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ORIGINAL RESEARCH

Therapeutic Effects of *Laetiporus Sulphureus* Culture Broth on Diabetic Foot Ulcer in Rats

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Purpose: To investigate the therapeutic effect of the culture broth of *Laetiporus sulphureus* on diabetic foot ulcer rats.

Patients and Methods: The diabetic foot ulcer model was established by combined treatment of streptozotocin (STZ) and hindfoot injury in rats. The rats were divided into groups: control, model, low-dose, and high-dose of *Laetiporus sulphureus* culture broth. Blood sugar and wound area were measured. ELISA determined levels of TNF-α, IL-6, and VEGF. H&E staining observed damaged skin tissues.

Results: Compared to the model group, the culture broth of *Laetiporus sulphureus* could lower blood glucose levels and decrease the wound area. It could notably decrease the concentration of TNF-α and IL-6, inhibit the expression of inflammatory factors in skin tissues, enhances the expression of VEGF and accelerate wound healing.

Conclusion: The *Laetiporus sulphureus* culture broth can effectively facilitate wound healing of foot injury in diabetic rats and has a certain therapeutic effect on diabetic foot. The study explores an alternative therapy from natural sources to address a gap in diabetic foot ulcer management. It provides a foundation for further research on Laetiporus sulphureus as a novel treatment.

Keywords: diabetic foot ulcer, *Laetiporus sulphureus*, TNF-α, IL-6, VEGF

Introduction

With the improvement of people's living standard in recent years, the prevalence of diabetes mellitus in China has been continuously increasing, and the diabetic foot ulcer has changed a common disease. Diabetic foot ulcer is an intractable complication of diabetes, which is a peripheral arterial vasculopathy of the foot caused by hyperglycemia leading to co-pathology of the peripheral nerves and motor nerves of the foot. The development and progression of diabetic foot are influenced by multiple factors, among which peripheral neuropathy, peripheral arterial disease, and local tissue infection are the most critical contributors to the onset of diabetic foot ulcers.[1]

Metabolic dysfunction and neurovascular disorders often lead to peripheral neuropathy in diabetic patients. Peripheral neuropathy indirectly triggers diabetic foot ulcers by causing distal limb hypoperfusion and impaired nerve function. Studies have shown that approximately 70% of diabetic foot ulcers are associated with lower extremity vascular lesions.[2] Peripheral arterial sclerosis or stenosis can impair vascular function and slow blood flow, leading to limb ischemia and hypoxia. This severely affects the proportion and function of inflammatory cells at the wound site, delaying wound healing. The damage caused by peripheral neuropathy and peripheral arterial disease also makes diabetic patients highly susceptible to local infections, which is another major reason for the slow healing of diabetic foot ulcers.[3,4]

According to the epidemiological surveys, the incidence of diabetic foot in people over 50 years old in China has reached 8.1%, and the annual mortality rate of diabetic foot ulcer has reached 11%,[5] which is one of the main causes of death and disability related to diabetes mellitus, at the same time, it also brings a heavy economic burden to society and families.

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Laetiporus sulphureus, also known as sulfur-colored Polyporus vermilion variant, red sulfur fungus, chicken crest fungus, belongs to the Basidiomycota, Agaricomycetes, Polyporales, Laetiporus.[6] It is widely distributed in regions such as Heilongjiang, Hebei, Sichuan, Tibet, Xinjiang, Taiwan in China, and is a large, rare, edible and medicinal fungus. The fruiting bodies are edible when young to mature, with delicious flavor and rich nutrition, while the older ones become cheese-like, not edible but can be used as medicine.

It is warm in nature, sweet in flavor, with various effects such as regulating function, promoting health and boosting body's immunity.[7,8]

Laetiporus sulphureus are easy to cultivate, and are rich in ingredients such as polyunsaturated fatty acids, oxalic acid, and boric acid. The toxic components are extremely low, and they cannot even detect.[9,10] It is an important food ingredient in China and parts of Japan where have the consumption records. In addition, studies have shown that there are a large amount of antioxidant, antibacterial and antitumor polysaccharide and active protein components in sulfuric bacteria, which has very important medicinal value.[9,11,12] In particular, the antioxidant and anti-inflammatory properties of Sulfuric bacteria may play an extremely important role in reducing oxidative stress levels and decreasing the release of inflammatory factors in diabetic foot.

