Hypoglycaemic effects of fermented mycelium of *Paecilomyces farinosus* (G30801) on high-fat fed rats with streptozotocin-induced diabetes


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**Background & objectives:** *Paecilomyces farinosus* is an entomogenous fungus with a powerful insecticidal activity against the larvae of Lipidoptera, Coleoptera and Hymenoptera. However, the hypoglycaemic activity of *P. farinosus* extract has not been studied. This study was undertaken to investigate the hypoglycaemic and anti-diabetic effects of *P. farinosus* (G30801) in rats with streptozotocin (STZ)-induced diabetes given a high-fat and compared with normal rats.

**Methods:** Rats fed with high fat diet for 2 months and injected with (30 or 50 mg STZ/kg bw) showed raised level of plasma triglyceride (TG), cholesterol, D-glucose concentration and glycosylated haemoglobin (HbA1C) %. The STZ-induced type 1 (T1DM) and type 2 diabetes (T2DM) in rats was further confirmed using glucose tolerance test and insulin-glucose tolerance test. *P. farinosus* (G30801) was fermented in different media [soybean (S), black bean (B), and rice (R)] and their extracts were tested for hypoglycaemic effect using T1DM and T2DM rats.

**Results:** STZ (30 and 50 mg/kg bw) could successfully induce T2DM and T1DM in rats, respectively. No change in blood glucose levels were noted in *P. farinosus* (R medium) treated normal rats (*P* < 0.05). In addition, STZ-high fat fed diabetic (T1DM and T2DM) rats when treated with *P. farinosus* (R medium) showed decreased blood glucose level as compared with *P. farinosus* extracted from B and S medium.

**Interpretation & conclusions:** Our findings showed hypoglycaemic effect of fermented *P. farinosus* (G30801) in experimental diabetes rat model fed with high fat diet.

**Key words** Diabetes - high-fat - hypoglycaemia - insulin - *Paecilomyces farinosus* - streptozotocin

It is estimated that 143 million people in the world live with diabetes and this number will probably double by the year 2030¹. Type 2 diabetes mellitus (T2DM) currently affects more than 200 million individuals worldwide². The characteristic features of T2DM include hyperglycaemia, near normal insulin levels, varying degrees of insulin resistance, slightly raised levels of glucagon and almost no ketoacidosis³.
Coronary artery disease (CAD) is markedly increased in subjects with T2DM; dyslipidemia in T2DM is often characterized by increased triglycerides, small, dense low-density lipoprotein (LDL), and low concentrations of high-density lipoprotein (HDL) cholesterol. Increased LDL and decreased HDL cholesterol have been shown to associate with the development of CAD in T2DM. Management of these complications represents a huge financial burden. Investigations with oral anti-hyperglycaemic agents derived from plants used in traditional medicine have shown these plants with good antidiabetic activity. Conventional drugs used have rigid and multiple dosing regimen, high-cost, and untoward effects. There are more than 1200 plant species broadly used in the treatment of DM and many of these showed effective hypoglycaemic activity after laboratory testing.

P. farinosus an entomogenous fungus, is a powerful insecticidal against the larvae of Lipidoptera, Coleoptera and Hymenoptera. Bioactive metabolites of P. farinosus have been investigated for antioxidant and anti-tumour properties. Studies on hypoglycaemic activity using P. farinosus polysaccharides are not yet reported. Thus the present study was undertaken to study the hypoglycaemic effect of P. farinosus extract from various fermented medium [soybean (S), black bean (B), and rice (R)] in rats with streptozotocin-induced diabetes and fed with high fat diet.

### Material & Methods

The study was carried out at Department of Life Science, National Dong-Hwa University, Hualien, Taiwan.

