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Research article/Araştırma makalesi

Hypoglycemic activity of a dietary mushroom *Pleurotus florida* on alloxan induced diabetic rats

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Abstract

Species of *Pleurotus* possess various medicinal properties including hypoglycemic and hypocholesterolemic activities. The feeding effect of the mushroom *Pleurotus florida* in powdered form was evaluated on growth, blood glucose and cholesterol level in alloxan-induced diabetic albino rats against control. The starch was estimated in dry mushroom and found to be 0.28% (dry weight). The result indicated no significant difference in respect of gaining body weights between Bengal gram-fed and mushroom-fed groups. For studying antidiabetic effect, the control group and half of alloxan-induced (100 mg/kg i.p) experimental groups were fed on normal diet with Bengal gram whereas the other half of alloxan-induced group was fed on same diet but with additional mushroom powder. Blood samples were analysed for glucose, serum cholesterol, triglycerides and uric acid levels on 7th, 14th, 21st and 28th days of alloxan treatment. Body weights and mortality were noted during the period of study. The diabetic group administered with mushroom diet showed significantly decreased level of blood glucose and cholesterol compared to the counterpart. Estimation of lipid profile revealed much lower LDL cholesterol in mushroom fed diabetic group than in non-mushroom fed diabetic control group. The study, thus, points out the prominent effect of feeding dried mushroom (*P. florida*) on growth of albino rat and positive effect of *P. florida* on lowering blood glucose and cholesterol level in diabetic rats thereby suggesting its hypoglycemic and hypocholesterolemic effect.

Key words: Alloxan induced diabetic, Blood glucose, LDL cholesterol, *Pleurotus florida*, Total cholesterol, Triglycerides

1. Introduction

The high prevalence of diabetes as well as its long-term complications have led to an ongoing search for hypoglycemic agents from natural sources (Nicasio et al., 2005). The major risk factors in development of coronary artery disease (CAD) have been identified as DM, increased blood levels of total cholesterol, low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol as well as lowered levels of high density lipoprotein (HDL) cholesterol (Francia et al., 1999). The initial step in the prevention and treatment of CAD and hypercholesterolemia is the modification of nutritional regime with a diet low in fats and fatty acids and rich in crude fibres. Mushrooms in general and *Pleurotus, Lentinus, Grifola* in particular, because of their high fibre content, proteins, microelements and low caloric value, are almost ideal for diets designed to prevent cardiovascular diseases as first suggested by traditional Chinese Medicine (Hobbs, 1995).

Oyster mushrooms have attracted much attention in human diet owing to having their good source of non-starchy carbohydrates, high content of dietary fibre, moderate quantities of proteins with most of the essential amino acids, minerals and vitamins (Croan, 2004). Many investigators have endeavoured to study the hypoglycemic effect from either the fruiting body or mycelia of various edible/medicinal fungi including *Tremella aurantia*, *Cordyceps sinensis*, and *Lentinus edodes* (Kiho et al., 1995, 1996; Yang et al., 2002). Lo et al. (Lo et al., 2004) reported that the fruiting body of *Cordyceps* has a potential to be a functional food for diabetes. Kiho et al. (Kiho et al., 1994) stated that the hot water extract of the fruiting bodies of *Agrocybe cylindracea* also has hypoglycemic effect. Yuan et al. (Yuan at al., 1998) investigated the hypoglycemic activity of a water-soluble polysaccharide from the fruiting bodies of

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Auricularia auricula-judae Que. The antidiabetic effect of the crude exopolysaccharides produced from submerged mycelial culture of *Phellinus baumii* in streptozotocin induced diabetic rats was investigated (Hwang et al., 2005). Earlier studies have reported insulin release and insulin like activity of other mushroom species like *Agaricus campestris* (Gray and Flatt, 1998; Talpur et al., 2002; Swanston-Flatt et al., 1989). Previous studies had shown hypoglycemic effect of aqueous extract of *P. pulmonarius* against alloxan-induced diabetic mice (Badole and Bodhankar, 2007), and cholesterol lowering effect of dietary *P. ostreatus* in hypercholesterolemic rats (Bobek et al., 1998).

