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Cultivation of *Pleurotus citrinopileatus* on water hyacinth and effect of dietary *Pleurotus* mushroom on lowering of blood glucose and cholesterol in diabetic model rats

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Oyster mushrooms (*Pleurotus* spp.) are widely used edible mushroom due to the ease of their cultivation and many favourable nutritional qualities as low calorie and fat content, but significantly high essential fatty acids, protein, vitamin, and mineral content. Diabetes and obesity are most widely found problem worldwide. Though there are several synthetic medicines to combat these diseases but people are more looking for inexpensive, natural herbal medicines to avoid any side effect. To promote awareness about medicinal importance of easily cultivable oyster mushroom among common people the present work was undertaken to produce *Pleurotus citrinopileatus* at minimum cost by cultivating on the aquatic weed water hyacinth and evaluation of the effect of feeding this mushroom (powder) supplemented diet on levels of blood glucose and cholesterol in alloxan-induced diabetic albino rats. *Pleurotus citrinopileatus* was cultivated on dry water hyacinth plants (without root). The harvested mushrooms were dried and powdered before feeding the test rats for 28 days. The control group of alloxan-induced diabetic rats were fed diet with powdered chick pea (Bengal gram) as the only protein source whereas the other group fed diet with powdered oyster mushroom as the protein source. After 28 days feeding no significant difference was observed between the control group and mushroom fed group of rats with respect to body weight. Analysis of blood samples for blood glucose and serum cholesterol showed significantly decreased level of blood glucose and low-density lipoprotein (LDL) cholesterol respectively in the mushroom fed diabetic group than in the non-mushroom fed diabetic group. This indicated the beneficial effect of oyster mushroom on lowering blood glucose and LDL cholesterol.

Keywords: blood glucose, diabetic rat, cultivation, *Pleurotus citrinopileatus*, LDL cholesterol. oyster mushroom, total cholesterol, water hyacinth.

INTRODUCTION

Diabetes is a global pandemic in recent times mainly due to widespread changes in lifestyles, and food habits. Approximately 537 million adults (20-79 years) worldwide have diabetes, the majority living in higher-income countries, and about 2 million deaths are directly attributed to diabetes each year. The total number of people living with diabetes is projected to rise to 643 million by 2030 (IDF, 2021).

Diabetes mellitus is a disorder of carbohydrate metabolism due to impaired insulin secretion and variable degrees of peripheral insulin resistance. Type 2 diabetes is becoming increasingly common among children and in 90% of adults with diabetes. Due to major risk of development of cardiovascular disease in diabetes, it is important to maintain healthy weight, blood pressure and cholesterol (low levels of low-density lipoprotein LDL and very low-density lipoprotein VLDL) in addition to maintaining blood sugar level. Diagnosis is by measuring plasma glucose and treatment is diet, exercise, and drug (Brutsaert, 2022). As food greatly impacts blood sugar, hence meal planning and choosing a healthy diet are the key aspects of diabetes management. The initial step in the prevention and treatment of hyperglycaemia and hypercholesterolemia is the modification of the nutritional regime with a diet low in fats and fatty acids and rich in crude fibre.

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In folklore mushroom consumption is linked to diabetes management. Mushrooms are fleshy spore bearing fruiting bodies of higher fungi, typically produced on dead organic matter. They have abundant insoluble carbohydrate, phenolic acid, flavonoids, alkaloids, and proteins (Agunloye *et al.* 2020). Various research outputs have shown that mushrooms possess functional nutraceuticals or medicinal properties and are being used on such purposes (Patel *et al.* 2012). Researchers have shown that mushrooms because of their high fibre, proteins, unsaturated fatty acids, microelements content, and low calorific value and low calorific value (Pardeshi and Pardeshi, 2009), are favourable for diets designed to prevent cardiovascular diseases. The production of oyster mushrooms (*Pleurotus* spp.) has been increasing at a rapid rate due to the ease of its cultivation on any lignocellulosic substrate. Unlike other mushrooms, the oyster mushroom can grow comfortably on a wide range of agricultural wastes, forest wastes or weeds available at minimum cost. These mushrooms are also attractive for human diet owing to their non-starchy carbohydrate, high dietary fibre, moderate protein contents with most of the essential amino acids, minerals, and vitamins (Khan and Tania, 2012). Previous researches have shown the antihyperglycemic effect of aqueous extracts of *P. pulmonarius* against alloxan-induced diabetic mice (Badole *et al*. 2008) and cholesterol lowering effect of dietary *P. ostreatus* in hypercholesterolemic rats (Alam *et al.* 2011).

