

Oyster mushroom (*Pleurotus ostreatus*) strain 238 ameliorates the oxidative stress in STZ-induced diabetic mice

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Abstract: The present study evaluates the biochemical effect of Oyster mushroom on the oxidative stress in diabetic mice. Thirty five male Swiss albino mice were divided into five groups, Group A; STZ induced diabetic mice, group B; STZ-induced diabetic mice treated with insulin, group C; STZ-induced diabetic mice fed on Oyster mushroom, group D; non diabetic non treated mice and group E; non diabetic fed on oyster mushroom mice. The levels of fasting blood glucose (FBG), malondialdehyde (MDA) and total antioxidant capacity (TAC), activities and genes expression of superoxide dismutase (CuZn SOD) and catalase (CAT) were determined. Our results revealed a significant increase of blood TAC levels, SOD and CAT activities and significant decrease of MDA level with an increase of SOD and CAT genes expression in liver tissues of the diabetic mice fed on mushroom. In conclusion; oyster mushroom has the ability to reduce the oxidative stress in diabetic mice.

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1. Introduction

Diabetes mellitus is a common metabolic problem; of no perfect treatment up till now (Jeong et al., 2010). It is characterized by disturbances in fuel and energy metabolism (Jawahar et al. 2006). One of the most consequences of diabetes mellitus is the generation of reactive oxygen species (ROS) due to the generation of advanced glycosylation end-products (AGEs) (Harrison et al. 2003). This is due to the deterioration of the antioxidant defense mechanisms in diabetic patients (Natheer 2011). On the other hand; the oxidative stress associated with diabetes has a great role in β -cell failure and the development of insulin resistance which is the main cause of type II diabetes mellitus (Jeffrey et al. 2009). Superoxide dismutase (SOD) and Catalase (CAT) are major enzymes involved in the protection of all the organisms against free radical damage (Tadhani et al. 2007). Involvement of SOD and/or CAT abnormalities in the induction of oxidative stress in diabetic models has been previously reported (Ime et al. 20011). There were several chemicals incorporated in treatment and/or control of diabetes, although they have harmful side-effects and fail to significantly alter the course of diabetic complications (Li et al. 2004). So tendency to use natural agents to control or treat diabetes gains a great attention in the last few years. Medicinal plants have been studied for the treatment of diabetes (Yeh et al. 2003), many agents also have been studied for their effect to lower blood glucose in diabetic models;

cinnamman (Kirkham et al. 2009), and chromium (Balk et al. 2007). But these trails for control diabetes are still in preliminary studies with weak effect. Mushrooms have been part of the normal human diet for thousands of years and, in recent times, the amounts consumed have risen greatly, involving a large number of species. Mushrooms are considered, all over the world, as valuable health foods since they are poor in calories, fat, and essential fatty acids, and rich in proteins, vitamin and minerals (Reis et al. 2012). Moreover, there are more medicinal properties have been reported previously by Filipa et al. (2012). Some of the mentioned properties are attributed to bioactive products with antioxidant activity such as phenolic compounds (Barros et al.2009).

This work was designed to evaluate the antioxidant power of one of the most common cultivated type of mushrooms in Egypt on the oxidative stress generated in diabetic mice model.

2. Material and methods:

Protocol

Thirty five male Swiss mice of an average age 4-5 months, weighted (at the beginning of the experiment 25 ± 5 grams), were housed in groups of seven in stainless steel cages in a room with a temperature of $22^\circ\text{C} \pm 2^\circ\text{C}$, a relative humidity of $55\% \pm 5\%$ and with a light-dark phase of 12 hours *ad libidum*. All experimental procedures of the present study were approved by the Medical Research Ethics Committee of Zagazig University, Egypt.

Induction of diabetes and animal diets

After acclimatization for 7 days, fourteen mice served as control were fed on a base diet. The remaining mice were fasted for 12 hours before intraperitoneal administration of a 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). Two days after STZ treatment, the mice were considered diabetic, as determined by non-fasting blood glucose levels of more than 200 mg/dL (Minshal-Brown *et al.* 2009) Then all experimental animals were classified into five groups from A to E: Group A; STZ induced diabetic mice, Group B; STZ-induced diabetic mice treated with insulin (0.3 units/50 gm body weight dissolved in 0.5 ml physiological saline according to Lee *et al.* (2001). Group C; STZ-induced diabetic mice fed on Oyster mushroom (*Pleurotus ostreatus*), Group D; Non diabetic Non treated mice; these mice were left as a control, Group E; non diabetic fed on oyster mushroom; they received the fresh fruiting bodies of *P. ostreatus* after subjecting to complete drying at 55-60 °C until complete dehydration (Walde *et al.* 2003) Mushrooms were incorporated into the diet by a dose of 62.5 g/kg (Sirag 2009). All experimental animals from all groups were sacrificed after 30 days.

