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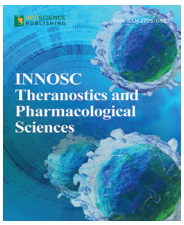

RESEARCH ARTICLE

Orally Administered Aqueous Extract of *Pleurotus ostreatus* Ameliorates Hyperglycemia in Streptozotocin-Induced Diabetic Rats

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Abstract:

Pleurotus ostreatus (Jacq.) P. Kumm. (Family: Pleurotaceae), commonly known as oyster mushroom, has been widely used to treat various ailments from simple headache to serious ones like diabetes. The mushroom has been shown to exert anti-inflammatory and antioxidant activities. In the present study, aqueous extract of *P. ostreatus* (AEPO) was examined for its antidiabetic activity in streptozotocin (STZ)-induced diabetic rats. STZ was administered to rats at 50 mg/kg body weight (i.p.), AEPO was orally administered at 100 and 200 mg/kg body weight, and metformin (500 mg/kg) was administered as positive control. The hypoglycemic effects of the AEPO were analyzed by assessing the blood glucose levels (oral glucose tolerance test, acute and postprandial antihyperglycemic activity), lipid parameters, and other hematological studies in comparison to standard drug (metformin). Results showed that AEPO caused a 26% reduction in blood glucose during fasting while 45% reduction in blood glucose during postprandial antihyperglycemic test in STZ-diabetic rats. It also helped in the normalizations of various complications associated with diabetes mellitus in rats. Observations from the current experiments indicate that *P. ostreatus* help in reduction of blood glucose level, thus confirming its antidiabetic activity in STZ-induced diabetes in rats. This study further advocates that supplementation of edible mushroom *P. ostreatus* could be a preventive approach in diabetes as well as in obesity management.

Keywords: Antidiabetic, *Pleurotus ostreatus*, Streptozotocin, Metformin

1. Introduction

Diabetes mellitus is known since ages as it has been mentioned in Ayurveda by Sushruta [1]. Diabetes mellitus is a chronic metabolic disease characterized by elevated plasma glucose concentration in fasting or postprandial state, or insulin resistance [2]. The World Health Organization and International Diabetes Federation reported an estimate of 10.5% (536.6 million people) global prevalence of diabetes, which is projected to rise to 643 million by 2030 and 783 million by 2045. An estimated 6.7 million deaths due to diabetes were reported in 2021. The incidences of diabetes are higher (3 in 4 adults) in low- and middle-income countries. Diabetes is characterized as a state of imbalance toward the factors that generate reactive oxygen radicals such as superoxide or hydroxyl radicals, and the level of antioxidant enzymes such as superoxide dismutases, catalase, and glutathione

peroxidases. The factors that generate reactive oxygen species (ROS) are both from the products of normal cellular physiology as well as from various exogenous sources. The excessive production of ROS and reactive nitrogen species (RNS) in or around the pancreatic beta cells is the major physiological reason that causes beta cells death and deficiency in insulin [3]. The development of insulin-dependent diabetes mellitus type 1 can be triggered by several risk factors, such as genetic, developmental, environmental and dietary factors. However, the ROS/RNS play central role in pancreatic β-cell death and disease progression, and both of the radical species play critical roles in cellular autoimmune-inflammatory responses [4]. Therefore, specific treatments with antioxidants and inflammatory drugs may inhibit the hyperglycemic response.

At present, treatments of diabetes mellitus include exercise, diet therapy, insulin therapy and oral antidiabetic agents such as sulfonylureas, biquanides, thiazolidinediones, and alpha glucosidase inhibitor. Despite of their effectiveness in reducing hyperglycemia, the use of these drugs is associated with non-desirable side effects [5]. Hence, herbal medicine can be used as an alternative therapy for treatment of diabetes. These herbal medicines help to reduce the complications associated with drugs and thus help in maintaining normal glucose level without triggering any complications [6]. Mushrooms are important food sources and represent a vast, untapped source of natural pharmaceutical products [7]. Mushrooms have low glycemic index which makes them low-calorie food, and contain high nutrients such as protein; therefore, they can be recommended for diabetics [7]. *Pleurotus* spp. or oyster mushrooms are rich in medicinal values such as effectiveness in reducing the total plasma cholesterol and triglyceride level and thus may reduce the chance of atherosclerosis and other cardiovascular and artery-related disorders [8]. These medicinal properties might be due to the presence of some important substance in dietary mushrooms [8]. *Pleurotus ostreatus* possessed potent antioxidant effects in the supplementation of corncobs with different herbs [9]. The antioxidant activity of *P. ostreatus* was found to be mechanistically associated with tyrosinase inhibitory effects tested in several antioxidant assays such as β-carotene-linoleic acid reduction, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, and ferrous chelating ability. It was further established that *P. ostreatus* has antioxidant properties by virtue of its phenolic composition and being enriched with selenium and zinc which serve as antioxidants [10]. Thus, this study aimed to utilize the antioxidant potential of *P. ostreatus* in an experimental model of diabetes. Streptozotocin (STZ), chemically an N-nitroso derivative of glucosamine, is a vast-spectrum antibiotic derived from *Streptomyces chromogens*. It acts as a pancreatic beta cell toxoid that stimulates and accelerates irreversible necrosis of pancreatic beta cells, making it an extensively used chemical inducer of diabetes in experimental animal models [11]. Thus, the present study determined the antidiabetic activity of an aqueous extract of *P. ostreatus* (AEPO) in an experimental model of diabetes induced by STZ in rats.

2. Materials and methods

2.1. Collection and preparation of AEPO

P. ostreatus was collected from Gorakhpur district, Uttar Pradesh, India. The sample was identified by relevant literature [12] and the sample was submitted with voucher number DDUNPL250 to the institutional repository. For extraction purpose, the fruiting bodies were dried under shade and ground to fine powder with the help of a grinder and stored in opaque screw top jar at room temperature. Aqueous extract of the macrofungal samples was prepared by the method of infusion. One gram of macrofungal sample as powder was mixed in 40 mL of boiling distilled water and allowed to infuse for 15 min. Then, it was filtered by Whatman no. 1 filter paper, and the volume was readjusted to 40 mL [13]. The final preparation named as AEPO was stored at 4°C until utilization.

