World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:7, No:7, 2013

Ameliorative Effect of *Calocybe indica*, a Tropical Indian Edible Mushroom on Hyperglycemia Induced Oxidative Stress

Shanmugasundaram Krishnakumari, Paramasivam Rajeswari, and Subramanian Kathiravan

***Abstract*—**Mushrooms are a group of fleshy macroscopic fungi. They have been valued throughout the world as both edible and medicine. They are highly nutritious with good amount of quality proteins, vitamins and minerals. An edible mushroom, *Calocybe indica* was selected to validate its nutritional and medicinal properties. Since tissue damage in hyperglycemia has been related to oxidative stress, we evaluated the enzymatic and non-enzymatic antioxidant status in the serum, liver and kidney since they are the target organs in diabetic complications. From the results, increased oxidative stress and decreased antioxidants might be related to the causation of diabetes mellitus. The treatment in the diabetic rats with the *Calocybe indica* showed an increase in the antioxidant system and decrease in the production of free radicals. The mushrooms which contain antioxidant phytochemicals has potential free radical scavenging capacity and hence can induce the antioxidant system in the body significantly reduces the generated free radicals thereby maintaining the normal levels of the antioxidants

***Keywords*—**Antioxidants, *Calocybe indica*, diabetes mellitus, edible mushroom, oxidative stress.

I. Introduction

FUNGI are well-known for their nutritional and medicinal value due to their content of a variety of bioactive substances with pharmacological properties [1]. The macro fungi, mushrooms have a history of traditional use in oriental therapies and modern clinical practices continue to rely on mushroom derived preparations [2]. They are appreciated all over the world not only by their texture and flavor, but also by their chemical, nutritional [3] and functional properties. Scientific research on mushrooms generally lags behind that of plants and animals [4]. Since ancient times, mushrooms have been consumed by humans in normal diet due to their highly desirable taste and aroma. In addition, the nutritional, toxic and medicinal properties of mushrooms have been recognized for a longer time [5].

Diabetes mellitus is a group of metabolic diseases [6] which affects the individuals of all ages [7]. It is characterized by hyperglycemia [8], [9], altered metabolism of lipids, carbohydrates, and proteins [6] which results from either the relative impairment in pancreatic insulin secretion or varying degrees of peripheral resistance to the action of insulin, or both [10]. Modern drugs, including insulin and other hypoglycemic agents control the blood glucose level only when they are regularly administered, but these treatments are tedious and have several undesirable side effects and fail to significantly alter the course of diabetic complications [11], [12].

Free radicals are chemical entities which are generated during normal cellular metabolism as a side product of oxidative phosphorylation which is a normal natural metabolism of aerobic cells both in animals and plants [13], [14]. However, antioxidant supplements or antioxidant containing foods may be used to help the human body to reduce oxidative damage or to protect food quality by preventing oxidative deterioration [15]. Identification of new antioxidants remains a highly active research area. From the literature of the previous research, it is evident that the mushrooms contain the activity against the free radicals.

Dr. Krishnakumari Shanmugasundaram, is Associate Professor with the Kongunadu Arts and Science College, Coimbatore - 64102, Tamil Nadu, India (mobile: 9942668270, office: 0422-2642095, e-mail: krishnashanmugambc@gmail.com).

II. Materials and Methods

A. Plant Material

1. Culture

The *Calocybe indica* species was collected from Sivasakthi mushrooms, a commercial mushroom unit at Erode, Tamilnadu, India. The mushrooms were surface sterilized and the spores from the mushrooms were inoculated into the Potato Dextrose Agar medium to obtain pure culture of the mushroom. The culture was maintained at room temperature for mycelial growth and the culture was sub cultured to multiply the mycelial culture.

2. Spawn Preparation

The mycelium was inoculated into the substrate (boiled and sterilized sorghum seeds) and maintained at room temperature for the growth of mycelium. The packs were checked then and there for any contamination and the contaminated packs were removed if present. After complete growth of the mycelium on the grains, the packs free from contaminations were selected and used further

3. Cultivation and Harvesting

The cultivation of *Calocybe indica* needs two steps. In the first step called mycelial running, the polythene bags were filled with soaked and sterilized paddy straw layer by layer with spawn on each layer, tied tightly and hanged in a room

which was maintained with optimum temperature and humidity. After the vegetative mycelial setting the bags were taken to the second step. In the second step called casing (i.e.) after mycelial setting, the bags were cut into two equal halves and cased with sterilized black soil. Then the bags were taken into the shed whose temperature was maintained at 40°C to 45°C. The buds of the mushroom were extruded and the matured mushrooms were harvested and used for the study.

