Ethyl acetate extracted from the Chinese medicinal plant Caesalpinia sappan L alleviates gustatory response in diabetes mellitus rats

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Ethyl acetate extracted from the Chinese medicinal plant Caesalpinia sappan L alleviates gustatory response in diabetes mellitus rats

Running title: H-EtOAc extracts of C. sappan relieved gustatory response in DM rats

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Abstract

Objective: The critical role of ethyl acetate (EtOAc) extracts of Caesalpinia sappan L (C. sappan) in disease control has been studied, while its potential function in diabetes mellitus (DM)-induced gustatory response remains unclear. Here, we focused on the regulatory role of EtOAc extracts of C. sappan in taste-regulatory peptides in vallate papillae and the hypothalamus.

Methods: DM rat models were established using high-glucose and high-fat feeding combined with intraperitoneal injection of streptozotocin followed by administration of high, medium, and low doses of EtOAc extracts of C. sappan. Then, fasting blood glucose, triglycerides, and total cholesterol of rats were measured, the morphology of tongue vallate papillae and apoptosis of taste cells were detected by hematoxylin-eosin and TUNEL staining, and the expression of taste-regulatory peptides (ghrelin, GLP-1, NUCB2, and DPP-IV) in tongue tissue and hypothalamus was detected using RT-qPCR, Western blot, and immunohistochemistry.

Results: A high dose of EtOAc extracts of C. sappan reduced blood glucose and lipids, improved the morphology of tongue vallate papillae and neurons in the hypothalamus, and suppressed apoptosis of taste cells. In addition, both in taste cells and the hypothalamus, a high dose of EtOAc extracts of C. sappan elevated the expression of ghrelin, GLP-1, and NUCB2 but reduced DPP-IV expression.

Conclusion: The current study highlighted the alleviative role of EtOAc extracts of C. sappan in gustatory response in DM rats via the regulation of taste-regulatory peptides in taste cells and the hypothalamus.

Keywords: Ethyl acetate extracts of Caesalpinia sappan L; diabetes mellitus; gustatory response; taste-regulatory peptides; vallate papillae; hypothalamus
Introduction

Diabetes mellitus (DM) represents a highly complex and heterogeneous disease, which can affect people at all stages of life (The Lancet, 2017). Taste disorders (dysgeusia) are a common issue in individuals with DM due to a reduction in saliva production, which can be exacerbated by neuropathy of taste-sensing nerves or microangiopathy that affects the taste buds (Borgnakke et al., 2015, Sinska et al., 2022). Notably, dysgeusia is a serious issue for patients with DM as it may lead to overeating and contribute to the progression of the disease (Kawakami et al., 2023). Thus, there is an urgent need to identify the underlying mechanisms behind gustatory response and develop effective treatment strategies.

Traditional herbal medicines have been used for centuries in the improvement and maintenance of health or the treatment of various types of human diseases (Bajic et al., 2016; Han et al., 2020; Kumar et al., 2021) due to their unique multi-target efficacy. Traditional herbal medicines can be also applied in the management of DM through their relevant mechanistic pathways, including improving insulin sensitivity, enhancing glucose uptake by adipose and muscle tissues, inhibiting glucose production from hepatocytes and glucose absorption from the intestine as well as suppressing inflammatory responses (Liu et al., 2004; Li et al., 2012; Sun et al., 2021). *Caesalpinia sappan Linn.* (*C. sappan*) belongs to the legume family (Leguminosae) and is distributed in Southeast Asia; it is used in traditional herbal medicines for the treatment of many diseases (Kim et al., 2015; Tran et al., 2015). The ethyl acetate (EtOAc) extract of *C. sappan* is considered a traditional Chinese medicinal extract that is commonly used in the treatment of atherosclerosis by upregulating D-mannose via the lysosome pathway, enhancing lysosomal function, mediating autophagy, and indirectly regulating the levels of lipid subtypes (Liu et al., 2022). In addition, a recent study has demonstrated the inhibitory effect of EtOAc extracts of *C. sappan* on acute myeloid leukemia by inducing mitochondrial apoptosis and promoting differentiation (Ma et al., 2020). A prior study showed that the Sappan Lignum extract can reduce
blood glucose levels and alleviate renal damage in rats with diabetic renal disease (Tong et al., 2013). To date, little research has focused on the role of EtOAc extracts of C. sappan in DM and the associated dysgeusia. However, the ethanolic extract of C. sappan exerts neuroprotective effects in a rat model of cerebral ischemia/reperfusion injury by inhibiting neuronal damage and activation of neutrophils, microglia, and astrocytes while promoting synaptic generation (Wan et al., 2019). Meanwhile, many studies have reported the association of DM with microglial activation (Ibrahim et al., 2011; Chandran et al., 2020; Kongtawelert et al., 2022). Taste bud cells express many peptide receptors and all these peptides have critical roles in nervous or endocrine tissues, affecting appetite or metabolism and physiological responses (Dotson et al., 2013). Many researchers report that streptozotocin (STZ) induces DM in vivo by specifically destroying pancreatic β-cells, and the STZ-diabetic rat model is well-established and commonly used (Baig and Panchal, 2019; Rais et al., 2022). Thus, in the present study, we developed a rat model of DM by injection with STZ, with the aim of investigating the possible role of EtOAc extracts of C. sappan in DM-associated gustatory response through taste-regulatory peptides and to elucidate the underlying mechanisms.