The aquatic perennial herb water hyacinth or *Eichhornia crassipes* (Mart.) Solms. of the family Pontederiaceae is a major freshwater weed in tropic and sub-tropic regions. Due to its intense rate of proliferation, the weed poses negative impact on biodiversity and ecological environment causing great socio-economic concern (Dechassa, 2020). On the positive side, water hyacinth has been used as one of the main components of aquatic system for waste water treatment throughout the world because of its phytoremediation ability to bioaccumulate hazardous pollutants and metals (Magar *et al.* 2017). This aquatic weed, which is naturally available round the year, has been found to support the cost-effective production of *P. citrinopileatus* significantly when it is used alone or in combination with paddy straw (Bandopadhyay, 2013; Bandopadhyay Mukhopadhyay, 2020). Previous studies have also shown that the nutritional qualities of the oyster mushrooms have not been adversely affected using water hyacinth as the substrate for mushroom bed preparation. Moreover, the heavy metals adsorbed in the plants (due to phytoremediation of waste water by water hyacinth), though translocated to the mushrooms from the substrate but have not been observed to accumulate beyond permissible limits (Bandopadhyay Mukhopadhyay, 2020).

To promote awareness among common people about the medicinal importance of thus produced oyster mushrooms (*P. citrinopileatus*), the present study has been undertaken to assess the effect of consumption of *P. citrinopileatus* supplemented diet on blood-glucose, serum total cholesterol, LDL and HDL cholesterol levels in normal and alloxan-induced diabetic rats. The dietary effect of *P. citrinopileatus* on the growth of normal rats has also been assessed.

MATERIALS AND METHODS

Cultivation of Mushroom

A pure culture of *Pleurotus citrinopileatus* Sing. (strain PI-100) was procured from NCMRT, Solan, India and was maintained on Potato-Dextrose-Agar. For mushroom seed (spawn), wheat grain spawn was prepared following standard method. Substrate used for cultivation of *P. citrinopileatus* was dead water hyacinth (*Eichhornia crassipes* Mart. Solms.) plants (obtained from the bank after clearing of water bodies infested with the weed) without roots. Cultivation was done in polypropylene bag method (Bandopadhyay, 2013). Fruit bodies were harvested from day 22-25 of spawning and upto 3rd flushes. Mushrooms were cut into slices, air dried at 45-50°C for 24 hrs. and homogenized to fine powder to use as protein supplement in diet of experimental rats.

Determination of starch and cholesterol

Starch content in dry mushroom *(P. citrinopileatus)* powder was estimated using anthrone reagent

following Sadashivam and Manickam (1996). Cholesterol was qualitatively determined following Salkowski Reaction for confirmative test of cholesterol.

Animal experiments

Albino Wistar rats of either sex weighing between 170 to 190g were used for the present study. The animals were housed in the Department of Physiology, Burdwan Medical College, Burdwan, West Bengal, India. They were allowed free access to food pellets containing ground wheat, oil, salt, milk powder, Bengal gram (*Cicer arietinum*) powder (5%) w/w) or *P. citrinopileatus* powder (5% w/w) and water ad libitum. Diabetes was induced in ten rats weighing between 170-190g by the administration of single intraperitoneal dose (100 mg/kg body weight) of alloxan monohydrate after overnight fasting of 12 h. Three days after alloxan injection, rats were screened for diabetes and those having fasting blood glucose level above 170 mg/dL were selected for the study of antihyperglycemic effect of *P. citrinopileatus*. A total of 20 rats (10 diabetic rats and 10 normal non-diabetic rats) weighing 170-190 g were divided into following four groups of 5 rats each:

Normal non-diabetic control: rats were given normal diet i.e. food pellets without mushroom but with gram powder (5% w/w).

Normal non-diabetic mushroom: rats were given food pellets supplemented with 5% (w/w) dry mushroom powder instead of gram powder.

Diabetic control: rats were given normal diet food pellets i.e., without mushroom supplementation.

Diabetic mushroom: rats were given mushroom supplemented (5% w/w) food pellets.

All the rats were given access to pellet diet and water ad libitum.

Biochemical analysis

All blood samples were collected by retro orbital plexus technique using heparinized capillary glass tubes at 7 days interval for one month i.e., on 7th, 14th, 21st and 28th day after stabilization of diabetic condition (Day 0). Collected blood samples were analyzed for fasting plasma glucose levels by the glucose oxidase peroxidase (GOD/POD) method using Glucose-Kit (Crest Biosystem, Goa, India) and were expressed in mg/dl. Blood samples collected after 28 days were analysed for serum total cholesterol (TC), HDL cholesterol (HDL-C) and LDL (LDL-C) cholesterol levels by CHOD / PAP method, PEG / CHOD-PAP method using diagnostic kit (Crest Biosystem, Goa, India). All the blood samples were analysed for the concerned parameters in the Department of Biochemistry, Burdwan Medical College, Burdwan, West Bengal, India.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) and were analysed by independent t-test using SP-SS software. For the statistical tests p Â 0.05 were taken as significant.

