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Original Research Article

Effect and mechanism of action of *Rhodiola rosea* L on diabetic peripheral neuropathy in rats based on Synapsin-I

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Abstract

Purpose: *To investigate the efficacy and underlying mechanism of action of Rhodiola rosea L. on diabetic gastroparesis (DGP) based on Synapsin-I.*
Methods: *The extract of Rhodiola rosea L. was obtained by preparing the crude medicine of traditional Chinese medicine. The Sprague-Dawley (SD) rats were randomly allocated to three groups: normal, DGP, and DGP+Rhodiola rosea L. groups. Rats in DGP+Rhodiola rosea L. group were treated with Rhodiola rosea L. once daily for 5 weeks at a dose of 150 mg·kg-1·day-1, while rats in DGP group received an equivalent volume of saline by oral gavage. Parameters were evaluated include body weight, fasting glucose level, gastric emptying rate and gastric acid secretion. Histopathological examination of the stomach was performed by hematoxylin-eosin (H&E) staining. Immunohistochemistry, cellular immunofluorescence, as well as western blot were employed to determine the expressions of Synapsin-I and protein gene product 9.5 (PGP9.5).*
Results: *Compared normal group, the body weight or rats in DGP group decreased, blood glucose was elevated, gastric acid secretion level decreased, and gastric emptying rate increased ($p < 0.05$). Besides, they also showed signs of misalignment of gastric mucosal glands, enlarged intercellular space, and significantly reduced protein expressions of PGP9.5 and Synapsin-I. Administration of Rhodiola rosea L. extract reversed the above changes in DGP rats, and also elevated the protein expression of both PGP9.5 and synapsin-I.*
Conclusion: *Rhodiola rosea L. extract exerts a protective effect in DGP by enhancing synaptic plasticity via upregulation of expression of synapsin-I. Thus, an experimental basis has been established for the potential clinical application of Rhodiola rosea L. extract in the management of diabetic peripheral neuropathy.*

Keywords: *Rhodiola rosea L, Diabetic gastroparesis, Synapsin-I, Synaptic plasticity, Diabetic peripheral neuropathy*

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INTRODUCTION

Diabetic gastroparesis (DGP) is one of the most common neuropathies of diabetes [1]. Approximately 30 – 50 % of individuals with long-term diabetes experience significantly delayed gastric emptying, which has a profound impact on their overall well-being and quality of life [2].

DGP is generally caused by a combination of factors, including neuropathy, hyperglycemia, gastrointestinal hormones, and microvascular disease. Enteric nervous system (ENS) lesions and severe hypofunction of synaptic transmission are important contributors to gastrointestinal dysfunction in DGP [3].

Impaired gastric emptying in individuals with diabetes is primarily attributed to the pathological changes in the ENS. Gastrointestinal motility disorders are caused by impaired transmission of neurotransmitters and electrical signals between neurocytes, or between neurocytes and smooth muscle cells. Understanding the pathological mechanisms involving the nerves and synapses in the ENS, as well as researching synaptic plasticity are highly relevant in developing effective therapeutic approaches for DGP. Synaptic proteins are a group of neuron-specific phosphoproteins closely associated with synaptic vesicles. They are distributed in almost all nerve terminals and are located on the surface of presynaptic vesicle membranes [4]. Synapsin-I is one of the main synapsins. It regulates the release of neurotransmitters through phosphorylation and non-phosphorylation, and plays an integral role in neuronal development and regeneration [5]. Protein gene product 9.5 (PGP9.5) is an ubiquitin hydroxyl hydrolase with specific effects, and it is the hallmark of neuronal fibers, and serves as a characteristic marker for assessing the function of autonomic nervous system [6].

Salidroside is the main compound found in Rhodiola rosea L. It has anti-hypoxia, anti-fatigue, anti-aging, sedative, anti-cold properties [7]. Rhodiola rosea L. glycosides, known for their neuroprotective effects, have shown promise as a neuroprotective agent in the treatment of peripheral nerve injury. However, there is limited research on their application specifically in diabetic neuropathy and gastroparesis till now.