In this study, the diabetic rat model was constructed by injecting streptozotocin (STZ). After successful modeling, skin damage was formed by skin perforation to simulate diabetic foot injury.

The effect of the culture broth of Laetiporus sulphureus (ttys-997) on diabetic foot ulcer rats was studied in order to find more effective treatment plans for diabetic foot ulcer.

Materials and Methods

Instruments and Materials

Blood glucometer (Sinocare Inc Co., Ltd)., Multi-mode plate reader (Thermo Scientific), Paraffin slicer (Leica, Germany), Upright microscope (NIKON), Vertical autoclave sterilizer (Shanghai Shen'an Medical Instrument Factory), Ultrasonic crusher (Shanghai Ouhe Mechanical Equipment Co., Ltd)., Low-temperature and high-speed centrifuge (Eppendorf), Electronic analytical balance (OHAUS International Trading Shanghai Co., Ltd)., Electric thermostatic drying oven (Shanghai One Instrument Science Instrument Co., Ltd).

The culture broth of *Laetiporus sulphureus* (ttys-997 culture broth) (Shenyang New Totem Biotechnology Co., Ltd)., Streptozotocin (STZ), Rat tumor necrosis factor-α (TNF-α) ELISA detection kit, Rat interleukin-6 (IL-6) ELISA detection kit, Rat vascular endothelial growth factor (VEGF) ELISA detection kit (Shanghai Enzyme-linked Biotechnology Co., Ltd)., H&E staining kit (Wuhan Servicebio Technology Co., Ltd).

Animal Model Construction and Grouping

50 male Wistar rats, 180–220 g, SPF grade, were maintained in temperature-controlled (22 ± 2 °C) and humidity-controlled (45~65%) rooms on a 12-h light/dark cycle with free access to water and food. All efforts were made to minimize the number of animals used and their suffering In compliance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, all animal experiments were approved by the Institutional Animal Care and Use Committee of Shenyang Pharmaceutical University (License number: SYXK (Liao) 2018–0009; Quality certificate number: 210726211101402541; Laboratory animal ethical review No. SYPU-IACUC-c2021-11-11-204).

10 rats were randomly selected as the control group, while the remaining 40 rats were fasted (water allowed) for 12~18 h and received an intraperitoneal injection of 60 mg/kg STZ (Day 0). After injecting, the rats were given enough food, and the drinking water was replaced by 5% glucose solution for 48 h. 72 hours after STZ injection (Day 3), random blood glucose (RBG) was measured by the tail-breaking method using a blood glucometer. RBG ≥16.7 mmol/L was considered as preliminary success of modeling.[13,14] The successfully modeled rats were randomly divided into a model group and two dosage groups of low and high *Laetiporus sulphureus* culture broth (ttys-997).

Day7, all rats would be prepared diabetic foot model.[15–18]The rats were anesthetized, the surgical site was disinfected, and then a 5mm*7mm wound was created with a scalpel on the right hind foot of the rats to establish a diabetic foot model. Starting the day after modeling, the low-dose (10 mL/kg) and high-dose (40 mL/kg) groups of ttys-997 culture

broth were given the corresponding ttys-997 culture broth by gavage, while the control group was given saline by gavage at 40 mL/kg, continuously for 14 day.

Detection Indicators

Blood glucose and wound area were measured on Day 3, 7, 10, 13 and 15 after modeling.

The length of the wound is determined by measuring the longest part of the wound along its long axis with a ruler made of centimeters. Similarly, the width of the wound is defined as the widest part along the vertical direction of the long axis. The area of the wound is the length (cm) ✕ width (cm).[19]

Take part of the injured skin for tissue homogenization, TNF-α, IL-6, and VEGF were detected using enzyme-linked immunosorbent assay (ELISA).

