



Hypoglycemic, Hepatoprotective and Hypolipidemic Effects of *Pleurotus ostreatus* in Alloxan-Induced Hyperglycemic Rats

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ABSTRACT

Pleurotus ostreatus (Oyster mushroom) is increasingly being recognized as an important food product with a significant role in human health and nutrition. The present study aims at evaluating the effect of *Pleurotus ostreatus* in Diabetes mellitus and its complications. Experimental diabetes was induced following an overnight fast, by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg) to experimental animals (Wistar rats). Normal saline (0.5 mL) was injected to control group animals. Hyperglycemia was confirmed three days after injection by measuring the blood glucose level. The Diabetic rats were fed with broiler feeds supplemented with dried Oyster mushroom powder at doses of 10 and 20 g daily for a period of four weeks whereas the negative control animals were given only broiler feeds. The results showed that intraperitoneal injection of alloxan significantly ($p < 0.05$) elevated blood glucose level accompanied by significant increase in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), sodium (Na^+), bicarbonate (HCO_3^-), potassium (K^+) and with a concomitant decrease in serum total protein, high-density lipoprotein and chloride (Cl^-). Oyster mushroom powder at doses of 10 and 20 g daily for four weeks resulted in a reversal of the above pathological conditions associated with diabetes. Between the two doses tested, 20 g daily of oyster mushroom showed more potency in ameliorating the scourge of diabetes and its complications. Therefore, the study has revealed the potential therapeutic effect of oyster mushroom in the management of diabetes and its complications.

Keywords: Diabetes, Impairment, Hypoglycemic, Intraperitoneal, *Pleurotus ostreatus*.

Introduction

Diabetes mellitus refers to a group of metabolic diseases characterized by high blood sugar (hyperglycemia) levels, which result from defects in insulin secretion, or action, or both. It is a potentially morbid condition with high prevalence worldwide, thus, the disease constitutes a major health concern.¹

According to Wild *et al.*, at least 171 million people worldwide suffered from diabetes as at the year 2000 (i.e about 2.8% of the population).^{2,3} In the year 2001, Beretta stated that according to the World Health Organization (WHO), there were approximately 160,000 diabetic patients worldwide, and the number has doubled in the last few years and is expected to double once again in year 2025.⁴ As a major health problem, diabetes is predisposed markedly by increased cardiovascular mortality and serious mortality related to development of nephropathy, neuropathy and retinopathy.⁵ Chemicals and biochemical hypoglycemic agents like insulin, tolbutamide, phenformin, troglitazone, metformin, rosiglitazone and repaglinide, are the mainstay of treatment of diabetes and are effective in controlling hyperglycemia but they have harmful side effects and fail to significantly alter the course of diabetic complication.² Reports have shown that hyperlipidemia which often lead to coronary heart disease are associated with diabetes mellitus.^{6,7} This accounts for the treatment of lipid disorders as a part of diabetes management. Studies have also revealed that the activities of liver damage markers such as serum alanine

aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) are tremendously increased in diabetic patients signifying liver damage.⁸⁻¹³ Alloxan, a β -cytotoxic glucose analogue is commonly used for the development of animal model of Type-1 Diabetes Mellitus/Insulin Dependent Diabetes Mellitus (IDDM).^{14,15} Alloxan is rapidly taken up by the pancreatic β -cells through GLUT2 receptors.¹⁶ The sudden release of insulin leading to severe hypoglycaemia and even mortality if glucose therapy is not given had been ascribed to its cytotoxic action.¹⁷⁻¹⁹