B. Sample Processing

The mushroom, *Calocybe indica* after washing were shade dried and was powdered in a mixer grinder.

1. Decoctions Preparation

- *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.

2. Induction of Diabetes Mellitus

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 administration of 65mg/kg streptozotocin, 15min after the intraperitoneal administration of 110mg/kg of nicotinamide. Hyperglycemia was confirmed by the elevated glucose levels in the plasma determined after 72 hours. The animals with blood glucose levels higher than 300mg/dl after 3 days of STZ injection were selected for the study.

3. Experimental Set Up

- Group 1 - Normal control
- Group 2 - Diabetes induced (Diabetic control)
- Group 3 - Diabetic animals treated with Glibenclamide at 5mg/kg body weight
- Group 4 - Diabetic animals treated with cold aqueous decoction at 120mg/100g body weight
- Group 5 - Diabetic animals treated with hot aqueous decoction at 120mg/100g body weight

The treatment was given orally once a day for 45 days. After the treatment regimen, the animals were sacrificed and their samples were subjected to different analyses. The blood was collected from the jugular vein and centrifuged at 4°C for 20 minutes and the serum was separated. Liver was removed and washed in ice cold saline. The tissues were homogenized in phosphate buffer of pH 7.5. The oxidative stress enzymes and the antioxidant status were assayed using standardized protocols.

III. Results and Discussion

A. Nutritional Constituents of Mushrooms

Mushrooms are rich sources of nutraceuticals [15] responsible for their pharmacological properties. These functional characteristics are mainly due to chemical composition [16]. Mushrooms are quite high in protein including all the essential amino acids and low in fat.

Mushrooms also contain relatively large amounts of carbohydrates and fiber and significant amounts of vitamins, as well as minerals [3].

Results from Table I reveals that the mushroom of the study *Calocybe indica* posses a comparable content of nutritional and chemical compounds which are essential in diet and hence proves that the mushrooms are rich in nutrients.

B. Antidiabetic Activity of Calocybe indica

In people with diabetes, glucose levels build up in the blood and urine, causing excessive urination, thirst, hunger, and problems with fat and protein metabolism [17]. Hyperglycemia is caused either by insufficient insulin secretion or insulin resistance [18]. Insulin deficiency in the diabetic state results in the impairment of glucose utilization leading to an increased generation of oxygen free radicals [19]. Insulin deficiency is manifested in a number of biochemical and physiological alterations. Insulin estimations are generally accepted as an index of β-cell function [20].

Results from Table II revealed a significant decrease in serum insulin level of diabetic rats caused decreased uptake of blood glucose and thus hyperglycemia in the diabetic rats. Administration of the mushrooms decoctions decreased the elevated blood glucose level and prolonged administration may stimulate the β-cells of islets of Langerhans to produce insulin. The increased level of insulin in mushrooms treated diabetic rats might be due to the activation of remnant β-cells of the pancreas [20]. The control of blood glucose in diabetic patients was achieved mainly by the use of oral hypoglycemic agents and insulin [21].

TABLE I
NUTRITIONAL VALUE OF *CALOCYBE INDICA*

Compounds		*Calocybe indica*
Metabolites	Total protein (%)	**32.61**
	Total carbohydrates (%)	34.00
	Total phenolics (A*)	6.95
	Total flavanoids (B*)	1.09
Vitamins	Ascorbic acid (mg/100g)	**125.00**
	Beta carotene (mg/100g)	108.79
Minerals	Sodium (mg/100g)	**1194.00**
	Potassium (mg/100g)	**5692.75**
	Calcium (mg/100g)	**88.43**
	Iron (mg/100g)	**111.37**
Others	Crude fibre (%)	**10.30**
	Total ash (%)	**14.14**

(A*): mg of gallic acid equivalents per gram of dried mushroom
(B*): mg of catechin equivalents per gram of dried mushroom