EXPERIMENTAL

Animals

For this study, eight-week-old male Sprague-Dawley (SD) rats weighing 180 – 220 g were used as experimental subjects. They were provided by Hangzhou Medical College Laboratory Animal Center. This animal experiment was approved by The Institutional Animal Care and Use Committee of Zhejiang Centre of Laboratory Animals (approval no. ZJCLA-IACUC-20030065), and all the experimental procedures were strictly in compliance with international guidelines 2.0 [8].

Establishment of DGP model

Following a one-week period of acclimation with standard diets, the SD rats were subjected to glycosuria test. The glucose in the collected urine samples were tested using glycosuria test paper. Any samples that indicated a positive result were excluded from the study. Following a 12-hour period of fasting, the rats were injected with 0.1 mol/L streptozotocin (STZ) (Sigma, The United States) citrate buffer (pH 4.5) at a dose of 50 mg/kg in the left lower abdomen. After a 72-hour interval, the samples of rats' blood were taken from their tail vein. The successful induction of diabetes in rats was confirmed if their immediate non-fasting blood glucose levels were detected ≥ 16.7 mmol/L. Intraperitoneal injection of citrate buffer solution (0.1 mol/L, pH 4.5) were given to rats in the normal group at an equivalent volume.

To establish the DGP model, the diabetic rats were fed with high-glucose and high-fat diets, whereas rats in the normal group were provided with regular diets. Following 8 weeks of continuous feeding, if the rats were observed to have symptoms of gastroparesis (abdominal distension, weight loss, and loose stool), the DGP rat model construction was successful.

Extraction of *Rhodiola rosea* L.

100 g of *Rhodiola rosea* L. was finely crushed, and then heated with 10 times the amount of water. The mixture was subjected to reflux for 1 h, and the process repeated three times. After filtration, the collected filtrates were combined and made up with distilled aqueous solution to 100 mL, thus giving a solution of *Rhodiola rosea* L. extract containing 1 g/mL of the crude drug.

Animal grouping

Rhodiola rosea L. was obtained from The Second Affiliated Hospital of Zhejiang Chinese Medical University and prepared into 1.0g crude medicine/milliliter. Following the establishment of the DGP rat model, *Rhodiola rosea* L. was used for treatment. The dose of *Rhodiola rosea* L. administered was 150 $mg \cdot kg^{-1} \cdot day^{-1}$, prepared using normal saline. Rats in DGP+*Rhodiola rosea* L. group received daily intragastric administration of *Rhodiola rosea* L. once a day for 5 weeks, while those in DGP group was given an equal volume of normal saline by gavage.

Determination of gastric acid secretion

Following a 24-hour fasting period, the rats were anesthetized and euthanized. The gastric cardia and pylorus of the rats were clamped, and their stomachs were taken out and placed in oxygen-saturated saline solution. Using Topfer's as an indicator, the solution containing gastric contents was centrifuged, then 0.1 mL of the supernatant was titrated with NaOH (0.01 mmol/L), and the total acid secretion was taken as the entire gastric acid secretion.

Determination of gastric emptying rate

The 1.5% methylene blue was added into the methyl cellulose solution (final concentration 1 mg/mL). Following a 24-hour fasting period, the rats were injected with 0.4 mL of methylene blue solution in the stomach, and euthanasia was performed 30 min after the injection. Subsequently, following the opening of the abdominal cavity, the gastric contents of the rats were flushed with 4 mL of normal saline, and the rinse solution was collected and centrifuged. The absorbance at 620 nm was determined, and gastric emptying rate computed by quantifying the methylene blue residues.

Determination of blood glucose

Following a 24-h fasting period, the SD rats were subjected to tail-tip blood sampling. The level of fasting blood glucose was measured using blood glucose assay kit (Biosino, China), and the corresponding serum concentration of fasting glucose was calculated.