Materials and methods

Animals

Animal experiments were carried out in accordance with the protocol approved by the Committee of Animal Research Institutions and NIH guidelines for the use and care of laboratory animals. All animal experiments comply with the rules and regulations of the management of experimental animals and the relevant ethical requirements of experimental animals (approved number: 2021114).

Eighty six-week-old male Wistar rats (specific pathogen free [SPF] level, weighing 160 ~ 200 g; Changchun Changsheng Biological, Liaoning, China, License No. SCXK (Liao) 2020-0001) were fed in a clean animal laboratory under standard conditions (20~25°C and 55% ± 15%
humidity) with 12 h light/dark cycles and free access to water and food. The padding was changed twice a week. All rats were subjected to acclimation for one week before the experiment. Notably, each rat was given a separate cage.

**Drug preparation**

Preparation of EtOAe extracts of *C. sappan*: crude *C. sappan* was purchased from Heilongjiang Medicinal Material Corporation (Heilongjiang, China). Identification and extraction of ethyl acetate extracts were performed by the Pharmacy College of Heilongjiang University of Chinese Medicine. In detail, crude *C. sappan* was crushed and turned into powder (40-mesh), which was soaked in 75% ethanol for 4 h in a four-neck beaker. Afterward, the sample was heated at 85°C and subjected to reflux extraction for 2 h, and then filtered. The process was repeated twice. The filtrates obtained were mixed, concentrated in a rotary evaporator, and oven-dried to a constant weight to obtain the ethanol, which was suspended with water and extracted with ethyl acetate. After the operation, the ethyl acetate extraction was recovered and dried to obtain a dry powder.

Epalrestat was purchased from Yangtze River Pharmaceutical Group (Taizhou, Jiangsu, national medicine permission number [NMPN]: H20040012).

**Animal model establishment**

Wistar rats weighed 200 ~ 250 g after one week of acclimation. After sorting according to body weight, 10 rats were selected as the control group according to a random number table, numbered, and fed with ordinary diet for eight weeks. The remaining 70 rats selected for experimental groups were fed with high-glucose and high-fat feed (10% egg yolk powder, 10% lard, 10% sugar, 10% cholesterol, 0.2% sodium cholate, and 59.8% common feed) for eight weeks and
then intraperitoneally injected with a low dose of STZ (30 mg/kg, prepared in 0.1 mmol/L citric acid buffer at pH 4.5) using a 6-gauge needle connected to a 1 mL syringe. Rats in the control group were intraperitoneally injected with an equivalent volume of citric acid buffer. About 72 h later, blood samples were collected from the tail vein, and rats with random blood glucose ≥16.7 mmol/L were used to establish the DM model. Rats with fasting blood glucose (FBG) ≥ 16.7 mmol/L were selected (n = 60). After ranking blood glucose values from high to low, the 60 rats were randomly divided into model groups: high-dose (H-EtOAc), medium-dose (M-EtOAc), low-dose (L-EtOAc), and the epalrestat group (n = 12 rats per group), and continued to be fed with a high-glucose and high-fat diet for eight weeks.

