Yidiyin, a Chinese Herbal Decoction, Improves Erectile Dysfunction in Diabetic Patients and Rats through the NO-cGMP Pathway

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The nitric-oxide (NO)-cyclic-guanosine-monophosphate (cGMP) pathway plays a key role in penile erection. Erectile dysfunction (ED) is a complication in male diabetic patients that impacts their quality of life. Recently, Yidiyin, a Chinese herbal decoction, is used to treat diabetic ED, but convincing evidence is lacking, and the potential mechanisms remain uncertain. In the study, diabetic ED patients had low scores on international index of erectile function-5 (IIEF-5), and administration of Yidiyin and hypoglycemic drugs for 16 weeks ameliorated patients’ scores on IIEF-5 more than the hypoglycemic drug alone. Moreover, streptozotocin-induced diabetes severely impaired rats’ erectile function and the activity of the NO-cGMP pathway in the corpora cavernosum, and treatment with Yidiyin for 4 weeks obviously increased the rats’ erectile function, remarkably enhanced the activity of nitric oxide synthase (NOS), and elevated the contents of NO and cGMP. Our findings indicate that Yidiyin improves diabetic ED probably by enhancing the NO-cGMP pathway.

Key words: diabetes; erectile dysfunction; international index of erectile function-5; nitric oxide synthase; traditional Chinese medicine

Erectile dysfunction (ED), defined as the consistent or recurrent inability to achieve or maintain penile erection sufficient for satisfactory sexual performance, is a common complication in male patients with diabetes. It has been reported that approximately 30–70% of diabetic men experience some form of ED, which is a much higher than for nondiabetic men.

Penile erection is a complex neurovascular process that requires both increased arterial inflow and restricted venous outflow, which is modulated by relaxation and contraction of the corpus cavernosum smooth muscle. This is regarded as the essence in the initiation and maintenance of normal erection. Substantial evidence indicates that nitric oxide (NO) plays a critical role in cavernosal smooth muscle relaxation and penile erection. In response to sexual stimulation, nitric oxide synthase (NOS) catalyzes l-arginine to generate NO. Then NO activates soluble guanylate cyclase (GC) in smooth muscle cells, which leads to the production of cyclic guanosine monophosphate (cGMP). Following the activation of protein kinases G (PKG) upon increased cGMP, actin-myosin system proteins and Ca$^{2+}$ channels are phosphorylated. These processes result in the decline of free cytoplasmatic Ca$^{2+}$ and ultimately relaxation of the cavernosal smooth muscle, triggering penile erection.

Previous reports have indicated that the development of ED originates mainly in dysfunction of the NO-cGMP pathway. Increasing evidence also indicates that diabetic ED also results from downregulation of the NO-cGMP signaling in the corpora cavernosum, including impaired NOS activity, low levels of NO and cGMP, and deficient activity of cGMP-dependent kinase. It is a remedy for diabetic ED to preserve or strengthen the activity of the NO-cGMP pathway. At present, inhibitors of phosphodiesterase type 5 (PDE5) are the most effective oral drugs. These include sildenafil, tadalafl, vardenafil, which arrest the breakdown of intracellular cGMP, which leads to relaxation of the cavernosal smooth muscle, thus arousing and sustaining erection. However, they also have side effects, including headache, cutaneous flushing, and even serious adverse events including chest pain, dyspnea, larynx edema, and asthma. Moreover, quite a number of diabetic ED patients and diabetic rats show a poor response to PDE5 inhibitor therapy. Hence the utilization of drugs is sharply limited, especially for patients with angiocardiopathy.

