Jiao-Tai-Wan Ameliorates the Insulin Resistance and Lipid Metabolism in T2DM Rats by Regulating Neurotransmitters

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Research Article

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by insulin resistance and β -cell dysfunction, and accompanied by neuroendocrine disorders. Recently, Jiao-Tai-Wan (JTW) has been reported to exert hypoglycemic effects against diabetes. However, its mechanism has not been clarified. Therefore, we attempted to explore the effect of JTW on alleviating insulin resistance and lipid metabolism disorder in T2DM rats by regulating the level of neurotransmitters.

Methods: Sprague-Dawley (SD) rats were treated with a high-fat diet/streptozotocin to induce T2DM and then gavaged with JTW for 4 weeks. Afterwards, endpoints including body weight, fasting blood glucose, glucose tolerance, serum insulin, and lipid index were determined, and we analyzed pathological changes in the liver and kidney. Meanwhile, the level of neurotransmitter neurotransmitters in the central nervous system and peripheral tissues was measured by UPLC-MS/MS. Furthermore, the expression of neurotransmitter transporter mRNA and protein levels in the brain and kidney of T2DM rats was analyzed by qRT-PCR and WB.

Results: The results showed that JTW ameliorated glucose homeostasis, insulin resistance, and lipid metabolism in T2DM rats by regulating the disorder of neurotransmitter distribution in the brain, kidney, intestine, adrenal gland, blood, and urine of T2DM rats. Mechanically, JTW may improve neurotransmitter disturbance by reducing mRNA and protein expression of SERT, DAT, and GAT-1 and increasing mRNA and protein expression of NET in the brain and kidney of T2DM rats.

Conclusion: Our findings confirm that JTW can play a hypoglycemic role by regulating the disorder level of neurotransmitter distribution in T2DM rats, which may have potential therapeutic implications for the treatment of T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a serious and chronic disease resulting from genetic, environmental and metabolic risk factors, etc [1]. T2DM and its complications have become one of the most serious threats to human health [2]. Actually, the treatment of T2DM has made great progress, such as insulin secretagogues, insulin sensitizers and biguanides [3]. However, the long-term usage of these hypoglycemic drugs may induce nausea, vomiting, and gastrointestinal discomfort, etc [4]. Therefore, it is urgent to find new drugs to treat T2DM and its complications.

Recent studies have reported that neurotransmitters in the body play an important role in the occurrence and development of diabetes [5, 6]. For instance, the level of serotonin (5-HT) in peripheral organs increased significantly, which is correlated with the glycated hemoglobin (HbA1c) in diabetic patients [7]. The increased intestinal and plasma 5-HT levels were observed in diet-induced obese mice [8]. Moreover, the absence of 5-HT protects the mice under a high fat diet against the development of their metabolic syndrome [9]. Therefore, the regulation of neurotransmitter levels may play a crucial in effect the treatment of T2DM and its complications.

At present, the evidence on the efficacy of using Traditional Chinese Medicine (TCM) for T2DM prevention and treatment has been found [10, 11]. Among these TCM, Jiao-Tai-Wan (JTW) is gaining a growing interest in the scientific community. JTW, which consists of *Rhizome Coptidis* (*Coptis chinensis* Franch, *Ranunculaceae*) and *Cortex Cinnamomi* (*Cinnamomum cassia* Presl, *Lauraceae*) at a ratio of 10:1 (g/g), is a classical traditional Chinese prescription for treating insomnia. Its mechanism might be related with the modulation of the circadian clock and inflammation genes expressions in peripheral blood monocyte cells [12]. In recent years, numerous reports have demonstrated that JTW not only has the effect on insomnia alleviation, but also has the anti-diabetic effect [13]. JTW can significantly reduce fasting blood glucose in rodents, improve glucose tolerance, and enhance insulin sensitivity. Mechanistically, it may be related to the regulation of AMPK signaling pathway [11, 14]. Nevertheless, the mechanism of JTW in the treatment of T2DM has not been clarified. Based on the above problems, our study aims to explore the anti-diabetic effect of JTW in T2DM rats, and illustrate that adjusting the content of neurotransmitters in brain and peripheral organs may be its major mechanism.