**Animal grouping and drug administration**

Administration intervention was conducted from the 16th week of the experiment; the duration of the intervention was 12 weeks, and gavage frequency was once daily during administration. The dry powder of the previously prepared EtOAc extracts of *C. sappan* was dissolved in 5‰ sodium carboxymethyl cellulose solution (Shijiazhuang Senlong Chemical, China) and fully stirred to prepare a suspension of EtOAc extracts of *C. sappan*. An 18-gauge needle (ball tip, Kyron Laboratories, Benrose, Johannesburg, South Africa) connected to a 1 mL syringe was used for gavage. Epalrestat treatment was used as the positive control group. The dosage was determined according to the animal's weight. The specific administration method and the final remaining number of rats in each group were as follows:

1) Control group (n = 10), rats in this group were subjected to intragastric administration of an equivalent volume of sodium carboxymethyl cellulose solution.

2) Model group (n = 7), intragastric administration of an equivalent volume of sodium
carboxymethyl cellulose solution.

3) High-dose group (H-EtOAc group, n = 7), intragastric administration of a suspension of EtOAc extracts of *C. sappan* (2.3 mg/kg/d).

4) Medium-dose group (M-EtOAc group, n = 7), intragastric administration of a suspension of EtOAc extracts of *C. sappan* (1.15 mg/kg/d).

5) Low-dose group (L-EtOAc group, n = 8), intragastric administration of a suspension of EtOAc extracts of *C. sappan* (0.575 mg/kg/d).

6) Epalrestat group (n = 8), intragastric administration of epalrestat (10 mg/kg/d).

**Sample collection**

After four weeks of drug treatment, the rats were subjected to fasting for 12-14 h and then weighed. The tail tip of the rat (about 0.3 ~ 0.5 cm) was cut off using surgical scissors to extract blood. FBG was measured using a glucose meter. The rats were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg). Blood was collected from the right ventricle below the xiphoid process and serum was separated and stored in a -80°C freezer for the detection of blood lipids. Tongue tissue and hypothalamus were collected from rats after euthanasia with 150 mg/mL of pentobarbital sodium. About 4-5 rats in each group (five in the control group and four in each of the other groups) were randomly selected for histopathological examination and immunohistochemistry. The remaining rat tissues were reserved for molecular biological detections (five rats in the control group, four rats in the L-EtOAc and epalrestat groups, and three rats in the other groups). Details are shown in Fig 1.
Measurement of lipid metabolism indexes

Triglyceride and total cholesterol kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu) were used for measurement of triglyceride and total cholesterol levels.

Scanning electron microscope

Fresh rat tongue tissues were quickly fixed in electron microscope fixative (Solarbio, Beijing, China) for 2 h. The fixed samples were rinsed with 0.1 M phosphoric acid buffer three times (15 min each), fixed with 1% osmic acid (pH 7.4) for 1-2 h (20°C), rinsed with 0.1 M PB (pH 7.4) three times (15 min each), and dehydrated with gradient alcohol. Then, samples were dried in a critical point dryer. Images were observed under a scanning electron microscope (Hitachi, Tokyo, Japan).

Hematoxylin-eosin (HE) staining

The collected rat tongue tissues were dehydrated by gradient alcohol (70% alcohol for 10 min; 90% alcohol for 10 min; and 100% alcohol for 20 min), cleared by xylene (Merck, Darmstadt, Germany), routinely embedded, and cut into continuous sections (7 µm). After routine dewaxing and hydration, the sections were stained with hematoxylin solution (Sigma-Aldrich, St. Louis, MO, USA) and 1% water-soluble eosin dye solution (Sinopharm Group, Beijing, China). The histopathological changes were observed under an optical microscope (BX53, Olympus, Tokyo, Japan). Neuron damage (%) = damaged neurons/all neurons.