Chinese herbs have been used to treat diabetes for thousands of years with few side effects. Recently, Yidiyin, a Chinese herbal decoction, was used to treat diabetic ED, but there has been limited research on the formula and the potential mechanisms are not clear. In this study, we investigated the effect of Yidiyin on erectile function and explored the underlying mechanisms of Yidiyin in diabetic ED patients and rats.
Materials and Methods

Drugs. Yidiyin was obtained from a mixture of the following plants: the tuberous root of Rehmannia glutinosa Steud., the rhizome of Dioscorea opposita Thunb. (Dioscoreaceae), the stem tubers of Alisma orientalis (Sam.) Juzep. (Alismataceae), the stem leaf of Epimedium brevicornum Maxim. (Cyzonimortaceae), the stem leaf of Paonosia sulfurifrons Andrews (Paoniosiaceae), the rhizome of Salvia miltiorrhiza Bge. (Labiatae), the seed of Achyranthes bidentata Blume (Amaranthaceae), the seed of Cuscuta chinensis Lam. (Convolvulaceae), the tuberous root of Pseudostellaria heterophylla (Muq.) Pax (Caryophyllaceae), the tuberous root of Curcuma wenyujin Y.H. Chen and C. Ling (Zingiberaceae), and the dried fruit of Citrus medica L. (Rutaceae). All these herbs were authenticated by Professor Enpei Lu, Faculty of Pharmacy, Guangxi Traditional Chinese Medical University, China, based on their microscopic and macroscopic characteristics. The proportion of the 15 herbs in the decoction were 9.22, 6.91, 6.91, 4.61, 9.22, 4.61, 6.91, 6.91, 5.53, 9.22, 6.91, 6.91, 4.61, and 4.61 respectively. To ensure the therapeutic effects of the active ingredients of each herb, all the raw herbs were harvested in compliance with Good Agricultural Practices (GAPs) for Chinese Crude Drugs, and the processed herbs and the decoction were prepared in accordance with Good Manufacturing Practices (GMP) specific to Chinese herbal medicine. In a clinical trial, one treatment contained 200 mL of the herbal preparation (0.55 g herbs/mL), while in animal experiments, the herbal preparation was divided into two samples weighing 50 mg each, which were immediately frozen in liquid nitrogen. A sample was homogenized in 1 mL of distilled water. Then the supernatant was collected, the remaining sediment was washed twice with 75% ethanol and centrifuged at 3,000 × g for 15 min at 4 °C. Each supernatant was assayed for cGMP by radioimmunoassay with an RIA Kit (Shanghai Traditional Chinese Medical University, Shanghai, China) following the manufacturer’s manual. Each sample was tested in duplicate.

Erectile function evaluation. In clinical practice, IIEF-5 is not only a diagnostic tool for ED, but also a valid specific, sensitive appraisal of ED. IIEF-5 is comprised of five questions. Each question is scored on a five-point ordinal scale. Lower values represent poorer erectile function. Thus IIEF-5 scores range from 5 to 25, and a score above 21 is considered to indicate normal sexual function, and at or below this threshold, ED. A penis erectile experiment with apomorphine was used mainly to evaluate erectile function in animal experiment. Briefly, the room light was dimmed except for some indirect light sufficient for observation. After a 10-min habituation period, the rat was injected with apomorphine (80 μg/kg) subcutaneously in the loose skin at the back of neck. It was observed for 30 min to record frequency of erection. An erection was only counted when the emergence of an engorged glans penis and distal shaft was seen.

NOS activity and NO contents in penile crus tissues. In rats anesthetized by intraperitoneal injection of 25 mg/kg of pentobarbital, the rat penis was amputated. After careful removal of the penis skin, subcutaneous tissue, and penile bone, the corpora cavernosum was divided into two samples weighing 50 mg each, which were immediately frozen in liquid nitrogen. A sample was homogenized in 1 mL of distilled water. Then the supernatant was collected, followed by centrifugation at 3,000 × g for 15 min at 4 °C. Each supernatant was assayed for NOS and NO by colorimetry with an NOS assay kit and nitrate reductase with an NO assay kit respectively (Nanjing Research Institute of Jiancheng Bio-Engineering, Nanjing, China). Each sample was tested in duplicate.

Determination of cavernous cGMP. The other frozen sample was homogenized in 2 mL of cold sodium acetate buffer (50 mmol/L, pH 4.75), and cGMP was extracted by the following method: Each homogenized sample was mixed with 2 mL of dehydrated alcohol and then centrifuged at 3,000 × g for 15 min at 4 °C. After the supernatant was collected, the remaining sediment was washed twice with 75% ethanol and centrifuged, and the ethanol phase was maintained. The two supernatants were mixed and dried at 60 °C. Following redissolution, the samples were assayed for cGMP by radiomunnoassay with an RIA Kit (Shanghai Traditional Chinese Medical University, Shanghai, China) following the manufacturer’s manual. Each sample was tested in duplicate.