However, there is paucity of reports on the hypoglycemic activity of *Pleurotus florida*. In the present study, effect of consumption of *Pleurotus florida* supplemented diet on blood glucose, serum total cholesterol, total triglycerides, uric acid and HDL cholesterol levels of normal and alloxan-induced diabetic rats was evaluated. Dietary effect of *P. florida* on growth of normal rats was also assessed..

2. Materials and methods

2.1. *Mushroom*

Pure culture of *Pleurotus florida* (P-1 strain) was procured from National Centre of Mushroom Research and Training, Solan, India and was maintained on Potato-Dextrose-Agar slants. For mushroom seeding, wheat grain spawn was prepared following standard method. Substrate used for cultivation of *P. florida* was combination (1:1) of paddy straw and water hyacinth. Cultivation was done in polypropylene bag method (Bandopadhyay and Chatterjee, 2009). Fruit bodies were harvested from day 22-25 of spawning and upto 3rd flushes. The fresh fruit bodies were weighed and then dried in hot air oven at 40 ºC upto 85-90% moisture loss. The dried mushroom were ground and sieved to use as supplement in diet of rats.

2.2. *Determination of starch and cholesterol*

Starch content in dry mushroom (*P. florida*) powder was estimated using anthrone reagent (Sadasivam and Manickam, 1996). Cholesterol was qualitatively determined following Salkowski's Reaction for confirmative test of cholesterol.

2.3. *Experimental animals*

Albino wistar rats of either sex weighing between 100 to 115 g and 170 to 190 g 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 starch, oil, salt, milk powder, Bengal gram (*Ciser arietinum*) powder and / or *P. florida* powder (5% w/w) and water ad libitum.

2.4. *Antidiabetic activity*

Diabetes was induced in 10 rats weighing between 170-190 g by the administration of single intraperitonial dose (100 mg/kg i.p) of alloxan monohydrate after base line blood glucose estimation. Two 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 hypoglycemic effect of *P. florida*. A total of 20 rats (10 diabetic rats and 10 normal non-diabetic rats) weighing 170-190 g was divided into following four groups of 5 rats each: Normal control; rats were given normal diet i.e., food pellets without mushroom. Normal mushroom; rats were given food pellets supplemented with (5% w/w) dry mushroom 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.

2.5. *Body weight activity*

To study the effect of dietary mushroom on body weight, rats weighing 100-115 g were randomly assigned to 2 groups of 5 rats each.

Normal control; rats fed with normal diet food pellets containing Bengal gram (*Ciser arietinum*) as the major source of vegetable protein (i.e., without mushroom powder). Normal mushroom; rats were fed with food pellets containing mushroom powder (5% w/w) as the major source of vegetable protein (i.e., without Bengal gram). All the rats were given access to pellet diet and water ad libitum.

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2.6. *Biochemical analysis*

All blood samples were collected by retro orbital plexus technique using heparinised capillary glass tubes at 7 days interval for one month i.e., on 7th, 14th, 21st and 28th day after stabilization of diabetic condition. 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, triglycerides (TG) and uric acid (UA) levels by CHOD/PAP method, PEG/CHOD-PAP method, GPO/PAP method and Uricase/PAP method (Roeschlau et al., 1974) 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. During the study period of 28 days, the rats were weighed at 7 days interval and their body weights were recorded to calculate mean increase in body weight.

2.7. *Statistical analysis*

Data were expressed as mean ± standard deviation (SD). Significant differences among the means were determined through one way ANOVA using Statistical Package for the Social Sciences (SPSS) 17.0 statistics software. For the statistical tests p value of less than 0.05 was taken as significant.

