Effect of phytosome based extract of oyster and button mushroom on liver function profile of streptozotocin induced diabetic Wistar rats

Short Title: ANTIDIABETIC EFFECT OF MUSHROOM LOADED PHYTOSOMES

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Diabetesmellitus, *Pleurotus ostreatus*&*Agaricus bisporus*extract,Phytosome,Glibenclamide, Streptozocin.

ABSTRACT:
Diabetesmellitus is a persistent, sometimes fatal illness that can impact many different organ systems, including the liver. The current study serves as the basis for evaluating the effects of phytosomes derived from mushroom extract (oyster and button mushroom)on liver functioning factors such SGPT,SGOT and Bilirubin as well as the glucose level in streptozocin (STZ) -induced rats with diabetes. According to the results, the groups treated with a combination of lactobacillus and mushroom extract (oyster and button mushroom) showed a substantial drop in blood sugar levels compared to the diabetic control group ($P \leq 0.05$).Similarly, the levels of glucose, SGPT, SGOT, and bilirubin($P \leq 0.05$) significantly decreased after treatment with phytosomes of oyster mushrooms combined with Lactobacillus, as well as phytosomes of button mushrooms with Lactobacillus.Therefore, these can be considered effective alternatives for the treatment of diabetesmellitus alongside allopathic medications.

INTRODUCTION

Diabetes mellitus (DM) is a severe endocrine disease for which prevention and/or treatment of its side effects are currently extremely challenging.[1]Type 2 diabetes is primarily associated with obesity and is correlated with high blood pressure and dyslipidaemia. Thus, the treatment's goals are to increase insulin secretion and lessen insulin resistance. Low levels of glucose absorption are caused by type 1 diabetes, which is characterised by a lack of insulin release to the muscles and adipose tissue.[2]Even though oral hypoglycemic medications have made significant advancements in treating diabetes, there is still a need for more modern therapeutic agents because the medications that are now being developed have certain limitations.[3]Long-term exposure to dyslipidaemia and unregulated hyperglycemia causes chronic consequences such neuropathy, nephropathy, and retinopathy.[4]Research shows that diabetes mellitus (DM) is linked to various liver abnormalities, including hepatocellular carcinoma (HCC), abnormal glycogen deposition, fibrosis, cirrhosis, elevated hepatic enzymes, non-alcoholic fatty liver disease (NAFLD), viral hepatitis and acute liver disease.A number of key pathways have been identified that contribute to liver damage in patients with diabetes. The primary causal reason for compensatory hyperinsulinemia and hyperglycemia is insulin resistance.The liver, as a key insulin-sensitive organ, is particularly vulnerable to the oxidative stress caused by hyperglycemia, which can result in liver tissue damage.[5]Type 2 diabetes mellitus (T2DM), a chronic non-communicable disease marked by elevated blood glucose levels, has garnered increased attention due to its high prevalence and significant health consequences. Type 2 diabetes has a complex etiology and development that involves numerous signaling pathways. From a pathological viewpoint, insulin resistance (IR) and the resultant insulin shortage produced by the loss of pancreatic β cells are the two main pathogenic characteristics of type 2 diabetes (T2DM). The complexity of T2DM and the range of patient circumstances are increased by their diverse combination.[6]Insulin therapy effectively controls blood sugar levels, but it has drawbacks as well. For instance, it is useless when taken orally, has a short shelf life, needs to be refrigerated often, and increases the risk of fetalhypoglycemia in the event of an overdose. There are drawbacks to using sulfonylurea and biguanide during treatment.[7]

Despite considerable efforts to manage the condition, the prevalence of diabetes continues to rise, accompanied by an alarming increase in fatalities and morbidity due to diabetes-relatedcomplications.While insulin, tolbutamide, phenformin, troglitazone, rosiglitazone, and repaglinide are key hypoglycemic agents that effectively manage hyperglycemia, they also have