2.2. Evaluation of antidiabetic potential in experimental model of diabetes in rats

In vivo experiment was designed according to method adopted by Kim *et al.* [14] with few modifications. The protocols for these experiments were approved by the Animal Ethical Committee of the Institute (IAEC/DDU/2021-22). Healthy adult male albino rats weighing approximately 60–160 g

were obtained and were kept at room temperature 25±5°C in large cage with polypropylene-coated wire gauze on all sides. Rats were exposed to a photoperiod of 12 h/day. The cages were cleaned regularly to avoid rat smell and to maintain proper hygienic conditions. The rats were acclimatized to laboratory conditions for 10 days and fed on rat pellets and water *ad libitum*. Each rat was weighed and assigned a number for convenience before the onset of experiment. Experimental animals were divided into five groups with three rats in each (n=3); the categorization plan is as follows:

(i) Group 1: Vehicle control (received 0.1 M citrate buffer, i.p.)
(ii) Group 2: Diabetic rat (received STZ 50 mg/kg b.w. in 0.1 M citrate buffer, i.p., single dose)
(iii) Group 3: Diabetic rat received metformin (500 mg/kg b.w. aqueous solution, p.o., per day, for 4 weeks)
(iv) Group 4: Diabetic rat received AEPO (100 mg/kg b.w., p.o., per day, for 4 weeks)
(v) Group 5: Diabetic rat received AEPO (200 mg/kg b.w., p.o., per day, for 4 weeks)

Studies have utilized a dose range of 100–500 mg/kg for investigating the biomedicinal effects of *P. ostreatus* against several disease models in rats [15-17]. Based on above observations and our previous studies with 100–300 mg/kg mushroom extracts [18-22] with no notable toxicity, we used 100 and 200 mg/kg AEPO in this study. STZ was administered as a single dose while metformin and AEPO per day for 4 weeks.

2.3. Induction of diabetes in rats

Diabetes mellitus in rats was induced by single intraperitoneal (i.p.) administration of freshly prepared solution of STZ (HiMedia, CMS 1758) dissolved in 0.1 M citrate buffer, pH = 4.5 (vehicle control). For diabetes induction, each test animal was injected with 50 mg/kg volume of freshly prepared STZ. At the same time, normal rats received the same volume of vehicle control through the same route. The animals were returned to their cages after injection and allowed for free access to food and water. After 3–4 days, the fasting blood glucose level was measured from the tail vein using glucometer (mylife Pura™ blood glucose monitoring system). Animals with blood glucose level higher than 180 mg/dL were considered diabetic and used for the experiment.

2.4. Assessment of body weight

Rats were weighed initially and after the experiment. The relative change in body weight (b.w.) of rat was determined in percentage using following equation:

$$\text{Percent relative change in b.w.} = \frac{\text{Pre-treatment b.w.} - \text{Post-treatment b.w.}}{\text{Pre-treatment b.w.}} \times 100$$

2.5. Oral glucose tolerance test (OGTT)

The experimental animals were fasted overnight (about 12 h) before commencing the experiment. Rats of all the groups were given D-glucose (500 mg/mL) solution after half an hour after metformin and AEPO administration. Blood samples were withdrawn from tail vein before and after the metformin and AEPO administration at 30, 60, 90, and 120 min.

2.6. Determination of serum glucose

Blood glucose level was measured in two steps; first, fasting blood glucose level was measured, and second, postprandial antihyperglycemic was tested.

2.6.1. Fasting blood glucose level

Experimental animals were deprived of food for about 16 h with free access to drinking water before the commencement of experiment. Experimental animals were tested for their blood glucose level at 0, 24, 48, and 72 h of treatment of AEPO. Blood samples were collected from the tail vein for glucose analysis.

2.6.2. Postprandial antihyperglycemic test

This study was done in two steps, that is, acute and chronic. For acute study, rats were fasted for 1 h before test. After the fasting period, rats were given either metformin or AEPO orally using intragastric gavage. Blood samples were collected from the tail vein just before (0 h) and after 2, 4, 6, and 24 h administration of metformin or AEPO. In chronic study, glucose level in blood was measured at 0 day and 5, 10, 15, and 30 days after treatment.

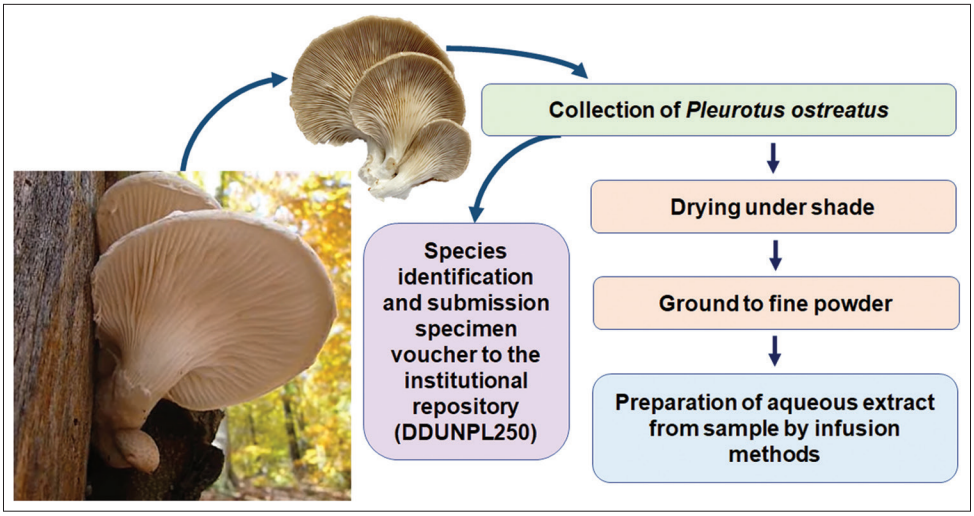

Figure 1. Schematic diagram for collection and preparation of the aqueous extract of *Pleurotus ostreatus*.

Metformin and AEPO were given to experimental rats every day.

2.7. Hematological assessment

Hematological analysis was performed from whole blood sample using the Hematological Analyzer Sysmex (KX 21, Japan). Erythrocyte count (red blood cell [RBC]), hemoglobin concentration (Hb), leukocyte count (white blood cell [WBC]), neutrophil percentage, and lymphocyte percentage in WBC were measured in control and treated rats. Biochemical Analyzer (Transasia ERA CHEM-5 PLUS, India) was used for analyzing blood biochemical parameters.

