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Inhibition of Jiang Tang Shu Xin (JTSX) Recipe in Endoplasmic Reticulum Stress CHOP Pathway and Improving Myocardial Remodeling in SD Diabetic Rats

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Abstract: *Objective* To explore the therapeutic molecular mechanism of Jiang Tang Shu Xin (JTSX) recipe with"invigorating Qi, nourishing Yin, activating blood circulation and detoxifying" in improving insulin resistance, inhibiting apoptotic bypass of endoplasmic reticulum stress (ERS) CCAAT enhance-binding protein homologous protein (CHOP), improving diabetic myocardial remodeling in SD diabetic rats. *Methods* SD rats were used to establish diabetes mellitus models, and after modeled successfully, were randomly divided into model group, western medicine group (Gliquidone+ Benazepril), low-dose JTSX group ($JTSX_1$), high-dose JTSX group ($JTSX_2$), and accepted corresponding drugs for 2 months respectively, taking the same batch of rats as normal control. After drugs administration finishing, blood lipid were measured by automatic biochemical analyzer, fasting serum insulin (FINS) and glycosylated hemoglobin (GHb) were measured by enzyme-linked immunosorbent assay (ELISA), insulin resistance index (IRI) was calculated. Masson staining was used to detect the expression of myocardial collagen fibers, immunohistochemistry to test the expression of myocardial nuclear factor-kB (NF-kB), tumor necrosis factor-alpha (TNF-α), TUNEL to check the apoptotic level of myocardial cells, RT-PCR to detect the transcription level of ERS molecules glucose regulated protein 78 (GRP78) and CHOP. *Results* Compared with the model group, the treatment groups could significantly reduce TG, LDL-C, GHb and IRI ($P<0.05$), increase FINS and HDL-C ($P<0.05$), decrease inflammatory factors NF-kB and TNF-α ($P<0.05$), down-regulate transcription of CHOP and GRP78 ($P<0.05$), and reduce cardiomyocyte apoptosis index (AI) ($P<0.05$); compared with the western medicine group, JTSX2 had more significant effect ($P<0.05$). *Conclusion* JTSX can inhibit insulin resistance, correct lipid metabolism disorder, restrain ERS-induced inflammatory reaction, suppress ERS-initiated apoptotic bypass of CHOP, and improve myocardial remodeling, with dose-dependent.

Keywords: Endoplasmic Reticulum Stress, Apoptosis, CCAAT Enhance-Binding Protein Homologous Protein, Myocardial Remodeling, Jiang Tang Shu Xin Recipe

1. Introduction

Diabetes mellitus, with its cardiovascular complication, seriously affects human health. Myocardial remodeling is a common pathological change in the development of heart diseases of different etiologies, including changes in cell structure and extracellular matrix of ventricular wall [1]. Apoptosis was found in both animal and human heart failure myocardial specimens, suggesting that apoptosis plays an important role in ventricular remodeling and progression of heart failure [2]. Various mechanisms of diabetes mellitus can participate in endoplasmic reticulum stress (ERS) and induce cardiomyocyte apoptosis [3]. Therefore, how to effectively restrain the ERS, reduce the apoptosis of myocardial cells and improve the heart remodeling is an important means to delay the occurrence and development of diabetic heart disease. According to Traditional Chinese medicine (TCM) theory, diabetic myocardial remodeling is essentially belonged to the

