Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(6):863-866

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Effect of aqueous extracts from *Cyclocarya paliurus* leaves on peripheral neuropathy in rats with type 2 diabetes mellitus

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ABSTRACT

The leaves of Cyclocarya paliurus (Batal.) Iljinsk (CP) have long been used in traditional Chinese medicine for the treatment of diabetic mellitus (DM). The aim of this study was to investigate the effect of aqueous extracts from CP leaves (ACP) on sciatic nerve conduction velocity (NCV) and several serum indicators in rats with type 2 diabetes. Male Wistar rats were used for inducing the DM model, the variation of body weight, blood glucose and general status showed the success of modeling. After treating by ACP, the sciatic NCV and contents of serum NGF-β , TNF-α and NO were ameliorated significantly ($P < 0.05$) compared with DM model. This research demonstrates that ACP offers potential for intervening diabetic peripheral neuropathy.

Keywords: *Cyclocarya paliurus*, nerve conduction velocity, NGF-*β*, TNF-*α*

INTRODUCTION

Diabetes mellitus (DM) is one of the most multiplex and common disease in clinical practice. Diabetic peripheral neuropathy (DPN) is the most commonly reported long term diabetic complication, affecting up to 50% of type 2 DM patients [1]. One prominent symptom of the disease is nerve conduction velocity (NCV) deficits especially longer nerve fibers [2]. The pathogenesis of DPN is complex and it is believed to be a multifactorial pathology involving hyperglycemia, oxidative stress, dyslipidemia and the polyol pathway, hexosamine pathway, accumulation of advanced glycation end products, loss of calcium homeostasis, et al [3-5]. A number of neuropoietic cytokines including interleukin-1 (IL-1), interleukin-6 (IL-6), ciliary neurotrophic factors(CNTF), tumor necrosis factor alpha (TNF-*α*), transforming growth factor beta 1 (TGF-*β*1) exhibit pleiotropic effects on glia cells and neurons and show importance for the homeostasis of the peripheral, central and autonomic nervous systems[6-8].

Cyclocarya paliurus (Batal.) Iljinsk (abbreviated as CP), commonly known as ‘sweet tea tree’, is a medicinal herb, which has been widely used in China as drug formulation in traditional Chinese medicine for the treatment of DM. In addition, CP antihyperglycemic herbal tea has been approved by the United States Food and Drug Administration (FDA), which was the first health tea from China certificated by FDA [9]. Many studies have demonstrated that CP possesses a variety of bioactivities, including antihypertensive, hypoglycemic, hypolipidemic, and antioxidant activity [10, 11].

In the present study, we investigated the effect of aqueous extracts from CP leaves (ACP) on sciatic NCV and several serum indicators in rats with type 2 diabetes, in the hope of finding their potential prevention of DNP.

EXPERIMENTAL SECTION

Chemicals and Instruments

STZ were purchased from Sigma-Aldrich (St. Louis. Mo., USA), Rat TNF-α ELISA Kit (R131029-12b) and Rat

NGF-β ELISA Kit (1909871108) were purchased from NEO Bioscience technology, China. Nitric oxide (NO) assay kit was purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China. All other chemicals and reagents used were of analytical grade.

Plant material and preparation of ACP

CP leaves were collected in Zhangjiajie, Hunan province, China and authenticated by Dr. Chongmei Xu from the Department of Pharmacognosy of Weifang Medical University. A voucher specimen (No. 20131102) was deposited in the herbarium of the university. The ACP was obtained by water extraction in previous study and the contents of polysaccharide and total polyphenol were (479.3 ± 19.8) mg/g and (38.3 ± 2.3) mg/g, respectively. The extracts were dissolved in ultra pure water before experiments.