Hematoxylin-eosin (H&E) staining

The gastric body was excised from the rats. The tissue samples were preserved using 4% paraformaldehyde fixation and subsequently embedded in paraffin. Afterwards, the paraffin-embedded tissues were sectioned into 5 μm-thick slices, subjected to H&E staining, and finally observed under a light microscope and photographed for further analysis.

Western blot

The gastric body tissues of rats were subjected to treatment with RIPA lysis buffer, followed by a 30-minute incubation on ice for tissue lysis. After centrifugation, the resulting supernatant was collected at 4 ℃ for 10 min. The samples were subjected to the SDS-PAGE gel (10 %) electrophoresis for 2 h, and then transferred to PVDF membrane (Thermo, The United States) using wet method. Membrane blocking was performed using the Tris-buffered saline (containing 5 % BSA) for 2 h at 37 ℃. The primary antibodies (Abcam, The United Kingdom) Anti-PGP9.5 and anti-Synapsin I (1:1000) were incubated overnight at 4 ℃. The secondary antibody (Abcam, The United Kingdom) (1:2000) was incubated at a temperature of 37 ℃ for 1 h. PVDF membrane was first subjected to a reaction with Enhanced Chemiluminescence (ECL) reagents (Protech, The United States), and then exposure imaging.

Immunohistochemistry

The paraffin-embedded gastric tissues were initially blocked with 5 % bovine serum, and then incubated with the primary antibody, anti-PGP9.5 (1:500), at a temperature of 37 ℃ for 1 h, followed by incubation with the secondary antibody at the same temperature for 1 h. Following staining with DAB kit (Boster，China), the tissue sections were dehydrated with gradient ethanol, made transparent with xylene, sealed, and finally examined under an optical microscope.

Immunofluorescence

The tissue sections were blocked as described above, and then subjected to an incubation with the primary antibody, anti-Synapsin I (1:500), at 37 ℃ for 1 h, followed by the addition of FITC-labelled secondary antibody at 37 ℃ for 1 h. Afterwards, the tissue sections underwent three washes with PBS, followed by imaging using a fluorescence microscope.

Statistical analysis

The data obtained in this study were processed using SPSS 10.0, and presented as mean ± standard deviation (SD). Group comparisons were conducted using Student's *t*-test, and $p < 0.05$ was considered statistically significant.

RESULTS

Effect of *Rhodiola rosea* L. on blood glucose and body weight of rats

In comparison to the normal group, the rats in the DGP group were revealed to have significant weight loss and elevated levels of blood glucose ($p < 0.05$). In DGP+*Rhodiola rosea* L. group, the rats that were administered with *Rhodiola rosea* L. solution, showed weight gain and decreased levels of blood glucose when compared with rats in t DGP group ($p < 0.05$). The results are shown in Figure 1.