harmful side effects and do not significantly alter the progression of diabetic complications.Mushrooms, which have historically been used to treat diabetes, present a promising field for the development of innovative therapeutics to manage diabetes and its complications. Certain mushrooms have showed potential to regulate blood glucose levels and modifying the progression of diabetes issues in clinical and/or experimental settings without creating unwanted effects.[8]Mushrooms consist of numerous bioactive components such as polysaccharides, lectins,ergosterols, fibre, proteins flavonoids and terpenoids. In addition to amino acids, vitamins, andminerals, they are rich in macro and micronutrients. In addition, mushrooms are high in water and fibre. Their abundance of naturally occurring insulin-like enzymes aids in the digestion of glucose in diet and lowers insulin resistance. Additionally, they include many substances that support the proper operation of the liver and pancreas, aiding in the release and synthesis of insulin and confirming normal metabolic processes.[9]
The current study made use of two significant therapeutic mushrooms: *Agaricus bisporus*, also known as white button mushroom and *Pleurotus ostreatus*, also known as oyster mushroom. The white button mushroom, or *Agaricus bisporus*, is one of the edible mushroom species. most prevalent in the world due to a unique chemical with both biological and therapeutic properties.[10]*Pleurotus ostreatus* mushrooms are both nutritional and medicinal, giving them a unique delicacy.
For diabetics, these mushrooms have a beneficial synergistic impact on both the kidneys and liver.[11]. After white button mushrooms (Agaricus bisporus), Pleurotus ostreatus (P. ostreatus), also known as oyster mushrooms, is one of the most widely farmed edible mushrooms in the world.P. ostreatus offers β-glucans, a dietary fibre that has been gaining attention for its potential benefits in preventing insulin resistance, dyslipidaemia, hypertension, and obesity. It provides almost twice as many β-glucans as A. bisporus.[12]

MATERIALS AND METHODS

Extraction of*Pleurotus ostreatus* and*Agaricus bisporus*Mushroom

Fresh mushrooms were carefully rinsed with fresh water. The mushroom's fruiting body was removed and bakedfor 48 hours in an oven at 60ºC before being ground into fine powder.The water extract was made by the hot percolation method using Soxhlet apparatus. About 30g of mushroom powder (1:10, w/v) was added to water (300ml), the solvent, and heated to a controlled 50ºC.Dried the extract in a rota evaporator. For later usage, the extracts were kept in a clean, sterile container at 4°C.[13]

Biosynthesis of phytosomes of mushroom

Through solvent evaporation techniques, oyster and button mushroom phytosomes were developed. To make phytosome, soy lecithin and mushrooms were utilised. Carefully weighed amounts of mushrooms were dissolved in dichloromethane and methanol, respectively, and in soy lecithin. Subsequently, the two solutions were refluxed at 60 degrees Celsius for two hours in a round-bottom flask. At this point, the solution had a distinct yellow colour. Subsequently, the blend entirely evaporated at 60ºC in a rotating evaporator, yielding a thin layer. The thin film was hydrated using phosphate buffer (7.4), and the resulting suspension was gathered, filtered, then vacuum-dried, and preserved.[14]

Induction of diabetes in Wistar rats

Streptozotocin, that had been freshlysynthesized in 0.1M citrate buffer (4.5 pH), was administered to overnight-starved Wrister rats (45 mg/kg body weight) through an intraperitoneal injection. Using ACCU-CHEK sensor glucometer, blood samples were taken 48 hours following streptozotocin injection to confirm hyperglycemia. Participants in research were rats with diabetes, which was defined as having a blood glucose level or GT higher than 200 mg/dl 48 hours after being injected with streptozotocin.

Division and distribution of animal

36 Wistar rats have been taken in the experiment, and further divided in following 6 groups: Group 1 consisted of diabetic Wistar rats that were induced with STZ and were not given any medication. Group 2 consisted of diabetic rats given an allopathic medication (Glibenclamide) at dose of 40 mg per kilograms of body weight. Diabetic rats are treated with lactobacillus and oyster mushroom phytosomes at dosage of 100mg/kg body weight in Group 3. Group 4 consists of lactobacillus-treated diabetic rats and white button mushroom phytosomes administered at a 100 mg/kg body weight dose.

A diabetic rat treated with lactobacillus suspension (100 mg/kg body weight) is part of group 5. Group 6 consisted of Wistar rats that were not given STZ therapy, serving as a normal, healthy control group.