Statistical analysis. The results are presented as means ± SD. For clinical trials, the paired-sample t-test was performed to compare the difference within groups from the baseline. The independent-sample t-test and analysis of covariance (ANCOVA) with a model that included the baseline value of the dependent variable as covariate were also used for comparison between groups. For animal experiments, erection number and body weight data were determined by repeated measures analysis of variance (ANOVA). The indexes from corpora cavernosum were analyzed by one-way ANOVA, and ANCOVA between groups. All statistical analyses were processed through the Statistical Package for the Social Sciences (SPSS version 16.0 for Windows). p < 0.05 was considered to be statistically significant.
Table 1. Comparison of Clinical Characteristics between Two Groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Yidiyin</th>
<th>△p value</th>
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<tbody>
<tr>
<td>N</td>
<td>20</td>
<td>21</td>
<td></td>
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<tr>
<td>Age (year)</td>
<td>46.05 ± 8.79</td>
<td>47.29 ± 10.10</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Duration of DM (year)</td>
<td>6.90 ± 3.82</td>
<td>6.95 ± 3.40</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Duration of ED (year)</td>
<td>3.08 ± 2.60</td>
<td>3.44 ± 2.47</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Use of hypoglycemic agents</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Metformin (N/%)</td>
<td>15/75.00%</td>
<td>15/71.43%</td>
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<tr>
<td>Glitazone (N/%)</td>
<td>4/20.00%</td>
<td>5/23.81%</td>
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<tr>
<td>Novolol R (N/%)</td>
<td>1/5.00%</td>
<td>1/4.76%</td>
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<tr>
<td>SBP (mmHg)</td>
<td></td>
<td></td>
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<tr>
<td>Before</td>
<td>136.80 ± 10.45</td>
<td>137.52 ± 10.14</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>After</td>
<td>133.65 ± 8.52</td>
<td>134.62 ± 8.28</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>△p value</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
<td></td>
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<tr>
<td>DBP (mmHg)</td>
<td></td>
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<tr>
<td>Before</td>
<td>84.50 ± 5.27</td>
<td>84.38 ± 5.89</td>
<td>p &gt; 0.05</td>
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<tr>
<td>After</td>
<td>83.20 ± 6.00</td>
<td>82.12 ± 4.73</td>
<td>p &gt; 0.05</td>
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<tr>
<td>△p value</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
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<tr>
<td>FPG (mmol/L)</td>
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<tr>
<td>Before</td>
<td>7.56 ± 1.35</td>
<td>7.50 ± 1.26</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>After</td>
<td>5.98 ± 0.89</td>
<td>5.97 ± 0.79</td>
<td>p &gt; 0.05</td>
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<tr>
<td>△p value</td>
<td>p &gt; 0.01</td>
<td>p &lt; 0.01</td>
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<tr>
<td>HbA1c (%)</td>
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<tr>
<td>Before</td>
<td>7.15 ± 0.69</td>
<td>7.16 ± 0.66</td>
<td>p &gt; 0.05</td>
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<tr>
<td>After</td>
<td>6.11 ± 0.65</td>
<td>6.01 ± 0.66</td>
<td>p &gt; 0.05</td>
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<tr>
<td>△p value</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
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<tr>
<td>TSH (mIU/L)</td>
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<tr>
<td>Before</td>
<td>2.35 ± 1.07</td>
<td>2.56 ± 1.14</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>After</td>
<td>2.59 ± 1.22</td>
<td>2.45 ± 1.06</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>△p value</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
<td></td>
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<tr>
<td>T (nmol/L)</td>
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<tr>
<td>Before</td>
<td>18.73 ± 6.90</td>
<td>18.02 ± 5.97</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>After</td>
<td>19.13 ± 6.08</td>
<td>20.41 ± 7.31</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>△p value</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
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<tr>
<td>FSH (U/L)</td>
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<tr>
<td>Before</td>
<td>7.03 ± 3.16</td>
<td>7.13 ± 2.91</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>After</td>
<td>7.61 ± 3.45</td>
<td>7.80 ± 3.24</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>△p value</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
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<tr>
<td>LH (U/L)</td>
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<tr>
<td>Before</td>
<td>4.89 ± 1.72</td>
<td>4.87 ± 1.84</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>After</td>
<td>4.96 ± 1.90</td>
<td>5.02 ± 1.70</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>△p value</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
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<tr>
<td>AST (U/L)</td>
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<tr>
<td>Before</td>
<td>47.85 ± 13.43</td>
<td>46.14 ± 13.64</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>After</td>
<td>43.95 ± 9.31</td>
<td>42.62 ± 7.98</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>△p value</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
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△p value refers to comparison between before vs. after treatment within each group.