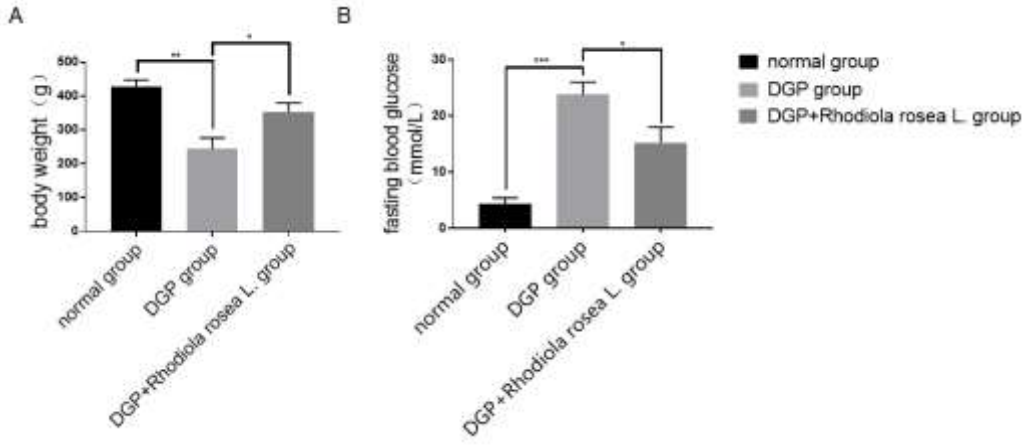

Figure 1: Effect of *Rhodiola rosea* L. on blood glucose level and body weight of DGP rats. *P < 0.05, **p < 0.01, ***p < 0.001

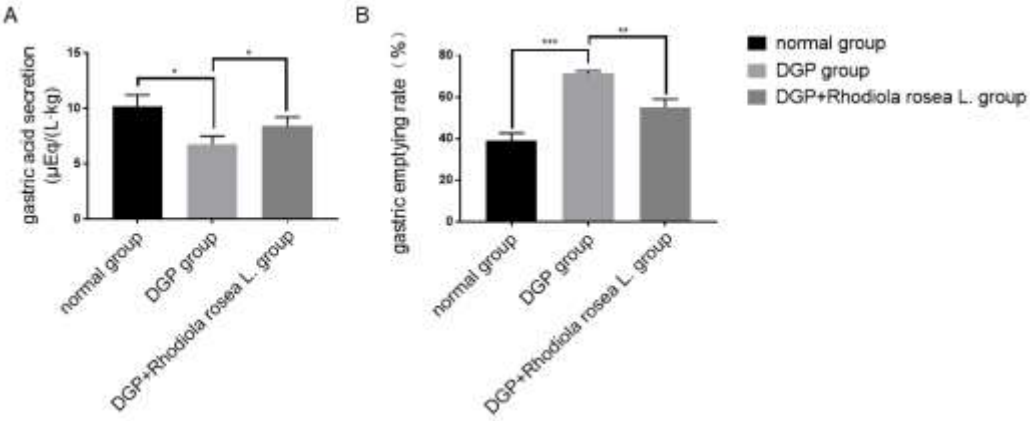

Figure 2: Effect of *Rhodiola rosea* L. on gastric acid secretion and gastric emptying rate of rats. A. Gastric acid secretion. B. Gastric emptying rate. *P < 0.05, **p < 0.01, ***p < 0.001

Effect of *Rhodiola rosea* L. on gastric acid secretion and gastric emptying rate of rats

The rats in the DGP group exhibited lower levels of gastric acid secretion, deficient gastric motility, and significantly delayed gastric emptying in contrast with normal controls (p < 0.05). In comparison with the rats in DGP group, those in the DGP+*Rhodiola rosea* L. group showed elevated levels of gastric acid secretion and decreased gastric emptying rate (p< 0.05). The results are shown in Figure 2.

Effect of *Rhodiola rosea* L. on the gastric histopathological status of rats

For rats in the normal group, the glandular cells and smooth muscle cells within the gastric mucosa were regularly and closely arranged, with moderate intercellular spaces, and no vacuolar degeneration or vasodilation (Figure 3). Conversely, for rats in the DGP group, the glands showed irregular alignment, along with enlarged intercellular spaces, and the gland cells had reduced cytoplasm, light-colored staining, mucosal vacuolar degeneration, and submucosal hyperemia. Furthermore, there was an observed vasodilation, and the cytoplasm of the smooth muscle cells within the gastric wall were light-stained, along with noticeable vacuolar degeneration. In comparison to the DGP group, the DGP+*Rhodiola rosea* L. group showed relieved mucosal and submucosal hyperemia, well-arranged mucosal gland cells and smooth muscle cells with reduced vacuolar degeneration, enlarged intercellular space, and decreased vacuolar degeneration in the stomach wall.