Test design

Following the induction of diabetes, Groups 3, 4, and 5 of diabetic rats were treated with lactobacillus suspension, oyster mushroom phytosomes (100 mg/Kg of body weight), and white button mushroom phytosomes, respectively. There were 21 days in the test period. Following this time, blood samples were taken on day 21 in order to test glucose,SGPT,SGOT and Bilirubin .Serums were separated and sent to a facility for measurement of the previously stated parameters following a 15-minute centrifugation of blood at 3000 revolutions per minute.

Assessment of biochemical parameters

All biochemical parameters were tested using commercially available kits from Erba Diagnostic Kit Mannheim GmbH, Mallaustr, Germany, including glucose,SGPT,SGOT and Bilirubin. Auto-analyzer Erba Chem-7.32 was utilized to ascertain the amounts of various biochemical parameters. An ACCU-CHEK sensor glucometer was used to measure the blood glucose levels.

Statistical analysis

The mean ± SEM was examined using one-way ANOVA with descriptive statistics. A significance criterion of P≤ 0.05 was used in different groups. Data was analyzed using SPSS 17.

Result

Table 1 &Figure (1) depicts the blood glucose profile of rats, presented as average±SEM. Diabetic rats administered streptozotocin exhibited a notable increase in blood glucose concentrations. Conversely, diabetic animals administered with Phytosomes of *Pleurotus ostreatus* combined with *Lactobacillus spp.* experienced a reduction of 39.7% in blood glucose concentrations, while those administered with *Agaricus bisporus* combined with *Lactobacillus spp.* showed a reduction of 37.6%, as illustrated in the accompanying figure.

The liver function indicators aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured in diabetic rats, along with bilirubin, a non-enzymatic marker, were notably elevated.

Regarding the comparative effects of POM and PBM on bilirubin levels, administering POM with *Lactobacillus spp.* for 21 days resulted in a reduction of 37.1% in bilirubin levels. Similarly, treatment with PBM with *Lactobacillus spp.* also led to a reduction of 37.1%.

In terms of aspartate aminotransferase activity, which was considerably higher in DC (Diabetic group) compared to NC (Normal control) rats, administering POM with *Lactobacillus spp.* for 21 days resulted in a reduction of 5.6%. Additionally, treatment with PBM with *Lactobacillus spp.* led to a reduction of 5.2%.

Similarly, alanine aminotransferase activity, elevated in diabetic rats, showed reductions after 21 days of treatment. Administering POM with *Lactobacillus spp.* resulted in a reduction of 9.7%, while treatment with PBM with Lactobacillus spp. led to a reduction of 7.5%.(table 2 &figure2)

Table :1 Comparison of Glucose between different groups with diabetic control group(*N*=6)

Parameters	**Glucose**	**P value**
Diabetic Control	284.9±12.6	
Glabenclamide	157.8±12.5	.000
L+O	171.7±12.8	.000
L+B	177.7±12.2	.000
Oysters Mushroom	185.9±12.6	.000
Button Mushroom	187.3±12.4	.000
Lactobacillus	191.9±12.6	.000
Normal control	90.9±9.5	.000

P> 0.05 # not significant, P< 0.05* significant, P<0.001&P < 0.0001*** highly significant"**