Functional foods such as mushrooms are increasingly being used for the treatment of certain health problems. Some mushrooms appear to be effective for both the control of blood glucose level and diabetic complications without side effects. Therefore, these edible mushrooms can be used as supplement in controlling hyperglycemia.²⁰ *Pleurotus ostreatus*, the Oyster mushroom, is increasingly being recognized as an important food product with a significant role in human health and nutrition.^{21,22} *Pleurotus* species are rich sources of proteins, minerals (Ca, P, Fe, K and Na), vitamin C and vitamin B complexes (thiamine, riboflavin, folic acid and niacin).²³ They contain high potassium to sodium ratio, which makes them an ideal food for patients suffering from hypertension and heart diseases. Treatment with mushroom, *P. ostreatus* (especially high level) has been shown to reduce the high blood glucose level in hyperglycemic rats.²⁴ *Pleurotus* species also possess blood-pressure-lowering activity and has been found to contain high amounts of lovastatin in the fruit-body, especially in the lamellae or gills.²⁵ *P. ostreatus* produces significant increases in liver glycogen when compared to diabetic controls and it was suggested that increase in liver glycogen may be due to enhanced rate of glycogenesis.²⁶ The purpose of this study is to evaluate the effect of *Pleurotus ostreatus* (Oyster mushroom) in Diabetes mellitus and its complications.

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Materials and Methods

Collection and identification of plant material

Oyster mushrooms were bought from No 9, 10 and 15 vegetable market, Museum street/opposite Nitel exchange terminus, Jos, Plateau State, Nigeria. The plant material was identified and authenticated in the Department of Botany, Nasarawa State University, Keffi. They were shade dried for six days and ground into fine powder. The dried ground mushrooms were stored in an air-tight container prior to commencement of study.

Animals

Adult rats (Wistar strain) weighing 220-300 g were procured from National Veterinary Research Institute (NVRI), Vom, Plateau State. Food and water were provided *ad libitum*. Animals were exposed to controlled environmental temperature ($28 \pm 2^\circ\text{C}$), relative humidity ($50 \pm 5\%$) and 12-hour light or dark. The principle governing the use of laboratory animals as laid out by the Department of Zoology, Nasarawa State University, Keffi were observed. The animals were allowed four weeks under these conditions to acclimatize before the commencement of the experiments.

Chemicals

Alloxan monohydrate was purchased from Sigma-Aldrich Co. (Taufkrichen, Germany). All chemicals used in this study were of analytical grades.

Drugs usage

Alloxan monohydrate and standard drug metformin used for the study were freshly prepared in normal saline at doses of 150 mg/kg body weight before each experiment.

Experimental induction of diabetes in rats

Experimental diabetes was induced following an overnight fast, by a single intraperitoneal injection of alloxan monohydrate at dose 150 mg/kg b.wt. Control animals were injected with 0.5 mL of normal saline. Hyperglycemia was confirmed three days after injection by measuring the serum glucose level. Only the animals with fasting blood glucose levels ≥ 120 mg/dL were selected for the study.

Experimental Design

The rats were divided into five groups, each group consisting of five animals. Group I: (Normal control) received broiler mash only for a period of four weeks. Group II: (Diabetic control) received broiler mash only for a period of four weeks. Group III: (Diabetic rats) received broiler mash supplemented with 10 g/day dried oyster mushroom powder. Group IV: (Diabetic rats) received broiler mash supplemented with 20 g/day dried oyster mushroom powder. Group V: (Diabetic rats) received broiler mash and standard drug metformin at a dose of 150 mg/kg body weight orally for a period of four weeks.

After 24 hours of the last treatment, all the animals were anaesthetized with chloroform and blood was collected via cardiac puncture into plain sample tubes and sera was obtained from it by allowing it to stand for 2 h at room temperature before centrifuging at 2000 rpm. The serum was used for estimation of various biochemical parameters.

Biochemical Analysis

The serum glucose and total cholesterol (TC) were determined by the method of Trinder and Searcy, respectively.^{27,28} High density Lipoprotein Cholesterol (HDL-C), Very Low density Lipoprotein Cholesterol (VLDL-C) and Triglyceride (TG) were determined by the method of Friedwald *et al.* and Assman, respectively.^{29,30} Low density Lipoprotein Cholesterol (LDL-C) level was calculated using formula below.³¹

$$\text{LDL} = \text{TC} - \text{TG}/5 - \text{HDL}$$

Marker Enzymes Analysis

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured spectrophotometrically by the method of Reitman and Frankel.³² ALP, ALT and total protein content of serum were determined by the method of King and Armstrong using disodium phenyl phosphate as substrate and by biuret method, respectively.^{33,34}