3. Results

3.1. *Antidiabetic activity*

Estimation of starch revealed low starch (0.28% on dry weight basis) content of *P. florida* which is preferable for diabetic persons. Moreover, qualitative estimation of cholesterol gave negative test in Salkowsky's reaction indicating presence of negligible amount of cholesterol in *P. florida*. Analysis of blood glucose levels revealed that administration of mushroom supplemented (5% w/w dry powder) diet to alloxan-induced diabetic rats significantly ($p < 0.05$) reduced blood glucose levels in 'diabetic mushroom' group of rats on 7th, 14th and 21st days of alloxan treatment as compared to 'diabetic control' group (Fig. 1).

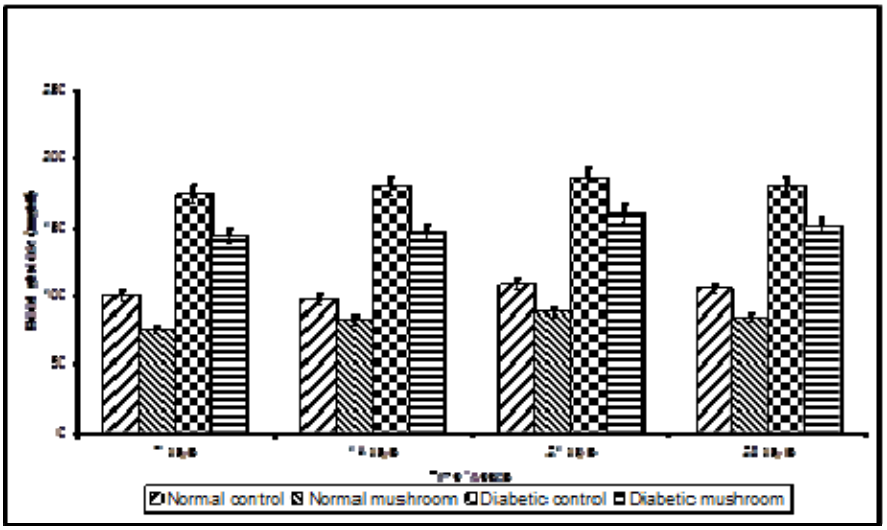

Figure 1. Effect of *P. florida* (5% w/w) supplemented diet on plasma blood glucose levels of normal and alloxan induced diabetic rats. Each value is the mean± standard deviation of five replicates. Bar represents standard deviation. Mushroom supplemented (5% w/w dry powder) diet to alloxan-induced diabetic rats significantly ($p < 0.05$) reduced blood glucose levels in 'diabetic mushroom' group of rats on 7th, 14th and 21st days of alloxan treatment as compared to 'diabetic control' group

Among the normal groups of rats, the group fed with *P. florida* supplemented diet (Normal mushroom) exhibited reduced level of blood glucose compared to its counterpart fed with normal diet (Normal control). These results indicate hypoglycemic effect of *P. florida* in alloxan-induced diabetic rats. Significant ($p < 0.05$) decline in total cholesterol (Fig. 2) and LDL-cholesterol (Fig. 3) was observed in ''diabetic mushroom'' rats as compared to their elevated levels in ''diabetic control'' rats after 28 days study period.

Although no significant changes were observed in serum triglycerides, uric acid and HDL cholesterol levels between ''diabetic control'' and ''diabetic mushroom'' groups of rats (Fig. 4).

