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Assessment of Antihyperglycemic Potential of Lyophilized and Oven-Dried Extract of *Calocybe indica* in Experimentally Streptozotocin-Nicotinamide Induced Diabetic Rats

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ABSTRACT

Uncontrolled diabetes can cause a number complication including heart disease, stroke, liver and kidney damage. Presently herbal formulations are mostly recommended to control diabetes due to lesser side effects compared to synthetic drug. Calocybe indica is rich in protein, flavonoids, lipid, fiber, carbohydrate and vitamin and incorporates an ample amount of essential amino acid and low fat product. The present study was aimed to evaluate the antihyperglycemic activity of lyophilized and oven-dried extract of Calocybe indica against STZ-induced diabetic complications as hepatotoxicity and nephropathy. The lyophilized extract (LE) and oven-dried extracts (ODE) of Calocybe indica were administered orally in Streptozotocin (STZ)-induced diabetic rats. After the administration of fractions, blood glucose levels were monitored at specific intervals and it was found that they were significant lowered. The effect of extracts on induced hyperlipidemia was evaluated and found significantly lowered the elevated total cholesterol, triglycerides (TGL) and low density lipoprotein (LDL) level while increased the high density lipoprotein (HDL). The increased level of aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), lactate dehydrogenase *(LDH), urea and creatinine in serum of diabetic rats were observed, and significantly decreased on administration of lyophilized and oven-dried extract of Calocybe indica. The results imply that extract could have a protective effect against hepatic and renal damage induced by oxidative stress in the diabetic rats. The lyophilized extract of Calocybe indica produces higher protective effect compared to oven-dried extract.*

Keywords: *Calocybe indica*, Antihyperglycemic, Streptozotocin, Liver, Kidney

INTRODUCTION

American Diabetes Association Expert Committee suggested that diabetes mellitus is a group of metabolic diseases identified by hyperglycemia, varied metabolism of carbohydrates, lipids and proteins. The inceptions of metabolic diseases are leading from imperfections in insulin secretion, insulin action or both. The hyperglycemia play important role in dysfunction and failure of various organs, especially eyes, kidneys, liver, nerves, heart and blood vessels thus covering a wide range of heterogeneous disease. Glucose metabolism may get severely hindered due to improper insulin production from the pancreatic β-cells. Glycogen catabolism in liver increases due to low insulin level resulting in low hepatic glycogen content in diabetes. Hepatic damage induced in such condition may demonstrate elevation of the liver marker enzymes such as transaminases and phosphatases. Diabetic nephropathy results in further increase of urea, uric acid and creatinine level in serum. Besides, hyperglycemia induces oxidative stress exacerbates the pathogenesis of diabetic complications.[1,2]

Presently, more than 285 million people of worldwide are affected from diabetes. The numbers of the diabetes patients are expanding in rural and poor populations throughout the world. It is assumed that diabetes will become

one of the world's killers within next two decades. Hence the appropriate and effective treatment of the diabetes patients is required to restraint over diabetes [3,4].

Calocybe indica belongs to class Basidiomycetes, order Agaricales and family Tricholomataceae. *Calocybe indica* is rich in protein, flavonoids, lipid, fiber, carbohydrate and vitamin and contains an abundant amount of essential amino acid and low fat product [5]. It is documented that the *Calocybe indica* accumulates a variety of secondary metabolites including phenolic compounds, terpenes, polyketides, sterols, ergosterol, flavanoids and steroids. These qualities make it suitable for food supplement in diet and also used in the form of medicines to alleviate various human disorders and diseases [6,7]. *Calocybe indica* are very effective in reducing the total plasma cholesterol and triglyceride level and thus reduce the chance of atherosclerosis, cardiovascular and artery related disorders. The antioxidant property of Calocybe indica prevent oxidative damage by free radical and reactive oxygen species may prevent the occurrence of diseases like carcinogenesis, ageing, physical injury, infection, obesity, diabetes, neurodegenerative diseases and cardiovascular disease[8,9].

