IN VITRO AND IN VIVO ANTIDIABETIC ACTIVITY OF CALOCYBE INDICA

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ABSTRACT

In vitro and *in vivo* antidiabetic activity of milky mushroom (*Calocybe indica*) exhibited significant results for its α-amylase (89.49 ± 3.54 % at 1.0 mg/ml) and α-glucosidase inhibitory activity (67.30 ± 2.93 % at 1.0 mg/ml) in a dose-dependent manner. The methanolic extract showed significant activity ($p < 0.05$) at the tested dose level (200 mg/kg b.w) which was comparable to glibenclamide, a standard antidiabetic drug. Presence of phytochemicals namely phenols, flavonoids, saponins and tannins which may be responsible for such antidiabetic activity.

Keywords: *Calocybe indica*, antidiabetic activity, phytochemicals, phenols, flavonoids.

INTRODUCTION

Diabetes mellitus is a life threatening chronic metabolic disorder caused by lack of insulin and insulin dysfunction, characterized by high levels of glucose in the blood. It is characterized by hyperglycemia and alteration in carbohydrate, protein and lipid metabolism caused by defects in insulin production or action [1,2]. Contributory factor in the pathogenesis of diabetes also comprises of oxidative stress [3,4].

Plants including fungi are the main source of natural compounds used as medicine and they have attracted considerable interest because of their wide variety of bioactive metabolites. Mushrooms are being developed as nutriceuticals which garner their essence and to make their consumption easy. Further, scientific validation of traditional knowledge bears evidence of the many positive effects of consuming mushrooms on human health [5-7]. The investigation of the local species may yield mycochemicals with novel medicinal properties that can be used for the development of therapeutic agents in diabetes and for other ailments [8, 9].

MATERIALS AND METHODS

The fruiting bodies of *Calocybe indica* were obtained from Mushroom Unit, Department of Biology, Gandhigram Rural Institute - Deemed University, Gandhigram, Dindigul, TamilNadu, India. Sample preparation [10], qualitative phytochemical analysis [11], *invitro* antidiabetic activity namely α-amylase [12] and á-glucosidase[12] inhibitory activity and *in vivo* antidiabetic activity namely evaluation of alloxan induced diabetic rats were carried [13].

Animal studies: Animal experiments were carried out according to the guidelines of the Committee for the purpose of control of experiments on animals and approved by the Institutional Animal Ethics Committee (Reg. No.: CPCSEA/265).

Statistical analysis: The results were expressed as mean values and standard deviation (SD). Linear regression analysis was used to calculate IC_{50} value. Data were analyzed using One-way Analysis of Variance (ANOVA) followed by Turkey's multiple comparison post hoc tests using SPSS software 16.0 versions. Values of $p<0.05$ were considered as statistically significant.

RESULTS

Qualitative phytochemical screening: Methanolic extract of *Calocybe indica* were qualitatively analyzed and presented in Table 1.Among the various phytochemicals assessed, maximum values of phenols and saponins; minimum values of flavonoids and lowest values of tannins were recorded.

Table 1. Qualitative phytochemical screening of *Calocybe indica*

Test for extract	Inference
Phenols	+++
Flavonoids	++
Saponins	+++
Tannins	+

Key: "+++" Occurrence very High concentration; "++" Occurrence High concentration; "+" Occurrence Low concentration.

***In vitro* antidiabetic activities:** The methanolic extract of *Calocybe indica* showed significant inhibition of á-amylase (89.49±3.54 at 1000 µg/ml) and á-glucosidase inhibitory activity (67.30±2.93 at 1000 µg/ml) in a dose-dependent manner and the concentrations required for 50% of the inhibition (IC_{50}) were 38.06 ± 0.82 and 281.27 ± 6.69 µg/ml, respectively (Table 2).