2. Materials And Methods

2.1 Preparation and analysis of JTW extracts

Rhizoma Coptidis and *Cortex Cinnamon* were purchased from Beijing Heyanling Pharmaceutical Development Co. LTD (Beijing China), certified by Professor Liu Chunsheng of School of Chinese Pharmacy, Beijing University of Chinese Medicine. First, the ratio of 10:1 *Rhizoma Coptidis* (800 g) and *Cortex Cinnamon* (80 g) was soaked in 10 times deionized water for 30 min. Secondly, the mixture of *Rhizoma Coptidis* and *Cortex Cinnamon* was extracted twice for 2 hours each time. Next, the JTW extract is filtered and concentrated to $6.83 \text{ g} \cdot \text{mL}^{-1}$. Then, a certain volume of JTW extract was added with 10 times deionized water, which was equivalent to $3.3 \text{ g} \cdot \text{mL}^{-1}$ of raw material per mL.

JTW dry powder was obtained by freeze-drying part of the extracts. The JTW dry powder was accurately weighed and ultrasonically dissolved in methanol, and then filtered to be tested. The chromatographic separation was performed on an Agilent HPLC 1290 series system (Agilent, Waldbronn, Germany). The detection conditions of HPLC were as follows: Agilent Extend-C18 column (4.6 mm×250 mm, 5 μm); The mobile phase was composed of acetonitrile (A) and 0.05 $\text{mol} \cdot \text{L}^{-1}$ potassium dihydrogen phosphate solution (B) (A: B = 45: 55); The flow rate was $1.0 \text{ mL} \cdot \text{min}^{-1}$; The column temperature was 30°C and the injection volume was 5 μL . The main components and contents of JTW were shown in Fig. 1a and Fig. 1b.

2.2 Animal and treatments

6-week-old male SD rats were purchased from SPF Laboratory Animal Science and Technology Ltd. (Beijing, China) (Certificate No. SCXK-2016-0002). The rats were maintained on a 12 h light/dark cycle in a humidity and temperature-controlled environment in a specific pathogen-free animal laboratory affiliated to the Experimental Animal Center of Beijing University of Chinese Medicine. This study was

approved by the Medical and Experimental Animal Ethics Committee of Beijing University of Chinese Medicine, number: BUCM-4-2018090201-3013.

The rats were randomly divided into two groups fed with normal control diet ($n = 8$) or a high-fat diet (including basic feed 72.7% (w/w), lard 20% (w/w), egg yolk powder 5% (w/w), cholesterol 2% (w/w), bile salt 0.3% (w/w)) for 4 weeks. After 4 weeks of dietary intervention, rats on high-fat diet were injected with streptomycin (STZ, sigma, USA) dissolved in citric acid buffer ($0.1 \text{ mmol} \cdot \text{L}^{-1}$, $\text{pH} = 4.5$, 4°C), with a dose of $35 \text{ mg} \cdot \text{kg}^{-1}$. Three days after injection, fasting blood glucose (FBG) of those rats was measured. The rats with fasting blood glucose level $\geq 11.1 \text{ mmol} \cdot \text{L}^{-1}$ were used in the further experiments, which were fed with high-fat diet throughout the whole study.

Thirty-two rats were randomly allocated into four equal groups as follows: normal control group, TZDM group (Model), JTW extract ($0.683 \text{ g} \cdot \text{mL}^{-1}$) treatment group (JTW) and metformin ($183 \text{ mg} \cdot \text{kg}^{-1}$) treatment group. Rats in normal control group and model group were given the equal amount of deionized water by gavage. All rats were administered once a day continuously for 4 weeks. The body weight, food intake, water intake and blood glucose of all rats were monitored weekly. In addition, urine was collected before the end of the experiment.

2.3 Oral glucose tolerance test (OGTT)

After 3 weeks of feeding, the rats of all groups were fasted overnight and orally loaded with glucose (2.0 g/kg). Blood glucose were measured in blood collected by venous bleeding from the tail vein immediately before and, 30, 60, 120 min after glucose administration.

2.4 Biochemical analysis

The serum insulin level was measured by rat insulin ELISA Kit (Jiangsu Kete, China) according to the manufacturer's instructions. The homeostatic model assessment of insulin resistance (HOMA-IR) index was calculated as $[\text{FBG (mmol/L)} \times \text{FIN (mU/L)}] / 22.5$ to assess insulin resistance.

The serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels and the liver TC, TG levels were determined using biochemical kits (Nanjing Jiancheng, China).

