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Research Article

POTENT ANTIHYPERGLYCAEMIC ACTIVITY OF *CALOCYBE INDICA* IN STREPTOZOTOCIN INDUCED DIABETIC RATS ANTIHYPERGLYCEMIC ACTIVITY OF *CALOCYBE INDICA*

PARAMASIVAM RAJESWARI AND SHANMUGASUNDARAM KRISHNAKUMARI*

Department of Biochemistry, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamilnadu, India.
Email: krishnashanmugambc@gmail.com

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ABSTRACT

Objectives: As vegetables, fruits and medicinal plants, mushrooms have become attractive as a functional food and as a source for the development of drugs and nutraceuticals. The present study was carried to find the antihyperglycemic effect of the milky mushroom, *Calocybe indica*.

Materials and methods: Female swiss albino rats weighing 200-250g were used for the study. Streptozotocin was used to induce diabetes mellitus. Hot and cold water decoctions of the milky mushroom, *Calocybe indica* (20ml/kg body weight) was orally administered to the diabetic rats for 45 days. The blood glucose, serum insulin, hemoglobin, glycosylated hemoglobin and total blood count and the survival and mortality rate were studied.

Results: The blood glucose level was significantly decreased in the decoction treated diabetic rats. Serum insulin level was increased and the glycosylated hemoglobin level was significantly altered back to the near normal level. The treatment has effect also in hematological parameters. The survival rate was significantly increased in the decoction treated rats.

Conclusion: The results suggests that both hot and cold water decoction of the mushroom possess antidiabetic activity but when compared, the cold water decoction showed more significant activity than the hot water decoction.

Keywords: Antidiabetic activity, *Calocybe indica*, Mushroom, Streptozotocin-nicotinamide induced diabetes mellitus.

INTRODUCTION

The medicine using fungal metabolites is now worldwide recognized [1]. Mushrooms have become attractive as a functional food and as a source for the development of drugs and nutraceuticals [2] while being devoid of undesirable side-effects [3]. Mushroom compounds would act in combination to influence cell surface receptors, and to trigger various downstream signaling events leading to high pharmacological efficiency and specificity [4]. The metabolic syndrome including subsequent pathologies is physiopathologically based on biochemical disturbances such as dyslipidemia, glucose intolerance, insulin resistance leading to progressive hyperglycemia, vascular and hematological perturbations [5]. Interestingly, several subsets of mushrooms produce metabolites able to influence positively one or several of these disturbances. Through acting on fundamental risk factors, mushrooms influence positively the very upstream events that may lead to cardiovascular diseases, diabetes, obesity and also neurodegenerative diseases [6]. Thus the use of mushrooms with potential therapeutic properties raises global interests from the scientific and clinical community.

Calocybe indica is a tropical edible mushroom of Indian origin. It is popularly known as milky mushroom or white summer mushroom /summer white mushroom. In some places they are called “Kuduk”, “dudhi chhata” and “dudha chhatu” [7]. The aims of the present study were to investigate the antihyperglycemic activity of *Calocybe indica* and to measure the comparative antihyperglycemic effect of hot and cold water decoction of the mushroom.

Currently the global prevalence of diabetes mellitus is estimated to be 150 million and this figure is expected to increase to over 300 million by 2025 [8]. More recently, a survey estimated that by 2030 more than 439 million people will suffer from diabetes mellitus (DM) which is one of the most common chronic diseases in nearly all countries [9]. It is characterized by hyperglycemia [10] and is associated with insulin deficiency, a relative impairment in pancreatic insulin secretion with varying degrees of peripheral resistance to the action of insulin [11]. It is a major degenerative disease in the world today [12], associated with complications which include hypertension, atherosclerosis, microcirculatory disorders [13] and generalized degenerative changes in large and small blood vessels and increased susceptibility to infection [14].

A key strategy in treating patients with diabetes is maintenance of blood glucose level. Current oral anti-diabetic agents, which include insulin releasers, insulin sensitizers and α-glucosidase inhibitors, have modest efficacy and limited of modes of action. In addition, current anti-diabetic drugs usually have adverse side effects, decreased efficacy over time, ineffectiveness against some long-term diabetic complications and low cost-effectiveness [15]. It has also been reported that before the advent of insulin injections and other pharmaceutical preparation, healers relied heavily upon medicinal plants and herbs to treat diabetes [16]. Therefore, discovery and development of novel drugs for diabetes is still needed. Although there are several modern facilities and advanced drugs are available to treat the diseases, due to their adverse causes, the world is looking for alternative medicines without harmful side effects. One such source is the macrofungi, called mushrooms. Since they possess edibility along with nutritional and medicinal properties, they could be taken as part of diet.