2.8. Statistical analysis

Data are presented as mean ± standard deviation (SD) or standard error of mean (SEM) from minimum three experimental repeats. The data were statistically analyzed by Students *t*-test and analysis of variance to compare parameters among groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of *P. ostreatus* on body weight of test animals

Body weight and behavioral change of the experimental animals were monitored as physiological markers of the health. Data showed that oral administration of AEPO caused no changes in gross behavior, and none of the animals died during the course of experiment. There were no harmful effects observed in rats due to treatment with AEPO, showing that it caused no changes in gross behavior of animals. Data presented in **Figure 2** demonstrated that control rats were more stable in their body weight. STZ-treated diabetic rats showed significant reduction in weight (39%) as compared to vehicle control ($P < 0.05$). Metformin-treated diabetic rats showed a greater effect on body weight with 17.3% reduction in body weight ($P < 0.05$). AEPO treatment to diabetic rats caused a dose-dependent increase in body weight by 16.3% and 20.2% increase in body weight at 100 and 200 mg/kg dosage, respectively. Diabetic rats treated with AEPO (200 mg/kg) showed a notable effect that helped to increase the body weight of experimental diabetic rat with a similar response to metformin (**Figure 1**). These results showed a preliminary protective effect of AEPO in diabetic rats as physiological marker of health.

3.2. Effect of *P. ostreatus* on fasting blood glucose level in STZ-induced diabetic rats

Reduction in blood glucose level is considered the biochemical marker of antidiabetic effect of a candidate drug agent. As it is evident from **Table 1**, fasting blood glucose level was elevated to a notable level (190 ± 1.38 mg/dL) in diabetic rats in comparison to vehicle control group (99 ± 2.32 mg/dL) at the time of starting the experiment (before treatment stage). STZ-induced diabetic rats showed a narrow range of blood glucose level (182 – 193 mg/dL) at the time of starting the experiment. Metformin treatment to

STZ-diabetic rats showed a reducing trend in the level of blood glucose by 170, 159, and 130 mg/dL at 24, 48, and 72 h, respectively. These reductions in the blood glucose levels accounted for 12%, 17%, and 32% at 24, 48, and 72 h, respectively. The data were significantly lowered ($P < 0.05$) as compared to vehicle control at different time points. AEPO at each concentration tested showed substantial results in lowering the blood glucose levels. Oral administration of AEPO to STZ-induced diabetic rats showed a dose- and time-dependent decrease in the blood glucose levels. AEPO (100 mg/kg) treatment to STZ-diabetic rats showed that blood glucose levels were reduced to 177, 165, and 145 mg/dL at 24, 48, and 72 h, respectively, which were about 3 – 20% reductions, whereas AEPO at 200 mg/kg dose led to a reduction oft blood glucose levels to 175, 161, and 139 mg/dL at 24, 48, and 72 h, respectively, with about 7 – 26% reductions. AEPO at 200 mg/kg dose showed a blood glucose-lowering effect in STZ-diabetic rats similar to that of metformin (500 mg/kg). Thus, these results showed potent antihyperglycemic effects of *P. ostreatus* in diabetic rats.

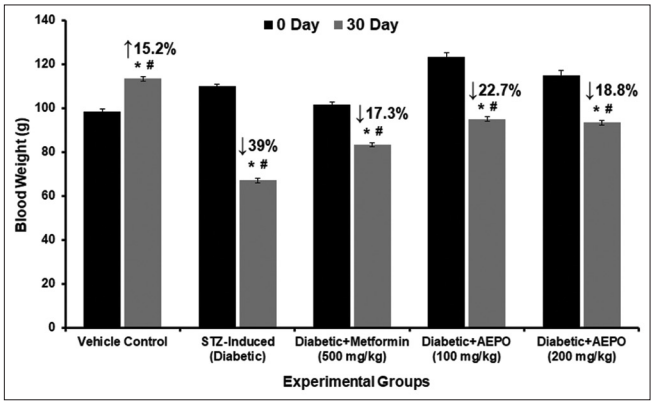

Figure 2. Effect of aqueous extract of *Pleurotus ostreatus* and metformin on body weight of diabetic rats. Values are mean ± standard error of mean for groups of 3 observations with their standard errors. ↓ decrease; ↑ increase in body weight. **P*<0.05 vs vehicle control 30 days; #*P*<0.05 vs. within group 30 min.

3.3. Postprandial antihyperglycemic effects of *P. ostreatus* on STZ-induced diabetic rats: acute and chronic assessment

Postprandial blood sugar levels are important indicator for diabetes and hyperglycemia, which is tested along with fasting blood sugar. Postprandial blood sugar measurements can give a clue about the metabolic health in a metabolically altered state of diabetes [23]. For postprandial antihyperglycemic test, two different criteria were used; first as acute study performed at different short intervals of 0, 2, 4, 6, and 24 h, and second as chronic study performed at 0, 5, 10, 15 and 30 days. In the acute study (**Table 2**), rats in vehicle control group showed a normal course of decline in postprandial blood glucose levels from 0 h to 24 h with total about 25 mg/dL reduction in 24 h, whereas STZ-induced diabetic rats showed an increasing trend in postprandial blood glucose levels from 0 h (270 ± 1.65 mg/dL) to 24 h (310 ± 2.92 mg/dL) with total about 40 mg/dL (14.8%) increment in 24 h. Treatment of STZ-induced diabetic rats with metformin (500 mg/kg) caused a time-dependent

Table 1. Effect of AEPO and metformin on fasting glucose level in STZ- induced diabetic rats

Experimental groups	Blood glucose level (mg/dL)			
	Before treatment	24 h after treatment	48 h after treatment	72 h after treatment
Vehicle control	99±2.32	95±1.95	98±2.77	98±2.98
STZ-induced (diabetic)	190±1.38*	193±2.84*	195±2.37*	195±2.85*
Diabetic+metformin (500 mg/kg)	193±2.33*	170±2.37# ^11.92%	159±1.19# ^17.61%	130±3.65# ^32.64%
Diabetic+AEPO (100 mg/kg)	182±2.72*	177±1.39# ^2.74%	165±0.99# ^9.34%	145±3.75# ^20.33%
Diabetic + AEPO (200 mg/kg)	188±2.84*	175±1.33# ^6.91%	161±1.37# ^14.36%	139±1.72# ^26.06%