categories of Xiao Ke complicated with cardiopathy. Yin debilitation and Qi deficiency, combined with dryness-heat, are the Xiao Ke cardiopathy basic constitution, which, with the passing of time, can result in blood stasis, phlegm turbidity, heat toxin depose and hold-up in the heart [4]. Early clinical studies have found that Jiang Tang Shu Xin (JTSX), which has the functions of "nourishing Yin and Qi, promoting blood circulation and detoxifying", can effectively inhibit insulin resistance, correct the disorder of lipid metabolism, suppress the RASS system activated by heart failure, reduce the ventricle secreting B-type brain natriuretic peptide (BNP), regulate the metabolism of myocardial energy, alleviate the filling pressure of the ventricle, reduce the morphological index of left ventricle end-diastolic volume (LVEDV), inhibit myocardial remodeling, and improve cardiac function [5]. In animal experiments, JTSX can down-regulate the ERS signal molecules transcription of c-Jun N-terminal kinases (c-JNK) and cysteine aspartic proteinase -12 (caspase12) mRNA, inhibit cardiomyocyte apoptosis, restrain myocardial fibrosis, reduce ventricular morphological indexes: left ventricular end-systolic dimension (LVESD), left ventricular end-diastolic dimension (LVEDD and fractional shortening (FS), increase ventricular functional indexes (E / A and EF), protect myocardial tissue and ultrastructure from damage, and enhance cardiac function [6-7]. In this study, from another apoptotic signal: CCAAT enhance-binding protein homologous protein (CHOP) bypass, the effect of JTSX on diabetic myocardial remodeling will be explored, further revealing the molecular mechanism of JTSX prevention and treatment of diabetic heart remodeling.

2. Materials and Methods

2.1. Modeling and Grouping

Male SD rats weighing 200–250g (provided from experimental animal center of Guangxi Medical University) were used for the experiment.100 rats were made models (Considering the death of model building, increase the number of 20%). After fed at room temperature 22-25°C for 7 days and fasted for 8 hours, rats were intraperitonealy injected streptozotoein (STZ) (Beijing Hua Yang Biotechnology Co., Ltd.)50mg / kg once.72 hours later, the fasting blood glucose (FBG) were tested with blood glucose test paper (Wuhan Boster Biological Technology, LTD). Rats with 2 consecutive times FBG≥16.7mmol / L were used for Diabetes models. Soon afterwards, the rats were fed with high fat diet (contain common feed 72.8%, lard 10%, cholesterol 1.5%, porcine bile salt 0.3%, custard powder 10%, provided by Jiangsu synergetic Biological Engineering Co., Ltd) for 3 months. In addition, 25 homology SD rats were taken as normal group, accepted injection of normal saline intraperitoneally, and fed with ordinary feed.

2.2. Administration

JTSX (comprise Ginseng, astragalus, Ophiopogon, Cornus officinalis, rehmannia, rhubarb, Rhizoma Coptidis, Salvia miltiorrhiza, yam, Schisandra, etc, suppled by Affiliated Hospital of Youjiang National Medical College) were made of extracts, 1g of which equivalent to 10g raw drug. group JTSX1 were treated with 1g·kg-1·d-1 JTSX suspensions, while group JTSX2 were administered by1.5kg-1·d-1 of it; the western medicine group were fed with Gliquidone (Beijing Wan Hui Shuanghe Pharmaceutical Co Ltd)7.50mg/kg and benazepril (Beijing Novartis Pharma Ltd) 2.50mg/kg, which were prepared for equal volume solution. drugs administration in rats lasted for 2 months, and continued to feed high fat feed, while the model group were fed with high fat only, and the normal group continued to feed on the normal diet. The operation of gavage, water exchange and add feed were carried out in the super-clean worktable.

2.3. Rat Sampling

Before and after two months drugs administration, rats were checked FBG (fasting 12 hours, drinking water freely), and subsequently were anesthetized by intraperitoneally injected with 8% pentobarbital sodium 2mL / kg, then 7-l0mL blood samples were collected by jugular vein puncture. Sera was separated by 3000r/ min 10min centrifugation, and kept in -20°C refrigerator. After blood sampling, rats were immediately executed, and their hearts were excised immediately on ice, and divided into two parts, one part fixed with 4% paraformaldehyde for Masson staining, immunohistochemistry and cell apoptosis analysis; the other stored in -80°C liquid nitrogen for RT-PCR experiment.