MATERIAL AND METHODS

Preparation of the decoction

The mushroom culture was maintained in Potato Dextrose Agar media. The spawn was prepared using white corn seeds as substrate. The mushroom was cultivated in our institute and harvested. The mushroom samples after washing were shade dried and the dried sample was powdered with a mixer grinder.

Since it is very economical and easy to prepare and consume the decoctions for every common people, instead of preparing extracts the decoctions were prepared out of the mushroom powder and used for the study.

Cold water decoction

10g of the powdered sample was dissolved in 100ml of distilled water which was continuously shaken for 24 hours in a mechanical shaker at 40°C. After 24 hours, it was filtered and used. The decoction was stored at 4°C for further usage.

Hot water decoction

10g of the powdered sample was dissolved in 100ml of distilled water which was boiled for one and half hours and filtered. The decoction was stored at 4°C for further usage.

Animals

Female albino wistar rats were obtained from Kerala Vertinary University. Animals were housed in polypropylene cages, maintained in a temperature- and humidity-controlled environment on a 12 h–12 h light–dark cycle with free access to food and water. The animals were grown to attain 200-250g weight. They were fed with standard rat pellet diet and water ad libitum. The Institutional Animal Ethical Committee, Department of Biochemistry, Kongunadu arts and science college (Autonomous), Bharathiar University, Coimbatore, Tamilnadu, India (No. 659/02/a/CPCSEA) has approved the study. Animals were acclimatized to laboratory conditions for a week before use.

Chemicals

All chemicals used in this study were of analytical reagent grade supplied by Himedia pvt Ltd, Mumbai, India.

Induction of diabetes

Streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline. Non-insulin-dependent diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection (ip) of streptozotocin (sigma) at 65 mg/kg body weight of the animals, 15 min after the (ip) administration of 110 mg/kg of nicotinamide. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h. The animals with blood glucose concentration more than 250 mg/dl were used for the study. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Experimental design

Survival Study

Animals were divided in to five groups, normal control (Group 1), diabetic control (Group 2), diabetic animals treated with glibenclamide (3 mg/kg) (a known hypoglycemic agent) (Group 3), diabetic animals treated with cold water decoction of the mushroom (20ml/kg body weight) (Group 4), diabetic animals treated with the hot water decoction of the mushroom (20ml/kg body weight) (Group 5). Each experimental animal group was orally administered daily for 45 days (treatment days) with the medicinal decoction. The survival and mortality were screened for 60 days. Based upon the survival and mortality rate of the animals in each group the average life span was calculated.

Antihyperglycemic study

Animals were divided in to five groups, normal control (Group 1), diabetic control (Group 2), diabetic animals treated with glibenclamide (5 mg/kg) (a known hypoglycemic agent) (Group 3), diabetic animals treated with cold water decoction of the mushroom (20ml/kg body weight) (Group 4), diabetic animals treated with the hot water decoction of the mushroom (20ml/kg body weight) (Group 5). Hypoglycemic potential of the decoction was studied estimating the blood glucose levels during treatment days (45 days) at regular intervals of 15 days. Each experimental animal group was orally administered daily for 45 days (treatment days) with the medicinal decoction. The animals were fasted overnight and sacrificed on day 46 by decapitation. The blood sample, used to estimate glucose, hemoglobin, glycosylated hemoglobin and total blood count was taken in the tubes coated with EDTA from the jugular vein and sera was separated by centrifugation for the analysis of insulin. The estimations were done using the respective kits provided by the Roche diagnostics pvt Ltd, Mumbai, India.

Statistical Analysis

The data of the diabetic parameters were analyzed for homogeneity of variance. Tests for of significant difference among means were performed using ANOVA analysis and the Post-hoc study was conducted using Least Significant Difference (LSD) test. Statistical analysis was conducted using SPSS (Version 16).

RESULTS AND DISCUSSION

On administration of glibenclamide and mushroom decoctions, the blood glucose levels of the diabetic rats showed a significant reduction from day 0 onwards (Table 1). From Table 2, it is noted that the glucose level was significantly ($p<0.01$) elevated and the insulin level was significantly ($p<0.01$) declined in the diabetic group than in the normal group. And it was clearly noted that the glycosylation of the Hb was taken place and the level of Hb was significantly decreased. After the treatment period, the glucose and glycosylated hemoglobin level have come down to the near normal level and the insulin and the hemoglobin levels were significantly increased when compared to the diabetic animals.

Table 1: Influence of the *Calocybe* indica decoction on fasting blood glucose levels in diabetic rats.

