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EFFECT OF *PLEUROTUS CITRINOPILEATUS* ON BLOOD GLUCOSE, INSULIN AND CATALASE OF STREPTOZOTOCIN-INDUCED TYPE 2 DIABETES MELLITUS RATS

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ABSTRACT

There is an increasing demand by patients to use natural products with hypoglycaemic activity for the treatment of diabetes mellitus. The present investigation aims at examining the hypoglycemic potential of methanolic extract of *P. citrinopileatus* mycelium on streptozotocin-induced type 2 diabetes mellitus rats. Oral treatment of control and diabetic rats with extract at 500 and 1000 mg/kg body weight were monitored for blood glucose, serum insulin and catalase activity. Results showed a significant reduction ($P<0.05$) in fasting blood glucose level and serum catalase activity but a significant increase in serum insulin level in the high dose treated group compared to untreated diabetes mellitus experimental group. Furthermore, some histopathological changes were noticed in the kidney tissue section. This study revealed *P. citrinopileatus* had excellent antidiabetic activity and thus have great potential as an ingredient in natural health products.

Key words: Yellow oyster mushroom, antidiabetic activity, hypoglycemic, mycelium

INTRODUCTION

Diabetes is the most common of the endocrine disorders, affecting more than 150 million people worldwide, with a projection of 300 million people by 2025 (Cockram, 2000). Diabetes mellitus (DM)is a metabolic disorder characterized by high blood glucose levels that results from defects in pancreatic insulin secretion and / or impaired target cell responsiveness to insulin (Groop and Tuomi, 1997). Despite numerous preventative strategies and armories of medication, the management of diabetes remains grossly unsatisfactory. Hypoglycemic agents, e.g., insulin, tolbutamide, phenformin, troglitazone, rosigitazone and repaglinide, are the mainstay of diabetes treatments and are effective in controlling hyperglycemia. However, they have harmful side-effects and fail to significantly alter the course of diabetic complications (Li *et al*., 2004). In addition to pharmacological treatment, hypoglycaemic effects can be obtained by nutritional approach. Naturally occurring compounds derived from foods have been proven to be effective in lowering blood glucose levels and can be further developed into nutraceuticals or health products for diabetes.

There are two main types of diabetes: type I and type II. Type I diabetes occurs as a result of insulin deficiency due to destructive lesions of pancreatic β-cells, and usually progresses to the stage of absolute insulin deficiency (Kuzuya *et al*., 2002). Typically, type I diabetes occurs in young subjects with acute onset, but may occur at any age, sometimes with slow progression.

Most patients previously called non insulin-dependent diabetes mellitus (NIDDM) belong to Type II diabetes category. In this type, mass of pancreatic β cells and their function are preserved to some extent, and insulin injection is seldom needed to sustain life. Both decreased insulin secretion and decreased insulin sensitivity (insulin resistance) are involved in its pathogenesis. Insulin resistance may not always be present. The relative role of these two factors varies between patients. With regard to insulin secretion, the acute insulin response to a glucose load is characteristically defective (Zimmet *et al*., 1997). Studies have also revealed that free radicals might also play vital role in the pathogenesis of this disease (Zimmet *et al*., 2001).

Mushrooms represent a major and as yet largest untapped source of powerful new pharmaceutical products and they are exemplary sources of natural medicines. *Pleurotus citrinopileatus,* an edible mushroom belonging to the *Pleurotaceae* family, has some physiological effects, including antitumor, immune enhancement, anti hyperglycemia (Lee *et al*., 2007; Hu *et al*., 2006), antioxidant (Beers and Sizer, 1952) and the reduction of blood sugar level (Willet, 1994). Nevertheless, little effort has been made to investigate antidiabetic activity of extracts obtained from either fruiting bodies or mycelium culture of *P. citrinopileatus*. Hence, the objective of this study was to elucidate, the effects of methanolic extract from mycelium of *P. citrinopileatus* on fasting blood glucose, serum insulin and catalase activity against streptozotocin-induced type 2 diabetes mellitus in rat model.

