Original Article

Oyster mushroom functions as an anti-hyperglycaemic through phosphorylation of AMPK and increased expression of GLUT4 in type 2 diabetic model rats

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Abstract

Objective: Traditionally, mushrooms have been used to reduce hyperglycaemia. However, the mechanism underlying this effect has not yet been explored. AMP-activated protein kinase (AMPK) is known to reduce hyperglycaemia through an insulin-independent pathway. This study aimed to observe the effect of oyster mushroom powder (OMP) on phosphorylation of AMPK (p-AMPK) and expression of GLUT4 mRNA in diabetic model rats.

Methods: Long-Evans rats were used to develop type 2 diabetic model rats through intraperitoneal induction of streptozotocin. The rats were then treated with oyster mushroom powder for 2 weeks. The effects on p-AMPK and GLUT4 mRNA expression were observed.

Results: The treatment with OMP significantly increased the levels of p-AMPK and GLUT4 mRNA expression in diabetic model rats.

Conclusion: Oyster mushroom can be used as an anti-hyperglycaemic agent through the activation of AMPK and increased expression of GLUT4.

Key words: Oyster mushroom, AMPK, GLUT4, Type 2 diabetes

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Introduction

Type 2 diabetes is a complex and heterogeneous disorder, which is characterized by impaired insulin sensitivity or decreased insulin secretion and is diagnosed as hyperglycaemia.1 A calorie-rich diet, obesity, and a sedentary lifestyle contribute to the rising number of individuals with type 2 diabetes worldwide.2 Insulin resistance and pancreatic β cell failure are defining metabolic parameters of type 2 diabetes.3 Moreover, in the majority cases, type 2 diabetes arises due to obesity and insulin resistance.4,5 However, type 2 diabetes is a chronic disease and leads to serious health complications.6 Therefore, in developed and developing countries, type 2 diabetes poses a major health threat.7 The prevalence and complications of type 2 diabetes are aggravating every day. Furthermore, the use of conventional, pharmacological, anti-diabetic drugs can sometimes increase the treatment complexity, due to drug side effects and high costs.8 Accordingly, natural products are alternatives, because these compounds are believed to have fewer side effects.

Mushrooms have been used as food and medicine for thousands of years. The mushroom serves as a natural source of medicine with antidiabetic potential.9 Oyster mushrooms (Pleurotus ostreatus) possess many valuable food qualities, e.g., low in calories, fats, and essential fatty acids, but rich in proteins, vitamins, and minerals.10,11 The oyster mushroom has a promising hypoglycaemic potential in an animal model.12 Although the acute and chronic, oral hypoglycaemic potential of the oyster mushroom has been already established in an animal model, the cellular mechanism is still unknown.

In mammals, AMP-activated protein kinase (AMPK) is a heteromeric enzyme complex, which is activated by phosphorylation of threonine 172, due to a variety of metabolic stressors.13 AMPK is activated in response to low levels of ATP, which results in an increase in the AMP:ATP ratio and also changes the cellular redox potential, resulting in a rise in the NAD/NADH ratio.13,14 In peripheral tissues, AMPK maintains a number of metabolic processes, such as glucose and lipid metabolism.14 Moreover, AMPK serves as a fuel gauge that responds to fluctuations in cellular energy levels and extracellular nutrient levels, such as glucose, hormones, and fatty acids. AMPK plays an important role in regulating whole body energy metabolism by responding to circulating hormones and by circulating the food intake.13

The glucose transporter 4 plays a key role in transporting extracellular glucose into insulin sensitive muscles and adipose tissues in vivo. Besides, skeletal muscles and adipose tissues are responsible for up to 50–80% of glucose transport in the body. GLUT4 expression in the skeletal muscle and adipose tissues of type 2 diabetic patients is significantly reduced, indicating that such patients have a lower capability to transport glucose.15 Therefore, the aim of this study was to observe the phosphorylation of AMPK and the expression of GLUT4 mRNA in mushroom-treated type 2 diabetic model rats.

