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# Hypoglycaemic Activity of Culinary *Pleurotus ostreatus* and *P. cystidiosus* Mushrooms in Healthy Volunteers and Type 2 Diabetic Patients on Diet Control and the Possible Mechanisms of Action

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**This study determined the oral hypoglycaemic effect of suspensions of freeze dried and powdered (SFDP) *Pleurotus ostreatus* (*P.o*) and *Pleurotus cystidiosus* (*P.c*), using healthy human volunteers and Type 2 diabetic patients on diet control at a dose of 50 mg/kg/body weight, followed by a glucose load. The possible hypoglycaemic mechanisms were evaluated using rats, by examining intestinal glucose absorption and serum levels of insulin, glucokinase (GK) and glycogen synthase kinase (GSK). The *P.o* and *P.c* showed a significant reduction ($P < 0.05$) in fasting and postprandial serum glucose levels of healthy volunteers and reduced the postprandial serum glucose levels and increased the serum insulin levels ($P < 0.05$) of Type 2 diabetic patients. The *P.o* and *P.c* increased the intestinal absorption of glucose but simultaneously reduced the serum glucose levels ($P < 0.05$) in rats. Both mushrooms reduced the serum GSK and promoted insulin secretion while *P.c* increased serum GK ($P < 0.05$). The hypoglycaemic activity of *P.o* and *P.c* makes mushrooms beneficial functional foods in diabetes mellitus. The mechanism of hypoglycaemic activity of *P.o* and *P.c* is possibly by increasing GK activity and promoting insulin secretion and thereby increasing the utilization of glucose by peripheral tissues, inhibiting GSK and promoting glycogen synthesis. Copyright © 2014 John Wiley & Sons, Ltd.**

*Keywords: Pleurotus ostreatus*; *Pleurotus cystidiosus*; hypoglycaemic; insulin; glucokinase; glycogen synthase kinase.

## INTRODUCTION

Mushrooms have been available throughout the world as both culinary products and medicines for many years (Wasser, 2011). Medicinal mushrooms have been established as remarkable therapeutic agents in traditional medicines, especially in Asian countries (Lindequist *et al.*, 2005). *Pleurotus* mushroom is distributed worldwide and is characterized by a cap which is convex and is also semicircular. Basidiomes are usually large and fleshy whereas stipe is short, solid and eccentric (Lechner *et al.*, 2004). They are called 'oyster mushrooms' comprising approximately 40 species (Spahr, 2009).

*Pleurotus ostreatus* (Jacq.:Fr.) P. Kumm. and *Pleurotus cystidiosus* O.K. Miller (Pleurotaceae, higher Basidiomycetes) are culinary-medicinal mushrooms grown worldwide. The *P.o* and *P.c* commonly known as American oyster and abalone, respectively, were shown to possess hypocholesterolaemic, antioxidant, antitumour, hepatoprotective, hypotensive, antinociceptive and antifungal activity (Abeytunga, 2011). In our previous studies, we demonstrated the promising acute and chronic oral hypoglycaemic potential of *P.o* and *P.c* in both normal and alloxan-induced diabetic animals as well as the anti-inflammatory activity of *P.o* (Jayasuriya *et al.*, 2012a; Jayasuriya *et al.*, 2012b).

Recently, medicinal mushrooms have become popular as functional foods which are also called as dietary supplements or 'mushroom nutriceuticals' (Wasser and Didukh, 2004). Functional food is similar in appearance to, or may be, a conventional food which affects beneficially one or more target functions in the body and promotes a state of well-being and health or reduces the risk of a chronic disease such as diabetes beyond basic nutritional function (Roberfroid, 1999; Rudkowska, 2009). The role of mushrooms as functional food in preventing diabetes was reviewed and summarized by De Silva *et al.*, (2012).