	Fasting Blood Glucose (mg/dl)			
	0rd day (i.e., after 72hrs from induction)	3rd day	15th day	30th day
Group1	99.50±7.45	100.33±9.50	101±9.25	101.50±3.99
Group 2	326.83±34.30^{a*}	404.50±23.05^{a*}	457.33±50.89^{a*}	529.50±49.74^{a*}
Group 3	300.17±20.07^{b*}	222.00±10.99^{b*}	186.00±7.77^{b*}	182.67±10.07^{b*}
Group 4	313.17±45.92^{c*}	281.33±42.08^{c*}	253.5±24.67^{c*}	215.33±21.30^{c*}
Group 5	310.67±28.72^{d*}	239.00±27.02^{d*}	218.83±29.40^{d*}	222.50±19.76^{d*}

Table 2: Protection by *Calocybe* indica decoction against changes in streptozotocin induced diabetic rats.

	Glucose (mg/dl)	Insulin (µIU/ml)	RBC (10^6/mm^3)	Hb (g/dl)	HbA1C (%)
Group 1	101.5±3.99	5.40±0.56	9.57±0.44	13.7±0.26	2.86±0.42
Group 2	529.50±49.74^{a*}	2.60±0.72^{a*}	7.97±0.80^{a*}	8.90±0.89^{a*}	8.93±0.70^{a*}
Group 3	182.67±10.07^{b*}	4.53±0.60^{b*}	9.22±0.23^{b*}	12.53±0.35^{b*}	3.57±0.86^{b*}
Group 4	215.33±21.30^{c*}	4.77±0.61^{c*}	9.07±0.25^{c*}	12.00±1.05^{c*}	3.63±0.47^{c*}
Group 5	222.50±19.76^{d*}	4.46±0.65^{d*}	8.88±0.31^{d*}	11.90±0.30^{d*}	4.03±0.75^{d*}

Values are expressed as mean ± SD (n=6) * - significant at $p < 0.01$

a – Group II compared to Group I; b – Group III compared to Group II; c – Group IV compared to Group II; d – Group V compared to Group II

The increase in blood glucose level called hyperglycemia in diabetes develops due to a decrease in insulin secretion, an impaired cellular sensitivity to insulin, or both [17]. Hemoglobin is the component of red blood cell which carries oxygen and carbondioxide. It can be observed that the level of the Hb is decreased due to increased glycosyltion by the blood glucose. This decrease in the Hb will lead to anemia. A1C is the nonenzymatic glycated product of the Hb beta chain at the valine terminal residue. The A1C constitutes about 60–80% of total glycated Hb. It is normally present in circulating red cells because of the glycosylation reaction between Hb and circulating glucose [18]. The Hemoglobin Glycosylation Index (HGI) is one of the factors that have been mentioned as a possible predictor of chronic complications of diabetes mellitus. The HGI is a measurement of the greater or lesser capacity of each individual to glycosylate Hb and thus other proteins, which has led it to be related with greater or lesser facility to develop chronic complications of diabetes [19, 20]. Anemia is a highly

prevalent condition in people with type 2 diabetes. According to the World Health Organization (WHO) definition, up to nearly 30% of patients with type 2 diabetes have anemia [21]. The formation of advanced glycation end-products (AGEs) on the surface of diabetic erythrocytes also enhances both the interaction and the binding with endothelial cells, thereby increasing their removal from the circulation [22]. From the results of this study, it is found that the mushroom has the ability to decrease the circulating blood glucose level and in turn can prevent the glycosylation of the Hb and also it has the ability to prevent anemia.

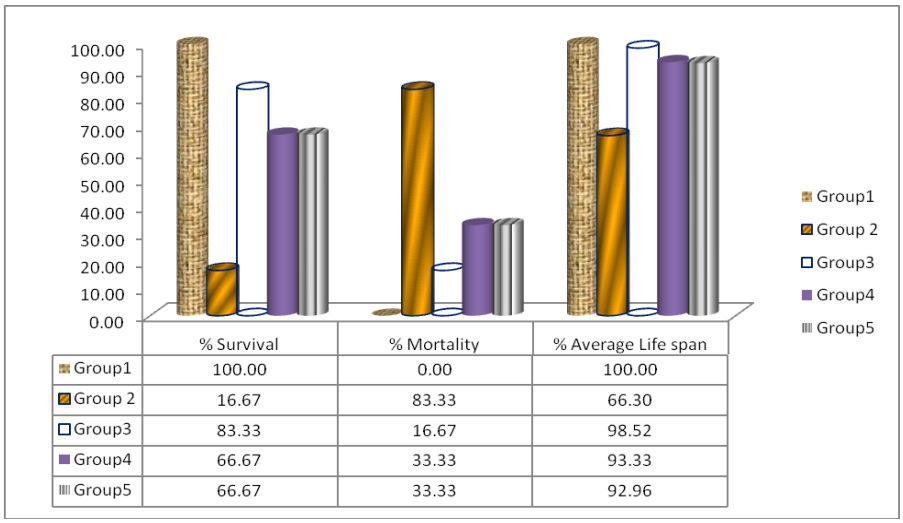
Table 3: Influence of *Calocybe indica* decoction on the alterations in haematological parameters in experimental rats.