Considering the wide use of *Calocybe indica* in folk therapeutics for the treatment of diabetes, the present study was conducted to investigate its antidiabetic activity in STZ-induced diabetic rats. Consequently, the potential therapeutic of *Calocybe indica* in hepatic and renal oxidative damage in diabetic rats was evaluated.

MATERIALS AND METHODS

2.1 Plant material

The *Calocybe indica* was obtained from Mushroom Research Centre, Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India and it was cultivated in Columbia Mushroom Centre, Raipur, Chhattisgarh, India. The mushroom was dried at room temperature until it was free from the moisture. Finally mushroom was subjected to size reduction to get coarse powder.

2.2 Preparation of oven-dried and lyophilized extracts

The powder of the *Calocybe indica*, was macerated with 70% ethanol for seven days. Each day solution was shaken continuously with orbital shaker for six hours. The extract was filtered, and the resultant extract was dried by oven-dried and lyophilized separately.

The half of the resultant extract was dried in oven by maintaining temperature 60 °C, and obtained oven-dried extract (ODE). The extracts were kept in air tight container for further study. Secondly, the remaining extracts were lyophilized (LE) and stored at -20 °C until further use.

2.3 Animals

Male Wistar albino rats having a weight of 170 – 220 gm were kept in quarantine for 10 days under standard husbandry conditions (27.3 ^{o}C, Relative humidity 65 ± 10%) for 12 hours in dark and light cycle, respectively, and were given standard food and water *ad libitum*. The study was permitted by the Institution Animal Ethical Committee and the approval number is CIP/IAEC/2014-15/049.

2.4 Induction of non-insulin dependent diabetes mellitus (NIDDM)

In overnight fasted adult male Wistar albino rats (170 – 220 g), Non-insulin dependent diabetes mellitus was induced by administration of a single dose of 60 mg/kg streptozotocin intraperitonally, 15 minutes after nicotinamide administration (120 mg/kg i.p.). Nicotinamide was dissolved in normal saline whereas STZ was in citrate buffer at a pH of 4.5. The elevated glucose level in plasma was determined at 72 hours and then on 7th day, after injection, this data confirmed hyperglycemia. To diagnose the diabetes, the threshold value of fasting plasma glucose level was taken as >126mg/dl. The rats which have permanent NIIDM were used only for the study[10,11].

2.5 Experimental design

The animals were divided into seven groups, each group containing six animals (n = 6). Group I were administered drinking water daily, served as normal control rats; Group II was Diabetic control rats; Group III albino rats were administered standard drug Glibenclamide (0.5 mg/kg); Group IV rats were administered LE (200 mg/kg); Group V rats were administered LE (400 mg/kg); and Group VI rats were administered ODE (200 mg/kg); and Group VII rats were administered ODE (400 mg/kg) for 28 days. On 0, 7th, 14th and 28th days of test sample administration, the

fasting glucose levels were determined. During the experimental period, the rats were weighed daily and the mean change in body weight was calculated.

2.6 Estimation of insulin level

The animals were segregated into seven groups of six rats each. The LE and ODE were administered for 28 days. Group I served as normal control rats, administered drinking water daily for 28 days; Group II had diabetic control rats, administered drinking water daily for 28 days; Group III diabetic rats were administered standard drug Glibenclamide (0.5 mg/kg); Group IV rats were administered LE (200 mg/kg); Group V rats were administered LE (400 mg/kg); and Group VI rats were administered ODE (200 mg/kg); and Group VII rats were administered ODE (400 mg/kg) for 28 days. The blood samples were withdrawn in order to examine the insulin levels. Serum insulin was measured using a GLAZYME INSULIN-EIA TEST18 [13,14].

2.7 Estimation of biochemical parameters

The animals were sacrificed by cervical dislocation on 12th day for determining biochemical parameters. Glucose oxidase method by using auto-analyzer was used to determine total cholesterol, triglycerides (TGL), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) [12-14].

The serum samples from all the groups were also used to study aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), lactate dehydrogenase (LDH), urea and creatinine levels using commercially available kits of Crest Biosystems (India)[4, 15, 16].