Effect of *Rhodiola rosea* L. on the neurons of rat gastric tissues

Figure 4 shows that, in contrast with the normal group, the protein expressions of the total neuronal marker PGP9.5 was significantly reduced. Following administration of *Rhodiola rosea* L. extract through gavage, there was an

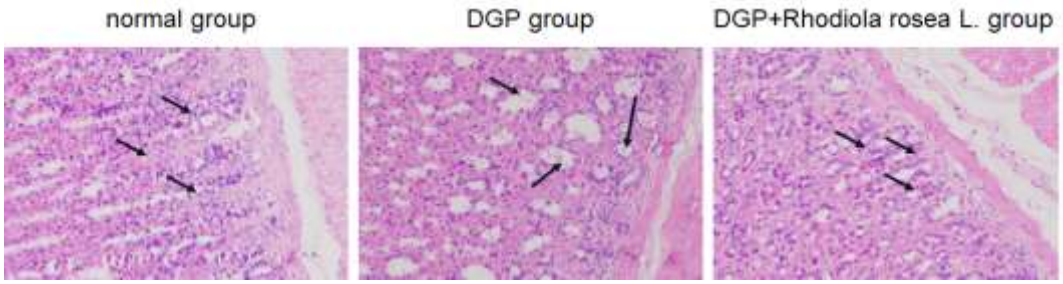

Figure 3: Effect of *Rhodiola rosea* L. on gastric histopathological status of rats

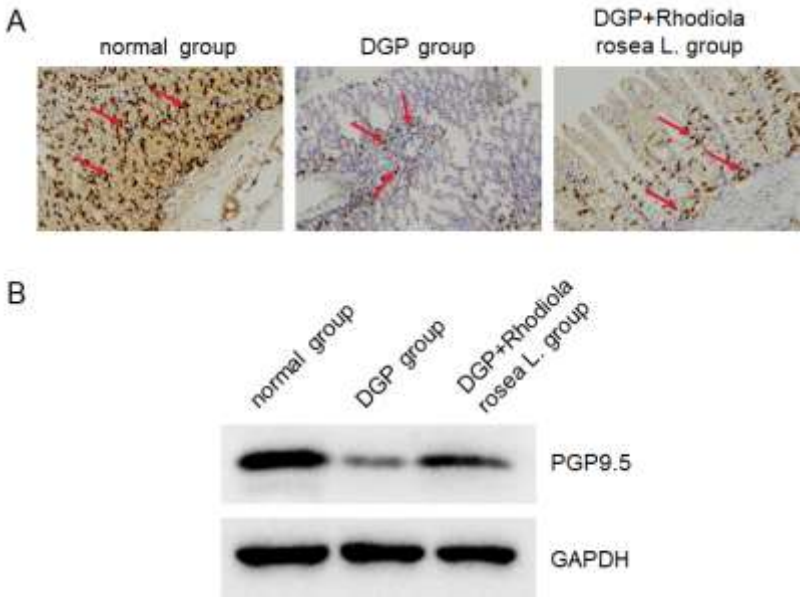

Figure 4: Effect of *Rhodiola rosea* L. on the neurons in the gastric tissues of rats. A. Immunohistochemistry for PGP9.5. B. Western blot for PGP9.5

increase in the intestinal neurons of the DGP rats. The PGP9.5 expression detected by western blot were consistent with that from immunohistochemistry.

Effect of *Rhodiola rosea* L. on Synapsin-I expression in rat gastric tissues

The expressions of Synapsin-I in rat gastric tissues detected by western blot and immunofluorescence are shown in Figure 5. In comparison to the normal controls, the DGP rats showed significantly reduced levels of Synapsin-I expression, whereas there were increased protein expression levels of Synapsin-I for rats in the DGP+*Rhodiola rosea* L. group as compared with the DGP group.