3. Index Determination

3.1. Detection of Blood Lipid, GHB, Fins and IRI

The contents of blood lipid, including triglyceride (TG), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C), were measured by automatic biochemical instrument. The glycosylated hemoglobin (GHb) and fasting insulin (FINS), which kits were purchased from Beijing Huayue Biotechnology Co., Ltd, were determined by enzyme-linked immunosorbent assay (ELISA), and the test procedures were strictly in accordance with the instructions. According to the HOMA-IR value (HOMA-IR=FINS×FPG / 22.5) proposed by Matthews, the insulin resistance index (IRI) was calculated.

3.2. Detection of Myocardial Collagen

The myocardial tissues fixed in 4% paraformaldehyde solution were taken out and embedded in paraffin. Routine 4um serial slices were made, and dried in oven at 60°C. Through dewaxing with 100% xylene, dehydrating with gradient alcohol, transparenting with xylene, and finally sealing with neutral gum, the myocardial tissue sections prepared above were stained with Masson trichrome in accordance with the instructions. Under the microscope, five non overlapping fields were selected for photography, and the collagen fiber were measured with Image Pro Plus version 6.0 software.

3.3. Detection of Cardiomyocyte Apoptosis by TUNEL

The operation were carried out according to the TUNEL kit instruction (Beijing Hua Yue Biotechnology Co., Ltd.). The heart tissues were embedded in paraffin and cut into 4um slices. Through dewaxing→rinsing→adding protease for 15 minutes, inactivating endogenous peroxidase by 3% H_2O_2 for 15 minutes before adding TUNEL reaction liquid, Then putting in 37°C temperature box for 1 hours, Adding peroxidase and DAB at room temperature for dyeing 10 minutes, gradient ethanol dehydrating, dripping Xylene for transparent and sealing sheets with resin, cardiomyocyte apoptosis index (AI) in five visual fields with more positive cells expression were examined and calculated with optical microscope.

3.4. Immunohistochemical Detection of NF-kB and TNF-α Expression in Myocardium

Having been immersed in paraformaldehyde for 24 hours, the heart tissue were embedded in paraffin, cut into 4um thin slices, then according to the instructions, dewaxed with xylene, rinsed with step-by-step ethanol, repaired with antigen, eliminated endogenous active oxide enzyme, dripped with blocking solution, added with anti NF-kB and TNF-α antibodies (purchased from Beijing Huayue Biotechnology Co., Ltd.), and then added biotin-labeled secondary antibody streptavidin peroxidase complex, DAB dyed, hematoxylin redyed. Under microscope, 5 non-overlapping images with more positive expression in myocardial sections were selected and photographed, and their integral optical density (IOD) were measured by Image-Pro Plus Version 6 image analysis software.

3.5. Detection of GRP78, CHOP mRNA Transcription in Myocardium by Rea1-time PCR

The reference and target gene primers were designed and synthesized by Shanghai Genechem Co., LTD. The sequence of primers is as follows: GAPDH upstream 5′-TTCAACGGCACAGTCAAGG-3′, downstream 5′-CTCAGCACCAGCATCACC-3′, amplified product 114 bp; CHOP up- stream 5′-TGCCTTTCGCCTTTGAGACAG-3′, downstream 5′-GCTTTGGGAGGTGCTTGT GAC-3′, amplified product 218 bp; GRP78 upstream 5′-CTTGGTATTGAAACTGTGGG-3′, downstream 5′-TGTTACGGTGGGCTGATTAT-3′, Amplified product 117 bp. Taking out the myocardium from -80 C refrigerator, grinding in a mortar, extracting of total RNA, conducting electrophoresis in agarose gel, and checking its integrity; The process of amplification of obtained cDNA is as follows (in accordance with the real-time fluorescence quantitative PCR kit instruction): 96°C 4min, one cycle, Subsequently 94°C 30 second, 58°C 30 second, 72°C 30 second, 40 cycle. With $2^{-\Delta\Delta Ct}$ representing the expression of target gene in experimental group, ΔΔCt=Experience group ($Ct_{target\ gene}$-Ct_{GAPDH}) -control group ($Ct_{target\ gene}$-Ct_{GAPDH}).

4. Statistical Analysis

Data acquired in this study were presented in a (mean ± standard deviation) manner and analyzed by SPSS 19 software. The diffffferences between groups were analyzed by One-way ANOVA. When P value<0.05, the diffffferences were considered statistically signifificant.