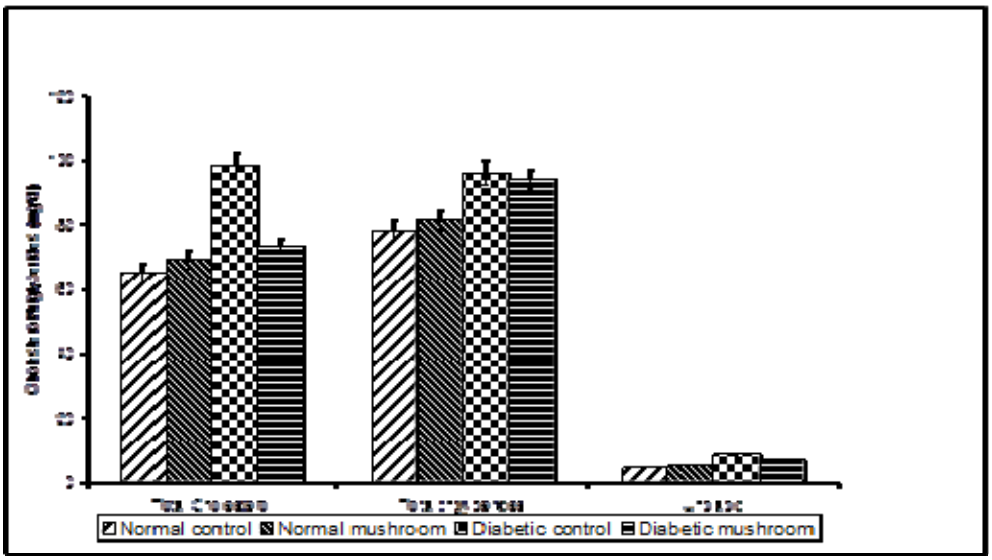

Figure 2. Effect of *P. florida* (5% w/w) supplemented diet on serum total cholesterol, total triglycerides and uric acid levels in normal and alloxan-induced diabetic rats after 28 days. Each value is the mean± standard deviation of five replicates. Bar represents standard deviation. Mushroom supplemented diet significantly ($p < 0.05$) declined the total cholesterol in diabetic mushroom groups as compared to diabetic control groups. No significant changes were observed in serum total triglycerides and uric acid levels between diabetic mushroom and diabetic control groups of rats

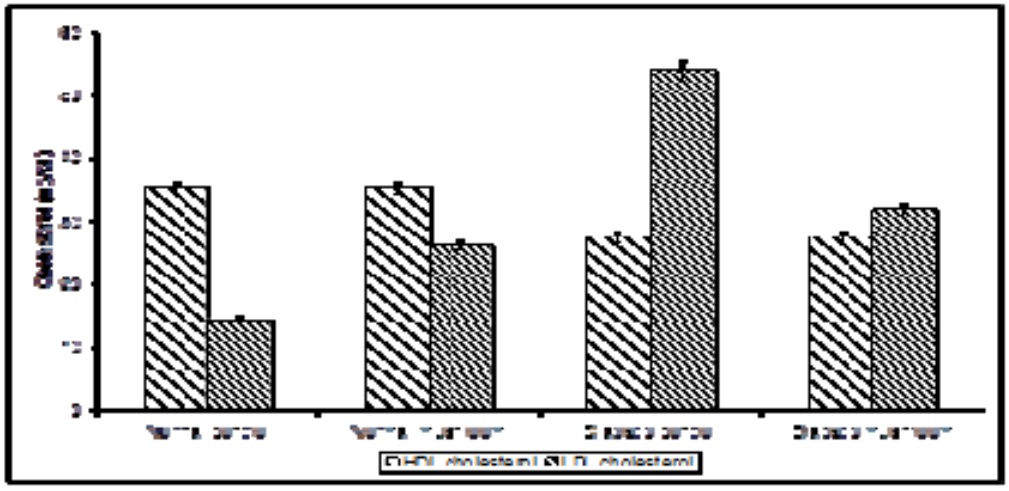

Figure 3. Effect of *P. florida* (5% w/w) supplemented diet on HDL and LDL cholesterol levels in normal and alloxan-induced diabetic rats after 28 days. Each value is the mean± standard deviation of five replicates. Bar represents standard deviation. Diabetic control and diabetic mushroom groups are significantly different at $p < 0.05$ for LDL cholesterol. HDL cholesterol did not change significantly