Table 2 shows rats’ erection numbers. Before the experiment, all the rats had normal erectile function, with no differences among the five groups (p > 0.05). Two weeks after STZ-induced diabetes (before treatment), erection numbers were visibly reduced in the model group, the insulin group, and the Yidiyin group as compared to before the experiment within each group (p < 0.01) without

Results

**Effects of Yidiyin on erectile function in diabetic patients with ED**

At baseline, there were no differences between the two groups in age, duration of diabetes and ED, blood pressure, including systolic blood pressure (SBP) and diastolic blood pressure (DBP), plasma glucose, including fasting plasma glucose (FPG) and postprandial plasma glucose (PPG), glycosylated hemoglobin (HbA1c), thyroid-stimulating hormone (TSH), testosterone (T), follicle stimulating hormone (FSH), luteinizing hormone (LH), the use of hypoglycemic agents (Table 1), or IIEF-5 scores (Fig. 1). Administration of Yidiyin and the hypoglycemic drug for 16 weeks significantly increased patients’ IIEF-5 scores, from 10.24 ± 3.33 to 19.43 ± 2.16 (p < 0.01), much higher than the hypoglycemic drug alone (19.43 ± 2.16 vs. 14.20 ± 2.14, p < 0.01). After treatment, although the levels of FPG, PPG and HbA1c obviously decreased as compared to before treatment (p < 0.01), there were no differences between the control group and the Yidiyin group in FPG (5.98 ± 0.89 vs. 5.97 ± 0.79), PPG (9.94 ± 1.45 vs. 9.79 ± 1.33), or HbA1c (6.11 ± 0.65 vs. 6.01 ± 0.66). After treatment, there were no differences between the two groups in TSH, T, FSH, or LH (all p > 0.05), and the indexes did not significantly decrease or increase than before treatment within each group (all p > 0.05).

**Safety of Yidiyin in clinical trial**

To ensure safety, blood counts and hepatic and renal function were measured at baseline and at the end of the trial. No severe side effects occurred, and no episode of hypoglycemia was reported by the patients. One patient in the Yidiyin group and two in the control group had slight diarrhea, but were improved after 3–7 d of treatment without dose change. One subject in the Yidiyin group suffered from abdominal distension, which was relieved after he started to take Yidiyin 4 times a day instead of twice a day (two treatments a day was not changed). There was no difference in frequency of side effects between the two groups.

**General data in the animal experiment**

Of 57 STZ-induced rats, 46 had blood glucose higher than 16.6 mmol/L with a increase in food and water intakes, hyperuresis, and a loss in weight as compared to the control group. After 2 weeks, 39 diabetic rats developed ED. During the experiment, six and seven rats died of infection or hyperglycemia in the model group and the Yidiyin group respectively.