Histological Analysis

H&E staining was performed to observe the condition of the injured skin tissue, which was used to evaluate the repair of diabetic foot wounds. The skin tissue was fixed in 4% paraformaldehyde, followed by paraffin embedding, sectioning, dewaxing, hydration, and H&E staining. The duration of hematoxylin and eosin (HE) staining was determined as follows: firstly, sections were stained with hematoxylin for a period of three to five minutes; secondly, they were subjected to eosin staining for 15 seconds; and finally, they were dehydrated and sealed. The samples were then observed under the microscope, and images were captured.

Statistical Analysis

All statistical analyses in this experiment were performed with SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA), and the experimental results were expressed as mean ± standard deviation (mean ± SD). Comparisons between the means of multiple groups were performed using one-way ANOVA to evaluate the overall variance differences, and LSD was used for multiple comparisons. For comparisons between two groups, the unpaired *t*-test was used. The inspection level was taken at $\alpha = 0.05$.

Results

Effect of Ttys-997 Culture Broth on Blood Glucose in Rats with Diabetic Foot Ulcer

The results are shown in Table 1 and Figure 1. The blood glucose level in the model group was significantly higher than that in the control group at each time point and was greater than 16.7 mmol/L, indicating that the diabetic rat model was successfully established. Compared with the model group, the blood glucose level of high-dose ttys-997 culture broth group reduced significantly at each time point, suggesting that the ttys-997 culture broth had the effect of reduce blood glucose.

Effect of Ttys-997 Culture Broth on Wound Healing of Diabetic Foot

From the experimental results, the wound area in the model group was significantly larger than in control group at each time point, indicating the wound healing was slow. The wound healing conditions of ttys-997 culture broth groups were better in the model group. The high-dose group demonstrating faster healing, and the wound area was significantly smaller than that in the model group at Day 3, 7, 10, 13 and 15. The above indicated that ttys-997 culture broth could promote the wound healing of diabetic foot. The results are shown in Figure 2.

Table 1 Effect of Ttys-997 Culture Broth on Blood Glucose in Rats with Diabetic Foot Ulcer (mmol/L)

Group	Day3	Day7	Day10	Day13	Day15
Control	7.18±1.11	6.03±1.13	7.23±1.36	7.27±1.43	5.81±0.70
Model	28.30±1.99$^{\#\#}$	23.13±4.17$^{\#}$	23.60±5.32$^{\#\#}$	22.19±3.57$^{\#\#}$	23.28±4.78$^{\#\#}$
Low dose	26.10±4.02	24.91±6.96	19.43±2.66*	18.80±3.61	19.83±1.54*
High dose	20.11±5.62**	14.23±4.10**	12.25±4.19**	12.33±6.34**	9.74±3.39**

Notes: $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs control group; *P<0.05, **P<0.01 vs model group. n=8.

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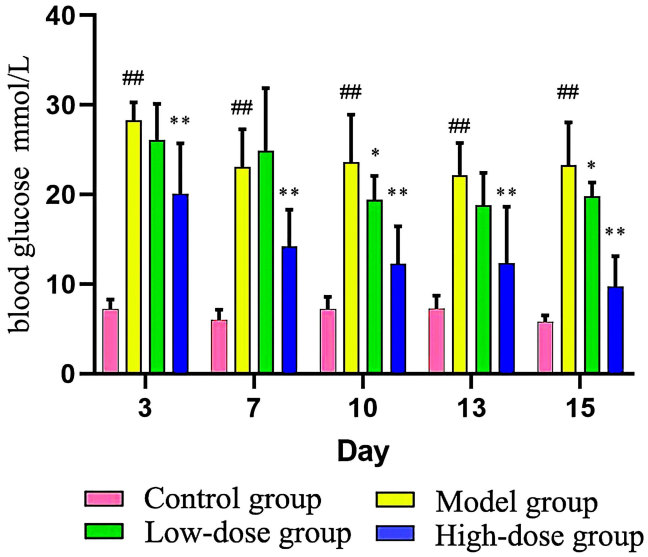

Figure 1 Effect of ttys-997 culture broth on blood glucose in rats with diabetic foot (mmol/L).
Notes: ##*P*<0.01 vs control group; **P*<0.05, ***P*<0.01 vs model group. n=8.