5. Result

5.1. Animal Experiment

After successful modeled, rats drank more, urinated more, with withered hair and weight loss, acting sluggish and slow. The process of intragastric administration was successful and no rat died.

5.2. Effect of JTSX on Lipid, GHb, FINS, IRI in Rats

The results showed that, compared with the normal control group, the GHB, IRI, TG and LDL-C contents in the model control group increased significantly (P<0.01), while HDL-C and fins decreased significantly (P<0.01). After administration, compared with the model group, the GHB, IRI, TG and LDL-C in each drug treatment groups decreased (P<0.05), while the HDL-C and FINS increased (P<0.05). Compared with western medicine group, JTSX2 were more effective (P<0.05). The effect of JTSX is better with the increase of dose. The results are shown in table 1.

Table 1. *Comparison of JTSX effect on lipid, GHb, FINS, IRI in rats ($\bar{x}$±S).*

Group	n	GHb (mmol/L)	FINS (mU/L)	IRI	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
normal group	25	5.97±1.21	21.12±1.38	4.89±1.21	0.95±0.15	0.95±0.59	0.97±0.09
model group	25	20.78±4.17^{a}	14.12±4.33^{a}	16.97±2.20^{a}	2.52±0.22^{a}	2.31±0.78^{a}	0.54±0.08^{a}
western medicine group	26	13.99±3.88^{ab}	16.85±4.34^{ab}	8.01±1.21^{ab}	1.91±0.28^{ab}	1.85±0.26^{ab}	0.78±0.09^{ab}
$JTSXR_1$ group	25	12.66±3.67^{bc}	17.76±4.77^{abc}	7.52±1.18^{abc}	1.69±0.25^{abc}	1.65±0.23^{abc}	0.84±0.08^{abc}
$JTSXR_2$ group	26	15.87±3.88^{ab}	16.77±4.67^{ab}	8.18±1.15^{ab}	1.97±0.24^{ab}	1.77±0.22^{ab}	0.75±0.07^{ab}

Notes: Compared with normal group, ^{a}P<0.01; Compared with model group, ^{b}P<0.05; Compared with western medicine group, ^{c}P<0.05

5.3. Effect of JTSX on Myocardial Histopathology

In Masson stained heart tissue, the normal myocardial fibers are red, while the collagen fibers are green (positive). The normal myocardial cells are closely arranged, orderly, bright red, and there is few or no fibroblastic proliferation in the stroma. In the model group, a large number of collagen

fibers were proliferated, while in each drug treatment group, the collagen fibers were significantly reduced (P<0.05). Compared with the western medicine group, the collagen fibers in JTSX2 group decreased significantly (P<0.05). But there was no significant difference between JTSX groups (P>0.05). The results are shown in Figure 1 and table 2.

5.4. Effect of JTSX on Cardiomyocyte Apoptosis

The normal nucleus is blue, and TUNEL positive staining is located in the nucleus, which is brownish yellow, and its volume is equal to or less than the normal nucleus. The results of apoptosis in situ of cardiomyocytes showed that, compared with the normal group, there is a large number of apoptosis in model group (P<0.01). Compared with the model group, the cell apoptosis index (CAI) of each drug treatment group were significantly lower (P<0.05). Compared with the western medicine group, the CAI of JTSX2 group decreased significantly (P<0.05). The effect of JTSX is related to increasing dose, but there was no significant difference between JTSX groups. The results are shown in Figure 1 and table 2.