Materials and Methods

Animals

Adult Long-Evans rats, weighing 170–220 g, were used in this study. The animals were bred at the Bangladesh University of Health Sciences animal house, in Dhaka, Bangladesh, and maintained at a constant room temperature of 22 °C, with a humidity of 40–70% and a natural 12 h day–night cycle. The experiment was conducted according to the ethical guidelines, approved by the Bangladesh University of Health Sciences. Type 2 diabetic model rats were created by a single intraperitoneal injection of streptozotocin (STZ) in citrate buffer (pH 4.5), at a dose of 90 mg/kg of the body weight, into rat pups (48 h old; average weight: 7 g).16,17 After 3 months, the STZ-injected rats were examined for their blood glucose level by an oral glucose tolerance test (OGTT), in which blood was collected from the tail tips.

Preparation of rat feed, supplemented with 5% oyster mushroom (P. ostreatus) powder

All of the standard rat pellet ingredients, i.e. flour, wheat bran, maize bran, rice bran, fish meal, beshon, powder milk, salt, oil, vitamins, molasses, and oil cake, were purchased from the market for poultry feed. Oyster mushrooms (P.
Oyster mushroom stimulates p-AMPK and GLUT4

Effect of the oyster mushroom on the body weight of type 2 diabetic model rats

Changes in body weight for the different rat groups are depicted in Figure 1. The initial body weights (g) were 176 ± 12, 196 ± 10, and 194 ± 22 for the control diabetic rats, the gliclazide-treated, and mushroom-treated diabetic model rats, respectively. Body weight was monitored every week, and after eight weeks, the bodyweight increases among the groups were similar. Therefore, the oyster mushroom did not have a significant effect on the body weight of type 2 diabetic model rats.
not show any effects on the body weight of the diabetic model rats (Figure 1).

Effect of the oyster mushroom on the serum glucose level of the type 2 diabetic model rats

To evaluate the effect of the oyster mushroom on glucose metabolism, fasting serum glucose (mmol/l) levels were measured for the different experimental groups. The fasting blood glucose on the first day was considered 100%, and the values on the 56th day were calculated based on the initial day. The untreated control group did not have any significant difference between day 0 and day 56. The fasting serum glucose level in the mushroom-treated (MT) group decreased significantly (p < 0.05; 29%) by the 56th day (day 0: 8.97 ± 1.31; day 56: 6.40 ± 1.41). For the gliclazide-treated (GT) group, the fasting glucose concentrations were 10.01 ± 1.44 and 6.70 ± 1.82 on day 0 and day 56, respectively, which indicates a 33% decline, compared to day 0 (Table 1).

Effect of the oyster mushroom on the serum lipid profile level of STZ-induced, type 2 diabetic model rats

Chronic effects of the oyster mushroom on the lipid profile were observed in the type 2 diabetic model rats. Both the serum triglycerides (TG) and total cholesterol (Chol) were measured, but did not show any differences among the control, the gliclazide-treated, or mushroom-treated groups between day 0 and day 56 (Figure 2).

Effect of the oyster mushroom on the phospho-AMPK (p-AMPK) protein in the muscle and adipose tissue of type 2 diabetic model rats

As shown in Figures 3 and 4, p-AMPK from the muscle and adipose tissue, respectively, of non-diabetic, diabetic control, mushroom-treated, and gliclazide-treated rats was detected using western blotting between the marker proteins 85 kDa and 50 kDa in size. These values are presented as ratios, compared to β-actin (a housekeeping protein) in bar diagrams. p-AMPK appeared to decrease more than 20% in the STZ-induced, diabetic model rats (the water control), compared to the non-diabetic rats. However, in the case of the mushroom-treated diabetic rats, p-AMPK increased about two-fold in the muscle tissues, compared to the control diabetic rats (Figure 3). Similar effects were also observed in the muscle tissues of gliclazide-treated diabetic model rats (Figure 3). In the case of the adipose tissue, p-AMPK seemed to increase more than three-fold in the mushroom-treated group and more than two-fold in the gliclazide-treated rats, compared to the control diabetic rats (Figure 4).

