Int J Pharma Res Health Sci. 2018; 6 (2): 2475-79
DOI:10.21276/ijprhs.2018. 2018. 02.24
Packialakshmi and Loganayagi
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Antihyperglycemic Activity of *Agaricus bisporus* Mushroom Extracts on Alloxan Induced Diabetic Rats

Packialakshmi Balakrishnan [1, 2, *], Loganayagi C T [2]

[1]Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore - 641029, Tamilnadu, India.
[2]Department of Biochemistry, J.J. College of Arts and Science, Pudukkottai - 622404, Tamil Nadu, India,

ARTICLE INFO

Received: 17 Apr 2018
Accepted: 27 Apr 2018

ABSTRACT

Mushrooms have been used for traditional foods and medicines in Asia. The aim of the present study was to evaluate the hypoglycemic effect of ethanol (ABEE) and methanol (ABME) extracts from the fruiting bodies of *Agaricus bisporus* (*A. bisporus*) on alloxan-induced diabetic rats. Serum glucose, protein and liver glycogen levels were measured. Lipid profile (Total cholesterol (TC), triglycerides (TG) and phospholipid (PL)) and marker enzymes (Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH)) were evaluated in serum and liver tissues of control and other experimental groups. The alloxan induced diabetic rats showed significant ($P<0.05$) increase in serum glucose, marker enzymes and lipid levels and decrease in serum protein and liver glycogen content when compared with normal control rats. Oral administration of ABEE and ABME to diabetic rats for 30 days significantly restored the levels of serum glucose, protein, lipids, marker enzymes and liver glycogen. ABEE (200 mg/kg b.wt) and ABME (200 mg/kg b.wt) are useful in controlling the blood glucose level, improve the lipid metabolism and is beneficial in preventing diabetic complications from lipid peroxidation and antioxidant systems in experimental diabetic rats. Among these two *A. bisporus* extracts, ethanol extract gave best result. Thus, these results suggest that *A. bisporus* mushroom could be useful for the prevention of diabetes.

Keywords: *A. bisporus,* Alloxan, Diabetes, Lipid profile, Hepatic glycogen, Marker enzymes

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and associated with increased free-radical activity. It is the major cause of premature mortality [1]. It comprises a group of chronic disorders characterized by hyperglycemia or diminished insulin secretion, or both. It is a metabolic disturbance of carbohydrate, fat and protein metabolism which leads to elevation of both fasting and postprandial blood glucose levels [2]. Moreover,

Corresponding author *
Dr. B. Packialakshmi
Department of Biochemistry, Kongunadu Arts and Science College,Coimbatore - 641029, Tamilnadu, India.
E-mail: biochem_bagya@yahoo.co.in

hyperglycemia is an important contributing factor for diabetic complications especially cardiovascular diseases [3]. Prolonged hyperglycemia with diabetes mellitus leads to the formation of advanced glycosylated end products which are involved in the generation of reactive oxygen species; these free radicals cause lipid oxidation and play role in the production of secondary complications in diabetes mellitus.

Oxidative stress is believed to be a common pathway linking diverse mechanisms for the pathogenesis of complications of diabetes [4]. The goals of managing diabetes mellitus are to optimize the control of blood glucose, reduce the effects of oxidative stress, and normalize disturbances in lipid metabolism. Drug management of diabetes without associated untoward effect has also remained a challenge for conventional medical practice. In many parts of the world, edible mushroom is one of the most popular foods, not only for texture and flavour but also for their chemical and medicinal characteristics. In recent years, much attention has been paid to the investigation of natural hypoglycemic drugs from various edible mushrooms [5].

A. bisporus is the most common edible cultivated mushroom in Western countries and is also known as white button mushroom [6]. It is mainly used in traditional medicine for diabetes, hypercholesterolemia and hypertension. There are many different substances in *Agaricus* mushroom that have potential medicinal properties including beta glucan, laccase, lectin, ergosterol, sodium pyroglutamate, etc. The lectin from *Agaricus* mushroom has potent antiproliferative effects on human epithelial cancer cells, without any apparent cytotoxicity. The antioxidant and cytotoxic properties of this mushroom were reported in many studies [7]. Till now there are no studies that have specifically addressed the efficacy of *A. bisporus* in diabetes. Thus, the present investigation aims to study the hypoglycemic effect of ethanol and methanol extracts of *A. bisporus* on alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1. Extract Preparation

A. bisporus was collected from the local market of Pudukkottai, Tamilnadu, India. Fresh mushrooms were chosen and air-dried in an oven (40 °C) for 48 h. The dried fruiting bodies were powdered (20 mesh) and stored in air-tight plastic bags for further analysis.