Table 2. *In vitro* antidiabetic activities of *Calocybe indica*- Inhibition of α-amylase and α-glucosidase inhibitory activity

Sample concentration (µg/ml)	á-amylase inhibition activity (%)	IC_{50} value (µg/ml)	α-glucosidase inhibition activity (%)	IC_{50} value (µg/ml)
200	31.34 ± 4.56		6.94 ± 0.33	
400	48.55 ± 1.66		20.77 ± 0.33	
600	56.88 ± 1.37	38.06 ± 0.82	41.49 ± 0.64	281.27 ± 6.69
800	81.70 ± 1.13		52.04 ± 0.33	
1000	89.49 ± 3.54		67.30 ± 2.93	

Data represent the mean $\pm$ S.E.M ($n = 3$) ($p < 0.05$)

***In vivo* antidiabetic study:** *In vivo* antidiabetic study revealed the significant reduction of blood glucose, serum cholesterol, serum triglyceride, LDL levels and significant increase of HDL level in *Calocybe indica* treated diabetic rats in14 days trials (Table 3).

Table 3. *In vivo* antidiabetic study - blood glucose, serum cholesterol, serum triglyceride, LDL and HDL levels in *Calocybe indica* treated diabetic rats in14 days trials

Experimental groups	Blood glucose (mg/dl)	Serum cholesterol (mg/dl)	Serum triglyceride (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Group - É	81.40 ± 3.22^{b}	34.00 ± 1.74^{b}	33.40 ± 3.45^{b}	22.00 ± 2.10^{b}	24.60 ± 1.47^{b}
Group-ÉÉ(120 mg/kg b.w)	512.0 ± 15.29	84.0 ± 4.94	123.00 ± 6.63	58.80 ± 3.22	10.20 ± 1.12
Group-ÉÉÉ(200 mg/kg b.w)	320.0 ± 14.40^{b}	99.20 ± 5.23	93.20 ± 4.61^{a}	42.20 ± 1.24	14.00 ± 0.65
Group - IV(400 mg/kg b.w)	124.40 ± 7.84^{b}	32.20 ± 2.49^{b}	38.20 ± 1.90^{b}	23.60 ± 1.90^{b}	25.80 ± 0.98^{b}

Each value represents the Mean $\pm$ SD (n = 5) [a]*p< 0.05*, [b]*p< 0.01* Vs Diabetic control (ANOVA followed by Turkey's multiple comparison test). Group I – Control; Group II – Diabetic; Group III – *Calocybeindica*; Group IV – Glibenclamide; Group V – HDL - High density lipoprotein; LDL - Low density lipoprotein; b.w - Body weight.

DISCUSSION

Calocybe indica is a good source of extractable phytochemicals with inhibitory potentials against key enzymes namely, á-amylase and á-glucosidase linked to Type 2 diabetes. *In vitro* tests can play a very important role in the evaluation of antidiabetic activity of drugs as initial screening tools, where the screening of large number of potential therapeutic candidates may be necessary. They might provide useful information on the mechanism of action of therapeutic agents [14-17].

Therapeutic approach for treating Type 2 diabetes mellitus is to decrease the post-prandial glucose levels. This could be done by retarding the absorption of glucose through the inhibition of the carbohydrates hydrolyzing enzymes, á-amylase and á-glucosidase, present in the small intestinal brush border that are responsible for the breakdown of oligosaccharides; disaccharides into monosaccharides suitable for absorption [14, 18-20]. Inhibitors of these enzymes, like acarbose, delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise [14, 15].

Jumepaeng *et al*. [21] reported á-amylase inhibitory activity was significantly higher as compared to acarbose drug currently administrated for controlling glucose levels in diabetic patients. Natural products from plants have shown lower inhibitory activity against á-amylase activity and stronger inhibitory activity against non insulin dependent diabetes mellitus (NIDDM) with minimal side effects. This is a positive result, since as explained earlier, the excessive inhibition of á-amylase results in abnormal bacterial fermentation of undigested carbohydrates in the colon, which inturn results in abdominal digestion, flatulence, meteorism and possibly diarrhea. On the other hand, it was found that dates extract strongly inhibits the activity of á-glucosidase [22, 23].