Immunohistochemistry

The collected rat tongue tissues were dehydrated by gradient alcohol, cleared by xylene, routinely embedded, and cut into continuous sections (7 µm). After being dewaxed and hydrated,
tissue sections were subjected to antigen retrieval. The prepared 3% hydrogen peroxide drops were added to the sections to block endogenous peroxidase. Then, tissue sections were incubated at room temperature for 15 min, washed with PBS three times (3 min each), sealed with diluted normal goat serum for 30 min, and incubated with dipeptidyl peptidase-IV (DPP-IV) (ab187048, 1:100, Abcam, Cambridge, UK), ghrelin (ab209790, 1:100, Abcam), nucleobindin 2 (NUCB2) (ab224348, 1:100, Abcam), and glucagon-like peptide-1 (GLP-1) (A14609, 1:100, ABclonal, Wuhan, China) at 4°C overnight. The sections were rinsed with PBS three times (3 min each), dried, and incubated with secondary antibody at 37°C for 30 min. After rinsing with PBS, the sections were developed with DAB color-developing solution, stained with hematoxylin (Sigma-Aldrich, St. Louis, MO, USA) for 2 min, sealed with neutral gum, and observed under a microscope (BX53, Olympus).

**TUNEL staining**

After routine dewaxing and hydration of rat tongue tissues, the sections were stained using the fluorescein-coupled TUNEL in situ Cell Death Assay kit (Roche Diagnostics, Mannheim, Germany), and examined under an Olympus IX-73 fluorescence microscope to obtain fluorescent images. Apoptotic rate (%) = apoptotic cells/total cells.

**RT-qPCR**

Total RNA was extracted by adding 1 mL Trizol reagent (Ambion, Austin, TX, USA) to 100 mg rat tongue tissue and was reverse-transcribed into cDNA using a reverse transcription kit. The samples were subjected to RT-qPCR in a real-time quantitative fluorescence PCR instrument (QuantStudio 6, ABI, Foster City, CA, USA). The reaction system included SYBR Mix 10 µL, forward primer 0.4 µL, reverse primer 0.4 µL, cDNA 4 µL, and RNase Free dH₂O 4.8 µL. Reaction
conditions consisted of 95°C for 10 min, 95°C for 15 s, 60°C for 60 s, and 72°C for 15 s, with a total of 40 cycles. Primer sequences are displayed in Table 1. Using β-actin as the internal parameter, the relative expression of the product was calculated with the $2^{-\Delta\Delta Ct}$ method.

**Western blot**

A small number of tongue tissue blocks were placed in a 2 mL EP tube and lysed with 300 µL RIPA lysate (Beyotime). After measurement of the protein concentration with the BCA kit (Beyotime), the corresponding volume of protein was added to the sample buffer (Beyotime), mixed, and heated in boiling water for 10 min to denature the protein. The protein was then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis for 1.5 h. Next, the protein was transferred to the membrane at 200 mA for 70-120 min. The membrane was blocked with blocking buffer at room temperature for 120 min and probed with the following primary antibodies at 4°C overnight: NUCB2, DPP-IV, β-actin (ab8226, 1:1000, Abcam), GLP-1, and ghrelin. Next, the membrane was incubated with the secondary antibody for 2 h. Following the addition of developing liquid, the band was scanned and the related grey value was analyzed by BandScan.

**Statistics**

Statistical analysis was performed using GraphPad prism8 software, and all data were described as mean ± standard deviation. For samples conforming to normal distribution, comparison between multiple groups was conducted by the one-way analysis of variance test, and post-hoc multiple comparisons were conducted with Tukey's multiple comparisons test. $P < 0.05$ was considered statistically significant.
Results

A high dose of EtOAc extracts of *C. sappan* improved blood glucose and blood lipid in DM rats

The rats were weighed before euthanasia, and the results showed that the body weight of rats in the model group was greatly reduced versus the control group. Compared with the model group, the body weight of rats in the L-EtOAc, M-EtOAc, H-EtOAc, and epalrestat groups had no significant change (Fig 2A). Measurement of FBG, triglyceride, and total cholesterol levels revealed that, compared with the control group, levels in the model group were markedly increased; relative to the model group, FBG, triglyceride, and total cholesterol levels in the H-EtOAc group were markedly lower, while there was no significant difference in the other groups (Fig 2B-D). Collectively, a high dose of EtOAc extracts of *C. sappan* may improve blood glucose and blood lipids in DM rats.

