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 streptozotocin (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
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 (annealing), 95 ºC for 3 min (extension) and chilled in ice for 5 minutes. RT-PCR was performed using GK specific oligonucleotide 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`-CAC CCA ACT GCG AAA TCACC-3` and reverse, 5`-GCCAAATGTGTAGATGACC-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 1.

### 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.

Acknowledgment: This work was carried out in Biochemistry labs of veterinary medicine and Medicine faculty, Zagazig University under the supervision of Prof. Yousef M Shehata and Prof. Youssri M Hussein. Authors are thankful for help of Dr Rasha Etewa in preparing the manuscript.
REFERENCES


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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Duration</th>
<th>Control group</th>
<th>Insulin treated</th>
<th>Oyster-treated</th>
<th>Button-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>30 days</td>
<td>118.71±6.26c</td>
<td>98.28±8.22de</td>
<td>143.71±10.76c</td>
<td>140.57±12.31c</td>
</tr>
<tr>
<td></td>
<td>60 days</td>
<td>116.20±15.76ab</td>
<td>81.57±11.13e</td>
<td>97.00±4.69d</td>
<td>96.71±6.26f</td>
</tr>
<tr>
<td>Hepatic glycogen (mg/gm tissue)</td>
<td>30 days</td>
<td>21.14±1.40bc</td>
<td>21.82±1.89bc</td>
<td>17.34±1.93c</td>
<td>13.85±0.73d</td>
</tr>
<tr>
<td></td>
<td>60 days</td>
<td>25.58±4.21b</td>
<td>41.08±7.66a</td>
<td>38.38±2.96a</td>
<td>39.10±9.13a</td>
</tr>
<tr>
<td>Hepatic glucokinase activity (U/L)</td>
<td>30 days</td>
<td>61.79±3.26e</td>
<td>104.25±3.24b</td>
<td>59.18±4.70e</td>
<td>44.76±8.73f</td>
</tr>
<tr>
<td></td>
<td>60 days</td>
<td>81.01±3.3d</td>
<td>98.38±5.77c</td>
<td>117.66±2.88a</td>
<td>95.08±3.46c</td>
</tr>
</tbody>
</table>

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

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 group.

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.