

Infused Oyster Mushroom (*Pleurotus ostreatus*) Inhibits Glucose Absorption through Intestinal Mucosal Membrane in Wistar Rats

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Abstract

Objective: To observe the effects of infused oyster mushroom (latin name: *Pleurotus ostreatus*) on glucose absorption through the intestinal mucosal membrane in Wistar rats.

Methods: This study used experimental design. Subjects of this study were six Wistar rats which were randomly divided into two groups; three rats were used for the analysis of the most effective time for glucose absorption and the other three were used to evaluate the effect of infused oyster mushroom on glucose absorption. The intestine of subjects was connected to in situ perfusion machine. Control glucose solution (Ctr) or glucose solution containing 5% (Oys 5%), 10% (Oys 10%), and 20% (Oys 20%) infused oyster mushroom was added into the perfusion machine and flowed back and forth in the lumen of intestine subsequently, accompanied by washing procedures using 0.9% NaCl between treatments. The glucose concentration was determined using deproteinization method followed by absorbance measurement. Student's t test was performed to analyze the difference between groups.

Results: The level of glucose absorbed through intestinal mucosal membrane in control glucose solution and glucose solution with different concentrations of infused oyster mushroom, i.e. 5%, 10% or 20%, was 25.19+3.4 mg/dL, 16.27+0.86 mg/dL, 13.22+1.58 mg/dL and 10.03+1.25 mg/dL, respectively. Student's t test showed significant differences between groups; Ctr and Oys 5% (p=0.03), Ctr and Oys 10% (p=0.02), and Ctr and Oys 20% (p=0.007).

Conclusions: Infused oyster mushroom inhibits glucose absorption through intestinal mucosal membrane in Wistar rats.

Keywords: Oyster mushroom (*Pleurotus ostreatus*), glucose absorption, intestine

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Introduction

Glucose is one of the primary sources of energy for living organisms that is mostly obtained from outside the body and is absorbed through small intestines. This absorption is mediated by transporter proteins by which the glucose can be transported from the intestinal lumen

into the intestinal mucosal cells and eventually into blood vessels. Intestinal glucose transport was mediated by Na⁺-D-glucose cotransporter SGLT1 and also by glucose transporter type 2 (GLUT2).¹⁻³ Some studies have revealed several factors that may affect the glucose transport through intestinal mucosal membrane, such as glucose concentration, temperature, inhibitors and activators, and bile.⁴

Diabetes mellitus is a metabolic disorder which is characterized by high blood glucose level. Diabetes mellitus prevalence is increasing rapidly and has reached about 1 in 11 adults in the world have diabetes mellitus.⁵ One of

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the strategies to lower blood glucose level in diabetic patients is through the inhibition of glucose absorption in intestine, an effect that can be achieved through the use of biguanide class of antidiabetic drugs.⁶

Previous studies have reported that several mushroom species had effects on metabolism as well as on glucose transporters. Giant oyster mushroom (*Pleurotus giganteus*) was found to accelerate adipocyte differentiation through activation of PPAR γ and glucose transporter type 1 and 4 (GLUT1 and GLUT4).⁷

Oyster mushroom (*Pleurotus ostreatus*) is one of the most famous edible mushrooms in Indonesia. It contains valuable nutritional substances including the following substances; polysaccharides, lipopolysaccharides, lipids, proteins, peptides, lectins, and glycoproteins.⁸ Moreover, oyster mushroom also has several benefits. A previous study has reported that oyster mushroom has an antidiabetic effect by increasing the translocation of GLUT4.⁹ Oyster mushroom extract can also protect major organs from oxidative damage by increasing several antioxidant enzymes.¹⁰ Another study revealed that oyster mushroom has the ability to inhibit the proliferation of breast and colon cancer cells.¹¹

Guanide, that is a known antihyperglycemic compound, has been reported to be associated with biguanide and it was also isolated from *Pleurotus* species.¹² Hence, it is important to investigate whether oyster mushroom has any effect on glucose absorption in intestine. The objective of this study was to know the effect of infused oyster mushroom on glucose absorption through the intestinal mucosal membrane in rats.

