DOI: 10.53469/jcmp.2025.07(11).18

The Effect of Wumei Pill on Atrogin-1, MURF1 in Diabetic Myopathy

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Abstract: ***Objective:*** ***Explore the effects of Wumei Pill on FBX32 (Atrogin-1), MURF1 related to muscle atrophy in diabeticopathy.*** ***Methods:*** ***(1) Male SD rats at 6-8 weeks of age were randomly divided into control group, model group, metformin group, andumei Pill group. Except for the control group, the rest of the rats were injected intraperitoneally with streptozotocin to establish a diabetic model. () The blood glucose of rats was monitored weekly, and the body weight was measured. (3) At the end of the 8-week experimental period, the rats were fast overnight and fasted for 12 hours before sacrifice, and the weight was weighed. The gastrocnemius muscle and soleus muscle tissues of the rats were taken, part the soleus muscle tissue was fixed with paraformaldehyde, and the rest of the tissues were frozen for storage. (4) The morphology of the soleus muscle of was observed by HE staining method, and the cross-sectional area of its fibers was measured; the expression levels of Atrogin-1 and MURF1 proteins rat skeletal muscle tissue were detected by real-time fluorescent quantitative PCR experiment and protein immunoblotting experiment.*** ***Results:*** ***(1) Wumei Pill can effectively control the trend of weight loss and the rise in blood sugar in diabetic rats. (2) The cross-section area of the soleus muscle fibers in the Wumei Pill group was significantly higher than that in the model group; and the expression levels of two proteins, Fbx32 MURF1, in their skeletal muscle tissue were significantly lower.*** ***Conclusion:*** ***Wumei Pill prevents muscle fiber structure damage, and reduces the expression of Fbx32 and MURF1 protein, thus playing its role in preventing musclerophy in diabetic myopathy.***

Keywords: Wumei Pill, Diabetic myopathy, Soleus muscle, Fbx32, MURF1.

1. Introduction

Skeletal muscle atrophy is one of the complications of diabetes mellitus, characterized by limited movement, pain, cramps, and decreased function. Diabetic myopathy is easily missed or misdiagnosed, and it is more common in type 1 diabetes patients with long disease duration and poor blood sugar control [1]. In the early stage of diabetic myopathy, there is often muscle pain, followed by local skin swelling and lumps, which eventually develop into muscle atrophy [2,3]. This not only directly affects the patient's personal health and quality of life, but also brings a heavy economic burden to the family and society. Therefore, researching and finding effective drugs to treat diabetic myopathy has become a hot topic of current research.

The use of Wumei pills in the treatment of diabetes has a long history and has been confirmed by a large number of clinical trials. However, there is currently a lack of relevant research on its treatment of diabetic myopathy. This article evaluates the therapeutic effect of Wumei pills on diabetic myopathy through animal experiments related to diabetic myopathy.

2. Materials and Methods

2.1 Materials

2.1.1 Experimental Animals

This experiment was reviewed and approved by the Animal Experiment Ethics Committee of Shaanxi University of Traditional Chinese Medicine and fully complied with the "Guidelines for the Breeding and Use of Experimental Animals". Male SD rats (180-200g) aged 6-8 weeks were purchased. The rats were kept in a controlled environment with a room temperature of (24±1)℃ and humidity of 50±20%, using a 12-hour light-dark cycle.

2.1.2 Drugs

Wumei Pills (provided by the Traditional Chinese Medicine Pharmacy of the Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine); Metformin Hydrochloride Tablets (Glucophage, H20023370); Streptozotocin (Biotopped, S6050F-100mg).

2.1.3 Reagents and Instruments

Primary antibody dilution buffer (Servicebio, G2025-100ML); Secondary antibody dilution buffer (Servicebio, G2009-100ML); Phosphorylated protease inhibitor (Servicebio, G2007-1ML); BCA protein quantification kit (Servicebio, G2026-200T); RNA extraction buffer (Wuhan Saiweier Biotechnology Co., Ltd., G3013); RNA lysis buffer (Wuhan Saiweier Biotechnology Co., Ltd., G3029); Microplate reader (Rayto, RT-6100); Electrophoresis apparatus (Servicebio, SVE-2); Panoramic slide scanner (3DHISTECH, PANNORAMIC DESK/MIDI/250/1000); Real-time PCR instrument (Bio-rad, CFX Connect).