	Differential count				*Platelets (10^3/mm^3)*	*HCT (%)*
	Neutrophils (%)	*Lymphocytes (%)*	*Monocytes (%)*	*Eosinophils (%)*		
Group 1	51.53±5.38	36.27±2.05	4.10±0.70	1.33±0.51	222.33±23.12	41.13±2.15
Group 2	84.73± 6.89 a*	18.13±4.27 a*	10.03±0.57 a*	4.30±0.72 a*	401.33±10.97 a*	53.23±2.90 a*
Group 3	53.63±4.91 b*	33.17±2.11 b*	4.27±0.80 b*	1.23±0.35 b*	227.33±14.57 b*	42.83±1.21 b*
Group 4	61.43±4.31 c*	30.47±1.99 c*	5.20±0.95 c*	1.50±0.72 c*	304.33±18.23 c*	44.97±4.61 c*
Group 5	62.47±3.23 d*	30.00±1.71 d*	5.57±1.27 d*	1.63±0.42 d*	292.67±18.15 d*	43.97±4.32 d*

Values are expressed as mean ± SD (n=6) * - significant at $p < 0.01$

a – Group II compared to Group I; b – Group III compared to Group II; c – Group IV compared to Group II; d – Group V compared to Group II

There was significant difference ($p<0.01$) in the total blood cells count in glibenclamide and decoction treated diabetic rats compared to the diabetic rats (Table 3). In the leukocyte formula, the neutrophils, monocytes and eosinophils and haematocrit (HCT) were found to be significantly increased in the diabetic animals when compared to the normal animals and this condition upon treatment with the decoctions of the mushroom was significantly reverted back to the near normal level. The lymphocytes count was significantly decreased in the diabetic group whereas in the treatment group, the count was increased significantly after treatment. Varying from the other groups, significant decrease in the lymphocytes, platelets and RBC was noted in the diabetic group. The decoctions treatment caused significant increase in these count. Peripheral white blood cell (WBC) count has been shown to be associated with insulin resistance, type 2 diabetes [23] and Polymorpho- and mononuclear leukocytes can be activated by advanced glycation end products [24]. The leukocytes are considered as the immune cells. The chronic infection causes alteration in the count of immune cells. Diabetes mellitus (DM) and high glycosylated hemoglobin (HA1c) have been associated with increased risk of infections [25]. The impaired immune system is common in DM patients, explaining the increased susceptibility to infection [26]. DM has been found to be associated with neutrophilic dysfunction [27], and lymphocyte function impairments [28]. Increased RBC has been shown to be associated with insulin resistance [29]. An increased haematocrit and blood viscosity are important determinants of blood rheology and are also associated with an increased risk of coronary heart disease [30]. The increased viscosity is due to the platelets which are responsible for blood coagulation. Diabetic patients have been shown to respond normally to haematocrit variation, suggesting that a raised haematocrit would also be associated with an increased risk of macrovascular disease [31].



	% Survival	% Mortality	% Average Life span
Group1	100.00	0.00	100.00
Group 2	16.67	83.33	66.30
Group3	83.33	16.67	98.52
Group4	66.67	33.33	93.33
Group5	66.67	33.33	92.96

Fig. 1: Survival, mortality and average life span of the experimental rats

The percentage life span of the diabetic and other groups is compared (Figure 1). In the diabetic group, the mortality was increased and hence reduction in the life span was noticed and the treatment with the glibenclamide and mushroom decoctions to the diabetic animals created reduction in the mortality rate and in turn significant increase in the life span was noticed.

Results obtained from the study revealed that the decoctions of the mushroom, *Calocybe indica* have shown antidiabetic activity by controlling the blood glucose level, serum insulin level and glycosylation reaction of the Hb. The medicinal decoction has counteracted the alterations in the blood cells count caused by diabetes mellitus. While comparing with the diabetic control, amongst the treatment with two decoctions, the cold water decoction has more effective capacity than the hot water decoction.

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