A high dose of EtOAc extracts of *C. sappan* improved the morphology of rat vallate papillae and inhibited the apoptosis of taste cells

As observed under the scanning electron microscope, the filiform papillae were significantly damaged and vallate papillae were atrophic in the model group (vs. the control group). Versus the model group, the L-EtOAc and M-EtOAc groups had reduced papilla injury, and the H-EtOAc and epalrestat groups had more obviously reduced papilla injury (Fig 3A). As displayed by HE staining, in comparison with the control group, the vallate papillae were atrophic and the number of taste buds was decreased in the model group; relative to the model group, the area of vallate papillae and number of taste cells in the L-EtOAc and M-EtOAc groups were increased, and these changes were more obvious in the H-EtOAc and epalrestat groups (Fig 3B). TUNEL staining revealed that,
compared with the control group (7.15%), the apoptosis of taste cells was greatly increased in the model group (19.32%); compared with the model group, the apoptosis of taste cells significantly declined in the H-EtOAc (12.65%) and epalrestat groups (11.35%) (Fig 3C). These results demonstrated that a high dose of EtOAc extracts of C. sappan improved the morphology of rat vallate papillae and inhibited the apoptosis of taste cells.

**A high dose of EtOAc extracts of C. sappan regulated the expression of taste-regulatory peptides in taste cells**

RT-qPCR and Western blot revealed that, compared with the control group, the expression of ghrelin, GLP-1, and NUCB2 was significantly reduced while DPP-IV expression was increased in the model group; relative to the model group, the expression of ghrelin, GLP-1, and NUCB2 was increased in the H-EtOAc, M-EtOAc, and epalrestat groups, while DPP-IV was significantly decreased (Fig 4A-B). A similar result was obtained by immunohistochemistry (Fig 4C). Altogether, a high dose of EtOAc extracts of C. sappan elevated the expression of ghrelin, GLP-1, and NUCB2 while reducing DPP-IV in taste cells.

**A high dose of EtOAc extracts of C. sappan regulated the expression of taste-regulatory peptides in the hypothalamus**

As seen by HE staining, neurons presented pyknosis, the number of damaged neurons in the model group was increased, and a few hemorrhagic spots were observed in the hypothalamus (vs. the control group); the number of injured neurons in the M-EtOAc, H-EtOAc, and epalrestat groups was notably reduced (vs. the model group) (Fig 5A). Compared with the control group, the expression of ghrelin, GLP-1, and NUCB2 was significantly reduced while DPP-IV expression was
increased in the model group; relative to the model group, the expression of ghrelin, GLP-1, and NUCB2 was visibly increased in the H-EtOAc, M-EtOAc, and epalrestat groups, while DPP-IV was significantly decreased (Fig 5B-C). A similar result was obtained via immunohistochemistry (Fig 5D). Altogether, a high dose of EtOAc extracts of *C. sappan* improved the morphology of neurons in the hypothalamus and elevated the expression of ghrelin, GLP-1, and NUCB2 while reducing DPP-IV in the hypothalamus.

**Discussion**

*C. sappan* has been widely applied in the treatment of DM-associated complications in Southeast Asia, specifically in traditional Chinese medicine (Adnan et al., 2022). Dysgeusia, a prevalent qualitative gustatory impairment, has been documented as a symptom of underlying systemic conditions, including DM (Jafari et al., 2021). The current study intends to investigate the potential role of EtOAc extracts of *C. sappan* in the DM-associated gustatory response in rats and explain the underlying mechanism. The results obtained suggest that a high dose of EtOAc extracts of *C. sappan* could improve DM-associated dysgeusia, which is related to its regulation of taste-regulatory peptides.

Initial results in the current study uncovered that a high dose of EtOAc extracts of *C. sappan* could remarkably lower blood glucose and lipids in rat models with DM. Consistently, previous results revealed that *C. sappan* can effectively reduce blood glucose levels in menopausal women with DM (Sudirman et al., 2020). Additionally, this study demonstrated that a high dose of EtOAc extracts of *C. sappan* inhibited taste bud cell apoptosis in diabetic rats. Taste buds are known to be a complex sensory organ distributed in the epithelium of circumvallate and fungiform papillae (Yamada et al., 2021). Each taste bud contains approximately 100 cells that process and integrate taste information with metabolic needs (Kikut-Ligaj and Trzcielinska-Lorych, 2015). Taste dysfunction has been reported in obese humans and animals, and mice with an obesogenic diet have...
fewer taste buds (Harnischfeger et al., 2021). In accordance with our results, a previous study revealed that taste bud cell apoptosis in the circumvallate papillae, which may be mediated by the intrinsic mitochondrial pathway, can induce a gustatory response in a rat model of type 2 DM, with downregulated Bcl-2 in circumvallate papilla, upregulated Bax, and cleaved caspase-9 and caspase-3 (Cheng et al., 2011). Inflammatory cytokines can trigger apoptotic cell death and thus cause abnormal cell turnover in taste buds (Wang et al., 2009). Therefore, we focused on the levels of apoptosis and inflammation-related genes to verify DM-induced taste bud cell apoptosis.