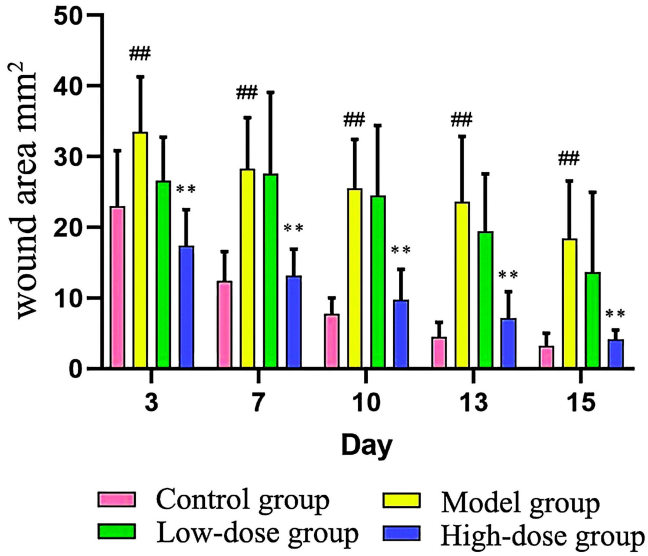

Figure 2 Effect of ttys-997 culture broth on wound healing of diabetic foot(unit: mm^2).
Notes: ##*P*<0.01 vs control group; ***P*<0.01 vs model group. n=8.

Histopathology of the Injured Skin

Pathological manifestations indicated that the repair of skin injury could be seen in each group. Except for the model group, recovery was better, with the new epidermal layer (black arrow), and the dermis layer was repaired by abundant proliferative connective tissue, fibroblasts had largely transformed into fibrocytes (yellow arrows), along with a number of newborn collagen fibers (red arrows) and neovascularization (blue arrows). In addition, the inflammatory reaction had subsided, with minimal lymphocyte infiltration observed (green arrows). In the control group, granuloma formation and multinucleated giant cells could be seen (orange arrows). The high-dose group showed better recovery relative to the low-dose group, and neonatal hair follicles were found in the dermis of the low-dose group (brown arrows). The model group recovered the worst, with scattered lymphocyte infiltration in multiple places (green arrows). The results are shown in Figure 3.