Values are mean±SEM for groups of three observations with their standard errors. AEPO: Aqueous extract of *Pleurotus ostreatus*; STZ: Streptozotocin. ^Values in percent indicate reductions in the blood glucose levels relative to 0 h (before treatment) of the respective treatment groups. **P*<0.05 versus control; #*P*<0.05 versus STZ group

reduction in postprandial blood glucose levels from 0 h (275 ± 0.99 mg/dL) to 24 h (220 ± 1.08 mg/dL). Metformin showed a total 20% reduction in the postprandial blood glucose level as compared to STZ-induced diabetes. Oral administration of AEPO showed a dose- and time-dependent decrease in postprandial blood glucose level in STZ-diabetic rats. AEPO showed a reduction of 8.7 and 14.5% postprandial blood glucose level at 100 and 200 mg/kg dosage.

In the chronic study (**Table 3**), rats in vehicle control group showed an almost constant level of postprandial blood glucose from day 0 to day 30. STZ-induced diabetic rats showed a gradual increase in postprandial blood glucose levels from day 5 to day 15 (about 22%), and a total of 69 mg/dL (25%) increase in postprandial blood glucose at day 30. Metformin (500 mg/kg) treatment to STZ-induced diabetic rats caused a time-dependent reduction in postprandial blood glucose levels from day 0 (275 ± 2.87 mg/dL) to day 30 (135 ± 2.38 mg/dL) with total 140 mg/dL (about 51%) reduction in postprandial blood glucose at day 30 which denotes a potent therapeutic reduction in hyperglycemia. Furthermore, oral administration of AEPO to STZ-induced diabetic rats showed a dose- and time-dependent decrease in postprandial blood glucose levels from day 0 to day 30. AEPO (100 mg/kg) treated diabetic rats showed about 80 mg/dL (about 30%) reduction in postprandial blood

Table 2. Effect of AEPO and metformin on postprandial blood glucose levels in STZ-induced diabetic rats (acute study within 24 h of treatment)

Experimental groups	Blood glucose level (mg/dL)				
	0	2 h	4 h	6 h	24 h
Vehicle control	115±2.99	110±1.98	106±2.55	102±2.32	90±1.03
STZ-induced (Diabetic)	270±1.65*	275±3.20* ^1.85%	280±2.74* ^3.70%	295±1.33* ^9.25%	310±2.92* ^14.8%
Diabetic+metformin (500 mg/kg)	275±0.99*	262±3.65 ^4.73%	257±2.39# ^6.54%	237±1.91# ^13.81%	220±1.08# ^20.0%
Diabetic+AEPO (100 mg/kg)	285±3.32*	276±2.39 ^3.16%	270±0.99 ^5.26%	269±2.87#,$ ^5.61%	260±2.61#,$ ^8.77%
Diabetic + AEPO (200 mg/kg)	275±0.98*	255±3.20# ^7.27%	247±1.87# ^10.18%	241±3.33# ^12.36%	235±1.87# ^14.54%

Values are mean±SEM for groups of three observations with their standard errors at different time points within 24 h. AEPO: Aqueous extract of *Pleurotus ostreatus*; STZ: Streptozotocin. ^Values in percent indicate the change in the blood glucose levels relative to 0 h (before treatment) of the respective treatment groups. *P<0.05 versus control; #P<0.05 versus STZ group; $$P$<0.05 versus metformin group

Table 3. Effect of AEPO and metformin on postprandial blood glucose levels in STZ-induced diabetic rats (chronic study up to 30 days of treatment)

Experimental groups	Blood glucose level (mg/dL)				
	0 day	5 day	10 day	15 day	30 day
Vehicle control	90±2.33	93±3.20	95±0.99	95±2.75	96±1.53
STZ-induced (diabetic)	270±2.54*	328±2.39* ^21.48%	330±1.54* ^22.22%	333±1.05* ^23.33%	339±0.99* ^25.55%
Diabetic+metformin (500 mg/kg)	275±2.87*	208±1.98# ^24.36%	195±1.85# ^29.09%	175±1.99# ^36.66%	135±2.38# ^50.90%
Diabetic+AEPO (100 mg/kg)	270±2.23*	235±1.32#,$ ^12.96%	226±2.45#,$ ^16.30%	220±1.56#,$ ^18.51%	190±1.89#,$ ^29.62%
Diabetic + AEPO (200 mg/kg)	275±0.98*	228±1.28#,$ ^17.09%	215±1.57#,$ ^29.81%	190±2.21# ^30.90%	150±2.63# ^45.45%

Values are mean±SEM for groups of three observations with their standard errors at different time points within 24 h. AEPO: Aqueous extract of *Pleurotus ostreatus*; STZ: Streptozotocin. ^Values in percent indicate the change in the blood glucose levels relative to 0 h (before treatment) of the respective treatment groups. *P<0.05 versus control; #P<0.05 versus STZ group; $$P$<0.05 versus metformin group.

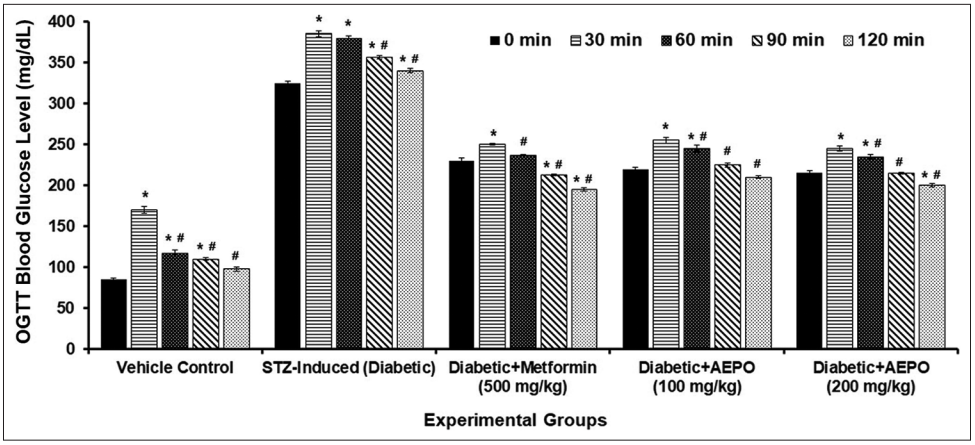

Figure 3. Effect of aqueous extract of *Pleurotus ostreatus* and metformin on oral glucose tolerance in diabetic rats. Values are mean ± standard error of mean for groups of three observations with their standard errors. *$P < 0.05$ versus 0-min within group; $^{\#}P < 0.05$ versus 30-min within group.

glucose, whereas diabetic rats treated with AEPO (200 mg/kg) showed about 125 mg/dL (about 45%) reduction in postprandial blood glucose.