**Effects of Yidiyin on erectile function in diabetic rats with ED**

Figure 2 shows rats’ erection numbers. Before the experiment, all the rats had normal erectile function, with no differences among the five groups (p > 0.05). Two weeks after STZ-induced diabetes (before treatment), erection numbers were visibly reduced in the model group, the insulin group, and the Yidiyin group as compared to before the experiment within each group (p < 0.01) without...
Diabetes was induced by STZ, those that had blood glucose levels higher than 16.6 mmol/L were regarded as diabetes, and the remainder were assigned to the STZ group. Diabetic ED rats were randomly divided into three groups based on different treatment for 4 weeks: the Yidiyin group, the insulin group, and the model group, receiving Yidiyin, insulin, and water respectively, and a control group and an STZ group receiving water. Erectile function was evaluated in a penile erectile experiment with apomorphine. *P < 0.01 vs. the control group or before the experiment within each group; **P < 0.01 vs. the model group or the insulin group after treatment.

Any differences among the model group, the insulin group, and the Yidiyin group (P > 0.05). Administration of Yidiyin for 4 weeks obviously increased erection numbers, from 0.75 ± 0.71 to 3.75 ± 0.71 (P < 0.01), much higher than in the model group (3.75 ± 0.71 vs. 0.78 ± 0.67, P < 0.01), similar to the control group (3.75 ± 0.71 vs. 3.50 ± 1.27, P > 0.05). Although insulin enhanced erection numbers, from 0.89 ± 0.78 to 1.22 ± 0.83, this difference was not statistically significant (P > 0.05). Compared to the control group, the STZ group showed no difference in erection numbers at the various stages (P > 0.05), and there was no statistical significance among the three stages (P > 0.05) in the STZ group.

Effects of Yidiyin on the NO-cGMP pathway in the corpora cavernosa of diabetic rats with ED

The activity of the NO-cGMP pathway in 5 groups is shown in Fig. 3. This study indicates that the activity of NOS and the contents of NO as well as cGMP in the model group decreased significantly, by 60.00, 45.27, 69.84% respectively, as compared to the control group (P < 0.01). Compared to the model group, administration of Yidiyin markedly enhanced the activity of NOS and the contents of NO as well as cGMP, by 100.00, 69.14, 125.03% respectively (P < 0.01 and P < 0.05). Even the levels of the three indexes in the Yidiyin group was similar to the control group (P > 0.05). Although insulin increased the activity of NOS and the content of NO as compared to the model group, the difference was not statistically significant (P > 0.05). There were no differences in the activity of NOS or the contents of NO or cGMP (P > 0.05) between the control group and the STZ group.

Discussion

In China and other Asian countries, it is very common to treat diabetic ED with Chinese herbs in Chinese
much higher than the hypoglycemic drug alone ($19$).

Epimedium brevicornum Maxim. and Cuscuta chinensis Lam.,22,23) and smooth muscle. 21,22) For example, neuronal action in medicine by activating the neuronal and blood systems philosophy of yin and yang in traditional Chinese

differentiation of signs and symptoms, and embodied the posed of 15 Chinese herbs, has been based on the

Yidiyin, a traditional Chinese medical decoction com-

convincing evidence is lacking. In clinical studies,

therapeutic outcomes with few side effects, but the

medical clinical practice, which has achieved beneficial therapeutic outcomes with few side effects, but the convincing evidence is lacking. In clinical studies, Yidiyin, a traditional Chinese medical decoction composed of 15 Chinese herbs, has been based on the differentiation of signs and symptoms, and embodied the philosophy of yin and yang in traditional Chinese medicine by activating the neuronal and blood systems and smooth muscle.21,22) For example, neuronal action in the hypothalamic-pituitary-adrenal axis is regulated by Epimedium brevicornum Maxim. and Cuscuta chinensis Lam.,22,23) Paeonia suffruticosa Andrews inhibits platelet aggregatory and blood coagulation,24) and Curcuma wenyujin Y.H. modulates the diastolic and contractile activity of isolated gastric muscle.25) After 16 weeks of treatment with Yidiyin and a hypoglycemic drug, IIEF-5 scores increased from $10.24 \pm 3.33$ to $19.43 \pm 2.16$, much higher than the hypoglycemic drug alone ($19.43 \pm 2.16$ vs. $14.20 \pm 2.14$), showing beneficial effects of the