Methods

This study was conducted using experimental design and was carried out at Laboratory of Biochemistry, Department of Biochemistry, Faculty of Medicine, Universitas Padjadjaran. The subjects of this study were six Wistar rats with age of 3–4 months and weight of 200–300 grams, which were divided into two groups. Three rats were used for the analysis of the most effective time for glucose absorption, while the other three were used to evaluate the effect of infused oyster mushroom on glucose absorption through intestinal mucosal membrane.

In order to identify the most effective time for glucose absorption in Wistar rats' intestinal mucosal membranes, the rats undergo fasting

first for 18–24 hours (only plain water was available). After being anesthetized, surgery was performed to take the intestine out. A cannula was gently fixed at the distance of 10 cm after gastric pylorus and another cannula was fixed at the distance of 25 cm after the first cannula. The intestine was then washed three times using 0.9% NaCl. Both cannulas that were already fixed to intestine were then connected to perfusion machine designed by Soedigdo and Marsongkohadi (Bandung Institute of Technology). This apparatus allowed the solution added into it to flow back and forth through the intestinal lumen six times per minute and the intestine was still functional for 6–8 hours.

The glucose concentration of the solution was then measured every 15 minutes for one hour period through deproteinization method using the peridochrome glucose/GOD-PAP reagent kit from ST Reagensia[®] and TCA 8% according to the manufacturer's instructions. Glucose concentration was measured based on the absorbance difference determined by spectrophotometer.

This experiment used old and fresh oyster mushroom with white body. The dried oyster mushroom was crushed to get very small pieces or powder form of mushroom. Five gram, 10 gram, or 20 gram of crushed mushroom was added with 100 mL of water to make 5%, 10%, or 20% infused oyster mushroom, respectively, in pot I. Pot I was then heated on hot water (90 °C)-containing pot II for 15 minutes. The suspension was stirred only once during this heating process and, finally, infused oyster mushroom was filtered.

Three Wistar rats were used to analyze the most effective time for glucose absorption through the intestinal mucosal membrane, while three other Wistar rats were used to analyze the effect of infused oyster mushroom on glucose absorption through the mucosal membrane. Solution used for measurement of absorbed glucose level in control condition was 24 mL of 3.0×10^{-3} M glucose solution (in 0.9% NaCl) added with 1 mL of 0.9% NaCl, while the solution used for analyzing the effect of infused oyster mushroom was 24 mL of 3.0×10^{-3} M glucose solution (in 0.9% NaCl) added with 1 mL of 5%, 10% or 20% infused oyster mushroom, respectively. Each of rat's intestine was treated with glucose only solution (as control) and 5%, 10%, and 20% infused oyster mushroom-containing glucose solution, respectively, followed by washing using 0.9% NaCl between treatments. Furthermore, the glucose concentrations was

measured using the deproteinization method, followed by absorbance determination using spectrophotometer as described previously.

Data were presented in mean±SEM and Student's t test was used to determine whether the difference between groups was significant.

The experimental procedures used in this study followed the Ethics Regulation of Animal Research in Faculty of Medicine, Universitas Padjadjaran.

Results

Firstly, in order to know in which time point glucose solution is absorbed most effectively through rat intestinal mucosal membrane, the rate of glucose absorption through rat intestinal mucosal membrane in 1 hour were measured (Fig. 1). By using in situ perfusion system, the level of glucose absorbed every 15 minutes was analyzed. The results showed that the absorbed glucose level at 15, 30, 45 and 60 minutes was 19.92±4.14 mg/dL, 30.25±6.19 mg/dL, 38.82±2.86 mg/dL and 41.99±4.01 mg/dL, respectively (Figure 1).

These data suggested that in Wistar rats the most effective time for glucose absorption through the intestinal mucosal membrane was 15 minutes of exposure which was evident from the higher glucose absorption rate during the first 15 minutes that was higher when compared to the same rate during minute 15–30, minute 30–45, and minute 45–60 (Fig. 1).

Next, the effect of infused oyster mushroom on the glucose absorption through intestinal mucosal membrane was analyzed and, taking advantage of the in situ perfusion system, the glucose absorbed level after addition of control glucose solution and after addition of infused oyster mushroom-containing glucose solution into the lumen of intestine was compared.

Based on the previous data showing that the most effective time for glucose absorption in this model was 15 minutes, in this experiment we measured the absorbed glucose level after 15 minutes of solution addition (Fig. 1).