Diabetes mellitus is a chronic endocrine disorder characterized by hyperglycaemia, resulting from deficiency in insulin secretion, action of insulin or both (Kumar and Clark, 2012). Promising hypoglycaemic activity of *P.o* and *P.c* in rats (Jayasuriya *et al.*, 2012a) has resulted in studies to establish the effect in humans. Hence, the purpose of the present study was to investigate the oral hypoglycaemic potential of *P.o* and *P.c* in healthy human volunteers and Type 2 diabetic patients on diet control and to identify the possible mechanisms underlying the said activity of both mushrooms. Further, this study examined the safety of long term consumption of both mushrooms. Hence, the major goal of this study is to explore the possibility of recommending *P.o* and *P.c* as functional foods.

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## MATERIALS AND METHODS

**Study setting.** The study with human subjects was conducted at the Family Practice Centre and Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka. The study with animals was conducted at the Animal House and Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka.

**Ethical clearance.** The protocol of the study was evaluated by the Ethics Review Committee of the Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka and ethical clearance (No. 380/8 and 599/11) was obtained. Written informed consent was obtained from healthy volunteers and diabetic patients on diet control. The human study has been registered in Sri Lanka Clinical Trials Registry (Trial Registration No: SLCTR/2013/021). Sri Lanka Clinical Trials Registry is linked to the Registry Network of the International Clinical Trials Registry platform of the World Health Organization (WHO-ICTRP).

**Experimental animals.** Healthy adult Wistar rats (200–250 g) were purchased from the Medical Research Institute Colombo. They were housed under standardized conditions at the Animal House and had access to food (WHO recommended food formula: Maize 40.1, Broken brown rice 10, Rice bran 2.5, Wheat bran 2, Wheat flour 13.5, Fish meal 8, Soya meal 8, Sugar 2.5, Soya oil 2, Grass powder 3, Bone meal 1.5, Mineral mix 0.4, Vitamin mix 0.24, NaCl 0.2, Beta mix E50 0.02, DL methionine 0.05, milk powder 6 spoons, B complex 600 tablets/100 kg) and water *ad libitum*, unless specified otherwise. In all animal experiments 6 rats were included per group.

**Collection of mushrooms.** Fresh *P.o* and *P.c* grown using the spawn provided by the Mushroom Cultivation Centre, Export Research Board (Ratmalana, Sri Lanka) were collected from a local farm. The identification and authentication were done by studying the spore print and the shape of the cap and the stipe.

**Preparation of mushrooms.** Fresh *P.o* and *P.c* were washed with water to remove soil particles and freeze-dried (Eyela, FD-5N, Japan) and ground with a commercial blender (Sonica, SA-317, China). Powdered samples of mushrooms were stored air-tight at 4 °C.

### Study on human subjects

**Effect of *P.o* and *P.c* in healthy volunteers.** Healthy human volunteers were recruited by an open advertisement. Subjects ($n$=22/group) were fasted overnight, and fasting serum glucose levels were measured using the glucose oxidase reagent kits (Biolabo reagents, France). Baseline values of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), gamma glutamyltransferase (γ GT) and creatinine levels were measured. Creatinine clearance was calculated using Cockroft–Gault equation (Kumar and Clark, 2012). Serum levels of ALT, AST, γ-GT and creatinine were measured by using the reagent kits (Biolabo reagents, France), and serum ALP levels were measured by using the reagent kits from Stanbio Laboratory, Texas.

All subjects received distilled water as the control. Thirty minutes later, 75 g of glucose in 300 mL of water was administered. Serum glucose levels were measured 2 h after the glucose load. Two groups of subjects received SFDP *P.o* and *P.c* at a dose of 50 mg/kg/body weight (b.w.) for 2 weeks, respectively. At the end of 2 weeks, following an overnight fast, blood was drawn for the determination of fasting serum glucose concentration. The same procedure was repeated with the administration of mushroom suspensions and glucose and postprandial serum glucose levels were measured. The subjects were monitored for 1 month for any adverse effects and at the end of one month serum levels of ALT, AST, ALP, γ GT and creatinine were determined. Creatinine clearance was calculated.