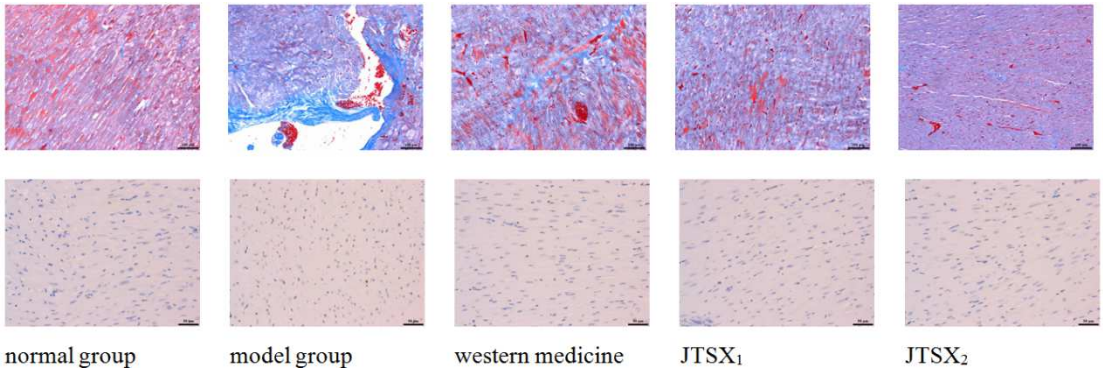

Figure 1. *Upstream (Masson staining): results of myocardial histopathology in five groups(×200); Downstream (TUNEL staining): cardiomyocyte apoptosis figure (×200).*

5.5. Effect of JTSX on the Expression of NF-kB and TNF-α in Rats Myocardium

Immunohistochemistry showed that, the positive expression were brownish yellow and distributed in the cytoplasm. The expression of NF-kB and TNF-α in the normal myocardium were low. Compared with the normal group, the positive protein in the model group were significantly increased (P<0.01). Compared with the model group, the NF-kB and TNF-α expression decreased significantly in each drug treatment group (P<0.05). Compared with western medicine group, the expression of NF-kB and TNF-α in JTSX2 group decreased (P<0.05). The positive expression decreased with the increase of JTSX dose, but there was no significant difference between JTSX groups (P>0.05). The results are shown in Figure 2 and table 2.

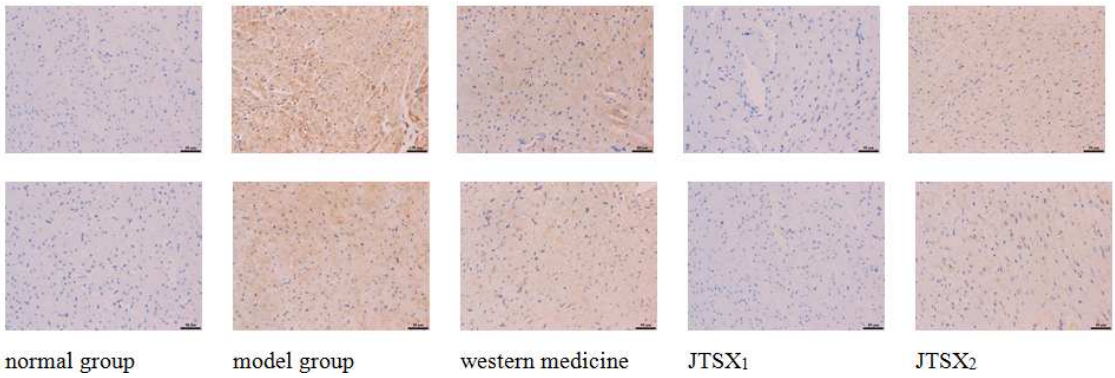

Figure 2. *Comparison of the results of myocardial immunohistochemistry in five groups(×200, Upstream NF-kB, Downstream TNF-α).*

Table 2. *The effect of JTSX on the cardiomyocytes apoptosis, collagen, NF-kB and TNF-α expression ($\bar{x}$±s).*

Group	n	CAI (%)	Collagen fiber (IOD)	TNF-α expression (%)	NF-kB expression (%)
normal group	25	2.8±0.27	364±51	1.71±0.30	1.77±0.29
model group	25	41.6±0.68[a]	9 102±885[a]	18.6±5.21[a]	20.7±6.66[a]
western medicine group	26	33.2±0.63[ab]	4 311±498[ab]	8.51±3.08[ab]	9.85±4.05[ab]
$JTSXR_1$ group	26	32.8±0.83[ab]	4 086±531[ab]	6.31±2.87[abc]	8.01±3.77[abc]
$JTSXR_2$ group	25	30.1±0.47[abc]	3 821±495[abc]	8.66±2.87[ab]	11.78±2.08[ab]