To prepare ethanol extract, mushroom powder (10 g) was extracted by stirring with 100 ml of ethanol at 25 °C for 24 h and filtering through Whatman No.1 filter paper. The residue was then extracted with two additional 100 ml portions of ethanol. The combined extracts were then rotary evaporated at 40 °C to dryness.

To prepare methanol extract, mushroom powder (10 g) was extracted by stirring with 100 ml of methanol at 25 °C for 24 h under dark condition. After centrifugation at 5000 g for 20 min, the residues were re-extracted twice with methanol. The supernatants were pooled together and the combined extracts were evaporated under reduced pressure at 45 °C for 30 min using a vacuum rotary evaporator. The extract obtained was dissolved in methanol at 100 mg/ml and stored at 4 °C for further use. Analyses were carried out in triplicates.

2.2. Animals

Male albino rats, weighing about 100-150 g were obtained from Trichy. The animals were kept at a room temperature of 26 ± 2 °C and humidity of 56 % – 58 %. They were fed with pellet diet manufactured by Sai Durga feeds & foods, Pranav Agro industries Ltd., Bangalore, India and drinking water was made available a libitum.

2.3. Drugs and Chemicals

Alloxan monohydrate (Loba Chemie Ltd., Mumbai, India), one touch horizon glucometer (Johnson & Johnson Ltd., Mumbai, India), and D-glucose (Qualigens Fine Chemicals, Mumbai, India) were purchased from respective companies. All other chemicals used in this study were obtained commercially and were of analytical grade.

2.4. Induction of Diabetes

Diabetes was induced by an intraperitoneal injection of freshly prepared solution of alloxan monohydrate at a concentration of 150 mg/kg body weight dissolved in sterile normal saline. After 48 h of induction, diabetes was confirmed by testing the blood glucose level (>200 mg/dl) and also by testing the presence of sugar in urine (glucosuria) using glucose indicator sticks.

In the experiment, a total of 24 rats were used. The animals were divided into 4 groups of 6 animals each.

Group 1: Normal untreated rats.

Group 2: Alloxan induced diabetic rats.

Groups 3: Alloxan induced diabetic rats treated with ABEE (200 mg/kg b.wt/day) in distilled water for 30 days.

Groups 4: Alloxan induced diabetic rats treated with ABME (200 mg/kg b.wt/day) in distilled water for 30 days.

After 30 days of treatment, animals were sacrificed. Blood was collected and serum was separated from clotted blood by centrifugation at 2500 rpm for 15 min and biochemical investigations were done. The liver of the animals from all groups were excised for biochemical analysis.

2.5. Biochemical parameters

Serum glucose was estimated by ortho-toludine method [8]. Hepatic glycogen content was estimated by the method of [9]. Marker enzymes namely, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were measured by commercial kits (Span Diagnostic, Surat). Determination of protein was done by Biuret method using diagnostic kit supplied by Agappe diagnostics, Maharashtra. TC, TG and PL levels were measured using assay kits (Sigma Aldrich, Bangalore, India).

2.6. Statistical analysis

All data are represented as mean ± SD. One-way analysis of variance (ANOVA) followed by Duncan multiple range test was done to determine significant differences in all parameters. $P<0.05$ was considered significant.

3. RESULTS AND DISCUSSION

3.1. Effect of *A. bisporus* extracts on serum glucose

The mechanism by which alloxan brings about its diabetic state includes selective destruction of pancreatic β cells which make cells less active, leading to poor sensitivity of insulin for glucose uptake by tissues, causes hyperglycemia when compared with normal rats [10]. The levels of glucose in normal control and alloxan induced animals are depicted in Fig. 1. The diabetic rats showed significant (P<0.05) increase in the levels of glucose when compared with normal control rats. Oral administration of ABEE (200 mg/kg b.wt) and ABME (200 mg/kg b.wt) to diabetic rats showed significant decrease in the level of glucose when compared with normal rats which may be due to the insulin like effect of ABEE and ABME on peripheral tissues, either by inhibiting hepatic gluconeogenesis or by promoting glucose uptake and metabolism [11].

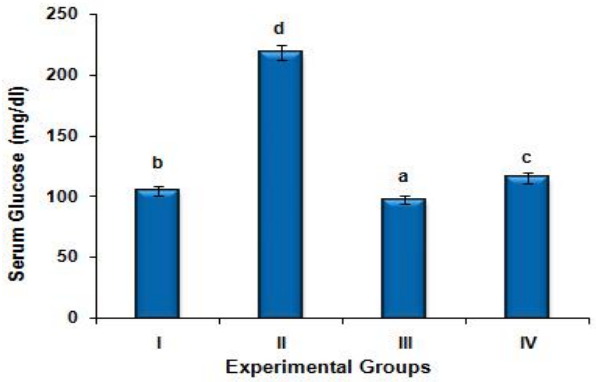

Fig 1: Effect of *A. bisporus* extracts on the levels of serum glucose in normal and alloxan induced diabetic rats. Values not sharing a common letter differ significantly at P<0.05 by DMRT.