**Effect of *P.o* and *P.c* in Type 2 diabetic patients, on diet control.** Type 2 diabetic patients on diet control and fasting serum glucose levels above 7.0 mmol/L were included in the study. After an overnight fast, the subjects ($n$=28) received distilled water as the control. Thirty minutes later, 75 g of glucose in 300 mL of water was administered. Serum glucose and insulin levels were measured 2 h after the glucose load. Baseline values of ALT, AST, ALP, γ GT and creatinine levels were also measured. Creatinine clearance was calculated. After a week, the subjects were divided to two groups as *P.o* and *P.c* by simple randomization. This was done by giving sequential numbers to subjects and allocating even numbers to one group (group 1) and alternate numbers to the other (Group 2). Prepacked freeze dried and powdered *P.o* and *P.c* were identical in appearance. Only the investigators were aware of the type of mushroom in each pack. These packs were numbered, and the codes were with the investigators. The patients allocated to each mushroom group were kept blinded to the allocated group.

The group SFDP *P.o* [($n$=14) (Wang and Chow, 2007)] received a single test dose of SFDP *P.o* (dose of 50 mg/kg/b.w) and the group SFDP *P.c* ($n$=14) received a single test dose of SFDP *P.c* (dose of 50 mg/kg/b.w). Thirty minutes later, 75 g of glucose in 300 mL of water was administered. Postprandial serum glucose and insulin levels were measured 2 h after the glucose load. The subjects were monitored for 1 month for any adverse effects and at the end of the 1-month period, serum levels of ALT, AST, ALP, γ GT and creatinine were determined. Creatinine clearance was also calculated.

### Studies on hypoglycaemic mechanisms

**Effect of *P.o* and *P.c* on intestinal glucose absorption of healthy and diabetic Wistar rats.** The rats were injected with alloxan monohydrate (Sigma Aldrich, USA) dissolved in normal sterile saline at a dose of 40mg/kg/b.w body weight intravenously to induce hyperglycaemia. Rats were grouped into six ($n$=6/group) viz Test 1: Normal *P.o*, Test 2: Normal *P.c*, Test 3: Diabetic *P.o*, Test 4: Diabetic *P.c*, Test 5: Normal control and Test 6:

Diabetic control. Following an overnight fast, groups Test 1 and 3 were orally administered the SFDP *P.o* whereas groups, Test 2 and 4 received SFDP *P.c* at a dose of 500 mg/kg, respectively. In our previous study, the dose of 500 mg/kg was identified as the maximally effective dose (Jayasuriya *et al.*, 2012a). Test 5 and 6 groups were fed with distilled water. A glucose load of 3.0 g/kg was administrated to each group, 30 min after respective administration of the suspension and water, respectively. Ninety minutes after the glucose load, rats were euthanized, and intestines were harvested. Blood was drawn by cardiac puncture for the determination of serum glucose level. The intestines were packed in ice and transported to the laboratory. These were opened up and washed repeatedly with distilled water, and the contents were brought to a final volume of 50 mL. The contents were centrifuged, and the glucose concentration was determined in the supernatants.

**Effect of *P.o* and *P.c* on serum levels of insulin, GK and GSK of diabetic Wistar rats.** Diabetic rats were fasted overnight. Test groups were administered with SFDP *P.o* and *P.c* at a dose of 500 mg/kg, respectively. Control group was fed with distilled water. A glucose load of 3.0 g/kg was administered to each group, 30 min after administering the suspension and water, respectively. Serum levels of insulin, GK and GSK were determined, using ELISA kits (Six C USA Co. Ltd, China) 90 min after the glucose load.

**Effect of long-term administration of *P.o* and *P.c* on healthy Wistar rats.** Normal rats were divided into two groups *viz*; SFDP *P.o* and *P.c* and control. Single daily oral administration of suspensions at a dose of 500 mg/kg was done for 6 weeks at prefixed times in addition to their normal diet and water. The control group received their normal diet and a dose of distilled water for the same period of time. At the end of the experimental period, serum levels of ALT, AST, γ GT, creatinine and haemoglobin (Hb) were measured using diagnostic kits. The rats were also observed for any abnormal behaviour, and the food and water intakes were measured daily.

**Statistical analysis.** Final results were presented as mean ± SEM. The results were analyzed for statistical significance using 'Student's *t* test. Statistical analysis was done using SPSS 17, and *P* values <0.05 were considered as significant.