RESULTS AND DISCUSSION

Cultivation of *P. citrinopileatus* Sing. on water hyacinth yielded 1447.4 ± 64g fresh fruit bodies out of 1kg dry substrate (water hyacinth) in 3 flushes (Bandopadhyay, 2013; Bandopadhyay Mukhopadhyay 2020). Estimation of starch of these mushrooms revealed low starch (0.28% on dry weight basis) content which is preferred for diabetic persons. Moreover, qualitative estimation of cholesterol gave negative test in Salkowski reaction indicating presence of negligible amount of cholesterol in *P. citrinopileatus*.

Analysis of blood glucose levels revealed that administration of mushroom supplemented (5% w/w dry powder) diet to the normal non-diabetic groups of rats, exhibited reduced level of blood glucose in the group fed with *P. citrinopileatus* supplemented diet ('Normal mushroom') compared to its counterpart (Normal control) fed with normal diet without mushroom (Fig. 1).

The alloxan-induced diabetic rats also showed significantly ($p<0.05$) reduced blood glucose level in 'diabetic mushroom' (fed with mushroom supplemented diets) group after 7th, 14th and 21st days of alloxan induced stabilized diabetic condition as compared to 'diabetic control' (fed with normal

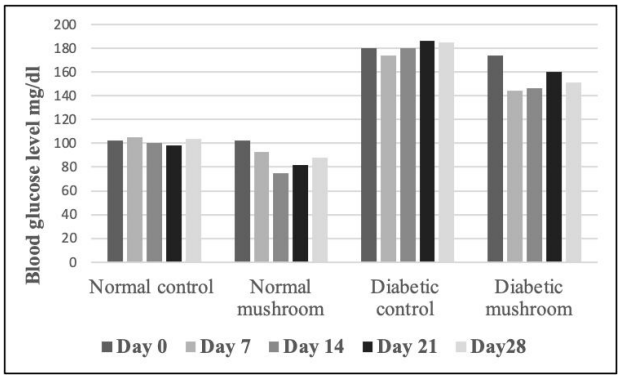

Fig.1 : Effect of *P. citrinopileatus* (5% w/w) supplemented diet on plasma blood glucose levels of normal non-diabetic and alloxan induced diabetic rats.

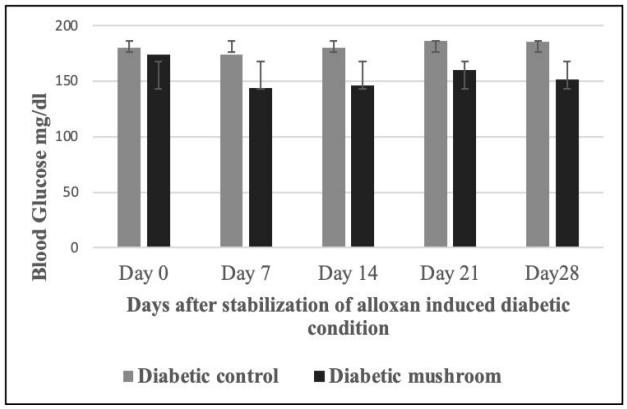

Fig. 2 : Effect of *P. citrinopileatus* (5% w/w) supplemented diet on plasma blood glucose levels of alloxan induced diabetic control and diabetic mushroom-fed rats.

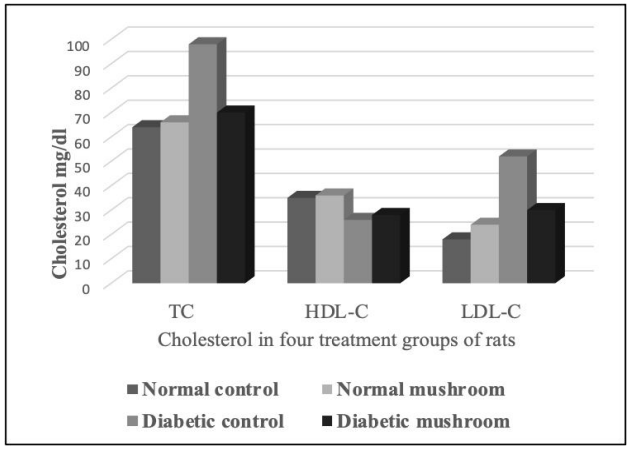

Fig. 3 : Effect of *P. citrinopileatus* supplemented (5% w/w) diet on serum total cholesterol (TC), HDL (HDL-C) and LDL (LDL-C) cholesterol in normal and alloxan induced diabetic rats.

diet) group (Fig.2). These results indicate antihyperglycemic effect of *P. citrinopileatus* in alloxan induced diabetic rats.