Yidiyin on diabetic ED. As is well known, hyperglycemia and non-enzymatic glycation of proteins are two pathophysiological causes of diabetic ED.26,27) However, the improvement in the erectile function of the diabetic ED patients due to Yidiyin appeared to have nothing to do with blood glucose or HbA1c, because the combination of Yidiyin and hypoglycemic agent did not further decrease blood glucose or HbA1c as compared to the hypoglycemic drug alone. It is believed that blood pressure is strongly linked to ED,20) but the blood pressure in two groups was about 140/90 mmHg, excluding primary hypertension, and Yidiyin did not change blood pressure. Although some studies have found that there are significant associations between low levels of sexual hormone and diabetic ED,29,30) the patients in our study had normal hormone levels of T, FSH, LH, and TSH, and treatment with Yidiyin for 16 weeks did not alter the levels of these hormones. It is likely that Yidiyin improved diabetic ED by another way independent of blood glucose, blood pressure, and sexual hormones. Additionally, Yidiyin, as a safe drug, was not found to have severe side effects, such as chest pain, dyspnea, larynx edema, or asthma in alleviating diabetic patients’ ED, quite differently from inhibitors of PDE5.11,12)

Although the pathophysiological causes of diabetic ED are multifactorial, including hyperglycemia and non-enzymatic glycation of proteins, increased oxidative stress and production of reactive oxygen species, altered angiogenesis, dyslipidaemia, psychological stress, impaired endothelial- and neurogenic-regulated relaxation of penile smooth muscle,31$^-$33) the endothelial- and neurogenic-mediated relaxation of the penile smooth muscle has been identified to be crucial to the initiation and maintenance of normal erectile function. A growing body of evidence indicates that the erectile tissue of diabetic men and experimental type 1 and 2 diabetes animals manifests an inability to relax in response to the activation of nerves or the endothelium.10,31,34,35)

It is well known that normal erectile function is a hemodynamic neurovascular process involving relaxation of the corpus cavernosum. Although several vaso-dilators have been implicated in the erectile response, including vasoactive intestinal peptides, prostaglandin, and acetylcholine, the principal neurotransmitter for penile erection is NO, which is produced by the endothelium of the arteries of the penis and nitrenergic neurons with the help of endothelial and neuronal NOS in response to sexual stimulation. Upon its release, NO enters smooth muscle cells and activates soluble GC, which prompts guanosine triphosphate (GTP) to generate cGMP, a second messenger specifically controlled by PDE5 that promotes smooth muscle tone and terminates the erection. Increased cGMP activates substrate PKG which has a higher affinity for cGMP, and then alters the intracellular calcium levels and opens calcium-dependent potassium channels, leading to a reduced influx of Ca$^{2+}$ across the plasma membrane and reduced Ca$^{2+}$ sequestration by cellular organelles, including the sarcoplasmic reticulum. These processes result in the reduction of free cytoplasmic Ca$^{2+}$ and ultimately relaxation of the smooth muscle. It is generally accepted that the major pathogenic mechanism of ED is deficiency in the relaxation of the penile

![Fig. 4.](image-url) Effects of Yidiyin on Blood Glucose in Diabetic ED Rats. Diabetes was induced by STZ, those that had blood glucose levels higher than 16.6 mmol/L were regarded as diabetes, and the remainder were assigned to the STZ group. Diabetic ED rats were chosen from diabetic rats by erectile function evaluation, and were randomly divided into three groups based on different treatment for 4 weeks: the Yidiyin group, the insulin group, and the model group, receiving Yidiyin, insulin, and water respectively, and a control group and an STZ group receiving water. *$p < 0.05$, **$p < 0.01$ vs. the control group.