2.5 Preparation of biological samples and detection of neurotransmitters

2.5.1 Biosample preparation

The tissue samples (heart, liver, brain, kidney, adrenal gland, intestinal) were homogenized in cold water-methanol (8:2) solution at a ratio of 1:2 (m: v). The protein concentration of tissue samples was determined by BCA protein detection kit (Beyotime, China). The serum was mixed with twice the amount of water-methanol (8:2), and urine with 9 times water-methanol (8:2). Above all biological samples were

performed as follows. Biological samples were centrifuged at 12000 rpm for 15 min at 4 °C, and then the supernatant was taken for 400 µL. Both 100 µL internal standard ($4 \mu\text{g}\cdot\text{mL}^{-1}$) and 1.5mL 0.1% formic acid-acetonitrile (4:6) were added to the supernatant, which centrifuged at 12000 rpm for 15 min at 4 °C. The supernatant was further used for UPLC-MS/MS analysis.

2.5.2 Preparation of standard solutions

A certain concentration of standard stock solution (5-Hydroxyindoleacetic acid (5-HIAA, sigma, USA), epinephrine (E), norepinephrine (NE), γ -aminobutyric acid (GABA) (Yuanye, China), dopamine (DA, National Institutes for Food and Drug Control, China)) was diluted with a water-methanol (8:2) solution to a concentration series of 0.05, 0.1, 1, 10, 40, 80, 100 $\mu\text{g}\cdot\text{mL}^{-1}$, and 5-hydroxytryptamine (5-HT, Yuanye, China) standard stock solution diluted with water-methanol (8:2) solution to a concentration series of 0.5, 1, 10, 20, 40, 80, 100 $\mu\text{g}\cdot\text{mL}^{-1}$ stored at 4°C for UPLC-MS/MS analysis. Furthermore, 4 $\mu\text{g}\cdot\text{mL}^{-1}$ standard stock solution of ISs was prepared by mixed 2-Pyridylacetic acid hydrochloride (IS, Yuanye, China) with water-methanol (8:2) solution.

2.5.3 Ultra-performance liquid chromatography tandem mass spectrometric analysis

Chromatographic separation was performed as follows: Chromatographic separation was achieved on Waters ACQUITY UPLC BEH C₁₈ (2.1× 100 mm, 1.7 µm); The mobile phase consisted of 0.3% (v/v) formic acid (A) - acetonitrile (B) with a flow rate of 0.30 mL·min⁻¹. The conditions of gradient elution were 0–2 min, 98% A; 2–3 min, 98% A-70% A; 3.0-3.5 min, 70% A-10% A; 3.5-5.0 min, 10% A; 5.0-5.1 min, 10% A-98% A; 5.1-7.0 min, 98% A; The injection volume was 2 µL and the column temperature was 25°C.

The optimal conditions of the APESI source of positive ion mode were employed as follows: capillary voltage of 4 kV, ion source temperature of 400°C, degassing flow rate of 800 L·hr⁻¹, cone flow rate of 150 L·hr⁻¹, spray pressure of 7.0 bar. The parameters and ion pairs of each neurotransmitter mass spectrum were shown in Table 1.

Table 1
Mass spectrometry information of 6 neurotransmitters and IS

Components	Chemical formula	Q1	Q3	CV	CE	Auxiliary qualitative ion
5-HT	$C_{10}H_{12}N_2O$	176.9	160.1	2	10	136.0
5-HIAA	$C_{10}H_9NO_3$	191.9	146.1	2	14	91.1
E	$C_9H_{13}NO_3$	184.3	166.0	2	10	107.0
NE	$C_8H_{11}NO_3$	169.9	152.0	72	6	106.9
DA	$C_8H_{11}NO_2$	154.3	137.1	2	10	91.1
GABA	$C_4H_9NO_2$	103.9	86.9	22	8	43.0
IS	$C_7H_8ClNO_2$	137.9	92.1	24	14	120.1

2.6 Histopathological analysis

Liver and kidney tissues fixed with 10% formalin were sectioned after being embedded in paraffin. Liver and kidney sections were prepared and stained with hematoxylin and eosin (H&E). Images were acquired using an inverted microscope (Olympus, Japan).

2.7 Quantitative real-time PCR analysis

The total RNA from brain and kidney tissues was extracted with Trizol reagent (Tiangen, China). One microgram of total RNA was converted to cDNA by using the Thermo fisher RT-PCR kit (Thermo, USA). Real-time quantitative PCR (qPCR) was performed with Power SYBR® Green PCR Master Mix (Thermo, USA). The PCR reaction was run in triplicate for each sample using the Step One Real-Time PCR System (Applied Biosystems, USA). The details of primer sequences were shown in Table 2.

