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Hypoglycemic Effect Of Button (*Agaricus Bisporus*) And Oyster (*Pleurotus Ostreatus*) Mushrooms On Streptozotocin Induced Diabetic Mice

In the present study, we examined the hypoglycemic effect of two species of edible mushrooms which are widely cultivated in Egypt. Fifty-six male Swiss mice of average weight 25±5 grams were used in the study. Mice were randomly divided into two main groups. Group A that served as control did not receive any treatment and group B was sterptozotocin (STZ)-induced diabetic group. The latter was divided into three subgroups, B1 diabetic–insulin treated, B2 diabetic–oyster and B3 diabetic–button agaricus. Blood glucose levels were routinely determined every week. After 30 and 60 days, mice from all groups were sacrificed. Blood samples and livers were collected for determination of hepatic glycogen, hepatic glucokinase activity and mRNA expression levels of GK. Our results revealed a significant hypoglycemic effect of the two species of mushrooms. There was a significant change in the hepatic glycogen content with a marked increase in the expression level of GK in mushroom feeding groups. We can conclude that Oyster and Button mushrooms have a potent hypoglycemic effect on diabetic mice which gives us a new prospect to face diabetes mellitus with high effect and low cost.

Key words: button, oyster, mushroom, glucokinase, glycogen, blood glucose, diabetes

INTRODUCTION

Currently, there is no perfect approach to treat diabetes as a common metabolic disorder {1}. Diet supplemented with fruit and vegetables had beneficial effects on diabetes {2}. Mushrooms are a nutritional goldmine and are one of nature's greatest providers of completely natural health benefits without toxicity {3}. White mushrooms contain high levels of dietary fibers and antioxidants; beta glycans; folate; ergothioneine; and polyphenols, {4-7} suggesting that the mushrooms may have potential hypoglycemic effects and may provide beneficial effects on Diabetes Mellitus. Mushrooms that are rich in trace elements were reported to lower blood glucose and increase glycogen {8, 9}. *Agaricus bisporus* can lower the blood glucose in STZ-induced diabetic rats {1}. Glucokinase (GK) is the principal determinant of both hepatic and islet glucose utilization (10). It promotes glycogen synthesis in the liver {11}. Low GK activity would cause diabetes, even though GK activity has long been thought to be a key determinant of hepatic glucose usage {12}. GK activators with optimized properties would be able to both blunt the postprandial glucose excursion and lower the fasting blood glucose in DM patients {11}. Few studies were performed on the possible hypoglycemic effect of oyster mushrooms which have been cultivated and produced extensively in Egypt and developing countries. The aim of this study was to clarify the hypoglycemic mechanism of Oyster and Button mushrooms through studying GK activity, its gene expression, hepatic glycogen content in normal and diabetic mice in comparison with insulin treatment in a trial to provide pre-clinical data of the effectiveness of mushrooms as GK activator. This represents a promising and new control option for DM.

MATERIALS AND METHODS

Protocol

Fifty-six male Swiss mice of average age 4-5 months and weighted at the beginning of the experiment (25±3 grams), were housed in groups of seven in stainless steel cages in a room with a temperature of 22°C ± 2°C, a relative humidity of 55% ± 5%, with a light-dark phase of 12 hours with free access to basal diets and water.

Induction of diabetes and animal diets

After acclimatization for 7 days, fourteen mice served as control group (group-A) and they were fed on base diet. The remaining mice were fasted for 12 hours before intra-peritoneal administration of single dose of STZ (Sigma Chemical Co., Poole, Dorset, UK) at 100 mg/kg body weight in 0.01 M sodium citrate buffer (pH 4.5){13}. Two days after STZ treatment, the mice were considered diabetic (B), as determined by non-fasting blood glucose levels of >200 mg/dL {14}. The diabetic group were sub-classified into 3 subgroups; 1, 2 and 3. sub-group B1(n=14): were treated by insulin (0.3 units/ 50 gm body weight dissolved in 0.5 ml physiological saline according to Lee et al., {15}; sub-group B2 (n=14): were fed on Oyster mushroom (*Pleurotus ostreatus)*; sub-group B3(n=14): were fed on Button mushroom (*Agaricus bisporus*). All animals were sacrificed after 30 and 60 days. The fresh fruiting bodies of *P. ostreatus and A. bisporus* were subjected to drying at a temperature of 55-60 ºC until complete dehydration {16}. Mushrooms were incorporated into the diet by dose of 62·5 g/kg {17}.

Blood glucose

Blood glucose was detected using one touch glucose strip (USA).

Blood and tissue sampling

Liver samples for RNA extraction were collected and preserved in liquid nitrogen until the RNA extraction. Samples for biochemical examinations were homogenized in physiological saline, none immediately examined samples were preserved at -20 until further investigations.

Biochemical Analysis

Hepatic glycogen contents were determined according to Roehring & Allred {19} and hepatic glucokinase activity was measured using the method of Pakoske et al., {20}.

Molecular Analysis

Total RNA was extracted from liver tissues using an RNA extraction solution (ISOGEN; Nippon Gene, Tokyo, Japan) according to the manufacturer's protocol.