Animal treatments

Male Wistar albino rats weighing between 180-220 g were provided by Weifang Medical Experimental Animal Center and bred in standard animal facility. All experiments on animals were conducted in accordance with and after approval by the Institution Animal Ethics Committees of Weifang Medical University. The animals were kept in controlled conditions of temperature (20-24 °C), relative humidity (60-70%) and 12/12 h light/dark cycle and fed with standard pellet diet and water *ad libitum*. Ten rats were selected randomly as a control group (group 1). The remaining rats were fed with high fat diet (HFD). After six weeks, the rats were injected intraperitoneally with a dose of 35 mg/kg streptozotocin (STZ) in citrate buffer (pH=4.5) and the rats in control group were injected with the same dose of citrate buffer. Blood was drawn from the tail vein after 72h to measure blood glucose levels. Animals with blood glucose under 10 mM were fasting and injected once again. Five weeks later, the animals with blood glucose >11.1 mM were selected and divided into three groups according to their blood glucose gradient, each as follows: Group 2 (n=20, DM model); Group 3 and 4 were DM rats treated with ACP: group 3 (n=12, 0.047 g/kg/day); group 4 (n=12, 0.094 g/kg/day). Each group was administered by gavage once a day for 8 weeks. Rats in group 1 and 2 were treated with an equal volume of distilled water.

General status of the rats

General status and food intake were observed every day, in addition, the values of body weight and blood glucose were determined regularly.

Blood sampling and biochemical analysis

Five weeks after the DM model was induced (the time before drug administration), five animals from group 2 was selected randomly and anesthetized by 10% chloral hydrate (0.3 mL/100 g) after overnight fasting. With the subjects prone, right sciatic nerve was dissected with a glass needle, and then the nerve conduction was made and recorded through an YSD-4GA Physiological and Pharmacological experiments more LAU (Huaibei Zhenghua Biologic Apparatus Facilities Co. Ltd., Anhui, China.). NCV was calculated using the following equations: NCV: s/t (m/s) = distance between stimulating and recording electrode / latency [12]. Then the blood samples from heart were collected and centrifuged (l7465 g × 10 min), the serum was separated and stored at -80°C until analysis. At the end of the treatment, all rats were anesthetized based on the method above, and blood samples were collected and NCV was detected. The serum NGF-*β* and TNF-*α* activity were determined by commercial ELISA kits using a BioTek microplate reader, Gene Co., Ltd. USA.

Statistical analysis

The experimental results were subjected to variance analysis using SPSS 16.0 and expressed as mean ± SD.

RESULTS

General features of experimental rats

Compared with the normal control, DM rats had tarnished fur and anabrotic tail with symptoms i.e. polyphagia, polydipsia, polyuria, thickened urine smell and other signs of DM. Treatment of ACP improved these general features. Blood glucose(BG) levels were similar in the four groups before the experiment, whereas the animals fed with HFD and injected with STZ developed high levels of BG. BG levels of animals in DM group didn't change much since molding and no significant differences were recorded throughout the administration period; however BG levels decreased significantly ($P < 0.05$) in treated groups. The value of body weights were decreased after a period of increase causing by the abundant nutrition of HFD. Drug administration groups prevented the body weight loss significantly. During the experiment period, rats in normal control group all survived however four animals died from intubations problems during drug administration, including one from group 2, two of them from group 3 and another one from group 4. Two animals died from the development of the disease. The animals were necropsied immediately after death, and no abnormality was observed in gross examination (Table 1).

Table 1 Effect of ACP on body weight, blood glucose and mortality. (mean ± SD)

	group	0 week	6w[a]	12w[b]	16w	20w
n	1	10	10	10	10	10
	2	20	20	14	14	13
	3	12	12	10	10	9
	4	12	12	11	11	11
BW (g)	1	200±20	268±25	328±39	367±46	412±50
	2	200±20	438±36*	476±66*	492±59*	466±62*
	3	200±20	453±43	485±61	526±68	548±68#
	4	200±20	417±39	471±47	517±58	560±66#
BG (mM)	1	3.3±0.5	3.4±0.4	3.2±0.7	3.6±0.5	3.8±0.6
	2	3.3±0.5	17.6±4.1*	15.9±3.8*	21.2±4.3*	21.1±5.0*
	3	3.3±0.5	17.1±3.7	14.6±4.1	13.8±2.9#	8.2±1.9#
	4	3.3±0.5	17.3±4.0	15.0±3.6	10.1±2.2#	4.4±0.9#