MATERIALS AND METHODS

Maintenance of culture and growth of mycelial biomass: Cultures of *P. citrinopileatus* (KUM 50093) were obtained from Mushroom Research Centre, Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia. Mycelium was grown on glucose, yeast, malt and peptone (GYMP) agar in plastic Petri dishes for 7 days at 25 ± 2 °C. Ten discs (5 mm in diameter) cut from the edge of the growing colony were transferred to 500 ml Erlenmeyer flasks containing 100 ml of sterilized GYMP liquid medium. The inoculated flasks were incubated under static condition for 14 days at 25 ± 2 °C.

Preparation of methanolic extract from *P. citrinopileatus* mycelium: Mycelium of *P. citrinopileatus* was mashed and soaked in methanol for 48 hours at 28 ± 2 °C. The methanol supernatant was then filtered through Whatman No. 1 filter paper and then evaporated to dryness under vacuum using rotary evaporator (BUCHI Rotavap or R-114) at 40 °C to form a crude extract. Crude extract was kept at 4 °C until further use for the treatment of Streptozotocin (STZ) induced diabetic rats. Chemicals used in the present study were of analytical reagent grade and were purchased from Sigma-Aldrich.

Animals and experimental design: Six to eight week old Sprague–Dawley rats (n=30) weighing 180–200g were purchased from the Centre for Animal Laboratory, Medical Faculty, University of Malaya, Malaysia. The rats were individually housed in cages in an air-conditioned room with controlled temperature of 22±2 °C and relative humidity of 45-55% under 12 hour light: 12 hour dark cycle. The animals had free access to pellets (Altromin 1324 FORTI, Germany) and water *ad libitum*. The experimental protocol was approved by the Animal Care and Use Ethics Committee, Faculty of Medicine, University of Malaya, Malaysia. The rats were divided into 5 groups (Group I to V), each group containing 6rats. The rats in Group I were control and were not treated with any drug. The rest of the rats were experimentally inoculated with streptozotocin to induce diabetes.

After acclimatization period of 48 hours, the diabetes was induced with streptozotocin (STZ) at a single dose of 70 mg/kg intraperitoneally. The STZ was dissolved in 0.1 M citrate buffer (pH 4.5) with an injection volume of1.0ml/kg. The control rats were injected with citrate buffer alone. The rats in group II were not treated, while Groups III and IV were orally administrated with 500 and 1000 mg/kg of *P. citrinopileatus* respectively. The rats in Group V were treated with 10 mg/kg glibenclamide dissolved in distilled water. The treatment lasted for 45 days. Two days after STZ administration, the glucose levels were determined to confirm diabetes. Rats exhibiting glucose level > 200 mg/dl were included in this study. On treatment day (two days after induction of diabetes), blood glucose level and body weight were measured in non-diabetic (control) and STZ-induced diabetic rats after 16 hours of fasting. The blood sample was collected at days 0, 2, 7, 15, 30, and 45 post treatment. All the samples were collected prior to16 hours of fasting. The fasting blood glucose levels were determined using ACCU-CHECK Active glucose monitoring system (Roche Diagnostics, Germany).

After 45 days of treatment, rats were fasted overnight and killed by decapitation. Blood was collected in plain tube, centrifuged at 3000 rpm for 15 minutes to obtain serum. The serum was kept at -70 °C until further analysis to determine catalase activity and insulin levels. The kidney was removed, washed in saline and fixed in 10% formalin for histology analysis.

Insulin assay: The insulin assay was done using the DSL-10-1600 ACTIVE Insulin ELISA kit (enzyme linked immunosorbent assay) which is an enzymatically “one step” sandwich-type immunoassay.

Catalase activity: The serum catalase activity was determined by the method of Beers and Sizer, (1952).

Histopathological evaluation: Kidney tissues were immediately fixed in 10% buffered neutral formalin solution for 24 hours. After fixation, tissues were embedded in paraffin and serial sections were cut and each section was stained with hematoxylin and eosin. The slides were examined under light microscope(Leica, Germany) and photographs were taken. The histopathological examination of kidneysample from each group was carried out to assess the architecture of the cells.

