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Effects of nuciferine on Nrf2/HO-1 signaling pathway in adipose tissue of obesity model rats

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Competing interests
The authors declare no conflicts of interest.
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Abbreviations
HE, hematoxylin and eosin; SOD, superoxide dismutase; TG, triglycerides; MDA, malondialdehyde; TC, total cholesterol; qPCR, quantitative polymerase chain reaction; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; SOD, superoxide dismutase; MDA, malondialdehyde; GSH-Px, glutathione peroxidase.
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Abstract
Objective: This study aimed to explore the therapeutic effect of nuciferine on high-fat diet-induced obesity in rats and the influence of nuciferine on nuclear factor erythroid 2–related factor 2 (Nrf2)/ heme oxygenase-1 (HO-1) signaling pathway in the adipose tissue. **Methods:** A total of 40 male Sprague Dawley (SD) rats were evenly divided into the normal, model, positive control, and nuciferine groups, using the random number table method. Except for the normal group, rats in the other groups were fed with high-fat diet for 12 weeks to establish the obesity model. During the model establishment, rats in the positive control group received atorvastatin calcium 2 mg/kg, rats in the nuciferine group received nuciferine 20 mg/kg, and rats in the normal and model groups received normal saline 2 mL, daily through intragastric administration for 12 consecutive weeks. After model establishment and administration, the body weight, Lee's index, and blood lipids of rats in each group were measured, and hematoxylin and eosin (HE) staining was performed on the liver and adipose tissues to evaluate the therapeutic effect of nuciferine on obesity rat model. Additionally, the levels of superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GSH-Px) in the serum of rats in each group were determined, and the gene expressions of Nrf2 and HO-1 in the adipose tissue of rats in each group were detected through quantitative polymerase chain reaction (qPCR) to investigate the mechanism of action of nuciferine in the treatment of obesity. **Results:** After 12 weeks of model establishment and administration, we observed that compared with the model group, nuciferine could significantly reduce the body weight, Lee's index, and serum triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) levels and increase the serum high-density lipoprotein cholesterol (HDL-C) level in obesity rat model ($P < 0.05$ or $P < 0.01$). HE staining revealed that nuciferine could significantly alleviate liver steatosis in obesity rat model and improve the cell morphology in epididymal adipose tissue. Moreover, nuciferine could elevate serum SOD and GSH-Px activities in obesity rat model and lower the serum MDA level ($P < 0.05$ or $P < 0.01$). The qPCR indicated that nuciferine could upregulate the gene expression of Nrf2 and HO-1 in the adipose tissue of obesity rat model ($P < 0.05$ or $P < 0.01$).

Keywords: obesity; nuciferine; antioxidant; Nrf2/HO-1 signaling pathway

Nowadays, as people's diet changes, the incidence of obesity is increasing annually. According to statistics, more than 30% of adults have a body mass index exceeding the standard value (≥24) [1]. Meanwhile, obesity can also develop into diseases, including type 2 diabetes, nonalcoholic fatty liver disease, and atherosclerosis, posing a serious threat to people's health [2]. Medication, surgery, diet control, and exercise are common methods to treat obesity, but most of them appeared to be problematic, including side effects, adverse reactions, and easy recurrence. In recent years, many studies have found that Chinese herbal extracts have obvious therapeutic effects on obesity. Berberine can significantly improve the serum high-sensitivity C-reactive protein (hsCRP) level in obese patients [3]. Rehmannia glutinosa polysaccharide can improve dyslipidemia among obese diabetic model rats by promoting GLP-1 secretion [4]. Studies have also found that astragalus polysaccharide can significantly improve the insulin resistance in obesity rat model [5]. As the main alkaloid in the lotus leaf, nuciferine has certain function in lowering blood lipids and has therapeutic effects on obesity [6, 7]. However, the mechanism of action of nuciferine in treating obesity remains unclear. In this study, the obesity rat model was induced by the high-fat diet. Firstly, the therapeutic effect of nuciferine on obesity rat model has been explored; secondly, the mechanism of action of nuciferine in mitigating obesity has been investigated from the perspective of regulating the oxidative damage and Nrf2/HO-1 pathway.

Materials and Methods

Laboratory animals

Male SD rats weighing at 200±20 g were purchased from Beijing Huafukang Bioscience Co., Ltd., with the animal production license number: SYXK (Jing) 2019-0037. The rats were raised in an SPF environment at a room temperature of 22 ± 2 ℃, and they were given access to food ad libitum.