Thus, results showed that AEPO act as potent antihyperglycemic agent and caused an active reduction in postprandial blood glucose in both acute and chronic studies. The antihyperglycemic effect of AEPO at 200 mg/kg was comparatively similar to that of metformin (500 mg/kg) in diabetic rats, suggesting its potent use in diabetes management.

3.4. Effect of *P. ostreatus* on oral glucose tolerance in STZ-induced diabetic rats

The OGTT is a vital preclinical test for characterizing the metabolic syndrome from prediabetes stage to type 2 diabetes. The oral glucose tolerance in both humans and animals acts as an indicator of the relative roles of insulin secretion and insulin resistance in the progression of glucose intolerance [24]. The OGTT was conducted at 0, 30, 60, 90, and 120 min, and results are presented in **Figure 3**. Results indicated interesting finding in observations from different treatment groups. Vehicle control group rats showed a response of OGTT as shown by a significant increase in blood glucose at 30 min that gradually reduced and retained a similar level to 0 min after 2 h ($P < 0.05$). STZ-induced diabetic rats showed a hyperglycemic response in OGTT with a significant increase in blood glucose at 30 min that remained similarly increased till 60 min ($P > 0.05$). The blood glucose level was moderately reduced at 90- and 120-min time intervals in STZ-diabetic rats that was significant as compared to 0-min ($P < 0.05$) as well as 30-min intervals ($P < 0.05$). This indicated that STZ-diabetic rats mimicked type 2 diabetes and that they serve as a suitable model for preclinical assessment of antihyperglycemic agents. Metformin treatment was very effective in controlling the blood glucose level and helped to reduce the OGTT glucose levels in very effective manner. STZ-diabetic rats treated with metformin (500 mg/kg) showed a promising therapeutic response in OGTT glucose levels. Metformin-treated diabetic rats showed that blood glucose level was significantly increased in first 30 min ($P < 0.05$) and that was gradually and significantly reduced at 120 min as compared to 0-min ($P < 0.05$) as well as 30-min ($P < 0.05$). Treatment of STZ-diabetic rats with AEPO showed a dose- and time-dependent reduction in blood glucose levels in OGTT with a response comparable to metformin. AEPO (100 mg/kg) group showed a significant increase in blood glucose at first 30 min ($P < 0.05$) and a gradual decrease that was significant as compared to 30-min ($P < 0.05$) and similar to 0-min ($P > 0.05$). AEPO at 200 mg/kg showed similar yet more potent response on blood glucose levels in OGTT with notable decrease at 120 min ($P > 0.05$). These observations suggested that AEPO has a potent antidiabetic effect as it could effectively regulate glucose tolerance in rats.

3.5. Effect of *P. ostreatus* on hematological parameters in STZ-induced diabetic rats

Diabetes causes elevated blood glucose level, which contributes to disturbed profile of blood cells and its

indices. Early normalization of glycemia has been suggested to inhibit pathological processes, which are meticulously associated with hyperglycemia such as increased oxidative stress and glycation of cellular proteins and lipids [25]. Thus, a good glycemic control remedy is recommended that could exert preventive effects on the blood profile as well. Results of the hematological analysis for hemoglobin levels are depicted in **Figure 4A**. Results indicated that hemoglobin level was significantly decreased in STZ-induced diabetic rats (13.9 ± 0.27 g/dL) as compared to vehicle control (15.7 ± 0.58 g/dL) with $P < 0.05$. Metformin (500 mg/kg) treatment to diabetic rats caused a notable increase in hemoglobin level (14.8 ± 0.75 g/dL) that was comparatively close to vehicle control ($P > 0.05$). Administration of AEPO to diabetic rats caused a dose-dependent restoration of hemoglobin toward normal range. Diabetic rats treated with AEPO at 100 mg/kg showed hemoglobin level 14 ± 0.55 g/dL, while AEPO at 200 mg/kg showed hemoglobin level 14.5 ± 0.81 g/dL with statistically similar values as compared to vehicle control. Total WBC count was estimated in experimental groups as another hematological parameter and the results are presented in **Figure 4B**. Results indicated that total WBC count was significantly increased in STZ-induced diabetic rats (5100/cc^3) as compared to vehicle control (4100/cc^3) with statistical significance ($P < 0.05$). Metformin (500 mg/kg) treatment to diabetic rats caused reduction in WBC count to a level (4200/cc^3) closely similar to vehicle control and significant to STZ-diabetic group ($P < 0.05$). AEPO treatment to diabetic rats showed a restorative effect on WBC count with 4500 and 4400/cc^3 at 100 and 200 mg/kg, respectively. WBC count restoration was significant as compared to STZ-diabetic group ($P < 0.05$) yet statistically similar as compared to vehicle control.