## RESULTS

### Study on human subjects

**Effect of *P.o* and *P.c* in healthy volunteers.** Serum glucose levels of healthy volunteers ($n = 22$/group) who received the two mushrooms are shown in Table 1. The percentage reduction in the fasting and postprandial serum glucose levels for the group given *P.o* was 6.1% and 16.4%, respectively. For healthy volunteers given *P.c*, these values were 6.4% and 12.1%, respectively. There were

**Table 1. Effect of multiple doses of suspensions of freeze dried and powdered *Pleurotus ostreatus* and *P. cystidiosus* on fasting and postprandial serum glucose levels in healthy volunteers**

| Treatment (50 mg/kg/b.w) | Fasting serum glucose concentration (mmol/L) | Postprandial serum glucose concentration (mmol/L) |
|---|---|---|
| Control group of *P. ostreatus* (water) | 4.5 ± 0.1 | 5.5 ± 0.2 |
| Test group of *P. ostreatus* (*P. ostreatus*) | 4.3 ± 0.1* | 4.6 ± 0.1** |
| Control group of *P. cystidiosus* (water) | 4.4 ± 0.1 | 5.3 ± 0.2 |
| Test group of *P. cystidiosus* (*P. cystidiosus*) | 4.1 ± 0.1* | 4.6 ± 0.1** |

Values are expressed as mean ± SEM.
Significantly different from Control at
*$p < 0.05$ and
**$p < 0.001$.

**Table 2. Effect of suspensions of freeze dried and powdered *Pleurotus ostreatus* and *P. cystidiosus* on serum levels of key hepatic enzymes (ALT, AST, ALP and γ-GT) and creatinine as well as creatinine clearance of healthy volunteers and Type 2 diabetic patients, on diet control**

| Treatment (50 mg/kg/b.w.) | | ALT (IU/L) | AST (IU/L) | ALP (U/L) | γ-GT (IU/L) | Creatinine (mg/dL) | Creatinine clearance (mL/min) |
|---|---|---|---|---|---|---|---|
| *P. ostreatus* (healthy volunteers) | Baseline | 24.8 ± 2.9 | 25.5 ± 2.9 | 30.7 ± 1.5 | 10.9 ± 1.2 | 0.85 ± 0.04 | 103.2 ± 9.3 |
| | After 1 month from the treatment | 25.3 ± 2.8 | 28.5 ± 3.9 | 28.5 ± 2.2 | 10.6 ± 1.5 | 0.89 ± 0.04 | 94.0 ± 5.8 |
| *P. cystidiosus* (healthy volunteers) | Baseline | 23.8 ± 2.8 | 29.8 ± 2.6 | 32.1 ± 2.4 | 15.8 ± 2.5 | 0.84 ± 0.06 | 117.6 ± 14.1 |
| | After 1 month from the treatment | 24.2 ± 2.6 | 29.5 ± 2.2 | 33.2 ± 2.9 | 15.6 ± 2.1 | 0.89 ± 0.05 | 104.2 ± 9.6 |
| *P. ostreatus* (diabetic patients) | Baseline | 32.2 ± 4.7 | 31.6 ± 2.8 | 43.5 ± 4.1 | 18.5 ± 2.6 | 1.01 ± 0.06 | 83.7 ± 8.3 |
| | After 1 month from the treatment | 36.9 ± 5.0 | 36.8 ± 4.0 | 42.2 ± 3.6 | 19.2 ± 2.9 | 1.02 ± 0.10 | 86.6 ± 11.7 |
| *P. cystidiosus* (diabetic patients) | Baseline | 32.2 ± 4.7 | 31.6 ± 2.8 | 43.5 ± 4.1 | 18.5 ± 2.6 | 1.01 ± 0.06 | 83.7 ± 8.3 |
| | After 1 month from the treatment | 34.1 ± 4.2 | 34.0 ± 3.3 | 47.4 ± 5.4 | 16.4 ± 1.9 | 1.05 ± 0.06 | 78.5 ± 6.9 |

Values are expressed as mean ± SEM.
No statistically significant differences were observed between the baseline and after one month values of tested parameters in each group.

no significant differences in serum levels of ALT, AST, ALP, γ-GT and creatinine as well as calculated creatinine clearance before and after 1 month from the treatment ($P > 0.05$). Table 2 shows the serum levels of key hepatic enzymes and creatinine as well as calculated creatinine clearance following the intervention.