Main reagents and instruments

Reagents included the following: Nuciferine (molecular formula: C19H21NO2; molecular weight: 295.38 Da; purity ≥ 98%) was purchased from Sichuan Victory Biological Technology Co., Ltd.; atorvastatin calcium (specification: 20 mg) was purchased from Pfizer Pharmaceutical Co., Ltd.; high-fat diet was purchased from Beijing Huafukang Bioscience Co., Ltd.; triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GSH-Px) assay kits were purchased from Nanjing Jiancheng Bioengineering Institute; RNA extraction, reverse transcription, and amplification kits were purchased from Tiangen Biotech (Beijing) Co., Ltd.

Instruments included the following: Multimode plate reader (Company: Thermo, model: Varioskan Flash), optical microscope (Company: Nikon, model: ECLIPSE TS100), fluorescence-based quantitative PCR instrument (Company: Bio-RAD, model: iQTM5).

1.3 Establishment of the obesity model and grouping

Method for the establishment of the obesity model: Rats were fed with high-fat diet (17.7% sucrose, 17.7% fructose, 19.4% protein, and 40% fat) for 12 weeks [8]. Grouping and dosage regimen were as follows: A total of 40 male SD rats were evenly divided into the normal, model, positive control, and nuciferine groups, using the random number table method. Except for the normal group, rats in the other groups were fed with high-fat diet for 12 weeks to establish the obesity model. During model establishment, rats in the positive control group received atorvastatin calcium 2 mg/kg, rats in the nuciferine group received nuciferine 20 mg/kg, and rats in the normal and model groups received normal saline 2 mL, once a day through intragastric administration for 12 consecutive weeks.

Observation indexes and detection methods

General conditions After 12 weeks of model establishment and administration, the body weight of rats in each group was measured, and the Lee's index of rats in each group was calculated according to the following formula: Lee's index = body weight (g)^(1/3)/body length (cm)*1000.

Detection of rat's serum indexes After 12 weeks of model establishment and administration, the rats in each group were anesthetized, blood was drawn from the abdominal aorta, and serum was collected. The serum TG, TC, LDL-C, HDL-C, SOD, MDA, and GSH-Px levels were detected using the assay kits.

HE staining of rat's liver tissue and epididymal adipose tissue An appropriate amount of epididymal adipose tissue and liver tissue was cut and placed in 10% formaldehyde solution for fixation; next, the tissues were embedded by a paraffin, sectioned at a thickness of 5 μm on a microtome, and stained with HE; finally, the section was transparentized and sealed and was placed under an optical microscope to observe for pathological changes of the adipose tissue.

qPCR The epididymal adipose tissue of rats in each group was cut, the total RNA was extracted, and the complementary DNA (cDNA) was synthesized by reverse transcription. With cDNA as the template, primers and SuperReal PreMix Plus, etc., were added according to the kit's manual. Conditions for amplification reaction were 95℃ for 15 min, 95℃ for 20 s, and 56℃ for 20 s, for a total of 40 cycles. The qPCR instrument was used to detect mRNA expressions of Nrf2 and HO-1 in the epididymal adipose tissue of rats in each group, with β-actin as the reference gene. The specific primer sequences are shown in Table 1.

Table 1 Primer sequences

Genes	Primer sequence (5'-3')
β-actin	Forward: ACC CGC GAG TAC AAC CTT CT
	Reverse: TCA GGG TCA GGA TGC CTC T
Nrf2	Forward: ATA TAC GCA GGA GCG GGA AG
	Reverse: TCC CAT CCT CAT CAC GTA AC
HO-1	Forward: GGG TCC TCA CAC TCA GTT TC
	Reverse: CCA GGC ATC TCC TTC CAT TC

Statistical analysis

Statistical analysis on detection indexes of rats in each group was performed using the SPSS Statistics 17.0. The results were expressed by ± s. Comparison of data between the two groups was performed using the independent samples t-test. P value was calculated, and $P < 0.05$ was considered statistically significant.

Results

Influence of nuciferine on the general conditions and blood lipid levels of obesity rat model

Observation of general conditions showed that compared with the normal group, the body weight and Lee's index increased remarkably in the model group ($P < 0.01$); compared with the model group, the body weight and Lee's index decreased remarkably in the positive control and nuciferine groups ($P < 0.01$, Table 2). Blood lipid level detection showed that compared with the normal group, the serum TG, TC, and LDL-C levels increased significantly ($P < 0.01$), while the HDL-C level decreased significantly ($P < 0.01$) in the model group; compared with the model group, the serum TG, TC, and LDL-C levels decreased significantly ($P < 0.05$ or $P < 0.01$), and the HDL-C level increased significantly ($P < 0.05$) in the positive control and nuciferine groups (Table 3).