2.2 Methods

2.2.1 Grouping and Model Preparation

After one week of acclimatization, rats were randomly divided into four groups: control group, diabetic group, metformin group, and Wumei pill group. Rats in the three groups other than the control group were intraperitoneally injected with a prepared streptozotocin (STZ) solution. The model group was considered successfully established when the fasting blood glucose level was greater than 11.1 mmol/L and remained stable for seven days; and the "three polys and one less" characteristics (polydipsia, polyphagia, polyuria, and weight loss) were observed.

2.2.2 Intervention Methods

The concentration of the umeboshi pill in the rat group was 20 g·kg^{-1}·d^{-1}; the concentration in the metformin group was 0.1 g/kg. Both the control and model groups were given deionized water. All groups were administered the medication by gavage.

Weight and blood glucose changes in each group were recorded weekly. After oral administration in the eighth week of the experiment, all rats were fasted overnight. The following day, they were anesthetized with sodium pentobarbital solution. The gastrocnemius and soleus muscles were harvested. Part of the soleus muscle tissue was fixed with paraformaldehyde, and the remaining tissue was frozen in liquid nitrogen and stored at -80℃ for later use.

2.2.3 Detection Indicators and Methods

Rat soleus muscle tissue was stained with hematoxylin and eosin, sections were prepared, and pathological changes were observed under an electron microscope. The expression concentrations of muscle atrophy-related proteins during diabetic myopathy were detected using quantitative real-time PCR and Western blotting experiments.

2.3 Statistical Methods

All data were analyzed using SPSS 26.0 statistical software and mapped using GraphPad Prism 9 software. One-way ANOVA was used to compare means for diversity; the LSD test was used for homogeneity of variance; and the t-test was used for heterogeneity of variance. Data are expressed as mean ± standard deviation ($\bar{x} \pm s$). A p-value < 0.05 was considered statistically significant.

3. Results

3.1 Effects of Wumei Pill on Body Weight and Blood Glucose in Type 2 Diabetic Rats

Compared with the control group, the model group rats showed significantly greater weight loss and faster blood glucose levels, demonstrating the successful establishment of the diabetes model. Compared with the model group, the Wumei Pill group and metformin group rats showed a significantly slower rate of weight loss and a significantly reduced rate of blood glucose increase, with blood glucose levels significantly lower than the model group (P<0.05), as shown in Figure 1.

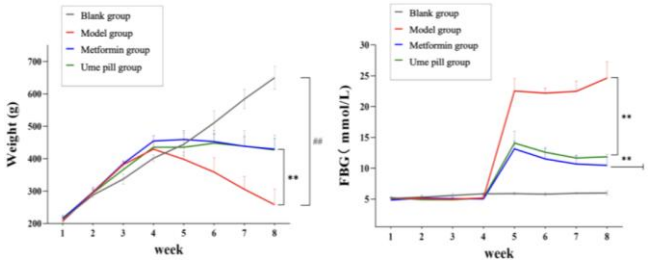

Figure 1: Changes in rat body weight and blood glucose levels.

Note: **Compared with the model group, P<0.05; ##Compared with the control group, P<0.05.

3.2 Pathological Results of Soleus Muscle Sections in Each Group of Rats

As shown in Figure 2, according to the HE staining results and Image-Pro Plus 6.0 software analysis, the area of soleus muscle fibers in the model group was significantly smaller than that in the control group (P<0.05). The area of soleus muscle fibers in the metformin group and the umeboshi pill group was higher than that in the model group, and the results were statistically significant (P<0.05).

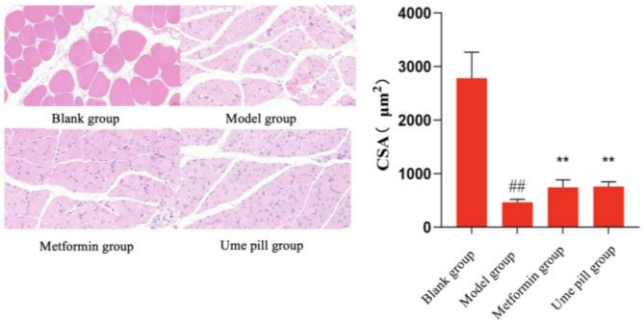

Figure 2: Pathological sections of soleus muscle of rats in each group (left); surface area of muscle fibers (right)

Note: **Compared with the model group, P<0.05; ##Compared with the control group, P<0.05.