Statistical analysis: Data are presented as mean ± SD. The means of the data were subjected to a one way analysis of variance (ANOVA) using the SPSS software (SPSS Inc.)and the significance of the difference between means was determined by the Duncan’s multiple range test at $p < 0.05$.

RESULTS AND DISCUSSION

Diabetes mellitus comprises a heterogeneous group of hyperglycemic disorders characterized by loss of glucose homeostasis and disturbances of carbohydrate and lipid metabolism. Patients with diabetes are at increased risk of atherosclerosis and in clinical sequelae: coronary, renal, peripheral vascular and cerebro vascular diseases. Concurrently, the most common cause of death in persons with diabetes is myocardial infarction. The pathogenesis, progression and epidemiology of atherosclerotic diseases are distinct in patients with diabetes.

Effect of *P. citrinopileatus*on blood glucose levels and body weights: Treatment with *P. citrinopileatus* extract showed reduction in blood glucose levels when compared with STZ induced diabetec rats. Figure 1 displayed fasting blood glucose levels in experimental rats for 45 days of treatment. There were significant increase ($p<0.05$) in blood glucose levels between day 2 (initial day) at 14.20 ± 1.20 mmol/L for low dose (500 mg/kg body weight) of *P. citrinopileatus* extract treated rats and 12.60 ± 1.68 mmol/L for high dose (1000 mg/kg body weight) of *P. citrinopileatus* extract treated rats compared to control group of rats at 5.05 ± 0.24 mmol/L due to the induction of diabetes at day 0.However, by day 30,there was no significant difference between control group of rats with 4.88 ± 0.24 mmol/L, 6.67 ± 1.55 mmol/L for group of diabetic rats receiving low dose and 6.23 ± 0.81mmol/L for group of diabetic rats receiving high dose treatment. However, the blood glucose levels were significantly lower between the concentrations of the extracts compared to the Glibenclamide drug that acts as standard drug on the same day.

Diabetic rats treated with both concentrations of *P. citrinopileatus* extracts showed an increase in the body weight as compared to the untreated diabetic rats, which may be due to its protective effect in controlling glycemia. At day 15, there was no significant difference in body weight between group of rats treated with low dose of extract at 233.33 ± 20.41 grams, high dose at 245.83 ± 18.82 grams, drug treated rats at 229.17 ± 18.82 grams and untreated rats at 225.00 ± 27.39 grams but at day 30, rats treated with low and high dose of extract gained weight significantly with 250.00 ± 31.62 grams and 275.00 ± 22.36 grams respectively, compared to glibenclamide treated rats at 216.67 ± 25.82 grams and untreated rats at 208.33 ± 12.91 grams. Further, at day 45, group of rats receiving high dose of extract gained significant body weight of 287.50 ± 13.69 grams compared to low dose treatment rats with 254.17 ± 29.23 grams(Figure 2). With regards to the changes in physical appearance, after being fed with methanolic extract for 45 days, the colour of the eyes of both low and high dose extract fed groups and glibenclamide fed group were normal when compared to untreated rats. After 45 days, eyes of the untreated rats become almost white. Their lenses also showed obvious lesions of pigmentary degeneration. The untreated rats were thin, the fur was rough and the rats were obviously losing fur.

Fasting insulin and catalase activity: The untreated diabetic rats showed significantly reduced fasting insulin levels (0.53±0.13µU/mL)as compared to the control rats (1.66±0.22µU/mL). There was no significant difference in fasting insulin levels of control group rats when compared with low dose and high dose treated rats at the end of 45 days. Controlrats and rats treated with high dose of mycelium extract had the same insulin level at 1.66 ± 0.22 µU/mL and 1.66 ± 0.16 µU/mL respectively, compared to drug treated rats with 1.04 ± 0.10 µU/mL (Table 1). The methanolic extract of *P. citrinopileatus* mycelium produced marked decrease in blood glucose levels at 500 mg/kg and 1000 mg/kg body weight in STZ induced diabetic rats over 45 days of treatment. Elevation of insulin by *P. citrinopileatus* extract treated STZ-induced diabetic rats could be due to the presence of insulinotropic substances that might have stimulatedmoreproduction of insulin fromfunctional β-cells.