DISCUSSION

DGP is often accompanied by autonomic neuropathy [9]. Currently, the therapeutic methods for DGP are far from ideal, as they mainly rely on the use of drugs for gastric emptying, and the effect is weakened after long-term use. In this study, compared with the normal controls, the DGP rats had significantly decreased body weight, elevated levels of blood glucose, reduced levels of gastric acid secretion, increased rgastric emptying rate, irregularly aligned gastric mucosal glands and enlarged intercellular space.

ENS lesions are gastrointestinal dysfunction of DGP caused by decreased function of synaptic transmission. This study investigated the protein closely associated with synaptic plasticity and protein impact on the synaptic plasticity in ENS in diabetic neuropathy. Synaptic vesicles formed by Synapsin-I with other isoforms such as synapsin-II and synapsin-III have found to be crucial in synaptogenesis and neurotransmission, axon formation and synaptic plasticity in the central and peripheral nervous systems [10]. Previous studies were mostly limited to cerebral ischemia-reperfusion, vascular dementia, depression, and other areas of neural differentiation [11]. Synapsin-I has also been shown to be abnormally expressed and down

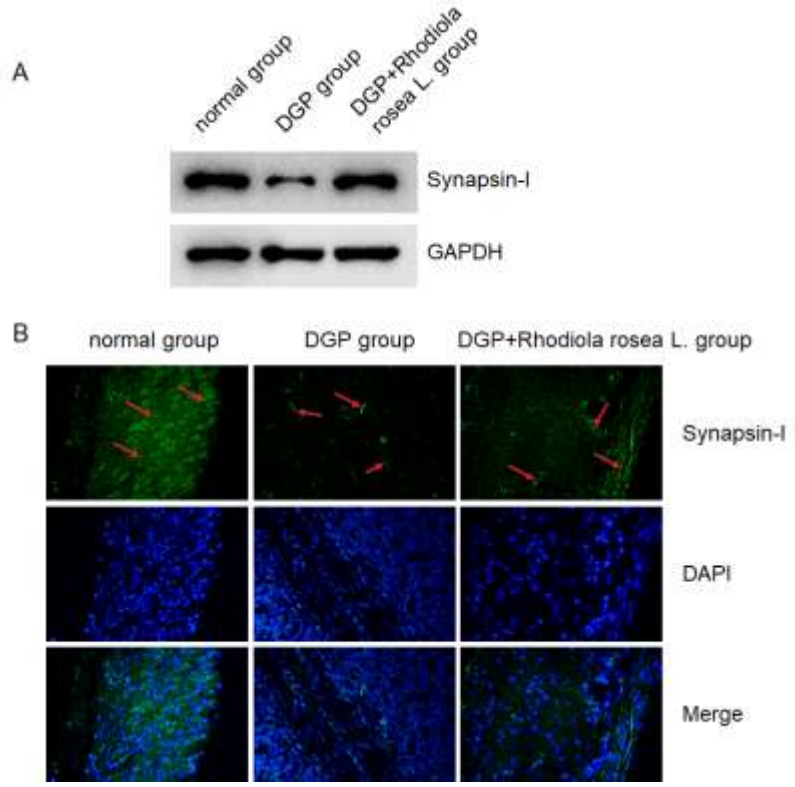

Figure 5: Effect of *Rhodiola rosea* L. on the expression of Synapsin-I in the gastric tissues of rats. A. Western blot for Synapsin-I. B. Immunofluorescence for Synapsin-I.

regulated in other complications of diabetes such as diabetic retinopathy and diabetic encephalopathy [12,13].

PGP9.5 staining is the most widely used technique in assessing the neurosome and nerve fibers in the gastric wall in DM. Some studies have shown that the nerve fibers in the longitudinal or circular muscle layers is significantly reduced in the gastric wall sections of patients with poorly-controlled DM [14]. Some researchers reported delayed gastric emptying in the established mouse model of ENS absorption, which may be directly associated with the loss of neurons [15]. Li *et al*. found severe neuronal damage, such as axonal depletion, mitochondrial swelling, and significantly reduced expression of PGP9.5 in the muscle layer of mice 12 weeks after the DM rat model was established [16]. High glucose-induced apoptosis, advanced glycosylation end products, and inflammatory responses induced by the interaction of neuropeptides with immune cells in ENS can lead to neuronal loss and gastrointestinal motility disorders [17]. In this study, compared with the normal controls, decreased expression of Synapsin-I and PGP9.5 was found in the gastric tissues of DGP rats.