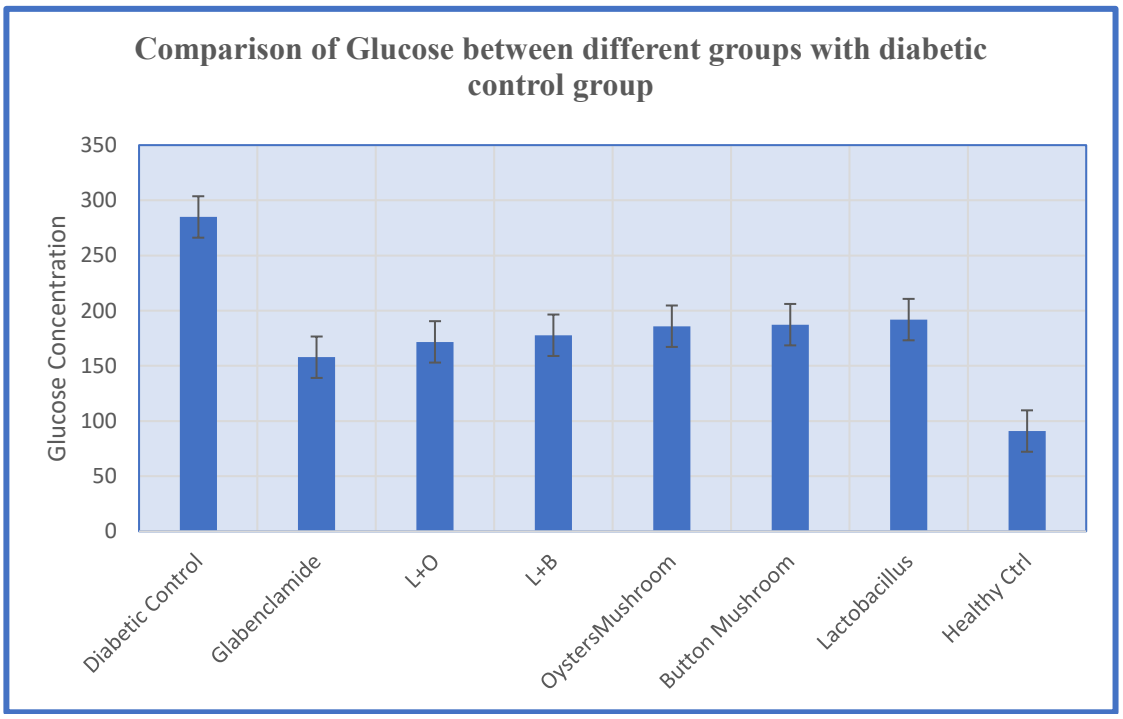

Figure :1 Comparison of Glucose between different groups with diabetic control group

Table:2Comparison of parameters of LFT between different groups with diabetic control group (*N*=6)

Parameters	**SGOT**	**SGPT**	**Bilirubin**
Diabetic Control	44.0±3.7	54.2±4.3	1.4±.1
Glabenclamide	39.8±1.8*	45.3±3.5***	.83±.2***
L+O	41.5±2.7$^{\#}$	48.9±2.9**	.88±.2**
L+B	41.7±2.8$^{\#}$	50.1±3.5$^{\#}$	.88±.2**
Oysters Mushroom	42.8±3.5$^{\#}$	51.6±4.5$^{\#}$	.96±.3**
Button Mushroom	43.1±3.2$^{\#}$	51.9±4.2$^{\#}$	1.0±.3*
Lactobacillus	43.2±3.4$^{\#}$	52.5±4.9$^{\#}$	1.3±.0$^{\#}$
Healthy Ctrl	22.7±5.1***	35.5±5.0***	.5±.1***

"P> 0.05 $^{\#}$ not significant, P< 0.05* significant, P<0.001&P < 0.0001*** highly significant"**

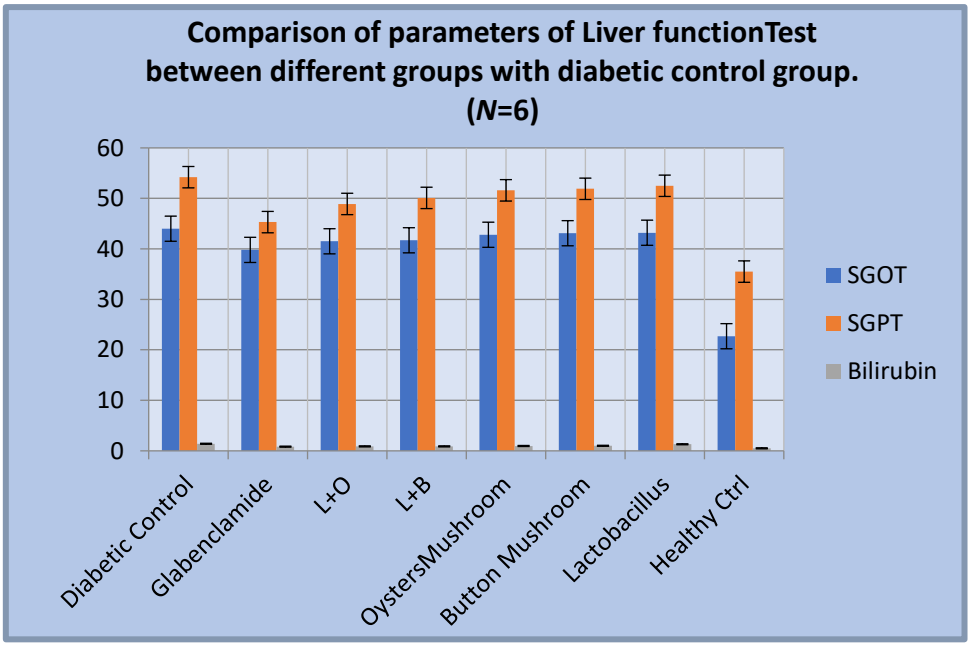

Figure:2Comparison of parameters of LFT between different groups with diabetic control