TABLE II
DIABETIC PROFILE OF THE UNTREATED AND TREATED DIABETIC RATS

Groups	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 (Δ#)	2.60±0.72 (Δ#)	7.97±0.80 (Δ#)	8.90±0.89 (Δ#)	8.93±0.70 (Δ#)
Group 3	182.67±10.07 (Φ#)	4.53±0.60 (Φ#)	9.22±0.23 (Φ#)	12.53±0.35 (Φ#)	3.57±0.86 (Φ#)
Group 4	208.17±18.84 (Φ#)	4.87±0.35 (Φ#)	8.98±0.20 (Φ#)	13.03±0.65 (Φ#)	3.27±0.60 (Φ#)
Group 5	211.67±19.68 (Φ#)	4.90±0.36 (Φ#)	9.08±0.37 (Φ#)	12.56±0.40 (Φ#)	4.17±0.47 (Φ#)

ANOVA was performed to compare the means of parameters- Glucose, Insulin, RBC, Hb and HbA1c in diabetic group (Group 2) with control (Group1) and also with the treatments (Groups 3,4,5). The test values are expressed as mean ± SD along with inference. In the table we use notations: Δ: Comparison with normal control group, Φ: comparison with diabetic control group, (ns): – no significant difference ($p>0.05$), (*): significant difference ($p<0.05$) and (#): significant difference ($p<0.01$)

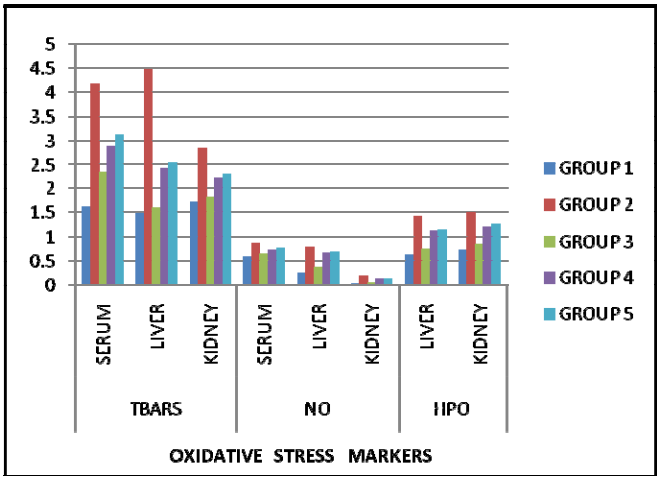

Fig. 1 Oxidative stress markers levels in control and *Calocybe indica* treated experimental rats

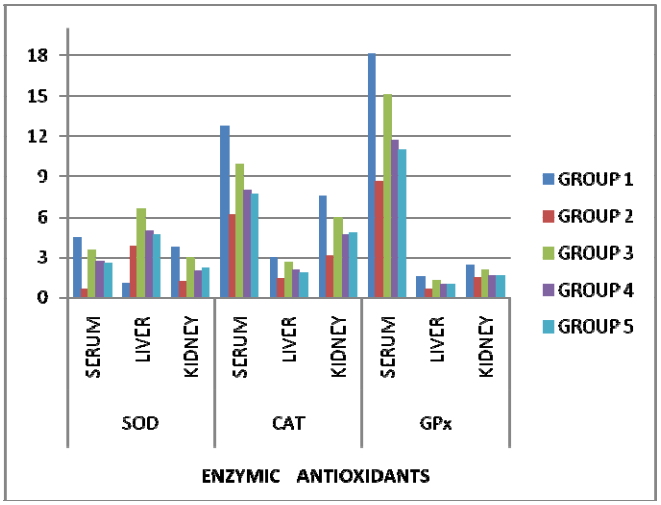

Fig. 2 Enzymatic antioxidants levels in control and *Calocybe indica* treated experimental rats