Table 2 Influence of nuciferine on the body weight and Lee's index of obese rats ($\bar{x} \pm s$, n = 10)

Group	Body weight (g)	Lee's index
Normal	370.52±31.60	286.81±13.39
Model	487.33±28.82##	334.18±8.17##
Positive control	405.37±15.15**	310.05±10.04**
Nuciferine	414.82±20.96**	308.83±15.99**

##$P < 0.01$, compared with the normal group; **$P < 0.01$ compared with the model group

Table 3 Influence of nuciferine on blood lipids of obese rats ($\bar{x} \pm s$, n = 10)

Group	TG (mmol/L)	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
Normal	1.68±0.22	5.03±0.91	0.82±0.42	2.18±0.06
Model	7.71±1.23##	8.22±0.34##	1.53±0.28##	1.57±0.18##
Positive control	3.19±0.51**	6.11±0.40**	1.04±0.16**	1.72±0.15*
Nuciferine	3.71±0.67**	5.96±0.77**	1.17±0.28*	1.83±0.29*

##$P < 0.01$, compared with the normal group; *$P < 0.05$, **$P < 0.01$, compared with the model group

Influence of nuciferine on the pathological changes of the liver and epididymal adipose tissues in obesity rat model

HE staining of the epididymal adipose tissue showed that the adipose cell had a moderate volume in the normal group but was enlarged significantly in the model group; compared with the model group, the adipose cell significantly shrunk in volume but increased in quantity in the positive control and nuciferine groups (Figure 1).

HE staining of the liver tissue showed that the liver cells in the normal group were arranged normally, with clear structure, and no obvious pathological changes. Significant steatosis occurred in the liver cells of rats in the model group. Compared with the model group, steatosis of the liver cells was improved significantly in rats of the positive control and nuciferine groups (Figure 2).

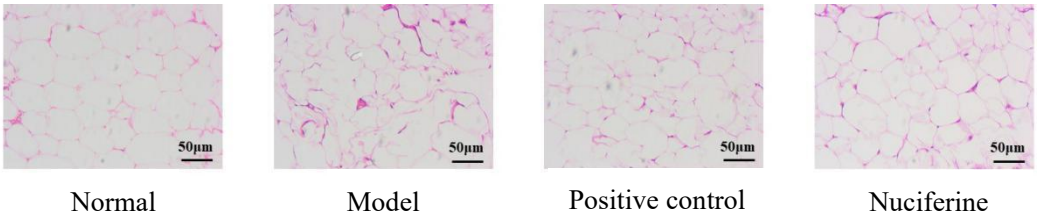

Figure 1 HE staining of the epididymal adipose tissue of rats in each group after model establishment and administration (n = 10)
Magnification: 100×

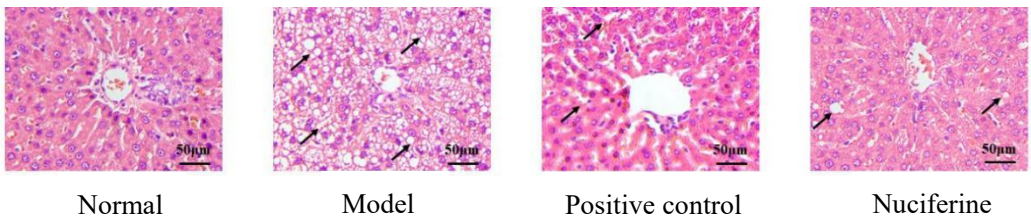

Figure 2 HE staining of the liver tissue of rats in each group after model establishment and administration (n = 10)
The black arrow in the figure indicates steatosis, magnification: 100×

Influence of nuciferine on the serum SOD, MDA, and GSH-Px levels in obesity rat model

Compared with the normal group, the serum SOD and GSH-Px activities were significantly decreased, and the MDA level was significantly elevated ($P < 0.01$) in the model group; compared with the model group, the serum SOD and GSH-Px activities were significantly elevated ($P < 0.05$ or $P < 0.01$), and the MDA level was significantly decreased ($P < 0.01$) in the positive control and nuciferine groups (Table 4).

Table 4 Influence of nuciferine on serum SOD, MDA, and GSH-Px levels in obese rats ($\bar{x} \pm$ s, n = 10)

Group	SOD （U/L）	MDA （mmol/L）	GSH-Px（U/L）
Normal	307.18±22.57	0.98±0.08	103.41±35.84
Model	230.17±31.90##	2.14±0.52##	62.37±39.52##
Positive control	288.64±15.87**	1.37±0.49**	85.41±17.77**
Nuciferine	269.33±37.05*	1.40±0.21**	75.27±8.60*

##$P < 0.01$, compared with the normal group; *$P < 0.05$, **$P < 0.01$, compared with the model group

Influence of nuciferine on the expression of genes related to Nrf2/HO-1 signaling pathway in the epididymal adipose tissue of obesity rat model

The qPCR revealed that compared with the normal group, mRNA expressions of Nrf2 and HO-1 in the epididymal adipose tissue were significantly downregulated in the model group ($P < 0.01$); compared with the model group, mRNA expressions of Nrf2 and HO-1 in the epididymal adipose tissue were significantly upregulated in the positive control and nuciferine groups ($P < 0.01$, Figure 3).