Glucokinase enzyme expression level determination

The expression level of glucokinase (GK) enzyme in mice livers was determined by Reverse transcriptase polymerase chain reaction (RT-PCR) {21}.

First strand cDNA was synthesized using two steps Superscript II kit (Invitrogen, Carlsbad, CA). The amplification was performed using thermal cycler (Takara MP, Japan) with cycling conditions of 25 ºC for 10 min (denaturation), 42 ºC for 90 min (anneling), 95 ºC for 3 min (extension) and chilled in ice for 5 minutes. RT-PCR was performed using GK specific oligoneuclotide primers pair, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as housekeeping gene. The primer pairs for amplification were designed as the following, Gk primers, forward, 5`-CAC CCA ACT GCG AAA TCACC-3`and reverse, 5`-CAT TTG TGG GGT GTG GAG TC-3`and for GAPDH, forward, 5`-CCCGTAGACAAAATGGTGAAGGTC-3`and reverse, 5`-GCCAAAGTTGTCATGGATGACC-3`with product sizes, 162 and 215 respectively. Then the amplified PCR products were electrophorised on 1.5% Agarose gel in 1X Tris acetate EDTA running buffer (1 x TAE) with condition of 100 Voltage/ 40 min as described by Uchida *et al*., {22}.

Statistical analysis: The data was processed using the statistical package for social science (SPSS Inc., Chicago, IL, version 13, USA). All results are expressed as mean ± SD. Comparison among groups was made by Student's *t*-test (unpaired), One-way analysis of variance (ANOVA). Duncan's test was used for testing the inter-grouping homogeneity. Statistical significance was set $P<0.05$.

RESULTS

The effects of mushrooms; Oyster (*Pleurotus ostreatus*), Button (*Agaricus bisporus*) feeding and insulin treatment on fasting blood glucose level (mg/dL), hepatic glycogen content (mg/gm tissue), and hepatic glucokinase activity (U/L) in STZ- induced diabetic mice are summarized in Table I.

Blood Glucose level

The blood glucose was significantly lowered in mushrooms feeding groups and insulin treated one compared to the control group (P <0.05) (Figure 1).

Hepatic glycogen content

Our results revealed that the hepatic glycogen contents show variation between groups at the two durations of the experiment. Hepatic glycogen content insignificantly declined after 30 days in diabetic treated groups, while it was shown to be significantly increased in diabetic treated groups after 60 days of the experiment (Table 1).

Hepatic glucokinase activity

There was an observed oscillation in the activity of the enzyme, while the activity increased in the second duration of experiment in all diabetic treated groups when compared with their control.

Hepatic glucokinase expression

There was a significant increase in the hepatic glucokinase expression level in insulin treated, Oyster and Button treated mice (P>0.05) (Figure 3).

DISCUSSION

In the present study, we have demonstrated that Oyster and Button mushrooms have significant hypoglycemic effects in diabetic mice. The hypoglycemic effects of Oyster and Button mushrooms were detected as the reduction ratio of fasting blood glucose (FBG) level in control groups to that of the diabetic treated groups with insulin and mushrooms. Different literatures have reviewed the hypoglycemic effects of various types of mushrooms {1, 8, 23, 24}. Compounds in mushrooms such as beta glucans are effective in lowering blood cholesterol levels and glycemic response *in vivo* {25-27, 30}. Although the hepatic glycogen contents showed variation between groups in the two durations of the experiment, hepatic glycogen concentration in insulin treated group increased as high blood glucose level stimulates insulin to activate both hepatic glucokinase and glycogen synthase to increase the rate of glucose uptake by the cell and glycogen synthesis respectively {28}. The level of hepatic glycogen in mushroom fed groups in the first duration of the experiment (30days) was insignificantly low if compared with their control group in the same duration. We can reveal this is due to the inability of mushrooms to induce significant changes in glycogen content through the first 30 days of experiment. Feeding mushrooms for 30 days may be insufficient to induce changes in hepatic glycogen. Previously, it was shown that 45 days feeding of mushrooms can increase hepatic glycogen contents {9}. The high level of hepatic glycogen in diabetic treated groups in second duration of the experiment was in the same line of Zhou & Han {29}. GK has high control strength on glucose metabolism by glycogen synthesis and glycolysis {10}. Accordingly, small changes in GK activity are associated with correspondingly large changes in glucose metabolism {31}. There are reports of decreased expression of GK in liver in human type-2 diabetes {12}. On the other hand, animal models which overexpress GK in their hepatic tissues, showed a good glucose tolerance and an increase in hepatic uptake of glucose {32-35}. Our results regarding GK expression level and activity in mice liver tissues indicated high mRNA expression level and activity in diabetic-insulin treated and in diabetic mushrooms fed ones if compared with their control groups. The ability of the two types of mushrooms used in our experiment to increase the activity of GK and its expression level in hepatic tissues explained the ability of mushrooms to induce glucose uptake by liver for subsequent metabolism {35}. The matter induces lowering of blood glucose.