Blood and tissue sampling

Blood samples were collected from all animals at the end of the experiment, the serum was separated in clean, dry, sterilized and labeled centrifuge tubes, stored at -20°C until performing biochemical investigations. Liver tissues were obtained immediately from all animals after slaughtering, for molecular biological examinations; the tissue samples were frozen in liquid nitrogen directly until further investigations.

Blood glucose level determination

Plasma glucose was measured by the glucose oxidase-peroxidase method using the kit supplied by SPINREACT, Spain (Ref: 1001190) following the manufacturer instructions.

Biochemical Analysis

Serum superoxide oxide dismutase (SOD) activity was determined according to Nishikimi *et al.* (1972), serum catalase (CAT) activity was determined according to Abei (1984), serum malondialdehyde (MDA) concentration was determined according to Ohkawa *et al.* (1979) and serum total antioxidant capacity (TAC) was determined according to Kusano and Ferrari (2008).

RNA extraction

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

Superoxide dismutase and Catalase mRNA expression level determination

The expression level of superoxide dismutase and catalase mRNA in mice liver was determined by reverse transcriptase polymerase chain reaction (RT-PCR) (Jackerott *et al.* 2002). First strand cDNA was synthesized using two steps Superscript II kit (Invitrogen, Carlsbad, CA). The amplification was performed using thermal cycler (Takara MP, Japan). The cycling conditions for SOD and CAT are initial denaturation for 3 minutes at 94 °C, then 35 cycles each cycle (denaturation at 94°C for 45sec, annealing at 60°C for SOD and GAPDH and 55°C for CAT, for 1 minute and extension at 72°C for 1 minute) the final extension at 72°C for 5 minutes. Specific oligonucleotides primers pairs were used; SOD (GenBank accession number NT_039638) forward primer 5'- ACCATCCA CTTCGAGCAGAAGG-3', backward primer 5'- GGGACACACCAGACTTCAGAGT-3' (Suzuki *et al.* 1993), CAT (GenBank accession number NM_009804) forward primer 5'- GCAGATACCTGTGAACTGTC-3', backward primer 5'- GATGAATGTCCGCACCTGAG-3' and GAPDH (GenBank accession number NM_008084.2) forward primer 5'- CCCGTAGACAAAATGGTGAAGGTC-3', backward primer 5'- GCCAAAGTTGTCATGGATGACC-3' (CAT and GAPDH primers were designed by the use of primer 3 web version 3) with product sizes, 441,229 and 215 respectively. Then the amplified PCR products were run on 1.5% agarose gel in 1X Tris acetate EDTA running buffer (1X TAE) with condition of 100 Voltage/ 40 min as described by Uchida *et al.* (2000).

Statistical analysis

The data was analyzed using the statistical package for social science (SPSS Inc., Version 13, Chicago, Illinois, 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 $p < 0.05$.

3. Results**Fasting blood glucose level (mg/dl)**

Blood glucose was significantly decreased in the blood of diabetic mice fed on oyster mushroom or treated with insulin when compared with their diabetic control groups ($p < 0.05$) (Table I).

Blood Superoxide dismutase activity (U/ml)

SOD activity was significantly increased in the normal mice fed on mushrooms, also the activity was higher in the diabetic groups fed on mushrooms or treated with insulin than in diabetic groups only (Table I).

Blood Catalase activity (U/L)

The activity of CAT was significantly higher in all diabetic groups fed on mushrooms or treated with insulin than that in the control groups while it significantly increased in control groups fed on mushrooms (Table I).

Blood Malondialdehyde level (nmol/l)

The MDA level was seen to be lowest in the serum of normal mice fed on mushrooms, the highest level was detected in the diabetic non-treated mice while its level was significantly lowered in diabetic mice fed on mushroom if compared with their control (Table I).

Blood Total antioxidant capacity (nmol/ μ l)

The TCA level was seen to be lowest in the diabetic-non-treated mice, while the highest level was observed in the normal mice fed on oyster

mushroom; the level was significantly high in the diabetic group fed on oyster mushroom if compared with diabetic non-treated groups (Table I).

Expression level of mRNA of Superoxide dismutase and Catalase genes

The expression level was semi quantitatively calculated as a fold increase in pixels using Image J software (<http://rsb.info.nih.gov/ij/>) to analyze the gel pictures. The expression level of mRNA of SOD was seen to be highest in the diabetic mice treated with insulin and fed on mushroom if compared with other groups fig. (1) and (2a); while the highest expression level of mRNA of CAT was observed in normal mice fed on mushrooms. The CAT expression level in the diabetic treated groups with mushrooms and insulin was higher than those diabetic nontreated groups. Figs. (1) and (2b).