Fasting catalase levels marks no significant difference between control rats and rats treated with both concentrations of mycelium extracts. Untreatedrats showed low catalase activity when compared to other groups (Table 1). Catalase is a hemoprotein which catalyzes the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals (Kohner *et al*., 1998). The catalase activity increased in myceliummethanolic extract treated rats and drug treated rats. Rats treated with extracts and drug enhances the oxidative stress due to sudden attack of free radicals caused by STZ where glucose level was increased above the renal threshold, gene expression and repairing mechanism may be in favour of synthesizing more catalase and antioxidants to compensate oxidative and carbonyl stress-mediated cellular damage and possibly the cells try to cope up with the adverse situation.

Table 1.Fasting insulin and catalase levels at the end of 45 days

Group	Fasting insulin levels (µU/mL)	Fasting catalase levels (U/mL)
Group I	1.66 ± 0.22^{c}	101.88 ± 4.07^{c}
Group II	0.53 ± 0.13^{a}	53.10 ± 1.53^{a}
Group III	1.60 ± 0.07^{c}	100.77 ± 2.34^{bc}
Group IV	1.66 ± 0.16^{c}	103.66 ± 2.69^{c}
Group V	1.04 ± 0.10^{b}	96.10 ± 2.85^{b}

Values are expressed by mean ± SD of six rats in each group
Means with different letter in the same column denotes significant ($p<0.05$).

Histological changes on kidney: Fig. 3 showed the STZ drug did damage all the glomeruli and interstitium stroma of cortex, but the cross section of cortex treated with glibenclamide drug (Fig 3d.) and mycelium extract (Fig 3c.) showed that the tissues suffered mild congestion compared to the cross section of the cortex of untreated diabetic rats.This indicates that the mycelium extract possessed wound healing property or perhaps an unknown enzyme that repairs the damage caused by STZ drug. Diffuse intercapillary glomerulosclerosis was observed in the histological study. It is characterized by an increased amount of eosinophilic material within the mesangial region and a widening of the mesangial matrix

in Fig. 3b, Fig. 3c but eventually the process becomes diffuse, involving the glomerulus and generalized, affecting all glomeruli within the kidney as seen in Fig. 3d. With increasing duration of diabetes, the mesangial material expands and coalesces, encroaching on adjacent capillary lumina and reducing the capillary surface area that is available for filtration. As this process progresses, entire glomeruli becomes hyalinised. Omara *et al*. (2012) also supported our study.