The presence of inhibition to á-glucosidase activity in the extracts of *Calocybe indica* mushroom could be caused by the presence of carbohydrate and protein (flavanols) which are suspected to be the competitive inhibitors for á-glucosidase enzyme. This is appropriate with the substrate of á-glucosidase, which is a food starch and carbohydrate (glycogen) [24].

In the present study, *in vitro* antidiabetic studies revealed the inhibition of á-amylase and á-glucosidase activity. The percentage inhibition at 200, 400, 600, 800 and 1000 µg/ml concentrations of *Calocybe indica* on á-amylase and á-glucosidase showed a concentration dependent reduction in percentage inhibition. Therefore, the antidiabetic effect of *C. indica* might attribute to its inhibitory effect against á-amylase and á-glucosidase that retarding the digestion of carbohydrate to delay the postprandial rise in blood glucose.

In the *in vivo* studies, blood glucose levels were assessed from 0 and 14th days in normal rats; diabetic induced rats; mushroom extracts treated rats and also glibenclamide treated rats. There is a significant reduction in all antidiabetic parameters on 14th day in the rats treated with *Calocybe indica* mushroom extracts. In the *in vivo* studies, alloxan induced diabetic rats showed significant increase in the levels of blood glucose than the diabetic rats (*p<0.05*). Blood glucose level was measured in normal and diabetic rats on day 0 and 14th day of drug treatment. After treatment with both species at 200 mg/kg b.w, the blood glucose levels on day 14th were significantly reduced compared to those on day 0 (*p<0.01*). The glibenclamide treated rats also showed significant reduction in serum glucose level (*p<0.05*). *C. indica* and glibenclamide administration attenuated hyperglycemia, while no significant changes were observed in normal and diabetic groups (*p>0.05*).

Mushrooms have been shown to be useful in supporting healthy cholesterol levels and have been shown to improve circulation; also they have been shown to help in maintaining blood sugar balance *via* blood sugar lowering effects, elevation of plasma insulin levels and enhanced liver metabolism of glucose and increase cellular insulin sensitivity [25]. Hyperglycemia caused by diabetes is known to be a cause of oxidative stress that leads mainly to enhanced production of mitochondrial ROS. Oxidative stress induced by hyperglycemia leads to the activation of stress sensitive signaling pathways, which worsen both insulin secretion and action, and promote the development of Type 2 diabetic mellitus [26-29]. Fasting hyperglycemia is a hallmark of diabetic mellitus. It has been postulated but is still debated that the fasting hyperglycemia in noninsulin dependent diabetic mellitus arises from the hepatic over production of glucose [30].

Wi *et al.* [31] suggested that the post absorptive hyperglycemia in Streptozotocin diabetic rats is largely due to decreased peripheral glucose clearance, while increased hepatic glucose output might also be a contributing factor at a very high Streptozotocin dose. Krishna *et al.* [32] stated that polysaccharide extracted from *Pleurotus citrinopileatus* showed blood glucose lowering effect in rats. These findings suggest that mushrooms are promising antidiabetic nutriceuticals, but there is lack of enough clinical evidences. Khan *et al.* [33] have reported that oral administration of *Pleurotus ostreatus* given to rats leads to blood glucose lowering effect in both insulin–dependent and insulin–independent diabetic conditions. Antidiabetic effects of ethanolic extract of *Pleurotus ostreatus* on alloxan induced diabetic rats was extensively studied and reported as an effective antidiabetic regimen [34].

CONCLUSION

The methanolic extract of *Calocybe indica* with its significant antidiabetic activity in rats, suggests its therapeutic potential for the prevention and control of diabetics; moreover, the mushroom species can be used as an easily accessible source of natural antidiabetic and as a possible food supplement or in pharmaceutical industry. However more intensive and extensive investigations are needed to exploit their valuable therapeutic potentials and the chemical characteristics of the antidiabetic components in the extracts should be further investigated.

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