Table I. The effect of oyster mushroom feeding on the fasting blood glucose level, serum superoxide dismutase activity, serum Catalase activity, serum Malondialdehyde level and serum total antioxidant capacity level in experimental mice.

Groups	Diabetic non-treated	Diabetic-treated with insulin	Diabetic- fed on oyster mushroom	Control non-treated	Control -fed on mushroom
Fasting blood glucose (mg/dl)	238 \pm 18.8 ^a	89.7 \pm 9.8 ^c	104 \pm 8 ^b	88.3 \pm 2.8 ^c	87.1 \pm 6.6 ^c
Superoxide dismutase activity (U/ml)	80.4 \pm 7.5 ^d	156.1 \pm 7.9 ^c	161.4 \pm 5.4 ^c	262.7 \pm 8.1 ^b	287.02 \pm 10.4 ^a
Catalase activity (U/L)	365.5 \pm 43.2 ^d	582.9 \pm 89.7 ^c	562.9 \pm 22.5 ^c	847.8 \pm 34.1 ^b	924.9 \pm 23.1 ^a
Malondialdehyde (nmol/L)	80.4 \pm 4.1 ^a	50.9 \pm 4.7 ^b	49.4 \pm 4.05 ^b	51.5 \pm 0.8 ^b	33.6 \pm 3.2 ^c
Total antioxidant capacity (nmol/ μ L)	1.4 \pm 0.21 ^d	2.7 \pm 0.18 ^c	2.4 \pm 0.26 ^c	3.5 \pm 0.4 ^b	4.5 \pm 0.36 ^a

Means within the same raw carrying different subscript are significant at $p < 0.05$

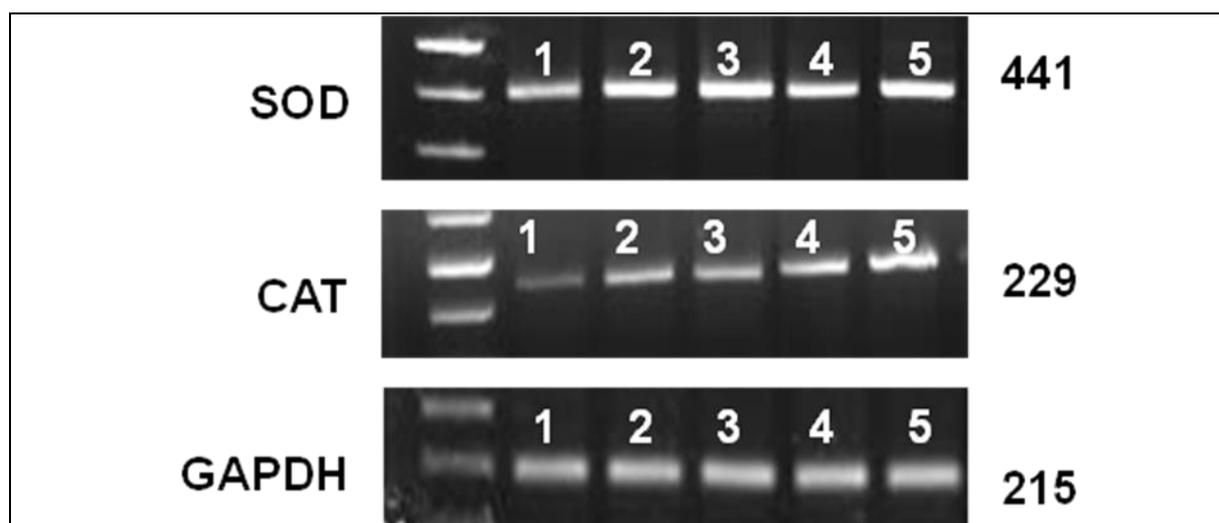


Figure 1. The expression level of mRNA of superoxide dismutase; Catalase and Glceraldehyde-3-phosphate dehydrogenase genes 1; Diabetic-non-treated, 2; Diabetic treated with insulin, 3; Diabetic fed on oyster mushroom, 4; control non treated and 5; control fed on mushroom.