Table 2
Primer sequences were used for quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

Target gene	Primer sequence	Product length
GAPDH	F CTGAGTATGTCGTGGAGTCTAC R AGTCTTCTGAGTGGCAGTGATG	287 bp
5-HTT	F GTCATTGGCTATGCCGTGGA R CACCCGTTTCGGTGGTACTG	167 bp
DAT	F TTCATGGTTATTGCTGGGATGCC R TGTAGAAGAAGCCCACGTAGAAAG	157 bp
NET	F ATCATCGCCTGGTCACTGTAC R GTTTGGGGTTCGGTACAGTTGG	106 bp
GAT-1	F ATCTTCTCCATCGTGGGCTTCAT R GATGAGCACCATGGAGGACAGAGC	467 bp

2.8 Western blotting assays

Total protein from brain and kidney tissues obtained to performed western blot. Brain and kidney tissues were homogenized in RIPA buffer. The protein concentration of tissue samples was determined by BCA protein detection kit (Beyotime, China). Total protein was separated by SDS-PAGE electrophoresis and transferred to a PVDF membrane which was blocked with 5% fat-free milk at 4°C for 60 min and incubated overnight with primary antibodies for serotonin transporter (SERT, 1:2000, ab181034, Abcam), norepinephrine transporter (NET, 1:1000, ab78190, Abcam), dopamine transporter (DAT, 1:200, ab128848, Abcam), γ -aminobutyric acid transporter subtype-1 (GAT-1, 1:1000, ab177483, Abcam) and β -actin (1:5000, AC001-M, Santa). The matching horseradish peroxidase (HRP) conjugated secondary antibodies (1:5000, proteintech) were used to evaluate the protein expression. The PVDF membrane was washed three times with washing solution TBST, and treated with chemiluminescence reagent. Western blot bands were quantified using Image J software (NIH, Bethesda, MD, USA).

2.9 Statistical analysis

Statistical analysis was performed by SAS 8.2. All values were expressed as means $\pm$ standard deviation. Comparisons among the three groups were achieved via one-way analysis of variance (ANOVA) followed by Dunnett's test., and the independent sample t-test was used for the comparison between the two groups. $P < 0.05$ was considered as a significant difference.

3. Results

3.1 JTW improves the weight body, food and water intake, blood glucose and insulin resistance of T2DM rats

The metabolic effects of JTW were evaluated by administering the compound to T2DM rats. As shown in Fig. 2a, after 4 weeks of JTW and metformin treatment, the rats were obviously protected from weight reduction. Further studies showed that compared with the normal group, the food and water intake of the model group rats were significantly increased, while JTW and metformin inhibited the food and water intake of T2DM rats, compared with the model group (Fig. 2b and Fig. 2c).

We next measured the blood glucose of all rats (Fig. 2d). As a result, the blood glucose of rats in the model group was significantly higher than that in the normal group. However, JTW and metformin treatment significantly reduced the blood glucose of rats compared with model group rats. To examine whether glucose metabolism of T2DM rats by JTW treatment, we investigated glucose homeostasis by oral glucose tolerance test (OGTT). JTW treatment greatly improved glucose homeostasis in T2DM rats compared with model group rats (Fig. 2e and Fig. 2f). Based on the above findings, we speculated that JTW improves insulin resistance. As expected, JTW significantly reduced the serum insulin level and HOMA-IR of T2DM rats compared with the model group (Fig. 2g and Fig. 2h). These results indicate that JTW ameliorates glucose homeostasis and insulin resistance in T2DM rats.

3.2 JTW reduces lipid levels in serum and liver of T2DM rats

T2DM is usually closely associated with hepatic steatosis. To investigate the beneficial effects of JTW on hepatic steatosis, we firstly observed the liver morphology of T2DM rats treated with and without JTW by H&E staining. The H&E staining of liver revealed that compared with the normal group, the model rats had more hepatocellular edema and lipid dripping. After being treated with JTW, the edema and steatosis of rat hepatocytes were reduced compared with the model group (Fig. 3a). In addition, we also determined the content of TC and TG in liver tissue (Fig. 3b and Fig. 3c). Consistently, compared with the model group, JTW significantly reduced the levels of TC and TG in the liver of T2DM rats, which suggests that JTW may exert some protective effects on the liver.

Moreover, the serum analysis shows that JTW and metformin treatment reduced TG, TG and LDL-C levels while increasing HDL-C levels of the serum in T2DM rats (Fig. 3d, 3e, 3f and Fig. 3g). Taken together, these results indicate that JTW can effectively ameliorate lipid metabolism in T2DM rats.