**Effect of *P.o* and *P.c* in Type 2 diabetic patients, on diet control.** Data from all participants were used for analysis. The effect of a single dose of *P.o* and *P.c* on postprandial serum glucose levels of Type 2 diabetic patients, who were on diet control, is given in Fig. 1A ($n = 14$/group). There was a significant reduction in postprandial serum glucose levels of *P.o* and *P.c* groups when compared with the control group (14.9%, $p < 0.01$ and 16.6%, $p < 0.001$, respectively). The findings on serum insulin are given in Fig. 1B. Postprandial serum insulin levels of *P.o* and *P.c* groups were significantly increased when compared with the diabetic control group (21.7%, $p < 0.01$ and 21.0%, $p < 0.05$, respectively). There were no significant differences in serum levels of ALT, AST, ALP, γ-GT and creatinine as well as calculated creatinine clearance before and after 1 month from the treatment ($P > 0.05$). No adverse effects were identified in both groups after 1 month from the treatment. Table 2 shows the serum levels of key hepatic enzymes and creatinine as well as calculated creatinine clearance following the intervention.

## Studies on hypoglycaemic mechanisms

**Effect of *P.o* and *P.c* on intestinal glucose absorption of healthy and diabetic Wistar rats.** The effect of SFDP *P.o* and *P.c* on intestinal glucose absorption of normal and diabetic rats is shown in Fig. 2. There was a significant reduction in postprandial glucose levels in serum and in the intestinal contents of normal and diabetic test groups

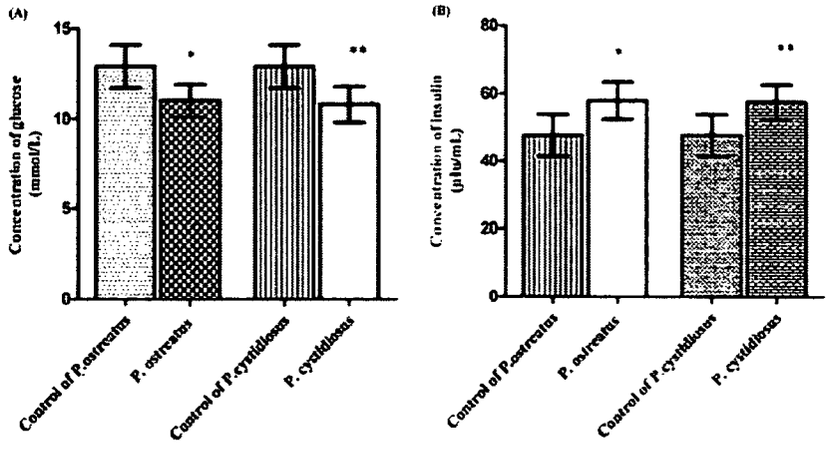


**Figure 1.** Hypoglycaemic effect of suspensions of freeze dried and powdered *P. ostreatus* and *P. cystidiosus* on Type 2 diabetic patients, on diet control A) Effect of *P. ostreatus* and *P. cystidiosus* on postprandial serum glucose levels of diabetic patients. Values are expressed as mean ± SEM. $*p < 0.01$ and $**p < 0.001$ when compared with the control group. B) Effect of *P. ostreatus* and *P. cystidiosus* on postprandial serum insulin levels of diabetic patients. Values are expressed as mean ± SEM. $*p < 0.01$ and $**p < 0.05$ when compared with the control group.