**Microorganism and media:** P. farinosus G30801 was kindly provided by Prof. Lee Son-Tay (Department of Biotechnology, Southern Taiwan University of Technology). The voucher specimens were deposited at the culture collection laboratory of Department of Biotechnology, Southern Taiwan University of Technology, Tainan County, Taiwan. P. farinosus G30801 was initially grown on potato dextrose agar (PDA) medium in a petri dish at 17°C, and then transferred to 250 ml flasks containing 100 ml of seed culture potato dextrose agar broth (PDB) medium and incubated on a rotary shaker (100 rpm) at 17°C for 7 days. Soybean (S), black bean (B), and rice (R) were evaluated for their potential as the major solid substrate in solid-state fermentation (SSF), respectively. Beans were soaked in 2-3 volume of water at room temperature for 4 h. Rice was boiled in water at 100°C. SSF was carried out by taking 300 g of the treated solid substrate in 1000 ml wide-mouth plastic bottle, moistening with liquid media solution containing 2 per cent peptone, 0.1 per cent KH₂PO₄ dissolved in distilled water. All plastic bottles were autoclaved at 121°C for 30 min and after cooling were inoculated with 10 ml of seed culture and incubated at 17°C for 20 days. The fermented product by SSF was freeze-dried (VirTis apparatus; Gardiner, NY, USA) and then ground into flour as the tested sample. The fermented products cultured from soybean, black bean and rice were named as S, B, and R powder, respectively.

**Quantification of crude water-soluble polysaccharides:** The fermented product of P. farinosus by solid-state fermentation was extracted with water (100°C, 4 h). The supernatant was obtained by centrifuging at 5000 xg to separate the solid from the crude extract. The supernatant were extracted by Sevag method to remove the dissociative protein. The polysaccharide content was taken as a index component for the quality control of batch fermentation. The polysaccharide contents of fermented products cultured from soybean (S), black bean (B), and rice (R) bore a resemblance to the original one (data not shown).

**Experimental animals:** Wistar rats (n=70 body weight 250 ± 20 g at 8 wk old) were obtained from National Laboratory Animal Center, Taipei, Taiwan. The rats were raised under a 12 h light/dark cycle and had free access to food and water and maintained on a standard laboratory diet (carbohydrates; 30%, proteins; 22%, lipids; 12%, vitamins; 3%) ad libitum. The protocol of animal care was recognized and approved from the Animal Ethics Committee of the institution.

**Induction of diabetes in rats:** Rats (n=40) were fed a high fat diet for 2 months and diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ, 30 or 50 mg/kg bw) (Sigma, St. Louis, Mo, USA) in 0.1 M citrate buffer (pH 4.5) to overnight fasted rats (modified from Zhang et al. via various dosage for treating different types DM). After 2 wk of STZ administration, animals with fasting blood glucose levels >200 mg/dl were considered diabetic and included in this study.
Body weights were determined gravimetrically, food and water intakes were recorded at 2 days and 2 wk following STZ or vehicle injection (saline), respectively. Blood glucose levels were determined using an automatic analyzer-Glucometer Elite XL (Bayer Incorporation, Toronto, Ont., Canada) with glucose oxidase/potassium ferricyanide reagent strips. Following plasma separation, an aliquot was taken for measuring triglyceride, cholesterol (CHOL), HDL, LDL, HbA1c by blood auto analyzer (Sysmex SF-3000, Sysmex Corp; Kobe, Japan).

**Experimental design**

Glucose tolerance test - Two weeks after STZ injection, rats were administrated (ip) 0.5 g glucose / kg bw after 15 h fasting. At approximately 0, 30, 60, 90 and 120 min following glucose injection, blood was sampled by venipuncture from the caudal vein for determining blood glucose.

Insulin-glucose tolerance test - To determine the response of the diabetic rats to insulin action, they were injected with 0.5 g glucose /kg bw, immediately followed by insulin (25.2 USP unit/mg, Sigma, MO, USA) at a dose of 0.2 U/kg bw. At approximately 0, 30, 60, 90 and 120 min following insulin injection, blood was sampled by venipuncture from the caudal vein and the percentage changes in blood glucose were calculated for each group. The STZ-induced diabetic rats were followed glucose tolerance test and insulin-glucose tolerance test and further defined as T1DM and T2DM based on amount of changed plasma glucose.

Assessment anti-diabetic activity of *P. farinosus* extract from various fermented medium in T1DM and T2DM rats - Two weeks after glucose tolerance and glucose-insulin tolerance tests, normal (n=30), T1DM (n=15) and T2DM (n=15) rats were fasted for 15 h and tested for blood glucose. Rat was orally given tested sample (R powder, S powder or B powder, 0.06 g/kg bw in water) and tested for blood glucose. After 30 min, rats were administrated (ip) 0.5 g glucose/kg bw. At approximately 0, 30, 60, 90 and 120 min following glucose injection, blood was sampled by venipuncture from the caudal vein for determining glucose. The percentage changes of glucose were calculated for each group.

