



Hypoglycemic Effect of Phansomba (*Phellinus Badius* Berk Ex Cooke) G. Cunn. on Alloxan-induced Diabetic Rats

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Abstract

Phansomba (*Phellinus badius* Berk ex Cooke) G. Cunn., family Hymenochetaceae, is a folk medicine used by local practitioners for various types of ailments. The hypoglycemic effect of aqueous extract of fruit body and mycelial biomass were investigated in alloxan-induced diabetic rats. The diabetic rats were orally administered with aqueous extracts of *Phellinus badius*, basidiocarp, and mycelial biomass at the doses of 800 mg/kg and 1000 mg/kg each. The blood glucose level changed at 6-hr interval at a dose of 1000 mg/kg in case of basidiocarp and mycelial biomass. The food intake of diabetic control was increased by 33.6% as compared to normal control, and the body weight gain was significantly decreased at a dose of 1000 mg/kg of both the samples. The blood glucose levels of the diabetic rats were significantly reduced after fourteen days. The plasma triglyceride and cholesterol level also significantly decreased as compared to diabetic rats (positive control). There was a considerable increase in the weight of body organs on administration of aqueous extract of Phansomba sample as compared to the normal rats (negative control). It also demonstrated a marked reduction in the level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The study reveals the potential of Phansomba samples as hypoglycemic agents with its aqueous extracts from fruit body and mycelial biomass.

Key words: Phansomba, hypoglycemic effect, alloxan-induced diabetic rats, *Phellinus*, biomass

1. Introduction

Diabetes mellitus (DM) is a very common endocrine disorder that affects more than 180 million people worldwide (6% of the population) and in 2030, it may double. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, insulin cannot be used orally whereas continuous use of the synthetic antidiabetic drugs causes side effects and toxicity^{1,2}; therefore, there is increasing demand of natural products with antidiabetic activity.

With this purview worldwide, research is being carried out to explore natural resource-based hypoglycemic agent³.

Mushrooms are also exemplary sources of natural medicines with antidiabetic activity⁴. Extracts of edible mushrooms have been proved to be the ideal food for diabetic prevention of hyperglycemia owing to their high content of fibre and protein and low fat content⁵. Various mushrooms have been reported to possess hypoglycemic activity⁵⁻⁸.

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Phansomba, a folk medicine from the Western Ghats of Maharashtra comprises different species from the genus *Phellinus* and are used to cure various ailments^{9,10}. Phansomba: Phanas – amba (Phanas = Jackfruit tree alambe = fungus) i.e. fungus growing on jackfruit (*Artocarpus heterophyllus* Lam.). *Phellinus badius* is one of the important species under the folk medicine Phansomba and has been noted to cure teeth, tongue, and throat-related ailments, to stop excessive salivation in case of children, against diarrhea¹¹.

Various mushrooms have been experimented for their efficacy against various ailments. Though there are few reports about the hypoglycemic activity of different *Phellinus* species^{4,12,13}. This study is carried out to assess the hypoglycemic potential of the folk medicine Phansomba from the Western Ghats of Maharashtra.

2. Materials and Methods

2.1 Organism and Growth

Phansomba samples, used as folk medicine, were collected from Western Ghats of Maharashtra, India, which were authenticated as *Phellinus badius* at Mycology Research Laboratory, Department of Botany, University of Pune. A specimen of this sample has been deposited at the Mycological Herbarium, Forest Research Institute, Dehradun, India. The fungus was also isolated in pure culture, and a copy was submitted to the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh and Forest Research Institute, Dehradun. In the present study, hot water extracts from fruit body and mycelia cultivated on finger millet grains (by solid state fermentation) of *Phellinus badius* were used.

2.1.1 Static solid state fermentation

The culture was isolated and maintained on PDA medium (extract from 200 g of peeled and diced potatoes, 20 g of dextrose, and 20 g of agar), aseptically transferred to precooked finger millet grains, and incubated at 27 ± 2 °C for fifteen days. After fifteen days, the complete biomass was harvested, dried in hot air oven at $47^\circ\text{C} \pm 5$ °C till the moisture content was below 2–3%, and powdered.