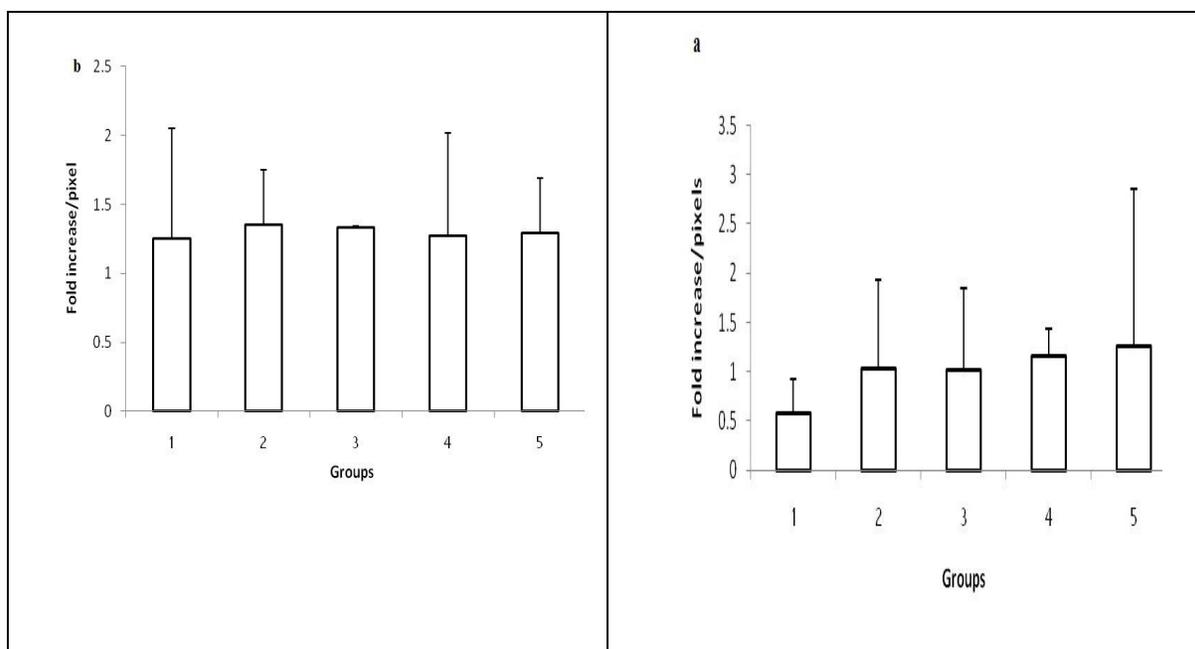


Figure 2. The expression level of (a) Superoxide dismutase and (b) Catalase genes analyzed by ImageJ software.

4. Discussion

In the present study the effect of oyster mushrooms on the oxidative stress resulted from diabetes mellitus in the experimental mice were examined. It is well-known that all diabetic models are suffering from chronic hypoglycemia (Kim *et al.* 2006). We tended to achieve this hyperglycemia using STZ as a strong chemical for inducing diabetes mellitus in experimental diabetic groups (Kim *et al.* 2001). The experimental animals from all diabetic groups were received a treatment with insulin and/or oyster mushroom. Our results revealed lowered blood glucose level in diabetic groups fed on both oyster and button mushrooms. This similar to the finding of many authors who reported the same effect of different types of edible mushrooms on diabetic models (Shehata *et al.* 2010). Several mechanisms have been proposed to explain the ability of mushrooms to induce lowering of blood glucose level in diabetic models; authors revealed the hypoglycemic power of mushrooms to its B-glucan contents (Jeong *et al.* 2010). It is known that the diabetes mellitus is usually associated with a deterioration in the antioxidant defense mechanisms; the matter which increase the oxidative stress and reactive oxygen species in the blood of diabetic models (Jeffrey *et al.* 2009 and Natheer 2011). This is what appeared in our results that the serum of diabetic mice showed the highest level of Malondialdehyde and the lowest levels of CAT, SOD and TAC. The expression level of SOD and CAT was calculated semi quantitatively in comparison to GAPDH as a house keeping gene. The expression

level was highest in the mushroom fed groups if compared with other diabetic groups. Our observations are similar to that of many reports which prove the antioxidant properties of mushrooms (Jayakumar *et al.* 2009). An increased oxidative stress not only resulted from hyperglycaemia associated with diabetes, but may also have an important causal role in β -cell failure and the development of insulin resistance which is the main cause of type II diabetes mellitus (Jeffrey *et al.* 2009). The antioxidant activities of mushrooms are previously regarded to their contents of many natural components of antioxidant properties like, phenols (Liu *et al.* 1997).; Vitamins such as A, C and β -carotenes (Murcia *et al.* 2002).; cystine, methionine and aspartic acid (Mattila *et al.* 2002) and Phenolic compounds, polyketides, terpenes and steroids (Cheung *et al.* 2003).

Conclusion

In conclusion mushrooms have the ability to reduce the oxidative stress and lower the level of MDA as an oxidative stress marker and increase the activity of antioxidant enzymes; SOD and CAT and TAC in blood as well as their expression levels in diabetic mice livers. This indicates the high antioxidant power of oyster mushroom in diabetic models.

Declaration of interest

The authors declare that they have no conflict of interest. All experiment materials and animals were self-funded from the authors.

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