3.3 JTW improves neurotransmitter endocrine disorder in T2DM rats

5-hydroxytryptamine (5-HT), also known as serotonin, is a major excitatory neurotransmitter and a strong vasoconstrictor. It is abundant in brain tissue, intestine and platelets, and plays a key role in learning, memory and emotion regulation [15]. It has been demonstrated that intestinal microorganisms can affect the host's glucose homeostasis through 5-HT biosynthesis in intestinal chromaffin cells; When the level of 5-HT in serum and colon decreased, the glucose tolerance of mice increased significantly [16]. However, it

has been finding that low brain serotonin levels are associated with poor memory and depressed mood [17]. In this study, we measured the contents of 5-HT, 5-HIAA, DA and other neurotransmitters in the central and peripheral of T2DM rats (Fig. 4a-4h). Compared with the normal group, the level of 5-HT in the brain of T2DM rats decreased significantly, while JTW treatment can dramatically increase the level of 5-HT in the brain of T2DM rats compared with the model group. Interestingly, the 5-HT levels in the adrenal glands, intestines, and serum of T2DM rats were markedly higher than those of normal control rats. After JTW treatment, the levels of 5-HT in the adrenal gland, intestine and serum of T2DM rats decreased significantly, which suggested that JTW could increase glucose tolerance in T2DM rats by regulating the level of peripheral 5-HT.

5-HIAA, or 5-hydroxyindole acetic acid, is an inactive acidic metabolite produced by serotonin (5-HT). The change of 5-HIAA content can indirectly reflect the change of 5-HT content [18, 19]. Consistent with the results of 5-HT content determination, after JTW treatment, 5-HIAA levels in the intestine and serum of T2DM rats dramatically decreased compared with the model group.

It is now recognized that norepinephrine (NE), which is a neurotransmitter in the sympathetic nervous system, is stored in the intracellular membrane-bound particles of adrenergic nerve endings [20]. Abnormal sympathetic activity is a predictor of insulin resistance, diabetes and cardiovascular events [21]. It has been found that the depletion of the norepinephrine stores in the heart in diabetic patients may in part be responsible for their reduced survival rate in acute myocardial infarction [22]. Moreover, Champaneri *et al*/reported that men with diabetes had significantly lower urinary catecholamines included epinephrine, norepinephrine, and dopamine compared with those without diabetes [23]. In this study, compared with the normal group, the NE levels in the heart and urine of the model group rats were significantly lower. After the treatment with JTW, the NE levels in the heart and urine of the rats were significantly higher than that in the model group. However, the results of NE determination showed that the NE level in serum of T2DM rats was dramatically higher than that in the normal control group. While compared with the model group, the JTW-treated significantly reduced the NE level in the serum of T2DM rats, which is consistent with the results of previous studies [21].

Epinephrine is now increasingly recognized as an important metabolic hormone that helps mobilize energy storage in the form of glucose and free fatty acids [24]. It was found that the levels of neurotransmitters in diabetic animals changed in varying degrees; Importantly, adrenaline was found to increase significantly in adrenal tissue and decrease in serum [25]. Strikingly, in this study, we also found that the levels of epinephrine in brain, kidney, blood and urine of T2DM rats were notably decreased, while those in adrenal gland were significantly increased compared with normal rats. While compared to T2DM rats, supplementation of JTW showed the levels of epinephrine in brain, kidney, blood and urine of T2DM rats were notably increased, meanwhile those in adrenal gland were remarkably decreased.

Dopamine is the main catecholamine neurotransmitter in the mammalian brain. It controls a variety of functions, including motor activity, cognition, mood, active reinforcement, food intake, and hormonal regulation [26]. Several lines of research indicate that abnormal dopaminergic neurotransmission could

be involved in pathophysiological processes leading to obesity and diabetes [27]. Studies demonstrated the intrarenal dopaminergic system was crucial for modulating the development and progression of diabetic kidney injury, and the decrease of renal dopamine production had negative effect on diabetic nephropathy [28]. Moreover, the increase of dopamine levels in the heart and brain could inhibit the occurrence and development of T2DM [29, 30]. Compared with the normal group, the levels of DA in the heart, brain kidney and urine of T2DM rats decreased significantly, while JTW treatment can dramatically increase the levels of DA above those of T2DM rats compared with the model group.

GABA is the main neurotransmitter of the central nervous system, and it also plays an important role in the peripheral nervous system [31]. Previous studies also shown that GABA plays a role in endocrine pancreas and diabetes mellitus [32]. In addition, more recent studies have revealed that GABA promotes human β -cell proliferation and improves glucose homeostasis [33, 34]. In this study, the levels of GABA in the liver, brain, adrenal gland, intestine and serum of the model group rats exhibited a remarkable reduction compared to those in the normal group. Importantly, intervention with JTW for 4 weeks obviously reversed the decrease of GABA levels in those of T2DM rats compared with the model group. These results suggest that JTW may promote islet β -cell proliferation and ameliorate glucose homeostasis by increasing the levels of GABA of T2DM rats.