3. Extraction

Around 200 g of powdered fruit body and powdered biomass were suspended in 500 ml of distilled water at 90 °C for five hours and filtered respectively/separately. The process was repeated twice. All the respective filtrates were combined, filtered through Whatman no. 1 filter paper, and evaporated to dryness under reduced pressure on rotary evaporator. This resulted into a brown powder (3% for fruit body and 4.5% for biomass) which was stored at -20 °C until used.

4. Animals and Breeding Condition

Wistar male rats were obtained from the National Toxicology Center, Pune, weighing 200–250 g and housed in stainless steel cages in a room with controlled temperature (22 ± 2 °C) and humidity ($55 \pm 5\%$) under a 12:12-hour light–dark cycle. The rats were fed with a commercial pellet diet (Amrut Rats and Mice Pellet, India) and were allowed water ad libitum throughout the experimental period.

5. Induction of Experimental Diabetes

The rats were subjected to a 12-hour fast. Diabetes was induced by intraperitoneal injection of alloxan monohydrate (Sigma Chemicals, India) dissolved in distilled water at a dose of 150 mg/kg body weight. Three days after induction of diabetogenic agent, blood glucose was determined with the help of glucometer (Contour TS). The rats were considered to be diabetic when blood glucose concentration was higher than 300 mg/dl. Thereafter, animals were used as insulin-dependent diabetes mellitus (IDDM) model.

6. Experimental Design

Animals were divided into six groups with six animals in each group: normal control (NC), alloxan treated (AT), FB 800 (alloxan treated + fruit body extract – 800 mg/kg), FB 1000 (alloxan treated + fruit body extract – 1000 mg/kg), MB 800 (alloxan treated + mycelial biomass extract

– 800 mg/kg), and MB 1000 (alloxan treated + mycelial biomass extract – 1000 mg/kg). Diabetic rats were treated with sample by using an oral zoned dose daily for fourteen days.

7. Analytical Measurements

The body weight gain and feed intake were periodically measured. Blood samples were collected in heparinised tubes, and the plasma was separated by centrifugation at 5000 ×g for 10 min. The plasma cholesterol, triglycerides levels were measured using an enzymatic colorimetric assay kit (Merck, India). The activities of alanine aminotransferase and aspartate aminotransferase were determined by using enzyme kits (Merck, India) based on Reitman-Frankel method¹⁴.

8. Statistical Analysis

The results were analysed for statistical significance by one-way analysis of variance (ANOVA) test using the statistical package of the social science (SPSS) programme. All data were expressed as mean±standard error (SE). Group means were compared by a one-way analysis of variance and Duncan's multiple range tests. Statistical differences were considered significant at $p < 0.05$.

9. Results

The effect of aqueous extract of *Phellinus badius* on food intake and body weight gain on alloxan-treated rats was investigated (Table 1). A significant difference in body weight gain was observed between diabetic group and

Table 1: Effect of *P. badius* fruit body and mycelial biomass aqueous extract on the food intake and body weight gain in alloxan induced diabetic rats for 14-days (n=6) $P < 0.05$.

Animals group	Food Intake (gms/day)	Body weight (gms/day)
NC	18.15 ± 0.83	4.93 ± 0.22
AT	24.25 ± 0.76	2.88 ± 0.02
FB 800	18.71 ± 0.29	3 ± 0.1
FB 1000	19.54 ± 0.42	2.94 ± 0.04
MB 800	19.23 ± 0.82	3.3 ± 0.26
MB 1000	20.1 ± 0.2	3.33 ± 0.27

Values are means± S.E, where n = 6, $p < 0.05$

non-diabetic animals, wherein the body weight gain was increased by 40.3% and 32.4% in FB 1000 and MB 1000 respectively as compared to normal control. Food intake of the diabetic control rats was increased by 41.1% as compared to normal control.

