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

Mohammad Asrafuzzaman, MSc [a], Mohammad M. Rahman, MSc [b], Manoj Mandal, MSc [c], Mohammad Marjuque, MSc [b], Amrita Bhowmik, MSc [b], Begum Rokeya, PhD [d], Zahid Hassan, PhD [a] and Mohammad Omar Faruque, PhD [e,*]

[a] *Department of Physiology and Molecular Biology, Bangladesh University of Health Sciences, Dhaka, Bangladesh*
[b] *Department of Applied Laboratory Science, Bangladesh University of Health Sciences, Dhaka, Bangladesh*
[c] *Department of Biochemistry and Molecular Biology, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalgonj, Bangladesh*
[d] *Department of Pharmacology, Bangladesh University of Health Sciences, Dhaka, Bangladesh*
[e] *Department of Nutrition and Food Technology, Jessore University of Science and Technology, Jessore, Bangladesh*

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الملخص

أهداف البحث: يستخدم الفطر للحد من ارتفاع السكر في الدم تقليديا. بالرغم من ذلك، لم يتم استكشاف الآلية الكامنة وراء هذا التأثير. ومن المعروف أن أحادي فسفات الأدينوزين المنشط البروتين كيناز يخفض من ارتفاع السكر في الدم من خلال مسار الأنسولين- المستقل. تهدف هذه الدراسة لمراقبة تأثير مسحوق فطر المحار على فسفرة أحادي فسفات الأدينوزين المنشط البروتين كيناز واستخراج مراسل الحمض النووي الريبي لناقل الجلوكوز النوع الرابع في الفئران المصابة بداء السكري.

طرق البحث: تم استخدام فئران ايفانز الطويلة لإستحداث فئران مصابة بداء السكري النوع ٢ من خلال تحريض الستربتوزوتوسين داخل الصفاق. تمت إضافة ٥٪ مسحوق فطر المحار مع التغذية المعتادة للفئران لمدة ٨ أسابيع متوالية. بعد ذلك، تم ذبح الفئران. واستخراج الحمض النووي الريبي بواسطة كاشف ترايزول، وتم استخلاص البروتينات من أنسجة مختلفة مع تحلل العازلة ريبا. كما تم قياس استخراج مراسل الحمض النووي الريبي لناقل الجلوكوز النوع الرابع من خلال تقنيات تفاعل البوليميراز المتسلسل للحمض النووي الريبوزي منقوص الأكسجين، وتم تحديد فسفرة أحادي فسفات الأدينوزين المنشط البروتين كيناز بواسطة النشاف الغربي. تم قياس كثافة شريط منتجات تفاعل البلمرة المتسلسل والبروتينات باستخدام برنامج جيه للصور.

النتائج: مكملات مسحوق فطر المحار لمدة ٨ أسابيع نتج عنه انخفاض مستوى السكر في دم الفئران المصابة بداء السكري النوع ٢ من خلال تحريض الستربتوزوتوسين داخل الصفاق. مستويات فسفرة أحادي فسفات الأدينوزين المنشط البروتين كيناز، كنسبة إلى بيتا-الاكتين، ارتفعت في العضلات والأنسجة الدهنية عند الفئران المصابة بداء السكري النوع ٢ المعالجة بالفطر، بالمقارنة بفئران مجموعة التحكم. ارتفع استخراج ناقل الجلوكوز النوع الرابع، كنسبة إلى جليسرالديهيد ٣-فوسفات ديهيدروجينيز، بشكل ملحوظ في كل من العضلات والأنسجة الدهنية للفئران المصابة بداء السكري المعالجة بالفطر.

الاستنتاجات: قد يقلل فطر المحار من ارتفاع السكر في الدم من خلال زيادة فسفرة أحادي فسفات الأدينوزين المنشط البروتين كيناز وأيضا استخراج ناقل الجلوكوز النوع الرابع في العضلات والأنسجة الدهنية.

الكلمات المفتاحية: أحادي فسفات الأدينوزين المنشط البروتين كيناز؛ ناقل الجلوكوز النوع الرابع؛ ارتفاع السكر في الدم؛ فطر المحار

Abstract

Objectives: 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

* Corresponding address: Department of Nutrition and Food Technology, Jessore University of Science and Technology, Jessore 7408, Bangladesh.
E-mail: faruque.nft.just@gmail.com (M. O. Faruque)
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streptozotocin (STZ). OMP was supplemented at 5% with the usual feed of rats for 8 consecutive weeks. Then, the rats were sacrificed. RNA was extracted by the TRIzol reagent, and proteins were extracted from different tissues with RIPA lysis buffer. Expression of GLUT4 mRNA was measured through cDNA-PCR techniques, and p-AMPK was detected using western blotting. The band intensities of the PCR products and proteins were measured using Image J software.

Results: Supplementation of OMP for 8 weeks resulted in a reduction of the serum glucose level in STZ-induced, type 2 diabetic model rats. The levels of p-AMPK, as a ratio relative to β-actin, increased in the muscle and adipose tissues of mushroom-treated type 2 diabetic model rats, compared to those in control diabetic model rats. Expression of GLUT4, as a ratio relative to GAPDH, increased significantly in both the muscle and adipose tissues of mushroom-treated diabetic rats.

Conclusion: Oyster mushroom may decrease hyperglycaemia through increased p-AMPK and also expression of GLUT4 in the muscle and adipose tissues.

Keywords: AMP-activated protein kinase; Glucose transporter 4; Hyperglycaemia; Oyster mushroom

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 aggrandizing 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 heterometric 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 transportation 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. Diabetic model rats with a blood glucose level ˃ 7.0 mmol/l under fasting conditions were selected to study the effect of white oyster mushroom powder.

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.*