Salidroside protects mice from diabetes-induced oxidative stress, and has hypoglycemic activity. Salidroside also significantly reduces the level of fasting blood glucose, the contents of triglyceride and total cholesterol [18]. Zhang *et al* reported that salidroside inhibited the c-Jun N-terminal kinase and p38 mitogen-activated protein kinase phosphorylation induced by amyloid beta peptide (25-35) in human neuroblastoma cells, thereby protecting against oxidative stress in the nerves [19]. Furthermore, some scholars have demonstrated that taxosin can remove intracellular reactive oxygen species and free radicals, improve damaged hippocampal neurons, and protect rat neural stem cells in an Alzheimer's disease rat model [20].

The foregoing studies indicate that salidroside can be used as a versatile agent to prevent or treat neuronal damage in patients with neurodegeneration. In the present study, *Rhodiola rosea* L. effectively improved the symptoms of gastroparesis in rats, mitigated the decrease in gastric acid secretion and body weight, increased the levels of blood glucose, and improved delayed gastric emptying, etc. At the same time, the expressions of synapsin-I and PGP9.5 in gastric tissues were elevated after intervention using *Rhodiola rosea* L.

CONCLUSION

The findings of this study indicate that *Rhodiola rosea* L. could relieve DGP-induced symptoms in rats and may have potential benefits for improving diabetic neuropathy in humans by enhancing synaptic plasticity through the upregulation of synapsin-I expression. However, clinical studies are required to validate findings.

DECLARATIONS

Acknowledgements

None provided.

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Ethical approval

This study was approved by The Institutional Animal Care and Use Committee of Zhejiang Centre of Laboratory Animals (approval no. ZJCLA-IACUC-20030065).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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REFERENCES

1. *Li. X, Zhang. S, Zhang. G, Yuan. J, Rong. S, Wang. M, Wu. J, Feng. X, He. H, Tian. L, et al. 3-Cinnamoyl-4-hydroxy-6-methyl-2H-pyran-2-one ameliorates diabetic peripheral neuropathy in type 2 diabetes mellitus rats via PI3K/Akt signaling pathway. Trop J Pharm Res. 2019; 18(12): 2509-2514.*
2. *Albarazanji K, Nawrocki AR, Gao B, Wang X, Wang YJ, Xiao YF. Effects of mixed meal tolerance test on gastric emptying, glucose and lipid homeostasis in obese nonhuman primates. Sci Rep. 2021; 11(1): 11866.*
3. *Song GQ, Chen JD. Synchronized gastric electrical stimulation improves delayed gastric emptying in nonobese mice with diabetic gastroparesis. J Appl Physiol (1985). 2007; 103(5): 1560-1564.*
4. *Maiole F, Tedeschi G, Candiani S, Maragliano L, Benfenati F, Zullo L. Synapsins are expressed at neuronal and non-neuronal locations in Octopus vulgaris. Sci Rep. 2019; 9(1): 15430.*
5. *Hernandez-Hernandez O, Guiraud-Dogan C, Sicot G, Huguet A, Luilier S, Steidl E, Saenger S, Marciniak E, Obriot H, Chevarin C, et al. Myotonic dystrophy CTG expansion affects synaptic vesicle proteins, neurotransmission and mouse behaviour. Brain. 2013; 136(Pt 3): 957-970.*
6. *Zhang M, Zhu P, Wang Y, Wu J, Yu Y, Wu X, Liu X, Gu Y. Bilateral sympathetic stellate ganglionectomy attenuates myocardial remodelling and fibrosis in a rat model of chronic volume overload. J Cell Mol Med. 2019; 23(2): 1001-1013.*
7. *Ding XJ, Zhang ZY, Jin J, Han JX, Wang Y, Yang K, Yang YY, Wang HQ, Dai XT, Yao C, et al. Salidroside can target both P4HB-mediated inflammation and melanogenesis of the skin. Theranostics. 2020; 10(24): 11110-11126.*
8. *Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. BMJ Open Sci. 2020;4(1):e100115.*
9. *Xia T, Yang Y, Li W, Tang Z, Li Z, Guo Y. Acupoint catgut embedding for diabetic gastroparesis: A protocol of systematic review. Medicine (Baltimore). 2019; 98(43): e17718.*
10. *Xiong J, Duan H, Chen S, Kessi M, He F, Deng X, Zhang C, Yang L, Peng J, Yin F. Familial SYN1 variants related neurodevelopmental disorders in Asian pediatric patients. BMC Med Genomics. 2021; 14(1): 182.*
11. *Xu L, Jia L, Wang Q, Hou J, Li S, Teng J. Salidroside attenuates hypoxia/reoxygenation-induced human brain vascular smooth muscle cell injury by activating the SIRT1/FOXO3alpha pathway. Exp Ther Med. 2018; 15(1): 822-830.*