From the present observation, it is evident that regular consumption of dried *P. florida* for 1-3 weeks by keeping its approximate dietary level at 5% (w/w) has beneficial effect on alloxan-induced diabetic rats in regard to decreased blood glucose level, serum total cholesterol, LDL cholesterol levels. Significant lowering of LDL cholesterol by dietary *P. florida* is a desirable biochemical state for prevention of complications of diabetes (Lue and Fruchart, 1991). Many mushroom varieties have been reported to possess hypoglycemic activities in animals (Gray and Flatt, 1998) as well as in diabetic patients (Konno et al., 2001). Several species of mushroom have been shown to have an effect on lipids in general and cholesterol in particular (Francia et al., 1999). Among *Pleurotus* spp., *P. pulmonarius* had been reported (Badole and Bodhankar, 2007) to show hypoglycemic activity in alloxan-induced diabetic mice and *P. ostreatus* had been reported to reduce total cholesterol level (Bobek et al., 1998) *Pleurotus* mushrooms had been recommended as a natural cholesterol lowering substance within the human diet (Gunde-Cimerman, 1999). Under normal physiological conditions, a wide range of antioxidant defense protects the body from diverse effects of free radicals produced *in vivo* (Halliwell and Gutteridge, 1990) but free radicals are generated more in diabetes (Jain et al., 1998) which is considered to be an important pathogenic factor in diabetes mellitus (Gumieniczek et al., 2005).

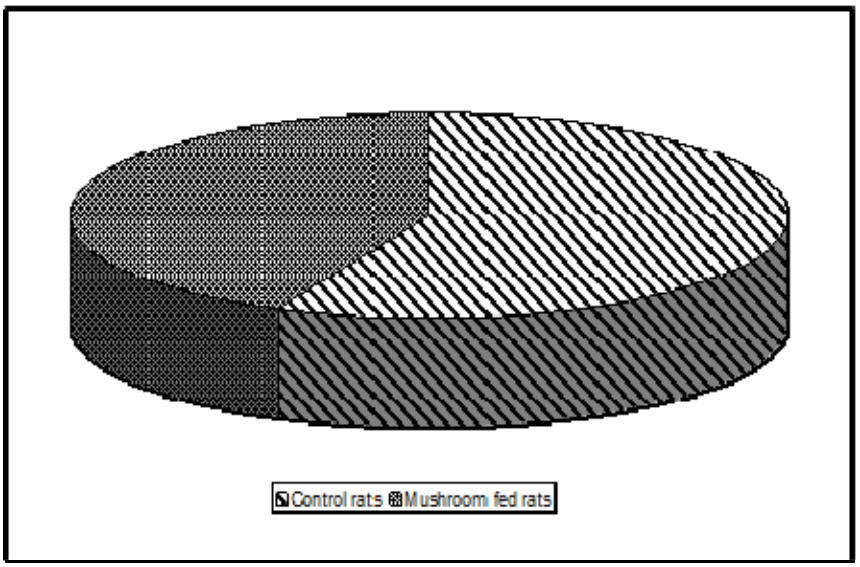

Figure 4. Average increase in body weight (g) in control rats and mushroom fed rats (insignificant as $p = 0.236$)

Nutritional factors including antioxidants have great influence in the management of diabetes mellitus and its complications (Alberti et al., 1997; Packer et al., 2000). Administration of appropriate antioxidants could prevent or retard diabetic complications to some extent (Packer et al., 2000)

3.2. *Body weight activity*

The study on increase in body weight shows almost similar increase in body weight of normal ‘‘control’’ rats and normal ‘‘mushroom fed’’ rats after 28 days study period revealing insignificant ($p = 0.236$) difference between the two groups. This indicates a comparable effect of *P. florida* with Bengal gram (*Ciser arietinum*) as the major vegetable protein source in rat diet.

4. Conclusions

On the basis of the present work, it may be stated that the consumption of *P. florida* supplemented diet renders hypoglycemic as well as hypocholesterolemic effect to alloxan induced diabetic rats. Hence dietary consumption of *P. florida* may serve as a cost effective additive measure in preventing diabetic complications against the present armamentarium of antidiabetic drugs. The present study also, emphasizes that there is no increase in body weight between the control and normal mushroom fed rats.

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