2.8 Statistical analysis

The results are expressed as mean ± SEM of six independent experiments. Statistical significance between the groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunet's test. A $P < 0.05$ value was considered as statistically significant.

RESULTS

3.1 Effect of lyophilized and oven-dried extract of Calocybe indica on hyperglycemia

The Antidiabetic activity of lyophilized and oven-dried extract of *Calocybe indica* was evaluated and the findings are presented in table 1. The blood glucose level of diabetic rat significantly enhanced compared to normal group of rats. The administration of LE and ODE at two different doses (200 mg/kg and 400 mg/kg) to STZ-induced diabetic rats caused significant reduction of blood glucose level. The Antidiabetic activity of extracts depends upon the dose and duration of the treatment. The maximum reduction of blood glucose in rats was observed on 28^{th} days. The blood glucose level of LE at the dose 200 mg/kg and 400 mg/kg group was noted 112.18±5.17 mg/dl and 92.72±3.75 mg/dl, respectively at the end of study. While ODE treated rats at the dose of 200 mg/kg and 400 mg/kg exhibited 123.89±4.43 mg/dl and 109.65±5.92 mg/dl blood glucose level, respectively. The findings of LE exhibited higher significant antidiabetic activity compared to ODE. The Glibenclamide treated rats significantly decrease in blood glucose level compared to diabetic control group.

Table 2

3.2 Effect of lyophilized and oven-dried extract of Calocybe indica on lipid profile

The effects of the LE, ODE and Glibenclamide on various serum lipid profiles of diabetic rats are demonstrated in table 2. The triglycerides, total cholesterol and LDL in serum were significantly enhanced in diabetic group compared to normal control group. However, HDL was significantly decreased in diabetic group in comparison to normal group. The administration of LE, ODE and Glibenclamide to diabetic rats resulting significantly decreased the triglycerides, total cholesterol and LDL in serum compared to diabetic control group. Consequently, the HDL was significantly increased in extract and standard drug treated group in comparison to diabetic control group.

3.3 Effect of lyophilized and oven-dried extract of Calocybe indica on body weight

The significant reduction in body weight of STZ-induced diabetic rats was noted on 28^{th} days. The body weight of the normal rats before and after completion of experiment was similar. The LE, ODE and Glibenclamide treated rat demonstrated no significant reduction in body weight on 28^{th} days. The little variations in body weight of rats between before induction of diabetes and after treatment with LE, ODE and Glibenclamide were observed (Table 3).

3.4 Effect of lyophilized and oven-dried extract of Calocybe indica on insulin

The decreased in serum insulin indicates the hyperglycemia in rats. The STZ-induced diabetic rats exhibited significant decreased in serum insulin on 28th days. The administrations of LE, ODE and Glibenclamide to diabetic rats for 28th days leading to significant increase in serum insulin were observed. The finding exhibited that the LE and ODE has capacity to monitor the serum insulin and it expressed the anthyperglycemia properties of *Calocybe indica*.

3.5 Effect of lyophilized and oven-dried extract of Calocybe indica on liver and renal serum

The increased level of AST, ALT, ALP, LDH, urea and Creatinine in serum of diabetic rats indicates the improper functioning of liver and kidney. The AST, ALT, ALP, LDH, urea and Creatinine in serum were significantly increased in diabetic control group compared to normal control group. Consequently, administration of LE (400 mg/kg), ODE (400 mg/kg) and Glibenclamide to diabetic rats resulted in significant decreased in biochemical parameters, representing the improvement in functioning of liver and kidney. Conversely, no significant increase in biochemical parameter was observed on administration of LE (200 mg/kg) and ODE (200 mg/kg) to diabetic group. The lower dose of LE and ODE were not so effective in restoring the function of liver and kidney.

