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Neuroprotective effect of Liuwei Dihuang decoction on cognition deficits of diabetic encephalopathy in streptozotocin-induced diabetic rat

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ABSTRACT

Ethnopharmacological relevance: Liuwei Dihuang decoction (LWDHD) is a well-known prescription of traditional Chinese medicine (TCM) and consists of six crude drugs including *Rehmannia glutinosa* Libosch. (family: *Scrophulariaceae*), *Cornus officinalis* Sieb. (family: *Cornaceae*), *Dioscorea oppositifolia* L. (family: *Dioscoreaceae*), *Paoenia ostii* (family: *Paeoniaceae*), *Alisma orientale* (G. Samuelsson) Juz (family: *Alismataceae*) and *Poria cocos* (Schw.) Wolf (family: *Polyporaceae*). It has been used for the treatment of "Kidney-Yin" deficiency syndrome in clinic in China for a long time. Recent studies found that LWDHD had a potential benefit for the treatment of diabetic complications. The aim of the present study is to investigate the neuroprotective effect of LWDHD on memory and cognition deficits in streptozotocin (STZ)-induced diabetic encephalopathy (DE) rats.

Materials and methods: Adult male Sprague Dawley (SD) rats were fed with high-glucose–fat diet for 50 days and then received an intraperitoneal injection of STZ (40 mg/kg) to induce DE model. Morris water maze test was used to evaluate the memory and cognition capability of DE rats. Choline acetyltransferase (ChAT), acetylcholinesterase (AChE), Na^+–K^+-ATP enzyme, iNOS and GSH kits were used to determine their activities or content in hippocampus. TUNEL staining, immunohistochemistry and Congo red staining were conducted to evaluate the apoptosis, caspase-3 protein expression, insulin-like growth factors 1 (IGF-1) and brain derived neurophic factor (BDNF) expressions, as well as Aβ deposition.

Results: The treatment with LWDHD (1 and 2 g/kg, p.o., once daily, 30 days) could significantly reduce the escape latency time and path length, and obviously enhance the spent time in the target quadrant and platform crossings in Morris water maze test compared with model group ($P<0.05$, $P<0.01$). LWDHD could also significantly decrease the level of fasting blood glucose, increase Na^+–K^+-ATP enzyme and ChAT activities, enhance remarkedly GSH level while decrease significantly AChE and iNOS activities in hippocampus ($P<0.05$, $P<0.01$). Furthermore, TUNEL staining, Congo red staining and immunohistochemistry showed that LWDHD significantly improved the expressions of IGF-1 and BDNF, attenuated the neural apoptosis, overexpression of caspase-3 and Aβ deposition in the hippocampus and cerebral cortex of STZ-induced DE rats ($P<0.01$).

Conclusion: Our findings suggested that LWDHD had a neuroprotective effect on DE rats. LWDHD may be of benefit in the treatment of DE.

Abbreviations: DE, diabetic encephalopathy; DM, diabetes mellitus; TCM, traditional Chinese medicine; LWDHD, Liuwei Dihuang decoction; STZ, streptozotocin; AChE, acetylcholinesterase; ChAT, choline acetyltransferase; AD, Alzheimer's disease; IGF-1, insulin-like growth factors 1; BDNF, brain derived neurophic factor; Aβ, amyloid beta; SD, Sprague Dawley; Na^+–K^+-ATPase, Na^+–K^+-adenosine triphosphatase; GSH, glutathione; ROS, reactive oxygen species; OGD, oxygen–glucose deprivation; NO, nitric oxide; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling assay; DAB, diaminobenzidine; ANOVA, one-way analysis of variance.

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1. Introduction

Diabetic encephalopathy (DE), one of the most common diabetic complications, is strongly correlated with the degeneration and dysfunction of the central nervous system (Northam et al., 2009; Knopman et al., 2011). There are many factors contributing to the pathogenesis of DE. Experimental and epidemiological studies suggest that chronic hyperglycemia is a major trigger factor for the initiation and development of DE (Cameron et al., 2001; Biessels et al., 2002). There is a growing body of evidence that the glycation-toxicity of glucose and lipids or/and proteins elicits the overgeneration of reactive oxygen species (ROS) and nitric oxide (NO) in the neurons (Nishikawa et al., 2000; Fukudome et al., 2008). The excess formation of ROS and inducible NO synthase can damage cellular proteins, lipids or DNA and then inhibit their normal functions and disturb homeostatics within the neuron, and ultimately result in cell death (Paradies et al., 2011). Additionally, glutathione peroxidase and Na^+–K^+-ATP enzyme activities were reduced in hippocampus of patients with cognition deficits (Mastrocola et al., 2005).

Liuwei Dihuang decoction (LWDHD), a traditional Chinese medicinal prescription, is first recorded in "Xiao er Yao Zheng Zhi Jue", and consists of six chinese crude drugs including *Rehmannia glutinosa* Libosch. (family: *Scrophulariaceae*), *Cornus officinalis* Sieb. (family: *Cornaceae*), *Dioscorea oppositifolia* L. (family: *Dioscoreaceae*), *Paoenia ostii* (family: *Paeoniaceae*), *Alisma orientale* (G. Samuelsson) Juz (family: *Alismataceae*) and *Poria cocos* (Schw.) Wolf (family: *Polyporaceae*). with a dose proportion of 8:4:4:3:3:3 (Wu et al., 2007; Wang et al., 2010; Huang et al., 2012). It has long been utilized clinically in the treatment of diabetic mellitus and its complications, such as DE. Recent studies have revealed that LWDHD processed multiple pharmacological activities such as modulating immune function and cognitive enhancement effect (Chen et al., 2001; Zhou, 2004). However, the underlying activity of LWDHD on protecting cognitive impairment and neurotoxicity in DE has been not fully understood. In this study, we investigated whether LWDHD could ameliorate cognition deficits of STZ-induced DE rat and explore the possible underlying mechanisms.