Notes: Compared with normal group, [a]P<0.01; Compared with model group, [b]P<0.05; Compared with western medicine group, [c]P<0.05

5.6. Effect of JTSX on GRP78 and CHOP mRNA Transcription in Rat Cardiomyocytes

Compared with the normal control group, the expression of GRP78 and CHOP mRNA in myocardial cells of the model group were significantly increased (P<0.01). Compared with the model group, GRP78 and CHOP mRNA transcription in each drug treatment groups decreased (P<0.05). Compared with the western medicine group, the GRP78 and CHOP mRNA in JTSX2 group decreased more significantly (P<0.05). The effect of JTSX was better with the increase of dose, but there was no significant difference between JTSX groups (P>0.05). The results are shown in Figure 3 and table 3.

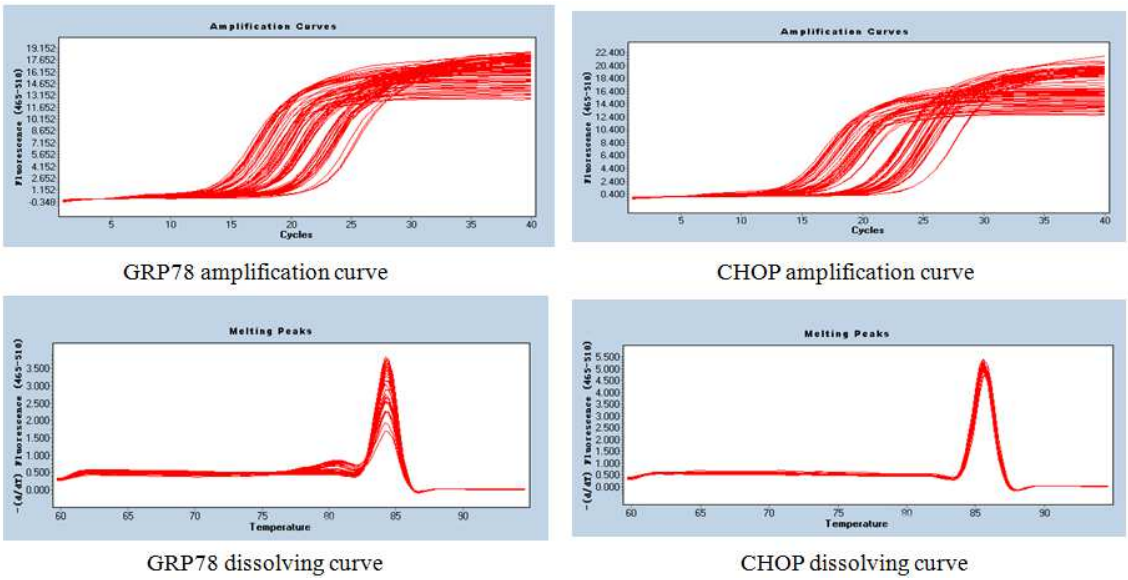

Figure 3. *Amplification and dissolution curves of GRP78 and CHOP mRNA in aorta.*

Table 3. *Comparison of relative transcription levels of GRP78 and chop mRNA in myocardial cells of rats in each group($2^{-\Delta\Delta Ct}$, $\bar{x}\pm S$).*

Group	n	GRP78	CHOP
normal group	25	1.07±0.23	1.06±0.27
model group	25	7.11±0.37[a]	7.75±0.25[a]
western medicine group	26	4.21±0.87[ab]	4.76±0.93[ab]
$JTSXR_1$ group	25	3.24±0.41[abc]	3.20±0.33[abc]
$JTSXR_2$ group	26	4.31±0.42[ab]	4.91±0.43[ab]

Notes: Compared with normal group, [a]P<0.01; Compared with model group, [b]P<0.05; Compared with western medicine group, [c]P<0.05