Table 2 shows the effect of *Phellinus badius* aqueous extract on the blood glucose level in alloxan-induced diabetic rats over a period of fourteen days. In the alloxan-treated group, the blood glucose level continuously increased during the experimental period reaching a final level of 594 mg/dl. In contrast, the administration of *Phellinus badius* FB 1000 and MB 1000 lowered the plasma glucose level by 47.97% and 51.68% respectively on day fourteen when compared to that of the diabetic control group.

Table 2 shows the blood glucose effects of two samples and their different doses (800 mg/kg and 1000 mg/kg) of the aqueous extract of *Phellinus badius* with standard (glibenclamide, Sigma) and control groups in

Table 2: Effect of *P. badius* fruitbody and mycelial biomass on the blood glucose level in alloxan-induced diabetic rats for 14 days (n=6) $P < 0.05$.

Animals group	Blood glucose level (mg/dl)					
	0 hours	2 hours	4 hours	6 hours	24 hours	14 th day
NC	102 ± 40	98.33 ± 2.51	95.33 ± 3.05	95.33 ± 2.30	97.33 ± 3.51	84.66 ± 4.04
AT	598 (± 3.46)	593.33 ± 7.02	596.66 ± 5.77	593.33 ± 8.32	591.33 ± 9.86	594.33 ± 4.72
FB 800	595.33 ± 5.03	575.66 ± 4.50	314.66 ± 3.05	324.33 ± 4.50	392.33 ± 2.51	354.00 ± 2.00
FB 1000	591.33 ± 3.05	490.66 ± 9.29	283.66 ± 4.72	193.33 ± 5.50	288.33 ± 7.09	309 ± 1.00
MB 800	517.33 ± 3.05	501.66 ± 4.72	359.33 ± 3.05	350.66 ± 9.81	380.33 ± 12.66	317.33 ± 3.05
MB 1000	586.66 ± 3.05	510.33 ± 10.26	305.66 ± 3.21	213.33 ± 14.46	350.66 ± 7.57	287.66 ± 1.52

Values are means± S.E, where n = 6, $p < 0.05$

alloxan diabetic wistar rats. The FB 1000 and MB 1000 showed a significant decrease in the blood glucose levels after six hours as compared to the diabetic control.

The plasma triglyceride and total cholesterol level were markedly reduced by oral administration of *Phellinus badius* aqueous extract of fruit body and mycelial biomass at 1000 mg/kg. Triglyceride level was reduced by 52.19% and 58.17% (MB 1000) and cholesterol level was reduced by 60.13% and 68.15% (FB 1000) (Table 3).

The weights of the body organs for the three experimental groups are listed in Table 4. There was no significant difference in the kidney weight and pancreas weight, whereas those of the liver, spleen, and heart were considerably increased in the diabetic groups as compared to the normal control group (Table 5).

10. Discussion

In the present study, we demonstrated that the fruiting bodies and mycelial biomass (SSF) of *Phellinus badius* have significant hypoglycemic activity in the alloxan-induced diabetic rats. Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus. It induces diabetes by partial destruction of the β -cells of islets of langerhans¹⁵. This results in hyperglycemia leading to type-1 diabetes mellitus. In the past, many mushroom varieties have been reported to possess hypoglycemic activities in animals^{16,17} as well as in diabetic patients¹⁸.

Generally, the body weights are reduced in diabetic rats and recovered when subjected to hypoglycemic treatment¹⁹. Yang also demonstrated that the body weight and feed intake were improved by administration

of edible mushroom *L. edodes* mycelia²⁰. In this study, *Phellinus badius* FB 800 and MB 1000 caused significantly increased in body weight. The cause of intake suppression may be due to the presence of a specific compound in extracts that has not yet been elucidated.

Table 3: Effect of *P. badius* fruit body and mycelial biomass aqueous extract on the plasma triglycerides and cholesterol level in alloxan-induced diabetic rats for 14-days (n=6) P< 0.05.