2. Materials and methods

2.1. Materials

The six individual medicinal materials of LWDHD, including the root of *Rehmannia glutinosa* Libosch., the fruit of *Cornus officinalis* Sieb., the tubers of *Dioscorea oppositifolia* L., the rhizomes of *Alisma orientale* (G. Samuelsson) Juz, the dried sclerotia of *Poria cocos* (Schw.) Wolf. and the root cortex of *Paoenia ostii*, were purchased from Jiangsu Medicine Company (Nangjing, China) and authenticated by Prof. Min-Jian Qin (Department of Medicinal Plants, China Pharmaceutical University, Nanjing, China). The voucher specimens, whose voucher numbers were CPU-LDW-RR-120315, CPU-LDW-FC-120315, CPU-LDW-RD-120315, CPU-LDW-RA-120315, CPU-LDW-CP-120315 and CPU-LDW-P-120315, were deposited in Department of Natural Medicinal Chemistry, China Pharmaceutical University. Reference substances gallic acid, morroniside, loganin, paeoniflorin and paeonol were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purity of each compound was analyzed to be higher than 98% by HPLC. Streptozotocin was obtained from Sigma (St. Louis, MO, USA). Rosiglitazone tablets and donepezil hydrochloride tablets were purchased from Chengdu HengRui Pharmaceutical Co., Ltd.(Chengdu, China); Congo red staining kits, GSH, Na^+–K^+-ATP, iNOS, ChAT, AChE and blood glucose kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China); Rabbit anti-BDNF (sc-20981), IGF-1 (sc-9013), caspase-3 (sc-8304) antibodies were purchased from Santa Cruz Biotechnology, Inc. (California, USA); Kit for terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) was purchased from Roche (Roche Diagnostics, Mannheim, Germany). The other reagents are of AR grade.

2.2. Preparation of herbal extracts

The six dried medicinal materials (2.5 kg) were mixed according to the weight ratio of 8:4:4:3:3:3 as described previously (Hsieh et al., 2003; Wang et al., 2010) and performed with a reflux extraction in 75% ethanol (v/v) for two times (2 h/time). After concentration, the yield of LWDHD was 21.8%. Then LWDHD was dissolved in distilled water solution for use.

2.3. HPLC analysis

The multi-components of the LWDHD were characterized by Agilent 1200 system equiped with an photodiode array detector (DAD), an quaternary pump, an autosampler, a column heater–cooler and an Agilent chemstation software. The samples were analyzed using Alltima C18 (150 × 4.6 mm, 5 μm) with the detector wavelength 242 nm, and the mobile phase consisted of CH3CN (A) and 0.1% (v/v) formic acid (B). A gradient program was performed as follows: 0–20 min, 5–10% A; 20–40 min, 10–15% A; 40–55 min, 15–15% A; 55–62 min, 15–20% A; 62–85 min, 20–35% A; 85–100 min, 35–45% A; 100–120 min, 45–90% A. The flow rate was 0.5 mL/min. The column temperature was kept at 25 °C.

2.4. Animals and drug administration

Adult male Sprague Dawley (SD) rats weighing 220–250 g (n=70) were purchased from the Experimental Animal Center in Jiangsu Province (Nanjing, China). Rats were kept in cages and maintained under standard housing conditions (room temperature 25 ± 1 °C and humidity 60–65%) with a 12-h light/12-h dark cycle. Food and tap water were available ad libitum. Rats were fasted 1 h prior to the administration of drugs. The rats were given a high-glucose–fat diet (formula: 15% lard, 10% sucrose, 3% cholesterol, 3% sodium chloride, 69% standard rat feed) for 50 days and then administered by a single intraperitoneal injection of STZ (40 mg/kg) (61 rats for the treatment of high-glucose–fat diet and STZ). After being injected for 7 days, the rats were fasted 12 h. The rats fed with standard feed (no-high-glucose–fat) were used for normal group (n=9). The blood samples were taken from the tail vein to evaluate the glycemia in serum using GOD-POD glucose estimation kit. Diabetic rats were characterized by glycemia indexes higher than 250 mg/dL (9 rats for each group). The diabetic rats were randomly divided into model, LWDHD-L (1 g/kg) and LWDHD-H (2 g/kg) (Wu et al., 2007) and positive control groups. Cholinesterase inhibitor donepezil (3 mg/kg) and hypoglycemic drugs rosiglitazone (5 mg/kg) were chosen for the positive control. The DE rats were administered with drug via oral gavage daily for one month from 10th day after STZ injection. The animal experiments were approved by the Science and Technology Department of Jiangsu Province and followed with the Provision and General Recommendation of Chinese Experimental Animals Administration Legislation.

2.5. Morris water maze test

After administration for 30 days, spatial memory ability was detected by Morris water maze test (Lee et al., 2010). The Morris water maze was carried out in a black circular pool (diameter: 180 cm; height: 55 cm). The pool was filled with a depth of 30 cm