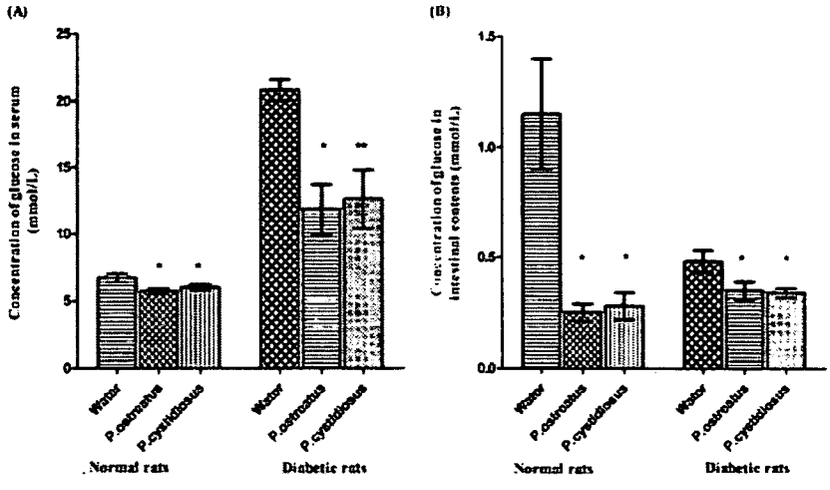


**Figure 2.** Effect of *P. ostreatus* and *P. cystidiosus* on intestinal glucose absorption of healthy and diabetic Wistar rats (A) Effect of *P. ostreatus* and *P. cystidiosus* on serum glucose levels of normal and diabetic rats following a glucose challenge. Values are expressed as mean ± SEM. Significantly different from control at $*p < 0.05$ and $**$ $p < 0.01$. (B) Effect of *P. ostreatus* and *P. cystidiosus* on intestinal glucose levels of normal and diabetic rats following a glucose challenge. Values are expressed as mean ± SEM. Significantly different from control at $*p < 0.05$.

(Test 1, 2, 3 and 4 groups) when compared with the respective control groups ($p < 0.05$). The percentage reductions of serum glucose levels of normal rats that received *P.o* and *P.c* were 14.4% and 10.3%, respectively whereas for diabetic rats those values were 43.4% and 39.5%, respectively. Percentage reductions of glucose concentrations in the intestinal contents of diabetic rats given *P.o* and *P.c* were 26.2% and 29.1%, respectively.

**Effect of *P.o* and *P.c* on serum levels of insulin, GK and GSK of diabetic Wistar rats.** The effect of SFDP *P.o* and *P.c* on serum levels of insulin, GK and GSK of diabetic rats is shown in Fig. 3. There was a significant increase in postprandial serum insulin levels of *P.o* and *P.c* groups when compared with the control group ($p < 0.05$) and the percentage increases were 32.6% and 38.3%, respectively. Postprandial serum GK level of *P.c* group was significantly increased when compared with the control group ($p < 0.05$) whereas the percentage increase was 23.1%. Percentage reductions of serum GSK levels of *P.o* and *P.c* groups were 25.6% and 12.5%, respectively ($P < 0.05$ when compared with the control group).

**Effect of long term administration of *P.o* and *P.c* on healthy Wistar rats.** There were no significant differences in serum levels of ALT, AST, γ-GT, creatinine and Hb of test and control groups following administration of SFDP *P.o* and *P.c* for 6 weeks. The serum levels of key hepatic enzymes, creatinine and Hb of *P.o* and *P.c* and respective control groups are given in Table 3. The *P.o* and *P.c* did not produce significant changes in behaviour, and the food and water consumption was constant throughout the study.

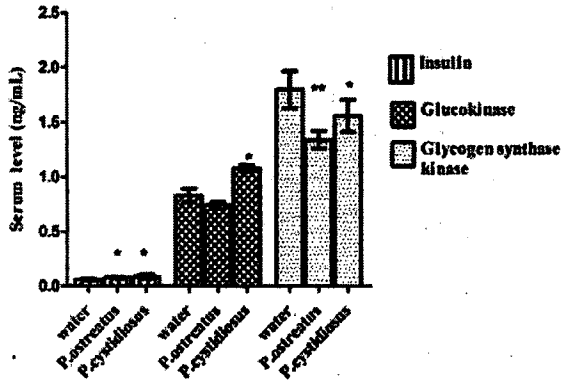


Figure 3. Effect of *P. ostreatus* and *P. cystidiosus* on postprandial serum levels of insulin, glucokinase and glycogen synthase kinase of diabetic rats Values are expressed as mean ± SEM. Significantly different from control at $^{*}p < 0.05$ and $^{**}p < 0.01$.