DISCUSSION

Phytoconstituents assists us in understanding the plant physiology and biochemical pathways. The therapeutic efficacy of medicinal plants depends upon nature and quantity of chemical constituents present in plants. Most of the phytoconstituents are thermolabile and sensitive to oven temperatures[1]. The drying process plays a critical role during evaporation of solvent from semi solid extracts. It has been documented that the high temperature may cause chemical degradation of heat labile phytoconstituents or change in colour of extracts[4]. Hence drying of extract by oven is not better option to get good quality of dried powder extracts. The freeze-dried (lyophilization) is a drying process in which water is sublimed from the extract after freezing[4]. The lyophilization process preserves the chemical ingredients and improves the stability of extract during long storage of periods[17]. In the present study the lyophilized and oven-dried extract of *Calocybe indica* was used to evaluate the antidiabetic activity against STZ-induced diabetics complications such as oxidative stress, hepatotoxicity and nephropathy of diabetes rat's model. Consequently, it also assists in understanding the restoring efficiency of chemical compounds in lyophilized and oven-dried extract of *Calocybe indica*.

STZ induces a series of biochemical events leading in the production of high level of reactive oxygen species (ROS) and subsequent oxidative stress (O_2). It is documented that the oxidative stress produced by STZ contributes to the development and progression of diabetes and its complications[1]. The ROS played an essential role to damage pancreatic islets of β-cells, which arising insulin deficiency and hyperglycemia[4]. Mostly, the ROS in STZ is produced by the xanthine oxidase system of pancreatic cells and excited H_2O_2 procreation. It caused to DNA fragmentation and necrosis in the pancreatic β-cells islets. As results, it leads to reduce the rate of insulin synthesis by pancreatic β-cells. The reactive oxygen species produced by STZ also leads to damage liver, kidney, and hematopoietic system. It is documented that the oxidative stress contributes to the development and progression of diabetes and its complications[1,18]. Considering this numerous experimental and clinical studies exhibited the potential usefulness of polyphenolic compounds as antioxidants therapeutic agents for the prevention of oxidative damage in diabetes. Thus, the effects of lyophilized and oven-dried extract of *Calocybe indica* on oxidative stress in liver and kidney, as well as on blood biochemical parameters of STZ-induced diabetic rats were investigated in this study.

In the present study, we observed antihyperglycaemic effect of lyophilized and oven-dried extract of *Calocybe indica* through the oral glucose tolerance test, it significantly improves glucose tolerance. It has been documented that hyperlipidemia is a complication associated with hyperglycemia. During study it was observed increase in total cholesterol, triglycerides, LDL, and decrease in HDL in streptozotocin induced diabetic rats. The increase in the total serum cholesterol, triglyceride and LDL levels in the diabetic rats is mainly due to increased mobilization of free fatty acids from peripheral deposits, as insulin inhibits the hormone-sensitive lipase. The increase in the serum LDL level may also result from glycosylation of the lysyl residues of apoprotein B, which leads to a decrease in LDL metabolism due to a decrease in the affinity of LDL for its receptors. The administration of lyophilized and oven-dried extract of *Calocybe indica* to diabetic rats caused significant reduction in total cholesterol, LDL, triglycerides and significant rise in HDL. The mechanism of the hypolipidemic actions of *Calocybe indica* are not known; however, they could be mediated by control of tissue metabolism and improved insulin secretion and action

because insulin lowers lipid levels and normalizes lipids in STZ-induced diabetic rats. The potent antidiabetic effect of the *Calocybe indica* extract suggests the presence of potent antidiabetic active principles, which produced antihyperglycemic effect in diabetic rats. The outcomes of lipid profile confirmed the potent antidiabetic activity of *Calocybe indica.*

Induced diabetes is marked by severe diminution of body weight, which may be attributed to loss or degradation of structural proteins. In our present study, we observed that diabetes-induced decrease in body weight was prevented by lyophilized and oven-dried extract of *Calocybe indica*, which is in accordance with earlier studies reporting, prevention of body weight loss in diabetic rats by the usage of phytochemicals or medicinal plant extracts. Insulin resistance is a condition where normal or elevated insulin level produces an attenuated biological response. In diabetes, insulin resistance is the central pathophysiological event.