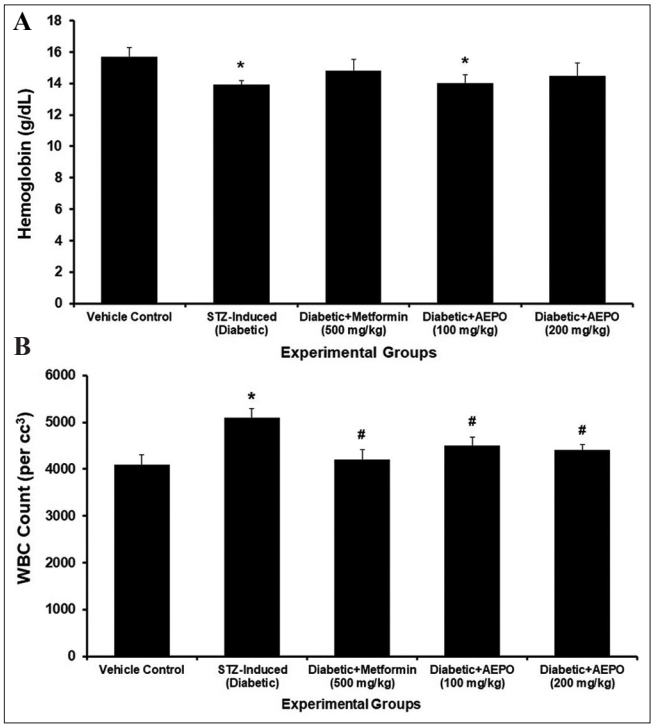

Figure 4. Effect of aqueous extract of *Pleurotus ostreatus* and metformin on hemoglobin and white blood cell (WBC) levels in diabetic rats. (A) Hemoglobin levels and (B) WBC count are presented as mean ± standard error of mean for groups of three observations with their standard errors. *$P < 0.05$ versus vehicle control; $^{\#}P < 0.05$ versus Streptozotocin-diabetic group.

The further analyzed hematological parameter was different leukocytes count (DLC) from different experimental groups (**Table 4**). Results showed notable variations in the DLC parameter in different experimental groups. The level of neutrophils was drastically increased (40%) in STZ-diabetic rats (84%) as compared to vehicle control (60%). Diabetic rats treated with metformin (500 mg/kg) showed 65% neutrophils which were comparatively close to vehicle control. Administration of AEPO caused a dose-dependent restoration in the neutrophils level in diabetic rats with 77% and 69% neutrophils at 100 and 200 mg/kg dosage, respectively. The level of lymphocytes was decreased by 35% in STZ-diabetic rats (26%) as compared to vehicle control (40%). Metformin (500 mg/kg) treatment could elevate the level of lymphocytes (33%) in diabetic rats yet not significant when compared with STZ-diabetic or vehicle control groups. Treatment of diabetic rats with AEPO caused a limited restoration of lymphocytes to 29% and 30% at 100 and 200 mg/kg, respectively. Estimation of RBC count showed that its level was reduced in STZ-diabetic rats to 4.25 million/mm^3 as compared to vehicle control (5.50 million/mm^3). Diabetic rats treated with metformin (500 mg/kg) showed RBC level of 5.22 million/mm^3, which was comparatively close to vehicle control ($P > 0.05$) and significantly elevated as compared to STZ-diabetic group ($P < 0.05$).

Table 4. Effect of AEPO and metformin on different leukocyte count (DLC) of experimental rats

Experimental groups	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)	Basophils (%)	Total RBC count (million/mm^3)	Platelet count (lac/mm^3)	Hematocrit (%)
Vehicle control	60±1.09	40±0.65	0	0	0	5.50±0.58	1.53±0.32	42.9±0.37
STZ-induced (diabetic)	84±1.22* ↑40%	26±0.8* ↓35%	1	2	0	4.25±0.27* ↓22.73%	2.1±0.25* ↑37.25%	47.1±1.38* ↑9.80%
Diabetic+metformin (500 mg/kg)	65±0.95$^{\#}$ ↑8.33%	33±1.9*$^{\#}$ ↓17.5%	0	0	0	5.22±0.36$^{\#}$ ↓5.10%	1.59±0.82$^{\#}$ ↑3.92%	44.2±1.12*$^{\#}$ ↑3.03%
Diabetic+AEPO (100 mg/kg)	77±1.32* ↑22.18%	29±0.3* ↓27.5%	0	0	0	4.82±0.72*$^{\#\$}$ ↓12.36%	1.85±0.35*$^{\#\$}$ ↑20.92%	45.3±1.33*$^{\#}$ ↑5.60%
Diabetic + AEPO (200 mg/kg)	69±1.94$^{\#}$ ↑15%	30±0.8* ↓25%	0	0	0	5.18±0.89*$^{\#}$ ↓5.82%	1.73±0.21*$^{\#}$ ↑13.07%	44.4±1.87*$^{\#}$ ↑4.66%

Values are mean±SEM for groups of three observations with their standard errors. Values in percent indicate increase (↑) or decrease (↓) in parameters. RBC: Red blood cell, erythrocyte count; AEPO: Aqueous extract of *Pleurotus ostreatus*; STZ: Streptozotocin. *P<0.05 versus control; $^{\#}P$<0.05 versus STZ group; $^{\$}P$<0.05 versus metformin group

Administration of AEPO to diabetic rats caused a dose-dependent restoration in the RBC levels with 4.82 and 5.18 million/mm^3 RBCs at 100 and 200 mg/kg dosages, respectively. Measurement of platelet count showed similar observations as RBC count. STZ-diabetic rats showed 2.1 lac/mm^3 as compared to vehicle control (1.53 lac/mm^3). Metformin (500 mg/kg) treatment to diabetic rats showed notable restoration in the platelet count (1.59 lac/mm^3) which was comparatively close to vehicle control (P > 0.05) and significantly elevated in comparison to STZ-diabetic group (P < 0.05). AEPO administration to diabetic rats caused a dose-dependent restoration in the platelet count by 1.85 and 1.73 lac/mm^3 at 100 and 200 mg/kg, respectively. Estimation of hematocrit levels showed that it was by about 10% in STZ-diabetic rats (47.1%) as compared to vehicle control (42.9%). Metformin (500 mg/kg) treatment to diabetic rats restored the level of hematocrit to 44.2% which was comparatively close to vehicle control (P > 0.05) and statistically significant as compared STZ-diabetic group (P < 0.05). AEPO administration to diabetic rats caused a dose-dependent restoration in the hematocrit level by 45.3 and 44.4% at 100 and 200 mg/kg, respectively. The change in hematocrit by AEPO (200 mg/kg) was statistically close to the level of vehicle control. The DLC profile of experimental groups showed that there was not much notable difference in the levels of eosinophils, monocytes, and basophils (**Table 4**).