6. Discussion

Endoplasmic reticulum (ER) is an organelle with important physiological functions in cells, where protein synthesis, folding, glycosylation, modifying and transporting are regulated, and where steroid, cholesterol and lipid are synthesized. Under pathological conditions, such as hypoxia, inflammatory and hyperglycemia induced by oxidative stress, lead to the imbalance of endoplasmic reticulum homeostasis, the corresponding signal pathway will be activated and a series of stress reactions in cells will be triggered, which is called ERS [8]. ERS is triggered by unfolded protein response (UPR), and glucose regulated protein 78 (GRP78) is the feeling molecular for endoplasmic reticulum homeostasis [9]. When ER is in steady state, GRP78 is combined with the domain of three transmembrane proteins: Activating transcription factor 6 (ATF6), inositol demand enzyme 1 (IRE-l) and protein kinase R like endoplasmic reticulum kinase (PERK)], which exposed to the endoplasmic reticulum. When ERS occurs, GRP78 dissociates from PERK, ATF6 and IRE1 due to its higher affinity with the unfolded proteins, and then binds with the unfolded proteins to promote folding correctly. After dissociation, PERK, ATF6 and IRE1 are activated respectively, to activate the protective signaling pathway of ERS and protect the cells. However, in the case of persistent and severe ERS, PERK, ATF6 and IRE1 activate the three downstream apoptotic signaling molecules: CHOP, (c-JNK), caspase12, and then trigger apoptosis [10]. After dissociation from GRP78, IRE1 binds with tumor necrosis fector receptor associated factor 2 (TRAF2) to form IRE1 α - TRAF2 complex, which can collect IkB kinase, phosphorylate and degrade IkB, release NF-kB into nucleus, activate transcription of pro-inflammatory gene, while PERK dissociating from GRP78, it induce the expression of CHOP, which in turn activated the expression of growth arrest and DNA damage inducing gene 34 (GADD 34), endoplasmic reticulum redox factor 1 (ERO1), death receptors (DRS) and other genes, thus promoting the synthesis of ERS apoptosis pathway related proteins [11]. In addition, CHOP can inhibit the expression of Bcl-2, deplete glutathione, induce the production of oxygen free radicals and promote apoptosis [12]. It has been shown that in $CHOP^{-/-}$ rats, ERS induced apoptosis is inhibited, so CHOP plays an important role in ERS induced apoptosis [13].

Cardiomyocytes are terminal differentiated cells without proliferative ability, consequently, apoptosis will reduce the number of cardiomyocytes, while myocardial interstitial cells

increase compensably, and produce too much extracellular matrix, which leads to myocardial fibrosis and myocardial remodeling, and ultimately leads to heart failure [14]. Therefore, cardiomyocyte apoptosis plays an important role in the development of myocardial remodeling. ERS often leads to dysfunction of cells with strong metabolism (such as cardiomyocytes), because these cells need endoplasmic reticulum to deal with a large number of energy metabolism substances, highly sensitive to the change of intracellular metabolism balance, where fat accumulation, glucose concentration increase and immune cytokine accumulation are likely to trigger ERS [15]. Hyperglycemia in diabetes damages the normal pathway of glucose metabolism in cells, while the four pathways including polyol, advanced glycation end products (AGEs), protein kinase C (PKC) and hexosamine pathway, are over activated, which produce excessive ROS in the cell, affect the homeostasis of endoplasmic reticulum, block the folding of protein spatial structure, initiate the accumulation of unfolded protein, as a result, induce ERS, which in turn can aggravate the inflammatory response and form a vicious circle [16]. In addition, due to insulin resistance, inhibition of the function of lipoprotein results in VLDL clearance rate reducing, lipid metabolism deranging, TG and LDL increasing, while HDL decreasing, and ROS promotes the increasing of ox-LDL, which can not be recognized by LDL receptor for normal degradation, resulting in ox-LDL accumulation in vivo, triggering ERS and apoptosis [17]. The lack of insulin (relative lack) increases the dependence of myocardial cells on lipid oxidation and energy supply, the enhancing of oxygen demand, the accumulating of lipid droplets such as triglycerides and free fatty acids (FFA) in cells, and the increasing of toxic intermediates in cells, all of which are easy to cause ERS [17-18]. Therefore, ERS, as a common pathway of multiple stressors, in the state of diabetic insulin resistance, through the coupling of UPR signal system with different types of metabolic abnormalities, a series of pathophysiological phenomena, can lead to endoplasmic reticulum dysfunction and induce ERS.