Simultaneously we discussed about the hepatic damage during diabetes, and it can determined by studying the serum aminotransferases activities. The liver cirrhosis alters their transport function and membrane permeability, leading to leakage of enzymes from the cells. The discharge of AST, ALT, ALP and LDH from liver cytosol into circulation exhibits severe damage to hepatic tissue membranes during the diabetes[15,16]. The study indicates increased activities of AST, ALT, ALP and LDH and it may be interpreted as a result of the liver cell destruction or changes in the membrane permeability demonstrating severe liver damage induced by diabetes. The administration of lyophilized and oven-dried extract of *Calocybe indica* able to protect against the increase in the activity of these enzymes in diabetic rats, demonstrating the protective effect of this polyphenol against hepatic damage induced by diabetic state. Additionally, it was observed that lyophilized extract of *Calocybe indica* produces higher protective effect compared to oven-dried extract. The outcomes of study revealed that *Calocybe indica* has potential to prevent the lipid peroxidation in hepatic tissue.

Similarly the diabetic rats were suffered from renal failure and it was confirmed by increased in serum urea and creatinine levels, which are considered as significant markers of renal dysfunction. The administration of lyophilized and oven-dried extract of *Calocybe indica* decreases the levels of urea and creatinine in diabetic rats. The outcomes suggested that *Calocybe indica* has capacity to reduce the renal injury caused by hyperglycemic state. The protective effect of renal failure of *Calocybe indica* is due to antioxidant property, which protects the kidneys against oxidative damage. In this study lyophilized extract of *Calocybe indica* produces higher protective effect compared to oven-dried extract.

Table 1: Effect of LE and ODE of *Calocybe indica* on fasting plasma glucose level in rats

Group	Fasting plasma glucose concentration (mg/dl)			
	Day 0	Day 7th	Day 14th	Day 28th
Normal Control	79.35±3.62	75.24±4.59	77.14±5.67	79.21±3.48
Diabetic control (Streptozotocin)	135.28±4.15[a]	213.82±5.20[a]	253.42±4.28[a]	296.58±5.26[a]
Diabetic + Standard Glibenclamide (0.50 mg/kg)	140.63±4.57	112.58±3.82*	91.42±4.81*	74.46±3.58*
Diabetic + LE (200 mg/kg)	132.49±3.35	149.21±5.14*	127.58±3.72*	112.18±5.17*
Diabetic + LE (400 mg/kg)	135.25±5.52	122.56±4.72*	108.42±5.34*	92.72±3.75*
Diabetic + ODE (200 mg/kg)	139.47±2.47	155.28±3.25*	139.15±4.54*	123.89±4.43*
Diabetic + ODE (400 mg/kg)	136.18±3.89	143.62±6.19*	125.48±3.47*	109.65±5.92*

*Values are expressed as mean ±SEM (Number of animals, n=6); significantly different at [a]P<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group*

Table 2: Determination of biochemical parameters after treatment with LE and ODE of *Calocybe indica*

Group	Lipid Profile (mg/dl)			
	Triglyceride	Total Cholesterol	HDL	LDL
Normal control	83.46±3.54	98.42±4.18	63.83±5.41	48.73±5.12
Diabetic control (Streptozotocin)	193.61±5.17[a]	181.75±4.76[a]	25.47±3.47[a]	173.92±3.45[a]
Diabetic + Standard Glibenclamide (0.50 mg/kg)	85.14±4.72*	89.54±3.52*	69.35±6.52*	59.41±4.63*
Diabetic + LE (200 mg/kg)	105.72±5.68*	115.43±5.67*	53.65±5.14	92.35±5.42*
Diabetic + LE (400 mg/kg)	90.24±3.51*	89.37±5.18*	61.92±4.82*	76.14±3.25*
Diabetic + ODE (200 mg/kg)	128.43±4.49*	142.35±4.28*	30.82±5.16	120.53±4.35*
Diabetic + ODE (400 mg/kg)	106.71±5.24*	119.24±3.76*	55.14±5.24*	95.59±3.68*