![Fig. 5.](image-url) Effects of Yidiyin on Body Weight in Diabetic ED Rats. Diabetes was induced by STZ, those that had blood glucose levels higher than 16.6 mmol/L were regarded as diabetes, and the remainder were assigned to the STZ group. Diabetic ED rats were chosen from diabetic rats by erectile function evaluation, and were randomly divided into three groups based on different treatment for 4 weeks: the Yidiyin group, the insulin group, and the model group, receiving Yidiyin, insulin, and water respectively, and a control group and an STZ group receiving water. Body weight decreased significantly in the three diabetic ED groups as compared to the control group before treatment. Administration of Yidiyin for 4 weeks obviously increased body weight as compared to the model group. *$p < 0.01$ vs. the control group; **$p < 0.05$ vs. the model group.
smooth muscle caused by an impaired NO-cGMP pathway. Studies on corpus cavernosum tissue from type 1 and type 2 diabetic patients and animals have also disclosed low activity of NOS, diminished NO-mediated function, impaired GC, reduced cGMP-generating capacity, and deficient PKG. Administration of Yidiyin thus was responsible for the impaired erectile function, as well as cGMP in the corpus cavernosum tissue, and decrease in the activity of NOS and the contents of NO.

According to reports, some herbs, including *Rehmannia glutinosa* Steud., *Epimedium brevicornum* Maxim., and *Cynomorium songaricum* Rupr. in the decoction, regulate the metabolism of NO and the penile intracavernous pressure of rats. Hence we inferred that Yidiyin has positive effects on the pathway of NO-cGMP. In order to confirm this, we designed an animal experiment. STZ-induced diabetic ED rats showed a decrease in the activity of NOS and the contents of NO as well as cGMP in the corpus cavernosum tissue, and thus was responsible for the impaired erectile function, consistently with the literature. Administration of Yidiyin increased diabetic ED rats’ erection numbers, enhanced NOS activity, and boosted the contents of NO and cGMP in the corpus cavernosum penis, and the indexes in the Yidiyin group were similar to that in the control group. In sum, increased NOS activity led to elevated NO levels, which resulted in increases in the content of cGMP, causing relaxation of the smooth muscle and triggering penile erection. Recent studies reported that *Epimedium brevicornum* Maxim. and extract of it increased the expression and the activity of NOS in the corpora cavernosum, and acted as a non-selective PDE5 inhibitor, and *Cascetta chinensis* Lam. also potentiated NOS expression and increased the contents of NO.

In a word, Yidiyin probably ameliorated diabetic ED through the activation of multiple targets in the NO-cGMP signaling. This calls for further study. The present study also indicates that Yidiyin led to a mild reduction in blood glucose, but there was no statistical significance as compared to the model group. These results suggest that Yidiyin improved ED caused by diabetes, probably through elevating the NO-cGMP pathway, which has little relationship with blood glucose.

Because treatment with the hypoglycemic drug for 16 weeks also improved patients’ IIEF-5 scores, we used insulin (Glargine) as control in the animal experiment. The results revealed that insulin obviously decreased the blood glucose of the diabetic ED rats, but did not significantly increase erection numbers, which conflicts with our clinical trial. Some reports have indicated that treatment with insulin for 16 weeks can ameliorate the erectile function of diabetic rats by decreasing HbA1C and inhibiting cell apoptosis. Allowing for the short course of treatment and the improvement to some extent in erection numbers and the activity of the NO-cGMP pathway, we speculate that insulin can improve diabetic ED as long as adequate treatment dose in animals.

Our study also indicates that Yidiyin prevented loss of body weight in the diabetic rats. We infer that weight gain had some relationship with the improvement in insulin resistance due to Yidiyin, because insulin resistance also exists type 1 diabetes, and Yidiyin reduced blood glucose to some extent, although there was no statistical significance.

We designed a placebo for Yidiyin as a control trial. As a Chinese herbal decoction, however, Yidiyin has a unique bitter flavor that is hard to replicate. Hence we had to give up on a placebo for Yidiyin, as is common in the study of the decoctions in traditional Chinese medicine. Additionally, we did not design an inhibitor of PDE5 for Yidiyin as a control trial in the animal experiment either, because the drug is not completely effective for diabetic ED and was not chosen as a treatment for human patients in our clinical trial. In view of insulin deficiency in the diabetic ED rats, we also used insulin (Glargine) as a substitute for Metformin or Gliquidone.

In summary, the present results indicate that Yidiyin can improve erectile dysfunction in diabetes, probably by enhancing the NO-cGMP pathway, suggesting potential uses of Yidiyin, or compounds derived from, against diabetic ED. Further studies are required to identify the active constituents of Yidiyin.

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