According to TCM theory, the basic constitution of diabetes mellitus (Xiao Ke) complicated cardiopathy, will final develop into Yin debilitation and Qi deficiency, blood stasis, heat toxin depose and hold-up in the heart [20]. Because ERS coupled with inflammatory response plays an important role in the occurrence and development of diabetic myocardial remodeling, and the essential characteristics of inflammatory response are similar to that of heat toxin, therefore, heat-clearing and detoxifying drugs can protect the heart through anti-inflammatory effect. Searching for treatment ideas from Tonifying Qi, Nourishing Yin, Aactivating circulation and Toxic-cleaning are the rich development of the ancient doctors' theory of diabetes treatment. JTSX consists of ten kinds of Chinese Medicine (ginseng, astragalus, cornus officinalis, ophiopogon, rehmannia, rhubarb, rhizoma coptidis, yam, salvia miltiorrhiza, schisandra), in which, cornus officinalis, yam and Rehmannia are the ingredients of Ming Dynasty *Jing Yue Quan Shu*' s Zuo Gui pill for treating true Yin deficiency; ginseng, ophiopogon root, Schisandra are Doctors of Jin-Yuan Dynasty Li Gao *Endogenous and Exogenous differentiation theory* Shengmai Powder ingredients, possessing nourishing Yin, tonifying Qi, generating fluid, and nourishing heart; Adding rhubarb, coptidis, to clear away heat toxin, and eliminate dampness; Adding salvia miltiorrhiza to promote blood circulation for removing blood stasis; Adding astragalus to nourish Qi and strengthen the positive for detoxification. All prescriptions own supporting, supplementing and attacking simultaneously.

7. Conclusion

ERS often occurs in cells with strong metabolism, which need endoplasmic reticulum to deal with a large number of energy metabolizing substances, and highly sensitive to the change of intracellular metabolic balance, Therefore, cardiomyocytes are firstly suffer from the dysfunction of endoplasmic reticulum. In addition, diabetic insulin resistance leads to damage of normal pathway of glucose metabolism, over activation of bypass metabolic pathway, oxidative stress, and disorder of lipid metabolism, which also seriously affects the homeostasis of endoplasmic reticulum. So the heart with diabetes is severely damaged. Continuous and intensive ERS can induce apoptosis, reduce the number of terminal cardiomyocytes with no reproductive ability, and promote myocardial fibrosis and remodeling. This study showed that, after the successful establishment of diabetes model, the fasting insulin level decreased, insulin sensitivity decreased, and insulin resistance were obvious. GRP78, a switch molecule upstream of endoplasmic reticulum stress signal, were abundantly expressed, indicating that ERS occurred in cardiac myocytes. After drug administration, GHb were significantly reduced, lipid metabolism disorder were corrected, IRI reduced, insulin sensitivity improved, insulin resistance improved, the levels of pro-inflammatory factors (NF-KB and TNF-α) were decreased, GRP78 and CHOP mRNA were down regulated, ERS mitigated, cardiomyocyte apoptosis decreased, and myocardial remodeling improved. The effect of JTSX increased significantly with increasing dose, with therapeutic effect of correcting insulin resistance, alleviating the coupling reaction of "oxidative stress- endoplasmic reticulum stress-cell apoptosis" in diabetes, reducing the myocardial apoptosis induced by ERS and improving cardiac remodeling, playing the multi-channel, multi link and multi-target regulatory role of "nourishing yin and Qi, activating blood and detoxifying" in TCM.

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