3.3 Expression of Atrophy-Related Proteins in Rat Muscle Tissue of Each Group

As shown in Figure 3, the expression levels of MuRF-1 and Fbx32, markers of muscle atrophy in skeletal muscle, were detected by RT-qPCR. The results showed that MuRF-1 and Fbx32 were significantly increased in the model group. Compared with the model group, the expression levels of MuRF-1 and Fbx32 were significantly decreased in the metformin group and the Wumei pill group, with statistically significant differences (P<0.05).

As shown in Figure 4, the above results were further verified by wb protein quantification experiments. The results showed that compared with the model group, the expression of MuRF-1 and Fbx32 in the skeletal muscle of rats in the metformin group and the Wumei pill group was significantly decreased, with statistically significant results (P<0.05).

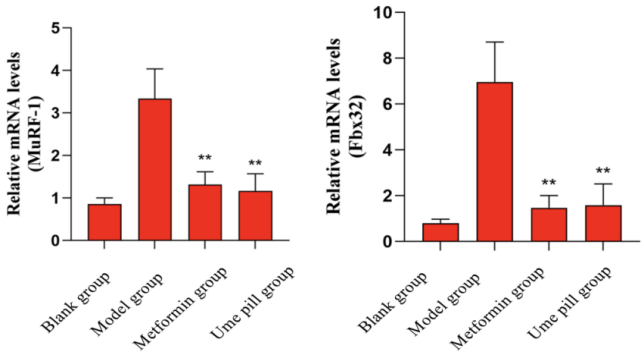

Figure 3: Gene expression levels of atrophy-related proteins in skeletal muscle of rats in each group.

Note: **Compared with the model group, P<0.05.

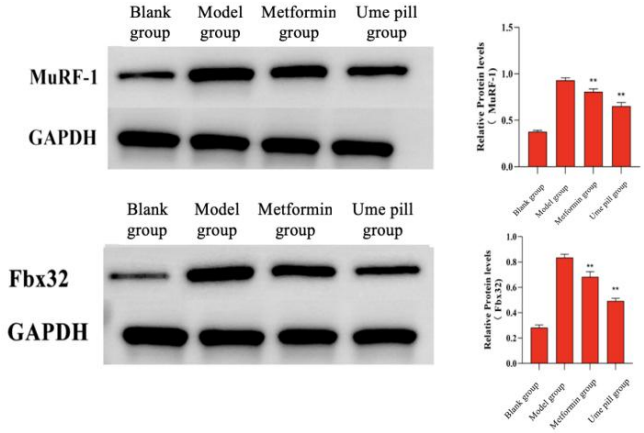

Figure 4: Expression levels of atrophy-related proteins in skeletal muscle of rats in each group.

Note: ***P*<0.05 compared with the model group.

4. Discussion

Diabetes belongs to the category of "Xiao Ke Bing" in traditional Chinese medicine [4]. According to the clinical symptoms of patients, it can be divided into "Shang Xiao", "Zhong Xiao" and "Xia Xiao". The organs involved are the lung, spleen and kidney. The research topic of this article is diabetic myopathy. According to the theory that "the spleen governs the muscles", diabetic muscular atrophy is closely related to the physiological dysfunction of the spleen [5]. Referring to the close relationship between "sweetness" and "spleen" in the Yellow Emperor's Inner Classic, "If a person has a sweet taste in their mouth, what is the name of the disease? What is the combination of the two? This person must eat sweet and fatty foods frequently. Fat causes internal heat, and sweetness causes fullness in the middle. Therefore, the qi overflows upward and turns into Xiao Ke". The spleen, with its function of transporting and transforming, distributing the essence of the whole body, and opening to the mouth, has the characteristic of being sweet in taste, and thus becomes the key organ for treating Xiao Ke. According to the theory of mutual restraint of the five elements in traditional Chinese medicine, sour medicines are used to restrain the evil of sweet turbidity in the body. When the evil of sweet turbidity is removed, the internal heat has no way to be generated, and Xiao Ke will be relieved. Zhu Dezeng et al. [6] treated 60 diabetic patients based on the theory of "sourness overcomes sweetness," achieving a total effective rate of 86.7%; Zhang Puying [7] treated diabetes based on the theory of "sourness overcomes sweetness," with significant clinical efficacy.