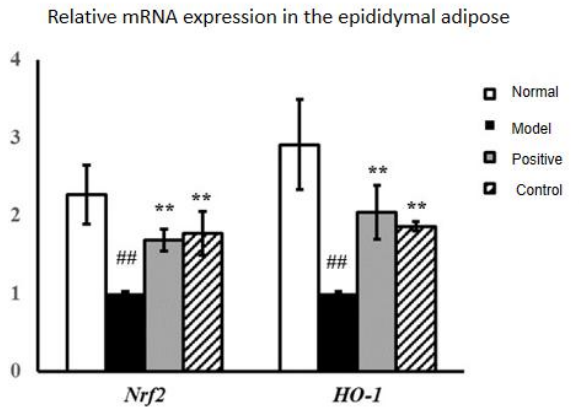

Figure 3 Influence of nuciferine on mRNA expressions of Nrf2 and HO-1 in the epididymal adipose tissue of obesity rat model (n = 10)

##$P < 0.01$, compared with the normal group; **$P < 0.01$ compared with the model group

Discussion

In this study, the obese rat model has been established by feeding the rats with a high-fat diet. The results suggested that compared with the normal group, the body weight of rats was significantly increased, and dyslipidemia was observed in the model group after 12 weeks of high-fat diet. The specific manifestations included elevated TG, TC, and LDL-C levels and lowered HDL-C level, which are consistent with the clinical manifestations of obesity and hyperlipidemia [9]. Additionally, HE staining has revealed that the epididymal adipose tissue cells were enlarged significantly and arranged disorderly, and typical steatosis was observed in the liver of rats in the model group, which are consistent with the pathological changes of obesity [10] and which indicate the successful establishment of the model. Nuciferine could significantly reduce the body weight of rats and improve dyslipidemia and pathological changes of the fat and liver tissues in obesity models. These results indicate that nuciferine has therapeutic effects on obesity. Atorvastatin calcium was selected as a positive control in the study. Atorvastatin calcium is a commonly used lipid-lowering drug in clinical practice, and it is widely used for treating obesity [11]. This study revealed that no significant differences were found in the body weight, blood lipids, and histopathological changes of rats between the nuciferine and atorvastatin calcium groups. However, whether nuciferine can replace atorvastatin calcium to treat obesity needs to be confirmed by further experiments.

Moreover, this study suggested that nuciferine could improve the activities of the oxidative stress-related end product MDA and key enzymes SOD and GSH-Px in the serum of obesity rat model. Oxidative damage is one of the important pathological manifestations of obesity. Excessive lipids will cause the body to produce massive reactive oxygen species (ROS). ROS can damage the structure of biological membranes in the form of O2-, leading to peroxidation of lipids in the cells, and ultimately damaging the cell function. Such process is called the oxidative damage [12]. As the end product of lipid peroxidation, MDA is an important indicator for evaluating oxidative damage of the body. The MDA level is significantly elevated in the obesity model. Therefore, lowering the MDA level can improve the oxidative damage and obesity [13]. SOD and GSH-Px are important antioxidant enzymes in the body. SOD catalyzes O2- to generate H2O2, which will generate H2O and O2 under the catalysis of GSH-Px. Studies have found that SOD and GSH-Px activities are decreased in obese patients, and increased SOD and GSH-Px activities can improve the oxidative damage caused by hyperlipidemia [13,14].

The qPCR revealed that nuciferine could significantly upregulate Nrf2 and HO-1 gene expressions in the adipose tissue of obesity rat model. Nrf2 is a nuclear transcription factor that is located in the cytoplasm and can form a dimer with Keap1. When cells are subjected to oxidative stress, ROS can inhibit the activity of Keap1 and promote the separation of Nrf2 from Keap1. After separation, Nrf2 will enter the cell nucleus to regulate the expression of its downstream antioxidant gene HO-1, promote the transcription of genes including antioxidant enzyme GSH-Px, and play the role of resisting the oxidative damage [15,16].

In summary, the findings of this study indicate that nuciferine has

therapeutic effects on obesity rat model, and its mechanism of action may be associated with the upregulation of Nrf2 and HO-1 expressions in the adipose tissue, thereby enhancing the body's antioxidant capacity.

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