** P < 0.05 compared with the normal control group; # P < 0.05 compared with the DM group. a, when STZ was injected; b, when gavage was conducted.*

Type 2 DM is a complex and heterogeneous disorder characterized by a persistent hyperglycemia. In the present study, we successfully induced DM rat models by feeding them HFD together with a twice injection of low dose STZ. This approach gained a success rate over 90% and would contribute to the efficient utilize of experiment animals. To assess the developments of DM, mental activity, fur condition, water intake, food intake and survival of the rats were observed every day. Body weight and blood glucose of the rats were determined every two weeks. Serum indicators and NCV was detected in two stage of DM, when before and after the treatment respectively. After 12 weeks feeding of HFD and 6 weeks after the injection of STZ, early functional abnormalities in the sciatic nerves were observed, including reduction in NCV and altered serum indicators.

Measurement of serum indicators

As shown in Table 2, the increased serum levels of NGF-*β* and TNF-*α* were found to be significant ($P < 0.05$) in DM rats. Contents of NO were found to be increased significantly ($P < 0.05$) at first few weeks, whereas decreased significantly ($P < 0.05$) about two months later. These serum indicators were ameliorated in ACP treatment groups versus DM model group.

Table 2 Effect of ACP on serum indicators in DM rats (mean ± S.D.)

group	1	2	3	4
n	10	13	9	11
NGF-β (ng/ml)	2.1±0.7	12.7±1.6*	10.6±1.4	8.5±1.1*
TNF-α (pg/ml)	20.0±1.5	38.3±3.1*	33.3±2.8*	28.1±3.5*
NO (μmmol/L)	23.1±1.9	13.4±2.5*	15.6±3.2	18.7±2.4*

** P < 0.05 compared with the normal control group; *P < 0.05 compared with the DM group.*

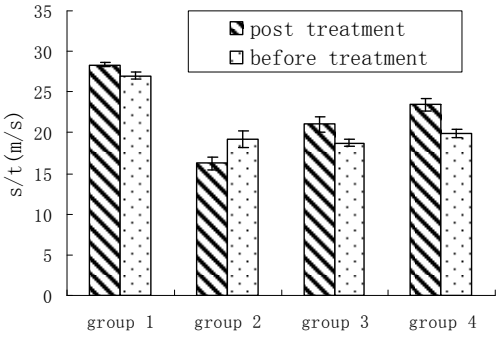

Fig 1 Effects of ACP on sciatic NCV in DM rats (mean ± S.D.)

It has been proven that hyperglycaemia and OS plays an important role in the pathogenesis of DM chronic complications [13, 14]. Meanwhile, hyperglycemia and OS have also been implicated as critical factors in the development of DPN. DPN develops as a result of hyperglycemia induced metabolic and microvascular changes. Insufficient function of peripheral nerve is manifested as slower NCV and disordered biomarker in serum including NGF-β, TNF-α and NO contents. In the present study, a typical characteristic of hyperglycemia was observed in the DM model group and we measured the serum levels of TNF-α, NGF-β and NO. Mean values of serum TNF-α and NGF-β level were found significantly increased in DM group. This result was consistent with previous studies and manifested the key roles of serum TNF-α and NGF-β level in DPN.

Effects of ACP on sciatic NCV in DN rats

The results are shown in Fig 1. Influenced by the diabetic, the NCV of DM rats in group 2 was much slowed compared with the normal subjects in group 1 ($P < 0.05$). However, the groups treated by ACP were notably improved than the DM group.

NCV studies can be used to quantify the nerve injury in DPN. In our study, ACP was found to reverse the deficiency of NCV significantly. Furthermore the results showed that the levels of TNF-α and NGF-β in different groups were correlated with NCV which shows significant negative correlation with NCV.

CONCLUSION

In summary, our findings suggested that ACP could significantly reduce serum biomarker TNF-α and NGF-β and alleviate NCV deficits, which might be the important protective mechanism against DPN in rats with type 2 DM.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (81274049), the Scientific and Technological Innovation Foundation of Weifang Medical University (K1302024) and the Natural Science Foundation of Shandong Province (2012CQ039).

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