The earliest record of Wumei Pill is found in Zhang Zhongjing's Treatise on Febrile and Miscellaneous Diseases in the Han Dynasty. Its composition is Wumei, dried ginger, ginseng, aconite, coptis, cinnamon twig, phellodendron bark, Sichuan pepper, asarum, and angelica. The whole formula combines attack and tonification, and balances cold and heat. It can be widely used in the treatment of various diseases in clinical practice. For example, Zhou Yan et al. [8] used modified Wumei Pill combined with esomeprazole magnesium enteric-coated tablets to significantly improve the clinical symptoms of patients with superficial gastritis. Tan Jun et al. [9] found that Wumei Pill can significantly inhibit the apoptosis of intestinal epithelial cells in rats with ulcerative colitis model, and can also inhibit the release of inflammatory factors and promote the repair of colonic mucosa. Huang Jiehua et al. [10] confirmed through clinical trials that Wumei Pill has a clear effect on reducing blood lipids, reducing blood sugar, and protecting pancreatic islet function. Modern research has shown that Wumei Pill can reduce the fasting blood glucose content of hyperglycemic mice induced by alloxan and has a certain repair effect on damaged pancreatic β cells [11]. Li Lan et al. [12] found in a clinical trial using modified Wumei Pill to treat moderate to severe diabetic peripheral neuropathy that Wumei Pill could improve nerve conduction velocity and alleviate symptoms and signs of qi in these patients. Zhang Xiaohuan et al. [13] found through animal experiments that Wumei Pill had definite efficacy in lowering blood sugar, blood lipids, and protecting the pancreas. Professor Liu Min of Guangzhou University of Traditional Chinese Medicine often used Wumei Pill in clinical practice to treat patients with diabetes due to spleen and kidney deficiency and cold, and heart and liver stagnation and heat syndrome, with significant efficacy [14].

This study referenced the highest effective dose of Wumei Pill for treating diabetes in previous literature [15] and observed its pharmacological effects on streptozotocin-induced type 1 diabetic myopathy model rats [16]. It was found that Wumei Pill significantly alleviated the degree of soleus muscle atrophy in the model rats and significantly reduced the expression of Fbx32 and MuRF-1, two muscle atrophy-related proteins, in the skeletal muscle tissue of the model rats.

Muscle atrophy is caused by the disruption of the balance between muscle anabolism and catabolism. When protein breakdown exceeds protein synthesis, it leads to the loss of muscle mass. Modern research shows that skeletal muscle atrophy caused by sepsis, cancer, burns, diabetes, etc., is mainly due to the ATP-dependent ubiquitin-proteasome pathway, which increases skeletal muscle protein breakdown [17-19]. Fbx32 and MuRF-1 are E3 ubiquitin ligases expressed in skeletal muscle [20], and studies have shown that increased expression of Fbx32 and MuRF-1 is closely related to muscle atrophy [21]. The severity of muscle atrophy can be determined by detecting the levels of Fbx32 and MuRF-1 in muscle tissue.

Based on the results of direct pathological sections and the concentration of microscopic muscle atrophy-related proteins, it can be clearly concluded that Wumei pill has a therapeutic effect on skeletal muscle atrophy caused by type 1 diabetes. It is speculated that its mechanism of action may be through affecting the ubiquitin-proteasome pathway to slow down muscle breakdown. This provides an experimental basis for studying the mechanism of action of Wumei pill in treating T1DM. However, the specific mechanism and dose-response relationship of Wumei pill in treating complications of T1DM still need further research.

References

[1] Ishida T, Iizuka M, et al. Juzentaihoto Suppresses Muscle Atrophy in Streptozotocin-Induced Diabetic Mice. Biol Pharm Bull. 2019; 42(7): 1128-1133.