## DISCUSSION

This study evaluated the oral hypoglycaemic activity of *P.o* and *P.c* using healthy human volunteers and Type 2 diabetic patients on diet control and attempted to elucidate the hypoglycaemic mechanisms. When consumed for 2 weeks, both fasting and postprandial serum glucose levels were decreased significantly in healthy volunteers, which suggest that long-term consumption of *P.o* and *P.c* may be beneficial to humans. Furthermore, *P.o* and *P.c* did not cause any hepato-renal damage in the healthy volunteers. These findings encouraged the evaluation of the hypoglycaemic potential of *P.o* and *P.c* in Type 2 diabetic patients on diet control.

*P. ostreatus* and *P.c* significantly reduced postprandial serum glucose levels and increased postprandial serum insulin levels in Type 2 diabetic patients on diet control. Moreover, as it is considered that tight glycaemic control is beneficial in lowering the risk of developing long-term complications of diabetes, *P.o* and *P.c* possess immense value as a functional food for improving postprandial glucose control in people with diabetes.

The observation of an increase of postprandial insulin levels in Type 2 diabetic patients led to the further evaluation of the hypoglycaemic mechanism of action of the two mushrooms. The significant fall in postprandial serum glucose levels in normal and alloxan-induced diabetic rats in the present study (Fig. 2A) are on par with our previous findings (Jayasuriya *et al.*, 2012a). The mushrooms had enhanced the absorption of glucose from the intestines as evident by the lower level of glucose in the intestinal contents of the mushroom groups. However, the serum glucose levels of the groups given the mushroom suspensions were also significantly lower than in the respective control groups. The reasons for reduced serum glucose despite an increase in intestinal absorption of glucose needed to be ascertained.

According to Fig. 2B, the mushrooms may have reduced the absorption of glucose into enterocytes, in diabetic rats. Some mushrooms such as *Ganoderma lucidum* effectively reduced glucose absorption through the intestine by inhibiting α glucosidase (Fatmawati *et al.*, 2011). There were no reported data on hypoglycaemic activity of *Pleurotus* produced via α glucosidase inhibition.

The effect of *P.o* and *P.c* on GK, a key hepatic enzyme in glucose metabolism, was examined in this study. Glucokinase is a Type IV isoenzyme, in the family of hexokinases (Cox and Nelson, 2008). Glucose enters into the pancreatic β cells and gets phosphorylated to glucose-6-phosphate by GK which in turn increases intracellular ATP. A cascade of reactions triggers the insulin secretion

**Table 3. Effect of long term administration of suspensions of freeze dried and powdered *Pleurotus ostreatus* and *P. cystidiosus* on serum levels of key hepatic enzymes (ALT, AST and γ-GT), creatinine and haemoglobin of healthy Wistar rats**

| Treatment | ALT (IU/L) | AST (IU/L) | γ-GT (IU/L) | Creatinine (mg/dL) | Haemoglobin (g/dL) |
|---|---|---|---|---|---|
| Control group of *P. ostreatus* (water) | 63.9±10.4 | 21.6±5.6 | 2.7±1.2 | 0.5±0.0 | 11.9±0.8 |
| Test group of *P. ostreatus* (*P. ostreatus*) | 66.6±5.2 | 24.1±4.8 | 3.2±0.3 | 0.4±0.0 | 12.0±0.8 |
| Control group of *P. cystidiosus* (water) | 36.4±4.2 | 20.1±1.8 | 4.1±0.7 | 0.8±0.0 | 11.9±0.8 |
| Test group of *P. cystidiosus* (*P. cystidiosus*) | 29.7±4.2 | 23.4±3.2 | 3.4±0.3 | 0.8±0.0 | 14.2±0.9 |

Values are expressed as mean ± SEM.