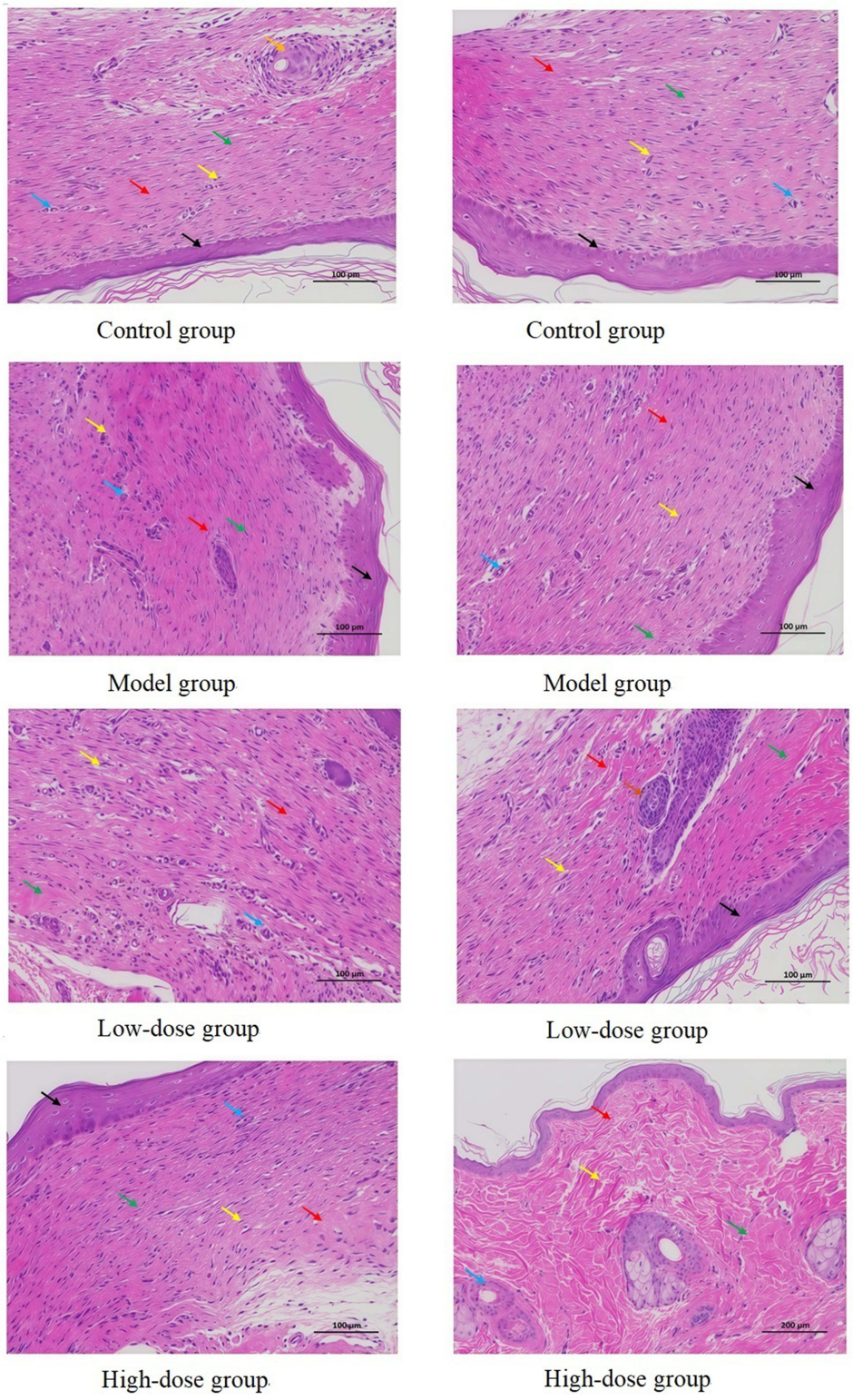

Figure 3 Histopathological changes of wound in each group of rats (HE, 200×) the new epidermal layer (black arrow); newborn collagen fibers (red arrows); fibrocytes (yellow arrows); neovascularization (blue arrows); lymphocyte infiltration (green arrows); multinucleated giant cells in the control group (Orange arrows); neonatal hair follicles in the low-dose group (brown arrows).

Effect of Ttys-997 Culture Broth on Skin TNF-α, IL-6 of Diabetic Foot Rat

The results are shown in Table 2. Compared with the model group, the high-dose group of ttys-997 culture broth significantly reduced the concentration of TNF-α in the skin. Compared with the control group, IL-6 concentration was

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Table 2 Effect of Ttys-997 Culture Broth on TNF-α, IL-6 in Foot Skin of Diabetic Rats

Group	TNF-α (pg/mg)	IL-6 (pg/mg)
Control	38.30±5.06	16.85±2.83
Model	48.05±5.17$^{\#}$	31.80±4.74$^{\#\#}$
Low dose	41.18±12.62	22.51±6.56**
High dose	32.81±4.81**	17.23±2.77**

Notes: $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs control group; **$P<0.01$ vs model group. n=8.

Table 3 Effect of Ttys-997 Culture Broth on VEGF Content in Foot Skin of Diabetic Rats

Group	Rat population	VEGF (pg/mg)
Control	8	40.78±9.89
Model	8	29.04±1.80$^{\#\#}$
Low dose	8	35.67±9.06
High dose	8	39.96±2.45**

Notes: $^{\#\#}P<0.01$ vs control group; **$P<0.01$ vs model group.

significantly higher in the model group. Both the low-dose and high-dose ttys-997 culture broth groups exhibited significantly lower IL-6 concentrations compared with the model group. The above showed that ttys-997 culture broth could inhibit the expression of inflammatory factors and promote wound healing by reducing inflammatory response.

Effect of Ttys-997 Culture Broth on VEGF Content in Foot Skin of Diabetic Rats

The content of VEGF in the model group was significantly lower compared with the control group. Compared with the model group, the high-dose group of ttys-997 culture broth was able to significantly elevate the content of VEGF. The above indicated that ttys-997 culture broth had the effect of promoting capillarogenesis. The results are shown in Table 3.