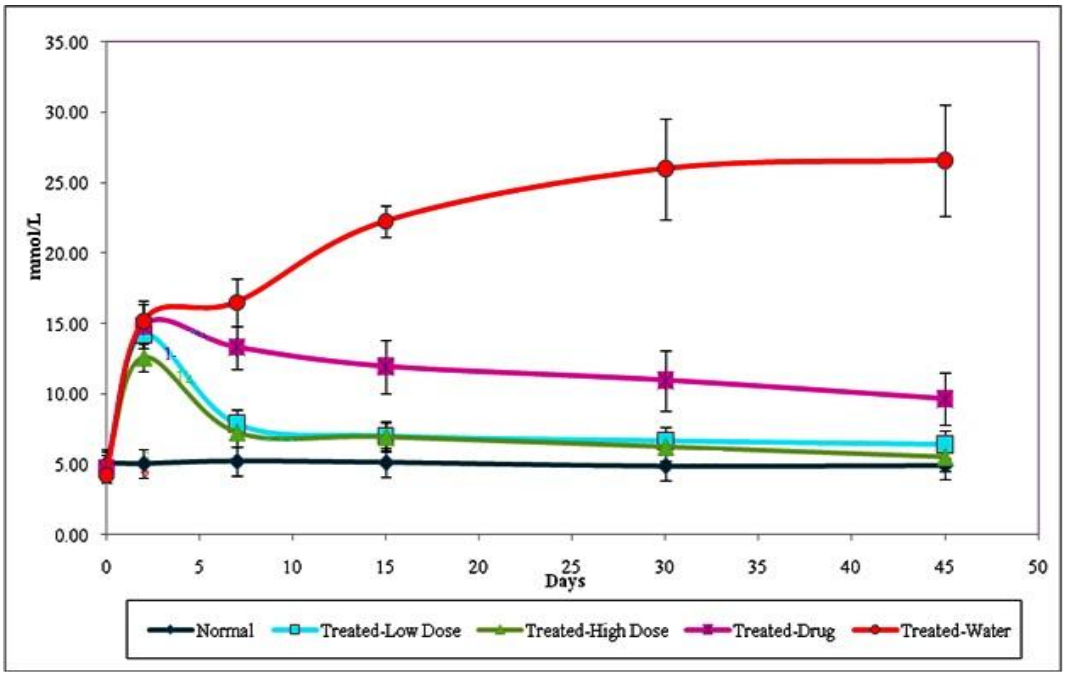

Fig. 1 Fasting blood glucose levels in STZ-induced diabetic and control rats for 45 days.

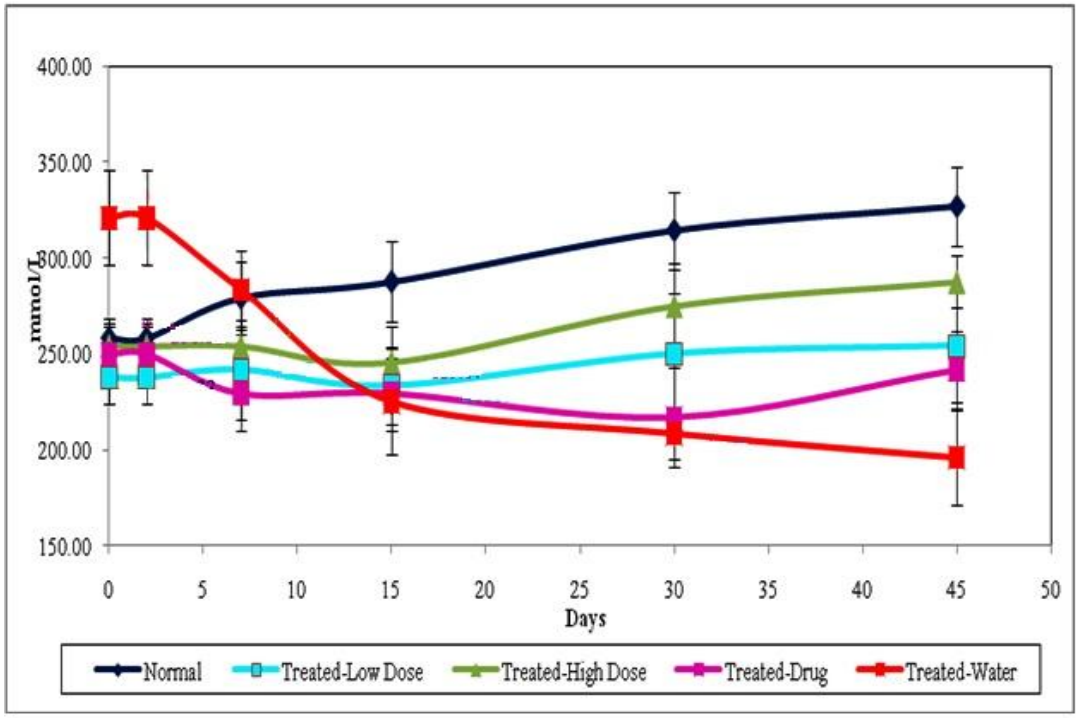

Fig. 2 Body weights of STZ-induced diabetic and control rats for 45 days.