3.4 PCA analysis of neurotransmitter distribution levels in Tissues, blood and urine

In view of the above result, the overall structural changes of neurotransmitters in tissues (heart, liver, brain, kidney, adrenal gland, intestinal), blood, and urine among the groups were analyzed by unsupervised principal component analysis (PCA). The results of the PCA were shown in Fig. 5a-5h. JTW treatment revised the variations of the levels of 6 neurotransmitters in the brain, kidney, adrenal gland, intestine, blood and urine of T2DM rats along [t1] to some extent, while moving the structure along [t2] markedly. Taken together, JTW treatment significantly restored the relative abundance of several neurotransmitters disturbed by T2DM, which was closely related to metabolic disorders.

3.5 JTW regulates the expression of neurotransmitter related transporter in brain tissue of T2DM rats

In accordance with the above findings, to explore whether JTW can regulate neurotransmitters to play a hypoglycemic effect, we also detected the expression of neurotransmitter related genes and proteins in brain tissue. The results of PCR and WB showed that compared with the normal group, the mRNA and protein levels of SERT, DAT and GAT-1 in the model group rats were significantly increased, while the mRNA and protein levels of NET were significantly decreased; After JTW treatment, the mRNA and protein levels of SERT, DAT and GAT-1 were significantly decreased, while the mRNA and protein levels of NET were significantly increased (Fig. 6a-6i).

3.6 JTW regulates the expression of neurotransmitter related transporter in kidney tissue of T2DM rats

According to the distribution level of neurotransmitters in different tissues, we found that neurotransmitters changed most significantly in brain and kidney tissues of T2DM rats. Therefore, we also detected the expression of neurotransmitter related gene and protein in renal tissue. Furthermore, we also observed the pathological morphology of rat kidney. Compared with the normal group, the basement membrane of the kidney tissue of type 2 diabetic rats was diffusely thickened, and part of the glomerular structure was destroyed. After treatment with JTW, the pathological damage of the kidney tissue was improved (Fig. 7a). Consistent with the expression of neurotransmitter related genes and proteins in brain tissue, the results of PCR and WB in kidney tissue showed that compared with the normal group, the mRNA and protein levels of SERT, DAT, and GAT-1 in the model group were markedly increased, and the mRNA and protein levels of NET were notably reduced; after JTW treatment, SERT, DAT, and GAT-1 were markedly increased, and the mRNA and protein levels of NET are notably increased (Fig. 7b-7j).

4. Discussion

T2DM is an increasingly serious global health problem, which is closely related to environmental factors and genetic factors, etc [35]. T2DM can cause serious complications, such as depression, anxiety [36], non-alcoholic fatty liver [37] and diabetic nephropathy [38], etc. Interestingly, neurotransmitter levels play a vital role in the pathogenesis of T2DM and its complications [39]. In diabetes, hyperglycemia and insulin resistance can lead to disorders of neurotransmitter levels [40]. Therefore, we speculate that regulating the level of neurotransmitters can be used to prevent and treat T2DM and its complications.

In this study, we investigated the effects of JTW on glycolipid metabolism and neurotransmitter levels in T2DM rats. It is demonstrated that JTW reduced the fasting blood glucose level, insulin resistance, food intake, water intake, serum lipids and hepatic lipids, and improved insulin sensitivity in T2DM rats. The above results are consistent with previous other studies [14, 41]. Hence, these results clearly showed that JTW has a potent anti-diabetic effect.

Our study showed that neurotransmitter levels were disordered owing to the high fat diet feeding in T2DM rats, which was dramatically improved by JTW treatment. In particular, the level of 5-HT in the brain of T2DM rats decreased significantly compared with the normal group, After the JTW treatment, the levels of 5-HT in the brain T2DM rats increased significantly. In contrast, the 5-HT levels in the adrenal glands, intestines, and serum of T2DM rats were markedly higher than those of normal control rats, While JTW dramatically decreased the level of 5-HT in the adrenal gland, intestine and serum of T2DM rats decreased significantly, which suggested that JTW could increase glucose tolerance in T2DM rats by regulating the level of peripheral 5-HT. These neurotransmitter disorders caused by HFD and T2DM have been reported in other studies [42, 43]. In addition, high plasma levels of 5-HT have been reported in T2DM patients, although the potential effect on insulin secretion is unclear [44]. It has been demonstrated that the nervous system can regulate insulin secretion from pancreatic β -cells through neurotransmitters

and their respective receptors expressed in β -cells [45]. Importantly, the increased expression of 5-HT receptor 2C in pancreatic β -cells might inhibit insulin secretion of db/db mice [43].