Total cholesterol (TC) and LDL-C were observed to be reduced significantly ($p<0.05$) (Fig.3) in

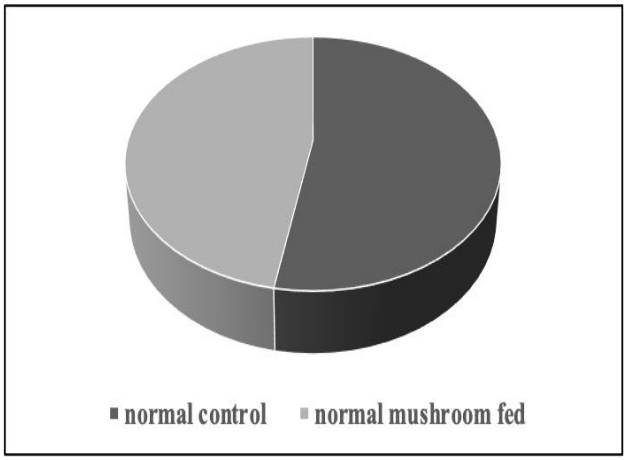

Fig. 4: Increase in body weight (g) in non-diabetic normal control and mushroom-fed rats (Change in body weights are insignificant; p=0.236).

'diabetic mushroom' rats as compared to their elevated levels in 'diabetic control' rats after 28 days study period. No significant changes were observed with respect to HDL-C levels between these two groups of rats (Fig. 3) after 28 days.

The study on body weight shows almost similar increase in body weight of 'normal control' (non-diabetic) rats and 'normal mushroom'-fed rats (Fig. 4) after 28 days study period revealing insignificant (p=0.236) difference between the two groups with respect to body weight. This indicates a comparable effect of *P. citrinopileatus* with Bengal gram (*Cicer arietinum*) as the major vegetable protein source in rat diet.

From the present observation, it is evident that regular consumption of dried *P. citrinopileatus* at approximate dietary level of 5% (w/w) for 3- 4 weeks has beneficial effect on alloxan-induced diabetic rats regarding decrease in blood glucose level, TC, and LDL-C levels. Significant lowering of LDL cholesterol after dietary consumption of *P. citrinopileatus* is a desirable biochemical state for prevention of complications of diabetes. Many mushroom varieties have been reported to possess hypoglycaemic activities in animals as well as in diabetic patients (Shamim *et al.*, 2023). Several species of mushroom have been shown to influence lipids in general and cholesterol. Abram et al. (2011) reported that *P. ostreatus* can reduce the LDL-cholesterol level but could not significantly affect HDL-cholesterol in human patient.

Suspensions of freeze dried and powered *P. ostreatus* and *P. cystidiosus* have shown significant reduction in fasting and postprandial (PP) serum glucose levels in healthy human volunteers and reduced PP serum glucose levels of type 2 diabetic patients (Banukie *et al.* 2015). *P. pulmonarius* had been reported to show hypoglycaemic activity in alloxan-induced diabetic mice (Badole *et al.* 2006).

Under normal physiological conditions, a wide range of antioxidant enzymes offer first line of defense and protects the body from diverse effects of free radicals (ROS) produced in vivo. Reactive oxygen species (ROS) is generated more in diabetes which is one of the major events that disrupt the endogenous antioxidant defense system in diabetes (Irondi *et al.* 2015). Previous investigation showed potent antioxidant activity of dry *P. citrinopileatus* in terms of Super Oxide Dismutase activity, lipid peroxidase activity, reducing power and total phenol (*in vitro and in vivo*) (Khatun *et al.* 2015). Hence restitution of antioxidant defense power by *P. citrinopileatus* supplemented diet might be one of the important contributory factors in antihyperglycemic effect of *P. citrinopileatus*. Agunloye and Oboh (2021) observed significantly higher antioxidant enzyme activities in diabetic rats fed with oyster mushroom supplemented diets which reduced lipid oxidation and offered protective shield against hyperglycaemia-induced ROS in diabetes. Oyster mushrooms which contain a natural lovastatin-like compound, have been shown to provide significant cholesterol reductions in animal models (Abrams *et al.* 2011). *Pleurotus* mushrooms had been recommended as a natural cholesterol lowering substance within the human diet.

The present study therefore, emphasizes the health benefit of oyster mushroom (*P. citrinopileatus*) supplemented (5%w/w) diet in reducing blood glucose level as well as LDL cholesterol rendering its anti-hyperglycaemic and anti-hypercholesterolemic effect to alloxan induced diabetic Wister rats. Oyster mushroom (*P. citrinopileatus*) could therefore be produced cost-effectively by small scale farmers or self-help groups using water hyacinth as substrate for mushroom cultivation and consumption of these oyster mushroom supplemented (5% w/w) diet could offer therapeutic effect against progression of diabetic complications.

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