Serum Electrolyte

Serum concentrations of Na^+ , K^+ , Cl^- and HCO_3^- were determined by the methods of Tietz, Skeggs and Hochstrasser.³⁵⁻³⁸

Statistical Analysis

Statistical analysis of the results was done by one way analysis of variance (ANOVA) using SPSS software followed by Dunnett's comparison test for significance. Significance was set at $p < 0.05$.

Results and Discussion

Alloxan is one of the commonly used beta-cytotoxic agents for the induction of Type-I diabetes mellitus in animal models. Its beta-cytotoxic action is associated with the sudden release of insulin leading to severe transient hypoglycaemia followed by chronic hyperglycemia resulting from the complete destruction of the pancreatic cells that secrete insulin.^{35,36}

In the present study, administration of alloxan at a dose of 150 mg/kg body weight to the rats caused significant ($p < 0.05$) elevation of blood glucose level as compared to normal rats in group I. Supplementation with dried oyster mushroom powder at doses of 10 and 20 g daily for a period of four weeks caused a significant reduction ($p < 0.05$) in blood glucose level as compared to diabetic rats in group II (Table 1). The results obtained from this study suggests that oyster mushroom has significant antidiabetic effect in alloxan-induced hyperglycemic rats. The lowering ability of the mushroom comes from four important antidiabetic proteins which are proflin-like protein, glyceraldehyde-3-phosphate dehydrogenase-like protein, trehalose phosphorylase-like protein (TP) and catalase-like protein which have potential of enhancing insulin action.³⁹ Other mechanism of action of these proteins from oyster mushroom might be in connection to its ability to delay the absorption of carbohydrate through inhibition of carbohydrate hydrolysing enzymes, α -amylase and α -glucosidase.⁴⁰ Our findings are in consonance to that of Ravi *et al.*⁴¹ and the findings suggested that oyster mushroom are promising as an antidiabetic nutraceutical.

The liver is an organ where metabolism, storage and detoxification of xenobiotic occur.⁴² The levels of AST, ALT and ALP have been reported to increase in alloxan-induced diabetic rats as a result of liver damages in chronic hyperglycemia.⁴³ Serum activities of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase and total protein level were used to assess the extent of liver damage in this study. The study has shown that there were significant ($p < 0.05$) increase in serum activities of AST, ALT and ALP and significant ($p < 0.05$) decrease in serum level of protein in alloxan-induced hyperglycemic rats as compared to normal rats in group I but these were however returned to normal levels by Supplementation with dried oyster mushroom powder at doses of 10 and 20 g daily for a period of four weeks (Table 2). Aspartate aminotransferase is an enzyme found mainly in the cells of the liver, heart, skeletal muscles, kidney, and pancreas while alanine aminotransferase is more specific for liver damage as the enzyme are found mainly in the liver.⁴⁴

Table 1: Effect of *Pleurotus ostreatus* on serum glucose level in alloxan-induced hyperglycaemic rats.

| Groups | Treatment | GLU (mg/dL) (day 3) | GLU (mg/dL) (day 28) |
|--------|---|----------------------------------|---------------------------------|
| I | CONTROL | 82.40 \pm 10.14 | 88.40 \pm 12.2 ^b |
| II | Diabetic control | 133.75 \pm 19.16 ^a | 276.56 \pm 12.24 |
| III | Diabetic treated with 10 g mushroom | 138.00 \pm 104.92 ^a | 106.25 \pm 44.16 ^b |
| IV | Diabetic treated with 20 g mushroom | 123.50 \pm 12.40 ^a | 60.75 \pm 4.92 ^b |
| V | Diabetic treated with 150 mg/kg Metformin | 142.01 \pm 7.55 ^a | 119.33 \pm 14.22 ^b |

Data is expressed as Mean \pm SD, n = 5.