Norepinephrine (NE), a neurotransmitter, plays an extensive role in the regulation of stress physiology, blood glucose levels and blood pressure [46]. However, the mechanism by which noradrenergic system is involved in the pathophysiology of diabetes has not yet been elucidated. Importantly, all diabetic rats, plasma norepinephrine was elevated and norepinephrine reuptake transporter expression reduced compared to non-diabetics [21]. Consistent with the above results, our results showed that the NE level in serum of T2DM rats was dramatically higher than that in the normal control group. Compared with the model group, JTW significantly reduced the NE level in the serum of T2DM rats. Moreover, we also measured NE levels in the heart and urine. Interestingly, the NE levels in the heart and urine of the model group rats were significantly lower compared with the normal group. After the treatment with JTW, the NE levels in the heart and urine of the rats were significantly higher than that in the model group. Meanwhile, we also determined the content of 5-HIAA, epinephrine, dopamine and γ -aminobutyric acid in brain and peripheral tissues of T2DM rats. However, the levels of these neurotransmitters change differently in different tissues. Therefore, we urgently need the further study to clarify its mechanism.

The high-affinity neurotransmitter transporter (5-HT [47], DAT [48], GAT-1 [49] and NET [50]) is the primary mechanism by which neurotransmitter (5-HT, DA, GABA and NE) synaptic transmission is terminated, which can control the duration and intensity of neurotransmitter signal through synaptic reuptake. Based on the result that changes in neurotransmitter levels are the most obvious in T2DM rats, we determined the genes and proteins expression of their related neurotransmitter transporter in the brain and kidney tissues. The results showed that compared with the normal group, the mRNA and protein levels of SERT, DAT, and GAT-1 in the model group were markedly increased, and the mRNA and protein levels of NET were notably reduced. After JTW treatment, SERT, DAT, and GAT-1 were markedly increased, and the mRNA and protein levels of NET are notably increased. These results are consistent with previous studies [47–50]. In addition, the levels of neurotransmitters in the central and peripheral are also affected by other factors such as synthetic enzymes and receptors, etc [51]. Hence, we need further research to reveal the mechanism of JTW on diabetes.

5. Conclusion

In summary, our study demonstrated that JTW ameliorated glucose homeostasis, insulin resistance, and lipid metabolism in T2DM rats by regulating the disorder level of neurotransmitter distribution in the brain, kidney, intestine, adrenal gland, blood, and urine of T2DM rats.

Declarations

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Author contributions

Jianhua Chen performed in pharmacodynamics experiments; Ziqi Jing and Xue Wang performed in neurotransmitter measurement; Chu Li and Yanyi Li performed in RT-PCR and WB experiments; Zhihui Li performed in data analysis; Yujie Zhang and Pengkai Ma conceived and designed the experiments.

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Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to limitations of ethical approval involving the patient data and anonymity but are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study processed under rules and regulations of the Medical and Experimental Animal Ethics Committee of Beijing University of Chinese Medicine with Approval no. BUCM-4-2018090201-3013. The study was carried out in compliance with the ARRIVE guidelines. For the plant study, we confirmed all the relevant institutional, national, and international guidelines and legislation are followed under the "Ethical approval and consent to participate" section.

Consent for publication

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest with regard to the contents of this article.

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Figures

a

Component	Component content (mg/g)
Cinnamic acid	0.058 ± 0.004
Magnoflorine	3.332 ± 0.085
Epiberberine	4.415 ± 0.112
Coptisine	8.317 ± 0.516
Palmatine	11.42 ± 1.456
Berberine	43.38 ± 6.002

Figure 1

Qualitative and quantitative analysis of main components of JTW. (a) The content of the main components of JTW. (b) HPLC chromatogram of main components in JTW. Note: a': Mixed standard solution; b': JTW solution; c': Methanol; 1: Cinnamic acid; 2: Magnoflorine; 3: Epiberberine; 4: Coptidine; 5: Palmatine; 6: Berberine.

Figure 2

Effects of JTW on body weight, food intake, water intake, blood glucose and serum insulin in T2DM rats. (a) Body weight. (b) Food intake. (c) Water intake. (d) Blood glucose. (e) Oral glucose tolerance test. Glucose levels were measured before (0) and at 30, 60 and 120 minutes after fed glucose. (f) Quantification of AUC from the OGTT. (g) Serum insulin concentration. (h) HOMA-IR. Data were shown as mean $\pm$ SD (n = 8). *P < 0.05, **P < 0.01 vs. Control group. #P < 0.05, ##P < 0.01 vs. T2DM group.