The assessment of the liver function test (LFT) parameters was analyzed in different experimental groups and presented in **Table 5**. In a nutshell, results showed that the levels of total cholesterol, triglyceride, low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), total cholesterol/high-density lipoprotein (HDL) ratio, and cholesterol/HDL ratio were elevated multifold in STZ-diabetic rats with statistical significance. Treatment with metformin (500 mg/kg) to diabetic rats was preventive in nature to larger extent yet statistically not closer to vehicle control or STZ-diabetic group. Administration of AEPO to diabetic rats showed a dose-dependent restoration of LFT parameters yet statistically not closer to vehicle control or STZ-diabetic group. The levels of HDL were reduced in STZ-diabetic rats as compared

Table 5. Effect of AEPO and metformin on lipid profile of experimental rats

Experimental groups	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL Ch. (mg/dL)	LDL Ch. (mg/dL)	VLDL Ch. (mg/dL)	Total Ch./HDL Ch. ratio (mg/dL)	LDL Ch./HDL Ch. ratio (mg/dL)
Vehicle control	58.5±1.02	24.1±1.54	42.1±1.05	17.76±1.22	4.82±0.86	1.73±0.09	0.45±0.02
STZ-induced (diabetic)	138.63±1.98*	134.7±1.22*	31.9±1.22*	45.16±2.01*	26.94±1.25*	2.84±0.27*	1.42±0.05*
Diabetic+metformin (500 mg/kg)	73.9±1.81*$^{\#}$	40.23±1.87*$^{\#}$	39.7±1.51*$^{\#}$	19.78±1.85*$^{\#}$	13.64±0.92*$^{\#}$	1.86±0.04*$^{\#}$	0.58±0.07*$^{\#}$
Diabetic+AEPO (100 mg/kg)	90.7±0.99*$^{\#\$}$	89.91±1.54*$^{\#\$}$	32.1±1.62*$^{\$}$	28.77±1.51*$^{\#\$}$	17.50±0.88*$^{\#\$}$	2.46±0.08*$^{\#\$}$	0.70±0.06*$^{\#\$}$
Diabetic + AEPO (200 mg/kg)	78.52±1.58*$^{\#\$}$	68.2±0.98*$^{\#\$}$	33.5±1.25*	26.75±1.85*$^{\#\$}$	16.44±0.42*$^{\#\$}$	2.31±0.09*$^{\#\$}$	0.67±0.02*$^{\#\$}$

Values are mean±SEM for groups of three observations with their standard errors. HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very-low-density lipoprotein; Ch.: Cholesterol; AEPO: Aqueous extract of *Pleurotus ostreatus*; STZ: Streptozotocin. *$P<0.05$ versus control; $^{\#}P<0.05$ versus STZ group; $^{\$}P<0.05$ versus metformin group

to vehicle control that was largely restored by metformin (500 mg/kg) treatment yet statistically reduced compared to vehicle control. Exceptionally AEPO was unable to show statistical alterations in the HDL level as compared to STZ-diabetic rats.

We further assessed the kidney function profile of different experimental groups and data are presented in **Table 6**. Results clearly indicate that all the diabetic rats showed altered levels of kidney function parameters. In a nutshell, results showed that the levels of serum creatinine, serum uric acid, serum urea, and serum blood urea nitrogen (BUN) were elevated multifold in STZ-diabetic rats with statistical significance. Treatment with metformin (500 mg/kg) to diabetic rats was preventive in nature to larger extent yet statistically not closer to vehicle control or STZ-diabetic group. Likewise, administration of diabetic rats with AEPO showed a dose-dependent restoration of kidney function parameters yet statistically not closer to vehicle control or STZ-diabetic group.

4. Discussion

Diabetes mellitus is one of the most common chronic diseases associated with carbohydrate metabolism. It is also an indication of co-morbidities such as obesity, hypertension, and hyperlipidemia, which are metabolic complications of both clinical and experimental diabetes [26]. At present, drug therapy either alone or in combination cannot restore normal blood glucose homeostasis, and many limitations exist in their use. While external insulin is necessary for control of type 1 diabetes mellitus, the use of drug therapy in type 2 diabetes is initiated only after dietary and lifestyle modifications [13]. Oyster mushroom (*Pleurotus* spp.) is known in the Indian traditional system of medicine for its antihyperglycemic and antihyperlipidemic potential. *P. ostreatus* is reported to contain several bioactive molecules that are attributed to its therapeutic effects. The major bioactive molecules are phenolics, flavonoids, polysaccharides, lectins, terpenoids, steroids, lipids, and glycoproteins. These phytochemical compounds act as exogenous antioxidants that can regulate oxidative stress, suppress inflammation, and regulate glycemic and lipidemic alterations in the human body [27]. By virtues of the benefits of oyster mushrooms, we attempted to explore the effects of an AEPO on

Table 6. Effect of AEPO and metformin on liver and function of experimental rats

Experimental groups	Serum creatinine (mg/dL)	Serum uric acid (mg/dL)	Serum urea (mg/dL)	Serum BUN (mg/dL)
Vehicle control	0.69±0.01	2.98±0.09	32.7±0.98	15.28±0.74
STZ-induced (diabetic)	1.11±0.07*	5.23±0.52*	85.5±0.81*	49.86±1.12*
Diabetic+metformin (500 mg/kg)	0.78±0.02*#	3.86±0.32*#	42.9±0.68*#	29.44±1.09*#
Diabetic+AEPO (100 mg/kg)	0.93±0.02*#$	4.88±0.55*#$	72.1±0.94*#$	40.22±0.98*#$
Diabetic + AEPO (200 mg/kg)	0.81±0.04*#	3.90±0.87*#	63.0±0.84*#$	32.52±0.85*#$

Values are mean±SEM for groups of three observations with their standard errors. BUN: Blood urea nitrogen; AEPO: Aqueous extract of *Pleurotus ostreatus*; STZ: Streptozotocin. *$P<0.05$ versus control; #$P<0.05$ versus STZ group; $$P<0.05$ versus metformin group

STZ-induced diabetes in rats through assessment of the antihyperglycemic mechanism.