12. VanGuilder HD, Brucklacher RM, Patel K, Ellis RW, Freeman WM, Barber AJ. Diabetes downregulates presynaptic proteins and reduces basal synapsin I phosphorylation in rat retina. Eur J Neurosci. 2008; 28(1): 1-11.
13. Liu Y, Fu X, Lan N, Li S, Zhang J, Wang S, Li C, Shang Y, Huang T, Zhang L. Luteolin protects against high fat diet-induced cognitive deficits in obesity mice. Behav Brain Res. 2014; 267: 178-188.
14. Pasricha PJ, Pehlivanov ND, Gomez G, Vittal H, Lurken MS, Farrugia G. Changes in the gastric enteric nervous system and muscle: a case report on two patients with diabetic gastroparesis. BMC Gastroenterol. 2008; 8: 21.
15. Baker C, Ahmed M, Cheng K, Arciero E, Bhave S, Ho WLN, Goldstein AM, Hotta R. Hypoganglionosis in the gastric antrum causes delayed gastric emptying. Neurogastroenterol Motil. 2020; 32(5): e13766.
16. Grover M, Bernard CE, Pasricha PJ, Lurken MS, Faussone-Pellegrini MS, Smyrk TC, Parkman HP, Abell TL, Snape WJ, Hasler WL, et al. Clinical-histological associations in gastroparesis: results from the Gastroparesis Clinical Research Consortium. Neurogastroenterol Motil. 2012; 24(6): 531-539, e249.
17. Bharucha AE, Kudva YC, Prichard DO. Diabetic Gastroparesis. Endocr Rev. 2019; 40(5): 1318-1352.
18. Zheng T, Bian F, Chen L, Wang Q, Jin S. Beneficial Effects of Rhodiola and Salidroside in Diabetes: Potential Role of AMP-Activated Protein Kinase. Mol Diagn Ther. 2019; 23(4): 489-498.
19. Zhang L, Yu H, Zhao X, Lin X, Tan C, Cao G, Wang Z. Neuroprotective effects of salidroside against beta-amyloid-induced oxidative stress in SH-SY5Y human neuroblastoma cells. Neurochem Int. 2010; 57(5): 547-555.
20. Qu ZQ, Zhou Y, Zeng YS, Lin YK, Li Y, Zhong ZQ, Chan WY. Protective effects of a Rhodiola crenulata extract and salidroside on hippocampal neurogenesis against streptozotocin-induced neural injury in the rat. PLoS One. 2012; 7(1): e29641.