Discussion

Diabetic foot ulcer is not only one of the serious complications of diabetes mellitus, but also one of the risk factors for diabetes-related mortality and disability. Currently, there is no effective treatment for diabetic foot in modern medicine, which typically involves controlling blood glucose, providing nutritional support and administering anti-infective medications. This study, we constructed the diabetic rat model combined with skin injury to simulate the condition of diabetic foot. Streptozotocin (STZ) was used to destroy rat pancreatic β-cells, leading to insufficient insulin secretion and the formation of a sustained hyperglycaemic state to simulate diabetes mellitus. Then, an incision was made in the foot, and wound healing was delayed due to the inhibition of angiogenesis, collagen synthesis and immune repair function by hyperglycaemia, thus simulating the chronic and poorly healed characteristics of diabetic foot ulcers.The model was able to better reflect the chronic inflammation and repair obstacles of diabetic foot ulcers through pathological mechanisms (hyperglycaemia + healing obstacles).By orally administering the ttys-997 culture broth, it was found that ttys-997 culture broth could lowered the blood glucose level of rats. Measurement of the wound skin area indicated that the culture broth of *Laetiporus sulphureus* could accelerate wound healing. Meanwhile, the levels of relevant inflammatory factors (TNF-α, IL-6) in the broken skin tissue decreased dramatically, while the level of VEGF, which is associated with the growth of vascular endothelial cells, increased. This suggested that *Laetiporus sulphureus* could inhibit the expression of inflammatory factors, improve the inflammatory response, and promote capillary formation in wounds. The results of H&E staining also showed that *Laetiporus sulphureus* culture broth could accelerate the repair of the connective tissues and promote the wound healing.

Based on the experimental results we have obtained, We hypothesize that these active compounds in Laetiporus sulphureus might achieve this therapeutic effect by effectively regulating blood glucose concentration in the body, thereby helping to maintain more stable glucose levels. This, in turn, could contribute to the overall improvement in the condition of diabetic foot ulcers. Additionally, we observed that these ingredients exhibit strong anti-inflammatory properties, capable of suppressing the inflammatory response in the affected areas. By inhibiting the release of various inflammatory factors, these active components could help alleviate the inflammation and associated symptoms commonly seen in diabetic foot ulcers. This dual action of regulating blood glucose levels and mitigating inflammation could potentially make Laetiporus sulphureus a promising natural therapeutic agent for the management and treatment of diabetic foot ulcers, warranting further extensive research and clinical trials to fully explore and validate its potential.

Our research still has certain limitations. First, we have not yet identified the active ingredients in Laetiporus sulphureus. *Laetiporus sulphureus* is an important edible and medicinal fungus. Regular consumption can strengthen the body, and it has various medicinal effects such as anti-inflammatory and anti-cancer properties. The Tujia ethnic group in China has a long history of using Laetiporus sulphureus to treat colitis, rheumatism, and rheumatoid arthritis. Studies have shown that eburicoic acid isolated from Laetiporus sulphureus has significant anti-inflammatory effects and may be the key active ingredient in Laetiporus sulphureus.[20] Second, the current experiment lacks positive control drugs. The treatment of diabetic foot mainly focuses on symptomatic treatment. Comprehensive treatment methods such as anti-inflammatory drugs and hypoglycemic drugs are adopted according to the specific conditions of patients. Due to the lack of drugs with clear therapeutic effects, this experiment failed to obtain suitable positive control drugs. Subsequently, we will consider selecting appropriate drugs for comparison to further clarify the therapeutic effect of Laetiporus sulphureus.

Conclusion

In summary, the culture broth of *Laetiporus sulphureus* can decrease blood glucose, improve inflammatory response, and promote wound capillary formation. In addition, the high-dose group demonstrated higher glucose-lowering, inflammatory suppression, and tissue repair. Thereby it can effectively facilitate wound healing of foot injury in diabetic rats and has a certain therapeutic effect on diabetic foot. The study addresses a critical gap in diabetic foot ulcer management by exploring an alternative therapy derived from natural sources. The findings provide a foundation for further exploration into *Laetiporus sulphureus* as a novel treatment option.Further research will be conducted to explore the underlying mechanisms of action, laying the foundation for clinical application in the near future.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

All authors declare no conflict of interest.

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