The levels of the glucose absorbed through the intestinal mucosal membrane in control glucose solution, 5% infused oyster mushroom-containing glucose solution (Oys 5%), 10% infused oyster mushroom-containing glucose solution (Oys 10%) and 20% infused oyster mushroom-containing glucose solution (Oys 20%) were 25.19±3.4 mg/dL, 16.27±0.86 mg/dL, 13.22±1.58 mg/dL and 10.03±1.25 mg/dL, respectively. Student's t test showed a significant difference between Ctr and Oys 5%

(p=0.03), Ctr and Oys 10% (p=0.02), and Ctr and Oys 20% (p=0.007) (Fig. 2).

These data seemed to suggest that infused oyster mushroom can significantly inhibited glucose absorption through intestinal mucosal membrane in a dose-dependent manner.

To evaluate whether washing the intestinal lumen using 0.9% NaCl could recover the ability of intestinal mucosal membrane to absorb glucose, the absorbed glucose level between glucose-only solution treatment before and after washing procedures-following treatment of infused oyster mushroom was compared.

The levels of glucose that is absorbed through intestinal mucosal membrane in the control glucose solution before treatment (Ctr pre), 10% infused oyster mushroom-containing glucose solution (Oys 10%), and control glucose solution after the washing procedures-following treatment (Ctr post) were 15.88±1.55 mg/dL, 2.65±1.19 mg/dL, and 23.11±1.16 mg/dL, respectively. Student's t test showed significant difference between Ctr (pre) and Oys 10% (p=0.01), and Oys 10% and Ctr (post) (p=0.003) (Fig. 3).

These data suggested that the inhibition of glucose transport through intestinal mucosal membrane by infused oyster mushroom in this system was reversible because washing the intestine several times using 0.9% NaCl solution after infused oyster mushroom treatment could recover the ability of mucosal membrane to absorb glucose (Fig. 3).

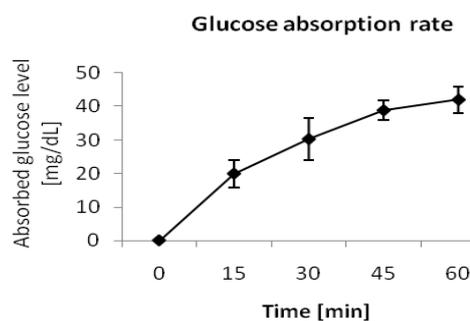


Fig. 1 Rate of Glucose Absorption through Intestinal Mucosal Membrane. The Absorbed Glucose Level was Measured at 15, 30, 45 and 60 Minutes after Addition of Glucose Solution. Data were Represented as Mean±SEM (n=3)

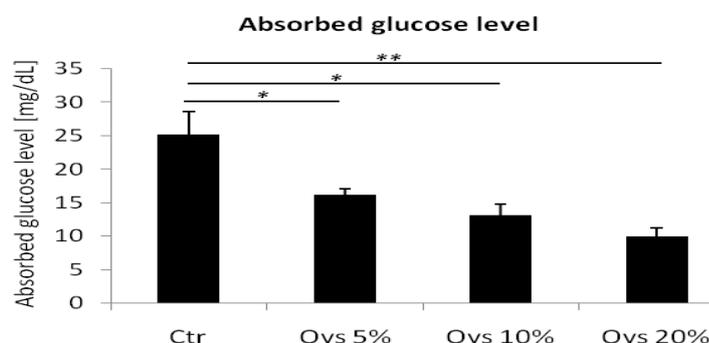


Fig. 2 Infused Oyster Mushroom Inhibited Glucose Absorption through Intestinal Mucosal Membrane. The Absorbed Glucose Level was Measured after 15 Minute-addition of Control Glucose Solution (24 mL of 3.0×10^{-3} M Glucose Solution (in 0.9% NaCl) Added with 1 mL of 0.9% NaCl), or Glucose Solution Containing Infused Oyster Mushroom (24 mL of 3.0×10^{-3} M Glucose Solution (in 0.9% NaCl) Added with 1 mL of 5% (Oys 5%), 10% (Oys 10%) or 20% (Oys 20%) Infused Oyster Mushroom). Data were Represented as Mean+SEM (n=3). Student's t test was Performed for Indicated Comparison. *p<0.05, ** p<0.01

Discussion

This study had revealed that infused oyster mushroom treatment had inhibition effect on glucose absorption through intestinal mucosal membrane in Wistar rats. This finding gives us an opportunity to find new strategy to control glucose absorption in the intestine, which is important for diabetic patients.