[2] Molinaro R, Dauscher C. Complications resulting from uncontrolled diabetes. MLO Med Lab Obs. 2017; 49(2): 20-22.
[3] Monaco CMF, Perry CGR, et al. Diabetic Myopathy: current molecular understanding of this novel neuromuscular disorder. Curr Opin Neurol. 2017; 30(5): 545-552.
[4] Tong Xiaolin. A review of the progress in TCM understanding and research on diabetes[J]. Beijing Journal of Traditional Chinese Medicine, 2016, 35(06): 509-512.
[5] Zhong Wen, Xie Chunguang, Gao Hong, et al. A study on the correlation between spleen-based treatment of diabetes mellitus and diabetic muscular atrophy based on the principle of "spleen governs muscles" [J]. New Journal of Traditional Chinese Medicine, 2017, 49(01): 196-199.
[6] Zhu Dezeng, Gu Limin. Treatment of 60 cases of type II diabetes mellitus using the method of "sour overcoming sweet" [J]. Liaoning Journal of Traditional Chinese Medicine, 1998, (01): 25.
[7] Zhang Puying. Experience in treating diabetes mellitus mainly using the principle of "sour overcoming sweet" [J]. Fujian Journal of Traditional Chinese Medicine, 1991, (01): 25.
[8] Zhou Yan. Clinical study on the treatment of chronic superficial gastritis of mixed cold and heat type using modified Wumei Wan formula [D]. Nanjing University of Traditional Chinese Medicine, 2017.
[9] Tan Jun, Huang Guihua, Lin Zhuangyan, et al. Clinical and experimental research progress of Wumei Pill in the treatment of ulcerative colitis [J]. Electronic Journal of Modern Medicine and Health Research, 2022, 6(03): 131-134.
[10] Huang Jiehua, Deng Xiaomin, Chen Cong, et al. Clinical observation on the treatment of obese type 2 diabetes mellitus with Qi and Yin deficiency and phlegm and blood stasis syndrome by Wumei Pill combined with Bazhen Decoction [J]. Chinese Journal of Experimental Traditional Medical Formulae, 2019, 25(16): 56-60.
[11] Lu Jian, Li Ying, Wang Lingzhi, et al. Discussion on the mechanism of Wumei Pill in lowering blood sugar [J]. Journal of Traditional Chinese Medicine, 2005, (05): 892-893.
[12] Li Lan. Clinical efficacy study of Wumei Pill in the treatment of moderate to severe diabetic peripheral neuropathy [D]. Guangzhou University of Traditional Chinese Medicine, 2012.
[13] Zhang Xiaohuan, Hu Jianping, Li Ying. Study on the decomposition of Wumei Pill for the treatment of diabetes [J]. Chinese Journal of Experimental Traditional Medical Formulae, 2006, (09): 41-44.
[14] Wu Zhijuan, Liu Min. Liu Min's experience in treating diabetes with classical formulas [J]. Journal of Guangzhou University of Traditional Chinese Medicine, 2019, 36 (02): 281-284.
[15] Zhou Guopei, Wu Fan, et al. Effects of Wumei Pill on intestinal flora, inflammatory factors and short-chain fatty acids in type 2 diabetic rats [J]. Chinese Journal of Experimental Traditional Medical Formulae, 2020, 26 (10): 8-15.
[16] Shen Yafei, Xu Yancheng. Study on the establishment of streptozotocin-induced experimental diabetic rat model [J]. Journal of Practical Diagnosis and Treatment, 2005, (02): 79-80.
[17] Mitch WE, Medina R, Grieber S, et al. Metabolic acidosis stimulates muscle protein degradation by activating the adenosine triphosphate-dependent pathway involving ubiquitin and proteasomes. J Clin Invest. 1994; 93(5): 2127-2133.
[18] Price SR, Bailey JL, Wang X, et al. Muscle wasting in insulinopenic rats results from activation of the ATP-dependent, ubiquitin-proteasome proteolytic pathway by a mechanism including gene transcription. J Clin Invest. 1996; 98(8): 1703-1708.
[19] Wing SS, Goldberg AL. Glucocorticoids activate the ATP-ubiquitin-dependent proteolytic system in skeletal muscle during fasting. Am J Physiol. 1993; 264(4 Pt 1): E668-E676.
[20] Bodine SC, Latres E, Baumhueter S, et al. Identification of ubiquitin ligases required for skeletal muscle atrophy. Science. 2001; 294(5547): 1704-1708.
[21] Gumucio JP, Mendias CL. Atrogin-1, MuRF-1, and sarcopenia. Endocrine. 2013; 43(1): 12-21.