Effect of the oyster mushroom on GLUT4 mRNA expression in type 2 diabetic rats

GLUT4 mRNA was extracted immediately after the animals were sacrificed using the TRIzol reagent. The cDNA was prepared and amplified for the GLUT4 gene, using equal amounts of cDNA. Figure 5 shows the expression of GLUT4 (glucose transporter type 4) as a ratio, relative to GAPDH (glyceraldehyde 3-phosphate dehydrogenase), which was also similarly amplified. The ratio increased significantly in the mushroom-treated rats for the muscle and adipose tissues, compared to the control diabetic rats, but no significant difference was observed for the liver tissues.
Discussion

Diabetes is a major public health problem worldwide, associated with serious complications and premature death, due to the continuous damage, dysfunction, and failure of various organs. To prevent acute problems and to reduce the risk of long-term complications from diabetes, glycaemic monitoring, self-management education, support, and medications are often required. At present, there are no drugs available that can cure the disease, and existing drugs are not complication-free for all individuals. Therefore, researchers are continuing their efforts to find new drugs for better management of the disease. However, diabetes is a metabolic disorder in humans, but not in animals. As such, development of a human-like, diabetic animal model for experimental purposes is a major challenge. In this study, STZ-induced, type 2 diabetic model rats were developed, as previously described. As found in other studies in humans, we also found that the oyster mushroom significantly reduced the blood glucose levels in model diabetic rats. The study did not find any effects on body weight or lipid profile levels with this regimen. In this study, glycated haemoglobin, the lipid profile, and advanced glycation end-products were not measured, but we plan to measure these in a future study. To obtain insights into the mechanisms behind the hyperglycaemic effects of oyster mushroom powder, we attempted to analyse an intracellular protein (p-AMPK) and the mRNA of GLUT4.

After 8 weeks of mushroom powder ingestion (5% of the usual feed), we sacrificed the animals in a fasting condition, and the tissues and organs were immediately collected, washed with ice-cold normal saline, and preserved at \(-70\,^\circ\text{C}\). Tissue mRNA was extracted within a day, and proteins were extracted within the week of the sacrifice. Equal amounts of total protein were also analysed for the housekeeping protein \(\beta\)-actin. The band intensity of p-AMPK is expressed as a bar diagram as ratios, relative to \(\beta\)-actin.
significantly in the muscle and adipose tissues of mushroom-treated diabetic model rats. As a positive control, we analysed the muscle and adipose tissues of gliclazide-treated rats, and similar effects were observed. Existing literature suggests that activated AMPK releases GLUT4 from the microvesicles in the cytosol to organize in the membranes of muscle and adipose tissues, which helps glucose enter the cells of those tissues. Therefore, it may be explained that the decreased hyperglycaemia in mushroom-induced type 2 diabetic model rats could be due to increased p-AMPK in the muscle and adipose tissues in these animals.

This study also explored whether oyster mushroom ingestion affected the expression of the GLUT4 gene in the muscle and adipose tissues of type 2 diabetic model rats. Equal amounts of mRNA (1 µg in all cases) were used to prepare the cDNA; then, 3 µl of cDNA was used for 35 cycles of amplification using polymerase chain reaction (PCR). Amplification of the housekeeping gene GAPDH was also conducted, and expression of GLUT4 was expressed as a ratio, relative to GAPDH. Our experiments showed that GLUT4 mRNA expression increased in both the muscle and adipose tissues of oyster mushroom-treated type 2 diabetic model rats. In a previous study, it was found that activation of AMPK regulates transcription of the GLUT4 gene in cultured human skeletal muscle cells. However, in our study, we found ample expression of GLUT4 in the livers of both model diabetic and non-diabetic rats. Therefore, GLUT4 mRNA expression in rat liver tissues may not be affected by STZ or the oyster mushroom; its expression is already in pick which may explained that rat liver tissue may not be a problem for the entry of glucose in diabetic condition.

**Conclusion**

Therefore, the hyperglycaemic effects of the oyster mushroom can be explained by the increased phosphorylation of AMPK and increased mRNA in muscle and adipose tissues.

**Limitations**

It would be better to understand the measured HbA1C, glycation end-products, and the total antioxidant status of the animals.

**Conflict of interest**

The authors have no conflict of interest to declare.

**Ethical approval**

We have taken Ethical Permission from the Institutional Ethical Review Committee of Bangladesh University of Health Sciences, Dhaka, Bangladesh

**Authors’ contributions**

MA: Animal experimentation, laboratory works (Western Blot) and Manuscript preparation. MMR: Lab works specially in mRNA extraction, cDNA preparations and PCR. MM: Band density of proteins and PCR products estimation and Result preparation. MM: Help in OGTT determination in model rats and tissue protein extraction. AB: Rat feed management and performs OGTT experiments to select diabetic rats. BR: Diabetic model rat development and tissue collection from model rats. ZH: Experiment design and Critical revision of the manuscript. MOF: Project design, fund hunting, supervise all experiments and finalize the manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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