Possible mechanism that can explain this finding was the one that makes use of guanide, which may be isolated from *Pleurotus* species that might also exist in oyster mushroom and that functionally inhibits glucose transport in intestinal mucosal membrane. Guanide is an antihyperglycemic compound associated with the biguanide class of antidiabetic drugs.¹² Several previous studies suggested that the

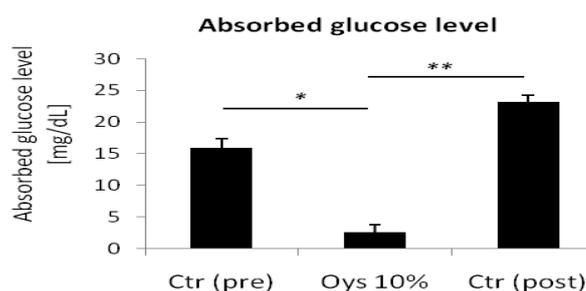


Fig. 3 Washing the Intestine Using 0.9% NaCl Recovered the Ability of Intestinal Mucosal Membrane to Absorb Glucose. The Absorbed Glucose Level was Measured after 15 Minute-addition of Control Glucose Solution (24 mL of 3.0×10^{-3} M Glucose Solution (in 0.9% NaCl) Added with 1 mL of 0.9% NaCl; Ctrl) Before (pre) and After (post) Treatment of Glucose Solution which Contained Infused Oyster Mushroom (24 mL of 3.0×10^{-3} M Glucose Solution (in 0.9% NaCl) Added with 1 mL of 10% Infused Oyster Mushroom; Oys 10%). Data were Represented as Mean+SEM (n=2). Student's t test was Performed for Indicated Comparison. * p<0.05, ** p<0.01.

biguanide derivatives could inhibit intestinal glucose absorption.^{6,13,14} Biguanide might have the ability to suppress the production of ATP required for active absorption of glucose in the wall of small intestine.¹³

Another possibility is that oyster mushroom might also contain other active compounds that act as the inhibitors for glucose transport in the intestine. The underlying mechanism might include the inhibition on Na⁺-D-glucose cotransporter SGLT1 or glucose transporter type 2, GLUT2. It is therefore very important to identify the active compound that might exhibit this effect through compound isolation techniques.

In a previous study, several factors have been suggested to affect glucose absorption through the intestinal mucosal membrane, including, among others, glucose concentration, bile, and temperature.⁴ In this study, several strategies to control these possible confounding factors were applied. To avoid glucose concentration bias, a standardized glucose solution and measurement kit were used in the study. To prevent bile contamination, only intestine connected to cannulas at both ends at the same length (25 cm) were used. All procedures in the same room at the same time to control the temperature difference between experiments.

Subsequent treatments of glucose solution only (as control) and glucose solution added

with different concentrations of infused oyster mushroom with washing procedure between treatments were performed. The experiments showed that after being exposed to infused oyster mushroom, which had an inhibition effect on glucose absorption, the ability of mucosal membrane to absorb glucose was recovered by washing the intestinal lumen using 0.9% NaCl solution (Fig. 3). Taking these data into account, it is possible for us to give several treatments subsequently as long as washing procedures is done between the treatments. Moreover, these data (Fig. 3) could partially exclude possibility that lower absorbed glucose level in infused oyster mushroom-treated intestine was due to declined function of intestine by time. This study, however, did not evaluate whether our subsequent treatments to the intestines lead to cells exhaustion.

Limitations in this study include the lack of supporting data that explain the underlying mechanisms how intestinal glucose absorption is inhibited by infused oyster mushroom. It is therefore necessary to do further studies such as a study to elaborate whether infused and extract of oyster mushroom as well as the active compound decrease the protein expression of glucose transporter type 2, GLUT2, and Na⁺-D-glucose cotransporter SGLT1, that primarily present in intestine.

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