^ap < 0.05 compared with control (group I)

^bp < 0.05 compared with alloxan-induced group control (group II)

Table 2: Effect of *Pleurotus ostreatus* on serum activities of liver enzymes in alloxan-induced hyperglycaemic rats.

| Groups | Treatment | AST(U/L) | ALT(U/L) | ALP(U/L) | Total Protein |
|--------|---|-----------------------------|----------------------------|----------------|---------------|
| I | Normal saline | 26.34 ± 12.25 | 5.18 ± 2.82 | 36.20 ± 16.41 | 6.21 ± 12.09 |
| II | Diabetic control | 47.63 ± 14.36 ^a | 29.05 ± 13.55 ^a | 149.00 ± 39.25 | 5.64 ± 02.07 |
| III | Diabetic treated with 10 g mushroom | 42.28 ± 11.86 ^{ab} | 15.75 ± 4.99 ^{ab} | 55.75 ± 17.9 | 6.81 ± 13.24 |
| IV | Diabetic treated with 20 g mushroom | 28.05 ± 10.06 ^b | 15.75 ± 4.99 ^{ab} | 55.75 ± 17.91 | 7.0 ± 05.16 |
| V | Diabetic treated 150 mg/kg Metformin | 31.33 ± 7.11 ^{ab} | 22.53 ± 5.89 ^{ab} | 85.67 ± 24.14 | 7.2 ± 4.26 |

Data is expressed as Mean ± SD, n = 5.

^ap < 0.05 compared with control (group I)

^bp < 0.05 compared with alloxan-induced diabetic control (group II)

Table 3: Effect of *Pleurotus ostreatus* on serum lipid profiles (mmol/L) in alloxan-induced hyperglycaemic rats.

| Groups | Treatment | Cholesterol | Triglyceride | HDL-C | LDL-C |
|--------|---|--------------------------|--------------------------|--------------------------|--------------------------|
| I | Normal saline | 3.00 ± 0.08 | 2.02 ± 0.03 | 1.99 ± 1.02 | 0.76 ± 0.19 |
| II | Diabetic control | 4.27 ± 0.15 ^a | 3.60 ± 0.11 ^a | 0.78 ± 0.04 ^a | 1.25 ± 0.11 ^a |
| III | Diabetic treated with 10 g mushroom | 3.87 ± 0.39 ^b | 3.25 ± 0.12 | 0.83 ± 0.16 | 1.05 ± 0.21 ^a |
| IV | Diabetic treated with 20 g mushroom | 3.42 ± 0.22 ^b | 2.55 ± 0.07 ^b | 1.94 ± 0.09 ^b | 0.89 ± 0.13 ^b |
| V | Diabetic treated 150 mg/kg Metformin | 3.87 ± 0.39 ^b | 3.25 ± 0.12 | 0.83 ± 0.16 | 1.05 ± 1.02 ^a |

Data is expressed as Mean ±SD, n = 5.

^ap < 0.05 compared with control (group I)

^bp < 0.05 compared with alloxan-induced diabetic control (group II)

Table 4: Effect of *Pleurotus ostreatus* on plasma electrolytes (Na⁺, Cl⁻, HCO₃⁻ and K⁺) in alloxan-induced hyperglycaemic rats.

| Groups | Treatment | Sodium (Na ⁺) (mmol/L) | Chloride (Cl ⁻) (mmol/L) | Bicarbonate (HCO ₃ ⁻) (mmo/L) | Potassium (K ⁺) (mmol/L) |
|--------|---|---------------------------------------|---|---|---|
| I | Normal saline | 47.00 ± 20.27 | 47.20 ± 38.73 | 34.80 ± 4.09 | 4.52 ± 0.67 |
| II | Diabetic control | 84.00 ± 13.37 ^a | 29.75 ± 10.87 ^{ab} | 37.50 ± 5.92 ^a | 5.13 ± 0.29 ^a |
| III | Diabetic treated with 10 g mushroom | 71.50 ± 22.43 ^{ab} | 52.25 ± 24.61 ^b | 38.25 ± 3.59 | 5.96 ± 1.56 ^a |
| IV | Diabetic treated with 20 g mushroom | 50.75 ± 22.52 ^b | 91.75 ± 7.89 ^{ab} | 42.00 ± 2.94 ^{ab} | 5.24 ± 0.42 ^a |
| V | Diabetic treated 150 mg/kg Metformin | 36.00 ± 31.24 ^{ab} | 94.67 ± 11.93 ^{ab} | 42.33 ± 5.51 ^{ab} | 4.99 ± 0.22 |