**Statistical analysis:** The control and treatment groups were compared by one-way ANOVA after performing the Duncan multiple range tests.

### Results

Rats fed with high fat diet showed increased body weight, CHOL, plasma TG levels (Table I) as compared to the normal rats (*P*<0.05). From the lipid profile it was evident that high fat fed rats showed decreased LDL-C, and increased HDL-C levels as compared with the control rats.

High-fat fed rat injected with STZ (30 and 50 mg/kg bw) were found to have (*P*<0.05) high TG levels in blood plasma. Contrary, high-fat fed rat with STZ induction (50 mg/kg bw) showed the significantly (*P*<0.05) higher percentage of HbA1c than that of the normal group (Table II). As compared with normal rat, high-fat fed rats showed significant difference (*P*<0.05) in the plasma total cholesterol.

To confirm the success of STZ-induced diabetes, rats were checked for glucose tolerance and glucose-insulin tolerance tests, respectively, at 2 wk following STZ administration. STZ-injected animals had blood glucose exceeding 200 mg/dl, compared to a normal range of between 50 and 135 mg/dl following glucose

#### Table I. Body weight and blood biochemistry in normal (n= 30) and high-fat fed rats (n=40) before STZ induction

<table>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>CHOL (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-fat fed rats (n= 40)</td>
<td>463.92 ± 32.01**</td>
<td>69.81 ± 9.80*</td>
<td>78.13 ± 26.07*</td>
<td>51.18 ± 9.92</td>
<td>5.16 ± 1.72**</td>
</tr>
<tr>
<td>Normal rats (n= 30)</td>
<td>357.50 ± 20.07</td>
<td>55.00 ± 6.49</td>
<td>37.25 ± 2.57</td>
<td>45.00 ± 4.69</td>
<td>8.30 ± 3.87</td>
</tr>
</tbody>
</table>

Values are mean ± SE. *P*< values*<0.05; **<0.01; *+<0.005 as compared to the normal rats

#### Table II. Blood biochemical parameters in normal, type I diabetic-high dosage and type II diabetic-low dosage STZ induced rats

<table>
<thead>
<tr>
<th></th>
<th>CHOL (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I diabetic rats (n=15)</td>
<td>67.30 ± 6.42</td>
<td>143.00 ± 32.41</td>
<td>51.33 ± 4.50*</td>
<td>6.00 ± 3.00</td>
<td>8.10 ± 0.65</td>
</tr>
<tr>
<td>Type II diabetic rats (n=15)</td>
<td>75.27 ± 19.08</td>
<td>124.68 ± 38.55</td>
<td>56.66 ± 16.50</td>
<td>6.00 ± 3.46</td>
<td>8.33 ± 1.06</td>
</tr>
<tr>
<td>Normal Rats (n=30)</td>
<td>56.33 ± 7.23</td>
<td>32.00 ± 5.56</td>
<td>46.00 ± 5.29</td>
<td>6.33 ± 2.88</td>
<td>4.16 ± 0.15</td>
</tr>
</tbody>
</table>

High and low dosages of streptozotocin (STZ, 50 and 30 mg/kg bw) were used in this experiment

Values are mean ± SE. *P*<0.05 presents the significantly different levels between type I and type II diabetic rats
injection at approximately 60 min in relation to 0 min (Fig. 1). Further, insulin-glucose tolerance test demonstrated that high-fat and STZ-induced rats had higher glucose level following insulin administration (Fig. 2).

To further investigate the hypoglycaemic effects of *P. farinosus* (G30801) fermented from various media (R, S and B) on diabetic rats, 30 min before glucose administration rats were treated with R, S or B extracts. Single oral administration of R, S or B extracts of *P. farinosus* (G30801) failed to alter the blood sugar in the normal rats (*P*<0.05; Fig. 3). Compared to the normal rats, *P. farinosus* extract from rice powder showed most significant hypoglycaemic effect at 120 min in T2DM (*P*<0.01) and in T1DM (*P*<0.05) rats, respectively (Fig. 4 and 5).

![Fig. 1](image1.png)  
*Fig. 1.* Glucose tolerance test in normal (n=30), type I and II diabetic (n=15, each group) rats. The diabetic animals had blood glucose exceeding 200 mg/dl, compared to a normal range of between 50 and 135 mg/dl following glucose injection at approximately 60 min in relation to 0 min.