*Values are expressed as mean ±SEM (Number of animals, n=6); significantly different at [a]P<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group*

Table 3: Effect of LE and ODE of *Calocybe indica* on changes in body weight in rats

Group	Change in Body weight (gm)		
	Before Induction	After Induction	After Treatment
Normal control	192.15±2.54	194.26±3.47	189.37±2.63
Diabetic control (Streptozotocin)	189.32±3.14	173.82±2.65	145.67±1.72*
Diabetic + Standard Glibenclamide (0.50 mg/kg)	181.68±2.69	162.54±3.25	175.92±2.43
Diabetic + LE (200 mg/kg)	193.27±1.83	158.31±4.92	176.62±3.48
Diabetic + LE (400 mg/kg)	201.57±3.72	179.43±3.47	195.71±3.14
Diabetic + ODE (200 mg/kg)	197.34±1.47	165.52±2.37	182.14±1.67
Diabetic + ODE (400 mg/kg)	192.65±2.43	155.74±1.92	181.45±2.15

Table 4: Effect of LE and ODE of *Calocybe indica* in insulin level of STZ induced diabetes in rats

Treatment Group	Insulin Level (Mean ±SEM) In mg/dl	
	Initial Reading	Final Reading
Normal control	0.75±0.14	0.79±0.73
Diabetic control (Streptozotocin)	0.83±0.25	0.25±0.54*
Diabetic + Standard Glibenclamide (0.50 mg/kg)	0.81±0.32	0.78±0.18*
Diabetic + LE (200 mg/kg)	0.79±0.09	0.65±0.25*
Diabetic + LE (400 mg/kg)	0.82±0.16	0.73±0.05*
Diabetic + ODE (200 mg/kg)	0.85±0.21	0.68±0.17*
Diabetic + ODE (400 mg/kg)	0.78±0.35	0.71±0.31*

Table 5: Effect of LE and ODE of *Calocybe indica* on liver and renal serum biomarkers of diabetic rats

Treatment Group	AST (U/L)	ALT (U/L)	ALP (U/L)	LDH (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Normal control	98.26±3.51	53.68±4.27	148.42±3.72	850.31±2.79	49.23±3.45	0.35±0.14
Diabetic control (Streptozotocin)	553.58±2.82	365.72±3.56	614.37±2.41	1532.49±4.17	123.58±2.48	0.82±0.05
Diabetic + Standard Glibenclamide (0.50 mg/kg)	82.35±5.14	78.25±4.15	126.48±5.37	828.19±3.65	43.47±4.31	0.46±0.08
Diabetic + LE (200 mg/kg)	132.42±3.48	145.16±3.72	193.57±3.62	953.73±4.83	79.61±3.63	0.55±0.24
Diabetic + LE (400 mg/kg)	110.67±4.19	66.73±5.34	135.61±2.79	886.28±2.62	56.15±5.24	0.47±0.18
Diabetic + ODE (200 mg/kg)	155.73±3.49	183.34±2.68	215.36±4.54	983.54±3.74	102.38±4.83	0.62±0.21
Diabetic + ODE (400 mg/kg)	123.92±4.26	99.18±3.73	167.24±3.41	902.37±3.57	65.48±4.52	0.51±0.02

CONCLUSION

In the present study generation and progression of diabetic complications during streptozotocin induced diabetes was investigated. The findings demonstrated that the administration of lyophilized and oven-dried extract of *Calocybe indica* provide effective protection against oxidative damage in liver and kidney STZ-induced diabetic rats. The results suggest that polyphenol compound present in extract improve the physiology of rats affected by diabetes. These polyphenol attributed to normalise the blood glucose, also improve the structural integrity of the kidney, liver and pancreas unlike hypoglycemic agent like Glibenclamide. The lyophilized extract of *Calocybe indica* produces higher protective effect compared to oven-dried extract. It indicates that freeze drying preserves the strength of phytochemicals quite well compared to oven oven-dried extract. The findings propose that regular intake of the *Calocybe indica* may be useful for diabetes mellitus.

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