescence intensity were significantly reduced, and triglyceride levels were also decreased, showing a dose-dependent trend. These findings demonstrate that Qiangli Dingxuan Tablets effectively inhibit lipid deposition and reduce triglyceride content, confirming their lipid-lowering effects at both phenotypic and biochemical levels.

At the molecular level, this study focused on the IIS signaling pathway and key genes involved in lipid metabolism. The results showed that in the model group, the expression of the upstream IIS receptor gene daf-2 was significantly upregulated, while downstream genes *daf-16* and *sod-2* were downregulated. Qiangli Dingxuan Tablets significantly downregulated *daf-2* and upregulated *daf-16* and *sod-2*, suggesting that they may alleviate energy metabolic imbalance and lipid accumulation by inhibiting overactivation of the IIS pathway [13]. In addition, the expression of the lipid synthesis regulator *sbp-1* and its downstream genes *fat-5* and *fat-6* was significantly increased in the model group, whereas Qiangli Dingxuan Tablets markedly reduced their expression, indicating suppression of lipid synthesis and reduced fatty acid production and storage. Meanwhile, Qiangli Dingxuan Tablets significantly upregulated *lipl-4* expression, promoting lipolysis and lipid degradation, thereby improving lipid metabolic disorders through both inhibition of lipid synthesis and enhancement of lipid breakdown.

Furthermore, obesity is often accompanied by elevated oxidative stress, which further exacerbates metabolic dysfunction [14]. In this study, Qiangli Dingxuan Tablets slightly upregulated stress-protective genes such as *hsp-16.1* and *hsp-12.6*, and significantly increased *sod-2* expression, thereby alleviating oxidative stress-induced damage and improving cellular stress and metabolic imbalance under obese conditions. These results suggest that the lipid-lowering effects of Qiangli Dingxuan Tablets are accompanied by antioxidant protective effects, acting through multiple pathways to ameliorate obesity.

6. Conclusion

In this study, a glucose-induced *C. elegans* obesity model was established. The results demonstrated that Qiangli Dingxuan Tablets significantly reduced lipid droplet accumulation and triglyceride levels in nematodes, improved obesity-induced locomotor impairment and body size abnormalities, and did not affect normal feeding or reproductive functions. The underlying mechanisms are closely associated with inhibition of the IIS pathway via downregulation of the key gene *daf-2*, upregulation of *daf-16* and *sod-2*, suppression of lipid synthesis-related genes (*sbp-1, fat-5,* and *fat-6*), and upregulation of the lipolysis-related gene lipl-4 as well as oxidative stress-related genes (*hsp-16.1* and *hsp-12.6*). Through coordinated regulation of lipid metabolism and oxidative stress-related pathways, Qiangli Dingxuan Tablets exert multi-target effects to ameliorate lipid metabolic disorders. These findings provide experimental evidence for the potential application of Qiangli Dingxuan Tablets in the prevention and treatment of obesity and lipid metabolism disorders, and also offer a reference for further mechanistic studies of its lipid-lowering effects.

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