![Fig. 2](image2.png)  
*Fig. 2.* Insulin-glucose tolerance test in normal (n=30), type I and II diabetic rats (n=15, each group). Insulin-glucose tolerance test demonstrated that high-fat and STZ-induced rats had higher glucose level following insulin administration.

![Fig. 3](image3.png)  
*Fig. 3.* Hypoglycaemic effects of various medium fermented *P. farinosus* (G30801) in normal rats. To investigate the hypoglycaemic effects of *P. farinosus* (G30801) on normal rats, rats were administered with R, S or B powder at 30 min before glucose. Data present as the changed amount of plasma glucose subtracted with the value of 0 min. Sample size is n=5 for each group. *P* <0.05; ** <0.01 and *** <0.005 present the significantly different level as compared to the glucose group.

![Fig. 4](image4.png)  
*Fig. 4.* Hypoglycaemic effects of various medium fermented *P. farinosus* (G30801) in T2DM rats. To investigate the hypoglycaemic effects of *P. farinosus* (G30801) on T2DM rats, rats were administered with R, S or B powder at 30 min before glucose. Data present as the changed amount of plasma glucose subtracted with the value of 0 min. Sample size is n=5 for each group. *P* <0.05 and ** <0.01 present the significantly different level as compared to the glucose group.
M. Projection of diabetes burden through 2050: significantly

**<0.01 present the significantly different level as compared to the value of 0 min. Sample size is n=5 for each group. **

**<0.05 and **<0.01 present the significantly different level as compared to the glucose group.

**Fig. 5.** Hypoglycaemic effects of various medium fermented P. farinosus (G30801) in T1DM rats. To investigate the hypoglycaemic effects of P. farinosus (G30801) on T1DM rats, rats were administered with R, S or B powder at 30 min before glucose. Data present as the changed amount of plasma glucose subtracted with the value of 0 min. Sample size is n=5 for each group. P<0.05 and **P<0.01 present the significantly different level as compared to the glucose group.

**Discussion**

The aim of the current work was to assess hypoglycaemic effect of P. farinosus in experimental diabetes model. Due to the high prevalence of diabetes worldwide, extensive research is still being performed to develop new antidiabetic agents and determine their mechanisms of action, consequently, a number of diabetic animal models have been developed and improved over the years.

Streptozotocin-induced diabetic rats are one of the animal models of human insulin-dependent diabetes mellitus. As in human type 2 DM, diet has a great influence on the development of overt diabetes as well as hypertension, hyperlipidaemia, and eventually nephropathy in experimental models. In the present study, rats were fed with high-fat diet and then induced with both low/high dose of STZ; high-fat diet fed rats showed higher plasma TG, HDL-C, and lower LDL-C concentration compared to normal rats. Similar phenomenon was previously reported. The clinical symptoms of T2DM rats are closer to those of diet and obesity related diabetes. STZ treatment induces weight loss related to diabetes severity. The reduction of body weight might be due to the low utilization of uptake blood sugar in cell.

Hyperlipidaemia has been reported to accompany hyperglycaemia states, high levels of TC; importantly LDL cholesterol is one of the major coronary risk factors which is the major cause of morbidity and deaths in diabetic subjects. A significance difference (P<0.05) in the plasma total cholesterol was observed in STZ induced diabetes rats as compared with the untreated group. The present experimental data demonstrate that a high-fat diet rat can successfully induce T2DM and T1DM via low/high dose STZ administration.

Some substances express anti-diabetic property by influencing cells to stimulate insulin secretion and restore insulin sensitivity. In our study, treatment with P. farinosus significantly reduced fasting blood glucose levels in high-fat fed/ (50 mg/kg bw) STZ-induced T1DM rats. Kiho et al demonstrated that the cultured mycelium of Cordyceps sinensis had hypoglycaemic activity and lowered plasma glucose concentration in normal, STZ-induced diabetic mice and epinephrine-induced hyperglycaemic mice. Total flavonoids of Polygonatum odoratum significantly reduced fasting blood glucose levels in STZ-induced T1DM mice and alloxan-induced T2DM rats. The hypoglycaemic mechanism of P. farinosus aqueous extract remains unclear and further studies are required to elucidate site(s), cellular and molecular mechanisms of P. farinosus extract. Our results demonstrate that fermented mycelia of P. farinosus (G30801) possess the hypoglycaemic effect in an experimental diabetes model and can be further evaluated for its use in alternative system of medicine.

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**References**


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