Animals group	Triglycerides (mg/dl)	Cholesterol (mg/dl)
NC	53.90 \pm 5.78	87.03 \pm 6.57
AT	117.85 \pm 16.62	157.96 \pm 24.70
FB 800	65.83 \pm 4.63	89.5 \pm 8.92
FB 1000	70.87 \pm 13.58	107 \pm 12.20
MB 800	61.48 \pm 18.46	98.06 \pm 10.35
MB 1000	61.51 \pm 19.35	91.33 \pm 5.22

Values are means \pm S.E, where n = 6, p < 0.05

Table 4: Effect of *P. badius* fruitbody and mycelial biomass aqueous extract on the plasma aspartate aminotransferase and alanine aminotransferase level in alloxan-induced diabetic rats for 14-days.

Animals group	AST (U/L)	ALT (U/L)
NC	66.3 \pm 2.47	46.83 \pm 1.51
AT	120.73 \pm 13.84	73.86 \pm 1.61
FB 800	80.06 \pm 3.88	43.26 \pm 1.70
FB 1000	79.86 \pm 2.01	48.9 \pm 0.60
MB 800	86.76 \pm 2.70	42.5 \pm 0.52
MB 1000	85.83 \pm 1.48	46.8 \pm 2.46

Values are means \pm S.E, where n = 6, p < 0.05

Table 5: Effect *P. badius* fruitbody and mycelial biomass aqueous extract on body organs weight/100 gm of rats.

Animals group	Body Organs weight per 100gms of rats				
	Liver (gms)	Spleen (gms)	Kidney (gms)	Pancreas (gms)	Heart (gms)
NC	4.479 \pm 0.60	0.378 \pm 0.05	0.773 \pm 0.13	0.379 \pm 0.01	0.411 \pm 0.02
AT	4.752 \pm 0.53	0.468 \pm 0.07	1.014 \pm 0.28	0.412 \pm 0.01	0.344 \pm 0.08
FB 800	4.211 \pm 0.10	0.462 \pm 0.005	0.812 \pm 0.07	0.401 \pm 0.01	0.453 \pm 0.05
FB 1000	4.791 \pm 0.006	0.456 \pm 0.09	0.797 \pm 0.11	0.301 \pm 0.05	0.390 \pm 0.01
MB 800	4.97 \pm 0.04	0.461 \pm 0.002	0.830 \pm 0.50	0.378 \pm 0.08	0.396 \pm 0.02
MB 1000	4.467 \pm 0.43	0.478 \pm 0.01	0.757 \pm 0.12	0.399 \pm 0.03	0.382 \pm 0.04

Values are means \pm S.E, where n = 6, p < 0.05

The increased viscosity of the gastrointestinal contents probably reduced the rate of emptying of the stomach and suppressed and delayed intestinal digestion²¹. This is associated with loss of appetite and thus reduced food intake.

The doses of FB 1000 and MB 1000 of the aqueous extract did significantly ($p < 0.05$) decrease the blood glucose levels of alloxan-induced diabetic rats at six hours.

The blood glucose lowering effect of extracellular polysaccharide can be explained by the fact that it might have increased utilisation in diabetic animals by promoting insulin secretion¹². Gray and flat concluded that the antidiabetic study using a fruiting body extract of *Agaricus campestris* and regulating plasma glucose level¹⁶. In the present study, FB 1000 and MB 1000 showed significant decrease in blood glucose level.

The plasma triglycerides and cholesterol levels were strongly related to the degree of diabetic control in IDDM rats²². The increased total cholesterol and triglyceride levels were observed in diabetic rats may be the result of impaired liver function in diabetes which acts either directly by enhancing the plasma glucose level^{23,24}. Increased immobilisation of free fatty acid and decreased clearance due to reduced LPL activity resulted in elevated levels of triglyceride and cholesterol in the blood plasma²⁵.

The AST and ALT levels are generally increased by metabolic changes in liver such as administration of toxin, cirrhosis of the liver, hepatitis, and liver cancer^{26,27}. These levels can be used as markers in identifying the extent of liver damage.

The body organs weight of the animals like heart, liver, spleen, kidney, and pancreas for their indirect diabetes diagnosis⁴. The weights of the liver and kidney were increased in diabetic rats reported by Kim²⁸.

The exact mechanism of action by which *Phellinus badius* exerted its hypoglycemic effect is not properly understood; we need further study with purification and combination for more efficient hypoglycemic effect.

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