In conclusion our study revealed that mushrooms are a potent source of high nutritional value as it keeps body weight constant during the time of the experiment. Both types of mushrooms have the ability to increase hepatic glycogen contents and activate GK activity in STZ-diabetic mice, so Oyster and Button mushrooms have a potent hypoglycemic effect which enables them to be a new prospect for the control of diabetes.

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Table I: The effect of mushrooms: Oyster (*Pleurotus ostreatus*), Button (*Agaricus bisporus*) feeding and insulin treatment on fasting blood glucose level (mg/dL), hepatic glycogen content (mg/gm tissue), and hepatic glucokinase activity (U/L) in STZ- induced diabetic mice.

Parameters	Duration	Control group	Insulin-treated	Oyster-treated	Button-treated
Blood glucose (mg/dL)	30 days	118.71± 6.26c	98.28± 8.22de	143.71± 10.76c	140.57± 12.31c
	60 days	116.20± 15.76ab	83.57± 11.13e	97.00± 4.69d	96.71± 6.26f
Hepatic glycogen (mg/gm tissue)	30 days	21.14± 1.40bc	21.82± 1.89bc	17.34± 1.19cd	13.85± 0.73d
	60 days	25.58± 4.21b	41.08± 7.66a	38.38± 2.96a	39.10± 9.13a
Hepatic glucokinase activity(U/L)	30 days	61.79± 3.26e	104.25± 3.24b	59.18± 4.70e	44.76± 8.73f
	60 days	81.01± 3.3d	98.38± 5.77c	117.66± 2.28a	95.08± 3.46c

Means within the same column carrying different superscripts are significant at (*P <0.05*)

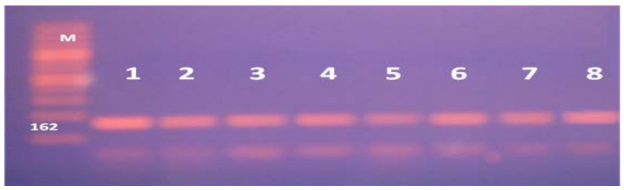

Figure 3: Effect of Mushrooms; Oyster (*Pleurotus ostreatus*), Button (*Agaricus bisporus*) feeding and insulin treatment GK gene expression in liver tissues of STZ-induced diabetic mice and control group.

Figure 4: Expression level of GAPDH as a house keeping gene in liver tissues of mice and control group.

1: Control group for 30 days; 2: Control group for 60 days; 3: Insulin treated group for 30 days; 4: Insulin treated group for 60 days; 5: Oyster fed group for 30 days; 6: Oyster fed group for 60 days; 7: Button fed group for 30 days; 8: Button fed group for 60 days; M: 100 bp Molecular marker.

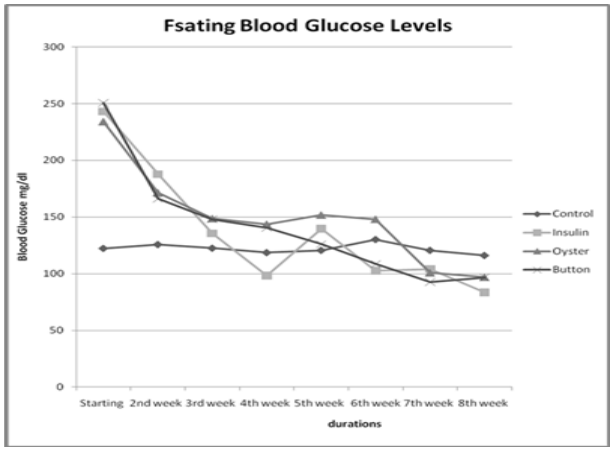

Figure 1: Effect of Mushrooms; Oyster (*Pleurotus ostreatus*), Button (*Agaricus bisporus*) feeding and insulin treatment on fasting blood glucose (mg/dL) in STZ- induced diabetic mice.

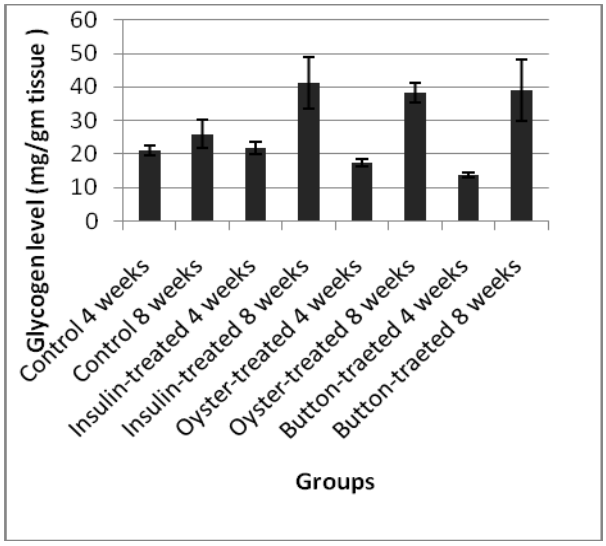

Figure 2: Effect of Mushrooms; Oyster (*Pleurotus ostreatus*), Button (*Agaricus bisporus*) feeding and insulin treatment on hepatic glycogen level (mg/gm tissue) in STZ- induced diabetic mice and control mice.