Figure 3

Effects of JTW on lipid level of serum and liver in T2DM rats. (a) H&E staining of liver tissues ($\times 20$ magnification; scale bar=100 μm). (b) Liver TC concentration. (c) Liver TG concentration. (d) Serum TC concentration. (e) Serum TG concentration. (f) Serum HDL-C concentration. (g) Serum LDL-C concentration. Data were shown as mean $\pm$ SD ($n = 8$). * $P < 0.05$, ** $P < 0.01$ vs. Control group. # $P < 0.05$, ## $P < 0.01$ vs. T2DM group.

Figure 4

Effects of JTW on six neurotransmitters in tissues, serum and urine of T2DM rats. (a) Neurotransmitter content in the heart. (b) Neurotransmitter content in the liver. (c) Neurotransmitter content in the brain. (d) Neurotransmitter content in the kidney. (e) Neurotransmitter content in the adrenal gland. (f) Neurotransmitter content in the intestinal. (g) Neurotransmitter content in the serum. (h) Neurotransmitter content in the urine. Note: 5-hydroxytryptamine (5-HT); 5-Hydroxyindoleacetic acid (5-HIAA);

Norepinephrine (NE); Epinephrine (E); Dopamine (DA); γ -aminobutyric acid (GABA). Data were shown as mean $\pm$ SD (n = 8). *P < 0.05, **P < 0.01 vs. Control group. #P < 0.05, ##P < 0.01 vs. T2DM group.

Figure 5

Principal component analysis (PCA) of six neurotransmitters in tissues, serum and urine of T2DM rats. (a) PCA of neurotransmitter in the heart. (b) PCA of neurotransmitter in the liver. (c) PCA of neurotransmitter in the brain. (d) PCA of neurotransmitter in the kidney. (e) PCA of neurotransmitter in the

adrenal gland. (f) PCA of neurotransmitter in the intestinal. (g) PCA of neurotransmitter in the serum. (h) PCA of neurotransmitter in the urine. Note: Z: control group; M: model group; J: JTW treatment group; Y: metformin treatment group. (n = 8).

Figure 6

Effects of JTW on mRNA expression and protein expression of SERT, DAT, NET and GAT-1 in brain of T2DM rats. (a) Relative expression of SERT mRNA in brain. (b) Relative expression of DAT mRNA in brain. (c) Relative expression of NET mRNA in brain. (d) Relative expression of GAT-1 mRNA in brain. (e) Western blotting assays were performed to measure the changes of SERT, DAT, NET and GAT-1 proteins in brain. (f) Relative expression of SERT protein in brain. (g) Relative expression of DAT protein in brain. (h)

Relative expression of NET protein in brain. (i) Relative expression of GAT-1 protein in brain. Note: Serotonin transporter (SERT); Dopamine transporter (DAT); Norepinephrine transporter (NET); γ -aminobutyric acid transporter subtype-1 (GAT-1). Data were shown as mean $\pm$ SD (n = 3). *P < 0.05, **P < 0.01 vs. Control group. #P < 0.05, ##P < 0.01 vs. T2DM group.

Figure 7

Effects of JTW on mRNA expression and protein expression of SERT, DAT, NET and GAT-1 in kidney of T2DM rats. (a) H&E staining of kidney tissues (Up: glomerulus; Down: renal tubule) ($\times 20$ magnification; scale bar=100 μm). (b) Relative expression of SERT mRNA in kidney. (c) Relative expression of DAT mRNA in kidney. (d) Relative expression of NET mRNA in kidney. (e) Relative expression of GAT-1 mRNA in kidney. (f) Western blotting assays were performed to measure the changes of SERT, DAT, NET and GAT-1 proteins in kidney. (g) Relative expression of SERT protein in kidney. (h) Relative expression of DAT protein in kidney. (i) Relative expression of NET protein in kidney. (j) Relative expression of GAT-1 protein in kidney. Note: Serotonin transporter (SERT); Dopamine transporter (DAT); Norepinephrine transporter (NET); γ -aminobutyric acid transporter subtype-1 (GAT-1). Data were shown as mean $\pm$ SD ($n = 3$). * $P < 0.05$, ** $P < 0.01$ vs. Control group. # $P < 0.05$, ## $P < 0.01$ vs. T2DM group.

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