DISCUSSION

The phytosome, which included button mushroom and oyster extract, had a substantial hypoglycemic impact at a dose of 100 mg/kg of body weight, according to the results. This study looked at the effects of Agaricus bisporus and Pleurotus ostreatus phytosomeson glucoseand liver function .These compounds use several pathways to demonstrate their anti-diabetic efficacy. Because the STZ-induced diabetic rat model exhibited diabetes characteristics, it was a useful experimental paradigm for our study.

In comparison to the diabetic control group in STZ-induced Wistar rats, the current study's findings showed a significant decrease in the serum levels of glucose,SGOT , SGPT and Bilirubin in both groups treated with a combination of lactobacillus with button mushroom phytosome, lactobacillus with oyster mushroom phytosome, and the allopathic drug Glibenclamide.

Diabetes requires the use of low-cost, non-toxic treatment to tackle the global health danger it poses. The negative effects of many synthetic hypoglycemia diabetic medications render them ineffective. Because mushrooms are known to contain naturally occurring bioactive components that may have anti-diabetic capabilities, scientists are currently concentrating on studying these Mushroom polyphenols have biological qualities that are beneficial to human health and medicine, such as anti-inflammatory, anti-cancer, anti-tyrosine, antihyperglycemic, and antioxidant effects.High concentrations of dietary fiber, antioxidants, beta glycans, folate, ergothioneine, and polyphenols have been found in white mushrooms,[15,16] indicating that the mushrooms may have hypoglycemic properties and be useful for those with diabetes mellitus .A. bisporus has been shown to lower blood glucose levels in diabetic rats given STZ.[17]The white button mushroom may have anti-inflammatory, hypoglycemic, and hypocholesterolemic properties due to its high level of acidic polysaccharides, dietary fibre, and antioxidants, which include vitamins C, B12, and D, folate, ergothioneine, and polyphenol.[18]. Many bioactive phytocompounds are polar and water-soluble, making them poorly absorbed by phospholipid structures. To enhance the bioavailability of these water-soluble BPCs, scientists have developed phytosomes.[19,20]

Glibenclamide, a second-generation sulfonylurea, works by stimulating pancreatic beta cells to produce insulin. It has a prolonged action, and its metabolites with hypoglycemic effects increase the risk of chronic hypoglycemia.[21]The challenges and limitations associated with commonly used synthetic pharmaceuticals are driving researchers to explore plant-derived antidiabetic therapies, which often offer improved safety and efficacy profiles. Plant-based medications, known for their success in treating diabetes mellitus and other conditions, provide advantages such as reduced

toxicity, fewer side effects, lower cost, and greater accessibility,[22]This study examined how phytosomes of Agaricus bisporus and Pleurotus ostreatus affect lipid profiles, renal and liver function, glucose tolerance, and glycemic control.[23]

CONCLUSION

Understanding the intricate relationship between liver health and diabetes is crucial for individuals with diabetes. Diabetic liver damage is a significant complication of diabetes that necessitates diligent monitoring and efficient treatment. Following a healthy lifestyle, keeping blood pressure under control, and consistently monitoring blood sugar and blood pressure can significantly reduce the chances of liver damage or slow down its advancement.The study demonstrates that administering phytosomes derived from oyster and button mushroom extract to STZ-treated rats results in significant improvements in their serum levels of blood glucose, SGOT, SGPT, and Bilirubin. Further investigation is required to identify and assess the specific molecular pathways responsible for the pharmacological effects of these antidiabetic phytochemicals. While plants and plant-based dietary components are generally considered safe for consumption, it is still essential to subject potential antidiabetic phytochemicals to toxicity testing to ensure the development of safe and efficacious phytomedicines.

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