The OGTT measures the body's ability to use a type of sugar, called glucose, that is the body's main source of energy. OGTT, a test of immense value and sentiment, in favor of using fasting plasma glucose concentration alone was seen as a practical attempt to simplify and facilitate the diagnosis of diabetes [28]. Badole and Bodhankar [29] reported that the combination treatment of aqueous extract of *P. pulmonarius* with acarbose produced a more synergistic antihyperglycemic effect than either drug alone. They explained that in the OGTT, administration of glucose load (2.5 g/kg) increased serum glucose levels significantly after 30 min in alloxan-treated diabetic mice. In OGTT of the present study, blood glucose concentration increases rapidly at 30 min. In the case of normal set, blood glucose returned to normal after 120 min. *P. ostreatus* was found to be effective in controlling blood glucose level in OGTT study, and after 120 min of study, blood glucose level was found to be 200 mg/dL at higher concentration (200 mg/kg) when compared to glucose at 0 h which was 215 mg/dL. Lower concentration (100 mg/kg) of *P. ostreatus* extract was also effective in reducing blood glucose. Similar result was also reported by Cha *et al.* [30] for *Fomitopsis pinicola* that showed a significant fall in fasting blood sugar and HbA1c, which may be attributed to the hypoglycemic potential of the oyster mushroom supplementation [31]. The previous studies on diabetes revealed that oral administration of macrofungal extracellular polysaccharides (EPS) or crude extract exhibits an excellent hypoglycemic effect, lowering the average plasma glucose level in EPS-fed rats to 55.1% with enhanced glucose tolerance [32]. In the present study, AEPO helped in the reduction of blood glucose when compared to diabetic control and metformin-treated animals. Significant fall in fasting blood sugar may be attributed to the hypoglycemic potential of the oyster mushroom supplement [31]. It was reported that macrofungi significantly reduced blood glucose level in diabetic subjects. It helps in the reduction of plasma glucose concentrations up to 24.7% in diabetic animal tested [33]. Badole and Bodhankar [29] stated that a single administration of aqueous extract of *Pleurotus pulmonarius* (500 mg/kg) significantly reduced serum glucose level at 2, 4, 6, and 24 h after administration. The macrofungi was reported to significantly reduce the blood glucose level in diabetic subjects [33]. *Agaricus bisporus* (white button mushroom) contains high levels of dietary fibers and antioxidants including Vitamins C, D, and B12, as well as folate and polyphenols that provide beneficial effects on cardiovascular and diabetic diseases. It helps in the reduction of plasma glucose concentrations up to 24.7% in diabetic animal tested [33].

Body weight was measured to confirm the effect of STZ on experimental animals. Rats of vehicle control were shown to be stable in their body weight. Diabetic rats (STZ-induced) showed decrease in body weight after 30 days of treatment. STZ-mediated decrease (39%) in body weight was significantly reversed by the AEPO administration in a dose-dependent manner. AEPO at 100 and 200 mg/kg dosage showed 22.7% and 18.8% decrease in body weight when compared against STZ-diabetic rats. On the other hand, there was 16.3% and 20.2% increment in body weight at 100 and 200 mg/kg AEPO as compared to STZ-diabetic rats. *P. ostreatus* was effective in maintaining body weight of STZ-induced diabetic mice and mimic the activity of metformin. Badole and Bodhankar [29] reported that the combination

treatment of *P. pulmonarius* with acarbose prevented a decrease in the body weight of the diabetic mice in a very effective manner. The ability to prevent body weight loss seems to be due to its ability to suppress hyperglycemia. STZ-induced diabetes was characterized by a severe loss in body weight and this reduction in body weight is due to the loss or degradation of structural proteins since structural proteins are known to contribute to the body weight. The previous reports showed that protein synthesis is decreased in all tissues due to decreased production of ATP and absolute or relative deficiency of insulin [34].

Diabetes mellitus has been found to be associated with neutrophilic dysfunction and lymphocyte function impairments. Leukocyte such as neutrophils, monocytes, and eosinophils as well as hematocrit was found to be significantly increased in the diabetic animals when compared to the normal animals, and this condition on treatment with the infusion of the mushroom was significantly reverted to the near normal level [35]. STZ-induced diabetic rats were treated with AEPO, and results obtained were compared with normal rats, diabetic rats (without treatment) and metformin-treated rats. The results from the present study showed the significant changes in biochemical parameters during the experimentally inducted diabetes. Blood glucose, hemoglobin, total WBC, leukocyte count, lipid profile, creatinine level, serum uric acid, serum urea, and serum BUN levels were determined in control and aqueous extract- and metformin-treated rats. The present study clearly showed that *P. ostreatus* lowers blood lipid levels by reducing the levels of total serum cholesterol, VLDL, LDL and serum triglyceride, and it increased serum HDL level, in keeping with work done by Agrawal *et al.* [31] on oyster mushroom (*Pleurotus* spp). According to Yang *et al.* [36], *Collybia confluens* mycelial powder (CCMP) lowered the plasma total cholesterol, triglyceride, and LDL by 22.9%, 19.9%, and 37.3%, respectively. In summary, the study presented the potential beneficial effects of an AEPO that were exerted through an antihyperglycemic mechanism in diabetic animal model.

5. Conclusions

While many plants that are known to be able to treat ailments in humans are emerging nowadays, mushrooms are becoming increasingly known for its potential in treating diseases. In diabetes, some herbal alternatives are proven to provide symptomatic relief and assist in the prevention of secondary complications of the disease. Alternative therapies for diabetes treatment are needed because of inability of current therapies to contribute to normoglycemia and prevent diabetic complications. Despite of their effectiveness in reducing hyperglycemia, the use of these drugs is associated with non-desirable side effects. Mushrooms are exemplary sources of natural medicines with antidiabetic potential. They serve as an ideal choice for diabetic patients due to their high content of fiber and protein along with low fat content. *P. ostreatus* has immense potential and can be developed as effective and safe antidiabetic drug. However, a further study is needed to identify the active fractions responsible for antidiabetic activity and to clarify the mechanism of the effect.

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Conflict of interest

The authors declare no conflict of interest related to this publication.

Author contributions

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Writing – review & editing: Siddhartha Kumar Mishra

Ethics approval and consent to participate

The protocols for these experiments were approved by the Animal Ethical Committee of the Institute (IAEC/DDU/2021-22).

Consent for publication

Not applicable.

Availability of data

Data used in this work can be made available to the readers.

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