Data is expressed as Mean ±SD, n = 5.

^ap < 0.05 compared with control (group I)

^bp < 0.05 compared with alloxan-induced diabetic control (group II)

Alkaline phosphates (ALP) is mostly used for evaluating the integrity of plasma membrane and increase in its activity can constitute threat to the life of cells that depend on a variety of phosphate esters for their vital process since there may be indiscriminate hydrolysis of phosphate ester of the tissue.⁴⁵ In diabetes, there is increase catabolic processes such as proteolysis, glycogenolysis and lipolysis as a result of a deficiency of insulin. The reduction in serum total protein level in alloxan-induced hyperglycemia in rats might be attributed to inhibition of protein synthesis with concomitant increase in protein catabolism. The serum lipids level is usually raised in diabetes and present a risk factor for the development of cardiovascular diseases most especially coronary heart diseases.⁴⁶ Lowering plasma lipids levels by phytochemical appears to be potent therapy in the management of cardiovascular diseases.⁴⁷ The study showed that there were significant ($p < 0.05$) increase in serum triglycerides, total cholesterol and low density lipoprotein cholesterol accompanied by significant ($p < 0.05$) decrease in high density lipoprotein cholesterol in alloxan-induced hyperglycemic rats. Supplementation with dried oyster mushroom powder at doses of 10 and 20 g daily for a period of four weeks restored the pathological alterations of these lipids profiles to normal level (Table 3). Hypolipidemic effect of the oyster mushroom may be related to the its ability to increase hepatic LDL receptor. Insulin can affect adipocytes by inhibiting lipolysis and promoting the storage of triacylglycerol in adipocytes. Its deficiency or inadequate utilization leads to the activations of hormone-sensitive lipases, glucagon and catecholamines which in turns increases the breakdown of lipids and mobilization of free fatty acids from the peripheral depots.⁴⁸ Activation of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a key rate-limiting enzyme responsible for the biosynthesis of cholesterol due to insulin deficiency or insulin might have been responsible for the observed hyperlipidemia.

The kidneys regulate the reabsorption of electrolytes into the blood at the renal tubules of the nephron.⁴⁹ Impairment of glomerular function, substances normally reabsorbed into the blood are excreted in the urine. The study also shows that administration of alloxan at dose of 150 mg/kg body weight to the rats caused significant ($p < 0.05$) increase in serum level of sodium, bicarbonate and potassium accompanied by a decrease in serum level of chloride. The increase in the levels of sodium, potassium and bicarbonate in alloxan-induced hyperglycemia indicates that the reabsorption at parietal cells of the distal and cortical collecting tubules of the nephrons are not compromised. The bicarbonate ion acts as a buffer to maintain the normal acidity in blood and other fluids in the body. Disruptions in the normal bicarbonate level may be due to kidney diseases or metabolic conditions. Supplementation with dried oyster mushroom powder at doses of 10 and 20 g daily for a period of four weeks led to significant ($p < 0.05$) reversal of these altered serum electrolytes (Table 4). This study has shown that Supplementation with dried oyster mushroom powder especially at dose of 20 g daily for a period of four weeks is more potent than standard drug metformin in ameliorating the scourge of diabetes and its complications.

Conclusion

The study has demonstrated that oyster mushroom besides its hypoglycemic and hypolipidemic effects could also protect the liver and kidney against impairment due to hyperglycemia.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that this study is original research work and that we shall be liable for any claims relating to the content of this article.

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