No statistically significant differences were observed in the tested parameters between the test and control groups.

from β cells (Cox and Nelson, 2008). Moreover, high levels of GK can cause an increase in utilization of blood glucose and promote glycogen storage in the liver whereas low levels of GK favours the release of glucose into the circulation via gluconeogenesis (Mahmoodi *et al.*, 2013). Therefore, it is advantageous to have increased activity of GK during the treatment of diabetes mellitus, and the role of glucokinase activators (GKA) such as piragliatin has been discussed (Bonadonna *et al.*, 2010; Matschinsky *et al.*, 2011). In the present study, *P.c.* has enhanced GK levels in serum. Hence, it appears that an acceleration of glucose metabolism via increased GK is one mechanism for the hypoglycaemic activity of *P.c.* Moreover, *P.c* might have components which act as GKA. The polysaccharides of *Cordyceps sinensis* significantly increased the activity of hepatic GK in diabetic mice (Kiho *et al.*, 1999).

Insulin binds to a specific receptor of the target cells and enhances glucose intake, utilization and storage in various tissues, and in the liver it suppresses glycogenolysis and gluconeogenesis while stimulating glycogen synthesis (Cox and Nelson, 2008). In muscle and adipose tissue, insulin increases facilitated transport of glucose and stimulates glycogen synthesis and glycolysis (Cox and Nelson, 2008). Increased levels of GK trigger insulin secretion (Prabhakar and Doble, 2011). The enhanced levels of GK in diabetic rats following administration of *P.c* would have acted as a stimulus for insulin secretion. By increasing insulin secretion in rats and in Type 2 diabetic patients on diet control, *P.o* and *P.c* may act as an insulin secretagogue. Mushrooms such as *Agaricus campestris* have demonstrated the insulin-releasing and insulin-like activity (Gray and Flatt, 1998). *Pleurotus* species such as *Pleurotus sajor-caju* and *Pleurotus citrinopileatus* are known to promote insulin levels in diabetic rats (Hu *et al.*, 2006; Kanagasabapathy *et al.*, 2012).

Alloxan causes selective necrosis of β cells (Lenzen, 2008). The increase in serum insulin level may be due to renewal of β cells in the pancreas by the mushrooms enabling pancreatic insulin secretion. *Agaricus bisporus* increased serum insulin levels, and the cellularity of islets of Langerhans of the pancreas and repopulation with β cells in diabetic rats as reported by Yamac *et al.* (2010). Speculation of this hypothesis on the effects of *P.o* and *P.c* on islet function needs further studies.

Inhibition of GSK leads to increased activity of glycogen synthase which in turn promotes glycogen synthesis in the liver (Force and Woodgett, 2009). Glycogen synthase kinase inhibitors (GSKI) have emerged recently as potential targets in the treatment of diabetes mellitus, e.g. maleimide compounds. The GSKI contribute to the glucose lowering effect by enhancing hepatic glycogen synthesis, reducing hepatic glucose output and promoting glucose uptake (Henriksen and Dokken, 2006). The *P.o* and *P.c* significantly reduced the serum levels of GSK. Thus, *P.o* and *P.c* may promote glycogen synthesis in liver by inhibiting GSK.

As evident by the findings of the present study, the two culinary mushrooms do not possibly exert any toxic effects in Wistar rats as well as human subjects.

In conclusion, the *P.o* and *P.c* exerted significant hypoglycaemic effect in healthy volunteers challenged with glucose and in Type 2 diabetic patients on diet control. The mushrooms are neither hepatotoxic nor nephrotoxic. Hence, this study confirms the suitability of *P.o* and *P.c* as a functional food for diabetic patients. The freeze dried suspensions of the two mushrooms exert their oral hypoglycaemic activity via several possible mechanisms *viz* increasing GK activity and promoting insulin secretion and thereby increasing the utilization of glucose by peripheral tissues, inhibiting GSK and thereby promoting glycogen synthesis.

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## Conflict of Interest

The authors have declared that there is no conflict of interest.

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