This study also revealed that a high dose of EtOAc extracts of C. sappan could increase the expression of the taste-regulatory peptides ghrelin, GLP-1 and NUCB2, while reducing that of DPP-IV both in taste cells and the hypothalamus. Ghrelin is a major appetite-stimulating neuropeptide found in the circulation and its growth hormone secretagogue receptor can enhance fat-taste responsiveness in female mice (Calder et al., 2021). Ghrelin can regulate adiposity, growth hormone release, food intake, and energy metabolism; ghrelin knockout mice exhibit increased plasma triglycerides and ketone bodies yet decreased food intake (Cai et al., 2013). GLP-1 is a pleiotropic hormone with beneficial potential due to its association with cardiovascular protection, islet function, diet control, inflammation relief, and glucose homeostasis (Xie et al., 2020). A previous study found that the peptide hormone GLP-1 and its receptor (GLP-1R) are expressed in mammalian taste buds; furthermore, GLP-1R knockout mice exhibit a dramatic reduction in sweet-taste sensitivity (Martin et al., 2009). For individualized treatment of type 2 DM, short-acting GLP-1R agonists can lower postprandial blood glucose levels by inhibiting gastric emptying, and long-acting GLP-1R agonists exhibit stronger effects on fasting glucose levels, mainly in correlation with their insulinotropic and glucagonostatic actions (Meier, 2012; Maselli and Camilleri, 2021). NUCB2 is a gut-brain peptide and the gastric mucosa may be one of its major sources (Li et al., 2013). NUCB2 is essential for integrating feeding, energy storage/expenditure, and glucose homeostasis; dysfunction of expression, secretion, and/or action of NUCB2 may be involved in obesity, type 2 DM, and metabolic syndrome (Nakata and Yada, 2013). In addition, a
significantly lower expression of NUCB2 mRNA has been found in the taste buds of DM rats than in normal controls (Cao et al., 2016). DPP-IV is a physiological enzyme solubilized in blood or membrane-anchored in tissues; it cleaves a wide range of physiological peptides and is related to disease processes, such as multiple sclerosis, eating disorders, rheumatoid arthritis, cancer, and type 2 DM (Flatt et al., 2008). Inhibitors of DPP-IV have been demonstrated to serve as a potential treatment strategy against type 2 DM (Kushwaha et al., 2014). In addition, inhibition of DPP-IV can enhance the activity of endogenous GLP-1 and d-glucose-dependent insulinotropic polypeptide, which are key regulators of blood glucose control and prandial stimulators of insulin secretion (Green et al., 2006).

Overall, this study demonstrates the protective effects of EtOAc extracts of C. sappan against the gustatory response in rat models of DM. This involved a decrease in DPP-IV expression and an increase in the expression of ghrelin, GLP-1, and NUCB2 both in taste cells and the hypothalamus. However, the specific mechanism by which EtOAc extracts of C. sappan affect the expression of the aforementioned taste-regulatory peptides remains unclear and requires further studies. In addition, three different doses of EtOAc extracts of C. sappan (high: 2.3 mg/kg/d; medium: 1.15 mg/kg/d; low: 0.575 mg/kg/d) were used in this study, with the finding that all could modulate peptide expression to improve the gustatory response in rats with DM, of which treatment with a high dose of EtOAc extracts of C. sappan had the most significant effect. Moreover, the high dose of C. sappan (2.3 mg/kg/d) corresponds to a human clinical dose of about 0.2 mg/kg/d, which provides a basis for solving the problem of gustatory response in DM patients in a clinical setting.

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**Declaration of interest**

The authors report no relationships that could be construed as a conflict of interest.