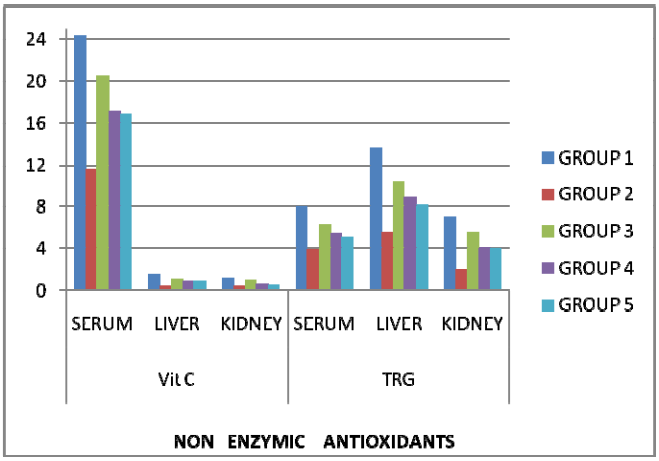

Fig. 3 Non enzymatic antioxidants levels in control and *Calocybe indica* treated experimental rats

C. Oxidative Stress Markers and Antioxidants in Diabetes

It was observed in the present study that the level of peroxides (LPO and NO) were significantly increased in hypoglycemic condition and the activity of enzymatic (SOD, CAT and GPx) and non-enzymatic (TRG and Vit C) antioxidants were decreased significantly in diabetic rats than the normal control rats in the serum, liver and kidney tissues, which are presented in Figs. 1-3. The treatment with the mushroom *Calocybe indica* and with the standard drug glibenclamide significantly reverted the level of these variants in diabetic animals.

From the results, the increase in the free radicals and decrease in the antioxidant system indicated that increased oxidative stress and accompanying decrease in antioxidants might be related to the causation of diabetes mellitus [22]. The decrease in antioxidants could be a result of decreased synthesis or increased degradation by oxidative stress in diabetes. Increased lipid peroxidation under diabetic conditions can be due to increased oxidative stress in the cell as a result of depletion of antioxidant protective systems, which can increase the deleterious effects of free radicals [23] such as loss of integrity and function of cell membranes [24].

The decline in the antioxidant status and increment in the free radical compounds in the diabetic rats are due to the chronic hyperglycemia which paves way to infer that the increased production of free radicals which automatically decrease the antioxidant system. Supplementation of

antioxidants could conceivably protect the human body from free radicals and ROS effects and retard the progress of many chronic diseases as well as lipid peroxidation [25], [26]. The treatment with the mushrooms to the diabetic rats shows increase in the antioxidant system and decrease in the production of free radicals. Maintenance of persistent normoglycemia by the administration of *Calocybe indica* decoctions may attenuate lipid peroxidation in tissues and thus prevent tissue damage [20]. The mushroom favored in the rats in lessening the oxidative stress mediated injury to the system and this property might be due to the presence of the antioxidants or its inducers such as phenols, flavanoids present in the mushrooms.

ACKNOWLEDGMENT

We are thankful to the Department of Science and Technology (DST), New Delhi, Tamil Nadu State Council for Science and Technology (TNSCST), Tamil Nadu for providing financial assistance and Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India for providing the facilities to carry out this work.