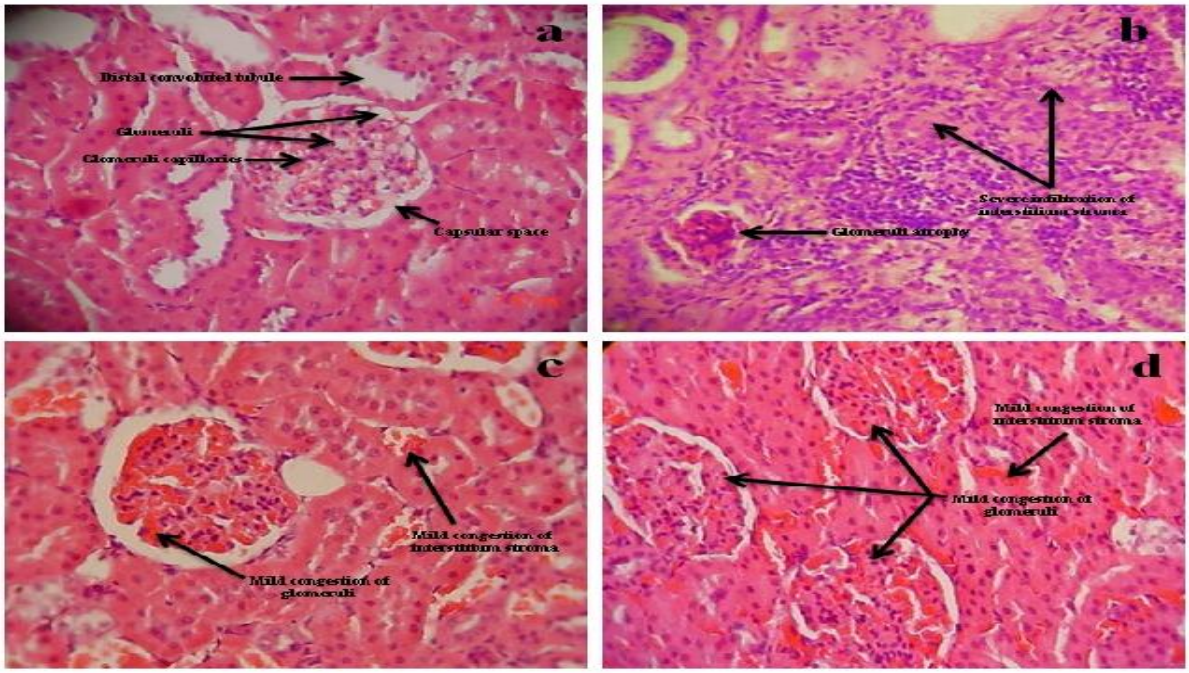

Fig.3 a. Histological section of kidney of control rats showed normal architectures of glomeruli and tubules and no abnormality detected (H & E stain, X80).

b. Histological section of kidney of STZ-induced diabetes untreated rats showed glomerular atrophy and severe infiltration of interstitium stroma of cortex with chronic inflammatory cells (H & E stain, X80).

c. Histological section of kidney in STZ-induced diabetes and treated with 1000 mg/kg of methanolic extract of *P. citrinopileatus* mycelium. Section showed a very mild congestion of the glomeruli and interstitium stroma of cortex (H & E stain, X80).

d. Histological section of kidney of STZ-induced diabetes rats and treated with 10 mg/kg Glibenclamide showed mild congestion of the glomeruli and interstitium stroma of cortex (H & E stain, X80).

Conclusion: In conclusion, the administration of methanolic extract of *P. citrinopileatus* mycelium exhibited significant regulation of blood glucose, insulin and catalase. The present study indicates that a significant antidiabetic effect of the extract to potentiate the β-cells of pancreas. *Pleurotus citrinopileatus* controls the blood glucose, insulin and catalase levels of STZ induced diabetic rats that lead to normo-glycemic. Therefore, *P. citrinopileatus* possessed antidiabetic property by stimulating the insulin production from the pancreas, extra-pancreatic action and its support to control the diabetes and their complications.

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