**Author contribution**

CX conceived the ideas. CX, CL, and YW designed the experiments. CL and YW performed the experiments. CX, CL, and YW analyzed the data. CX, CL, and YW provided critical materials. CL and YW wrote the manuscript. CX supervised the study. All authors have read and approved the final version for publication.

**Availability of data and materials**

The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.
References


**Legends**

**Figure 1** A timeline.

**Figure 2** A high dose of EtOAc extracts of *C. sappan* may improve blood glucose and blood lipids in DM rats.

Note: (A), Comparison of rat weight in each group. (B), Measurement of FBG in each group. (C), Measurement of triglycerides in each group. (D), Measurement of total cholesterol levels in each group. * P < 0.05 compared with the control group, # P < 0.05 compared with the model group.

**Figure 3** A high dose of EtOAc extracts of *C. sappan* ameliorated the morphology of rat vallate papillae and inhibited the apoptosis of taste cells.
Note: (A), Scanning electron microscopy of rat tongue tissue, red arrows mark the filamentous papillae and green arrows mark the vallate papillae. (B), HE staining of rat tongue tissue. (C) TUNEL staining of rat tongue tissue. * $P < 0.05$ compared with the control group, # $P < 0.05$ compared with the model group.

**Figure 4** A high dose of EtOAc extracts of *C. sappan* regulated the expression of taste-regulatory peptides in taste cells.

Note: (A-C), The expression of ghrelin, GLP-1, NUCB2, and DPP-IV in tongue tissue was detected by RT-qPCR (A), Western blot (B), and immunohistochemistry (C). * $P < 0.05$ compared with the control group, # $P < 0.05$ compared with the model group.

**Figure 5** Regulatory role of a high dose of EtOAc extracts of *C. sappan* in the expression of taste-regulatory peptides in the hypothalamus

Note: (A), HE staining of hypothalamus. (B-D), The expression of ghrelin, GLP-1, NUCB2, and DPP-IV in tongue tissue was detected by RT-qPCR (B), Western blot (C), and immunohistochemistry (D). * $P < 0.05$ compared with the control group, # $P < 0.05$ compared with the model group.
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Wistar rats

Intraperitoneally injected with streptozotocin

The rats were sacrificed after tail amputation for blood collection, and the hypothalamus and tongue tissues were collected

After rats were sacrificed, tongue tissues were collected

Establish the DM model

High-glucose and high-fat diet

Administration intervention

Control group (n = 10)
Model group (n = 7)
H-EtOAc group (n = 7)
M-EtOAc group (n = 7)
L-EtOAc group (n = 8)
Epalrestat group (n = 8)

5 in the control group and 4 in each of the other groups
HISTOLOGY AND HISTOPATHOLOGY

Aa control

Ba

cd L-EtOAc

ef epalrestat

B a control b model c H-EtOAc d M-EtOAc
e L-EtOAc f epalrestat
g Mean length (µm) Mean width (µm)

C a control b model c H-EtOAc d M-EtOAc
e L-EtOAc f epalrestat
g Apoptotic taste cell (%)
**HISTOLOGY AND HISTOPATHOLOGY**

Panel A shows images of tissue samples under different conditions. Each image is labeled with a letter:

- **a** control
- **b** model
- **c** H-EtOAc
- **d** M-EtOAc
- **e** L-EtOAc
- **f** eparrestat

Panel B contains a graph with bars representing protein expression levels for ghrelin, GLP-1, NUCB2, and DPP-IV under various conditions. The graph includes a legend with different conditions labeled as:

- control
- H-EtOAc
- M-EtOAc
- L-EtOAc
- epalrestat

Panel C shows a histogram with bars for protein expression levels under the same conditions as in Panel B.

Panel D presents micrographs of tissue sections stained for ghrelin, GLP-1, NUCB2, and DPP-IV under different conditions.

Panel E includes a table with numerical data for comparision of protein expression levels across different treatments.

**Figures and Tables:**

- **ghrelin**
- **GLP-1**
- **NUCB2**
- **DPP-IV**
- **β-actin**

**Legend:**

- Control
- H-EtOAc
- M-EtOAc
- L-EtOAc
- Eparrestat

**Units:**

- g
- mg
- µm

**Measurements:**

- 20 µm