REFERENCES

[1] Chang, R., 1996. Functional properties of edible mushrooms, Nutr. Rev. 54: S91–S93.
[2] Ferreira, I.C.F.R., Vaz, J.A., Vasconcelos, M.A. and Martins, A., 2010. Compounds from wild mushrooms with antitumor potential. Anti Cancer Agents Medicinal Chemistry 10, 424–436.
[3] Kalac, P., 2009. Chemical composition and nutritional value of European species of wild growing mushrooms: A review. Food Chemistry 113, 9–16.
[4] Sonnenberg, A.S.M., Baars, J.J.P., Hendrickx, P.M., and Kerrigan, R.W., 2005. Breeding mushroom: State of the art, Acta Edulis Fungi, 1: 163–173.
[5] Mattila, P., Suonpaa, K., and Piironen, V. 2000. Functional properties of edible mushrooms. Nutrition, 16, 694–696.
[6] Davis, S., 2006. Insulin, oral hypoglycemic agents and the pharmacology of the endocrine pancreas. In: Brunton, L., Lazo, J., Parker, K. (Eds.), Goodman and Gilman's. The Pharmacological Basis of Therapeutics, 11th ed. McGraw Hill Publishing, pp. 1613–1646.
[7] Lefebvre, P., 2005. Diabetes yesterday, today and tomorrow. The action of the International Diabetes Federation. Revue Medicale de Liege 60, 273– 277.
[8] Panunti, B., Jawa, A.A. and Fonseca, V.A., 2004. Mechanisms and therapeutic targets in type 2 diabetes mellitus. Drug Discovery Today: Disease Mechanisms 1, 151–157.
[9] Davi, G., Falco, A. and Patrono, C., 2005. Lipid peroxidation in diabetes mellitus. Antioxid. Redox Signal 7, 256–268.
[10] Obaid, M. and Turtle, J.R., 2004. Type 2 diabetes: an epidemic in the making. American Diabetes Foundation. 5, 29-34.
[11] Upadhyay, O.P., Singh, R.M. and Dutta, K., 1996. Studies on antidiabetic medicinal plants used in Indian folk-lore. Aryavaidyan 9 (3), 159–167.
[12] Rang, H.P. and Dale, M.M., 1991. The Endocrine System Pharmacology, second ed. Longman Group Ltd, United Kingdom pp. 504–508.
[13] Ajith, T. A. and Janardhanan, K. K. 2007. Indian medicinal mushrooms as a source of antioxidant and antitumor agents. Journal of Clinical Biochemistry and Nutrition, 40, 157–162.
[14] De la Fuente, M. (2002). Effects of antioxidants on immune system ageing. European Journal of Clinical Nutrition, 56(3), S5–S8.
[15] Elmastas, M., Isildak, O., Turkekul, I. and Temur, N., 2007. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. Journal of Food Composition and Analysis 20, 337–345.
[16] Manzi, P., Aguzzi, A. and Pizzoferrato, L., 2001. Nutritional value of mushrooms widely consumed in Italy. Food Chemistry 73, 321–325.
[17] Kathleen, A.H., 1996. Type 1-diabetes: Prevention of the disease and its complications. Alternative Med Rev 2, 256-281.
[18] Mahalingam Gayathri and Krishnan Kannabiran., 2008. Antidiabetic and ameliorative potential of ficus bengalensis bark extract in streptozotocin induced diabetic rats. Indian Journal of Clinical Biochemistry 23 (4), 394-400.
[19] Baynes, J.W., 1995. Reactive oxygen in the aetiology and complications of diabetes. Drug, diet and disease, Mechanistic approach to diabetes. In: Ioannides C, Flatt PR editors. Ellis Horwood Limited, Hertfordshire 2, 2003-2231.
[20] Hayath Basha, S.K. and Subramanian, S., 2011. Biochemical evaluation of antidiabetic and antioxidant potentials of Annona squamosa leaves extracts studied in STZ induced diabetic rats. IJPSR 2(3), 643-655.
[21] Campbell, R.K., White, J.R. and Saulie, B.A., 1996. Metformin: a new oral biguanide. Clinical Therapeutics 18, 360–71.
[22] Saxena, A.K., Srivastava, P., Kale, R.K. and Baquer, N.Z., 1993. Impaired anti-oxidant status in diabetic rat liver: Effect of vanadate. Biochem. Pharmacol 45, 539-542.
[23] Picton, S.F., Flatt, P.R., and Mcclenghan, N.H., 2001. Differential acute and long term actions of succinic acid monomethyl ester exposure on insulin secreting BRAIN-BD 11 cells. Int. J. Exp. Diabetes Res. 2, 19–27.
[24] Sheela, C.G., and Angusti, K., 1995. Antiperoxide effects of S-allyl cystein sulphoxide isolated from Allium sativum Linn and gugulipid in chlosterol diet fed rats. Ind. J. Exp. Biol. 33, 337–341.
[25] Lai, L.S., Chou, S.T. and Chao, W.W., 2001. Studies on the antioxidative activities of Hsian-tsao (Mesona procumbens Hemsl) leaf gum. Journal of Agricultural and Food Chemistry 49, 963−968.
[26] Gulcin, Y., Buyukokuroglu, M.E., Oktay, M. and Kufrevioglu, O.Y., 2003. Antioxidant and analgesic activities of turpentine of Pinus nigra Arn. Subsp pallsiana (Lamb.) Holmboe. Journal of Ethnopharmacology 86, 51-58.