3.2. Effect of *A. bisporus* extracts on serum protein

Fig. 2 shows the levels of serum protein in normal and alloxan induced rats. Rats induced with alloxan showed significant (P<0.05) decrease in serum protein compared to normal control rats which might be due to progressive proteinuria [12]. Treatment with ABEE (200 mg/kg b.wt) and ABME (200 mg/kg b.wt) to alloxan induced rats restored the biochemical changes to normal.

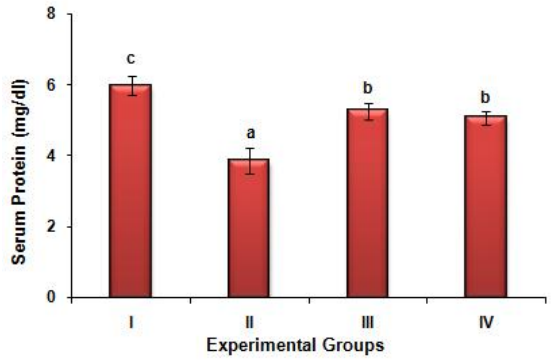

Fig 2: Effect of *A. bisporus* extracts on the levels of serum protein in normal and alloxan induced diabetic rats. Values not sharing a common letter differ significantly at P<0.05 by DMRT.

3.3. Effect of *A. bisporus* extracts on liver glycogen

Liver glycogen is considered as a best marker for assessing the hypoglycemic activity of any drug [13]. The peripheral free glucose is being stored in the liver in the form of glycogen by increasing glycogenesis. Diabetic rats showed decreased liver glycogen and treatment with ABEE (200 mg/kg b.wt) and ABME (200 mg/kg b.wt) increased hepatic glycogen content (Fig. 3). As a result of hepatic damage, the glycogen level in liver was significantly (P<0.05) reduced in diabetic animals which may be due to the lack of insulin in the diabetic state, which inturn inactivate glycogen synthase system. Restoration of hepatic glycogen by *A. bisporus* extracts could be due to insulin secretion or inhibition of glucose-6-phosphatase in the liver there by preventing conversion of glucose-6-phosphate to glucose.

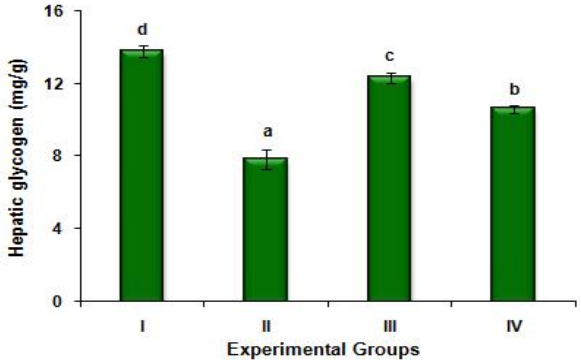

Fig 3: Effect of *A. bisporus* extracts on the levels of hepatic glycogen in normal and alloxan induced diabetic rats. Values not sharing a common letter differ significantly at P<0.05 by DMRT.

Table 1: Effect of *A. bisporus* extracts on the activities of marker enzymes in serum and liver of normal and alloxan induced diabetic rats.

Parameters	Normal control	Alloxan (150 mg/kg)	ABEE (200 mg/kg) + Alloxan	ABME (200 mg/kg) + Alloxan
Serum				
AST	53.68 ± 1.03^{a}	103.27 ± 1.39^{d}	58.17 ± 0.89^{b}	62.01 ± 1.81^{c}
ALT	81.22 ± 1.93^{a}	142.16 ± 2.28^{d}	88.42 ± 1.42^{b}	94.73 ± 2.73^{c}
LDH	132.4 ± 2.89^{a}	295.40 ± 5.96^{d}	143.50 ± 3.56^{b}	160.14 ± 5.13^{c}
ALP	53.59 ± 1.51^{a}	112.31 ± 1.84^{d}	61.93 ± 0.35^{c}	59.25 ± 2.81^{b}
Liver				
AST	19.72 ± 1.76^{d}	11.86 ± 1.01^{a}	17.19 ± 1.80^{c}	15.27 ± 0.85^{b}
ALT	21.14 ± 1.63^{d}	10.61 ± 1.72^{a}	20.79 ± 1.62^{c}	17.92 ± 1.62^{b}
LDH	23.72 ± 0.91^{d}	14.38 ± 1.70^{a}	18.29 ± 0.77^{b}	20.06 ± 0.96^{c}
ALP	18.67 ± 1.73^{d}	11.81 ± 0.08^{a}	17.67 ± 1.34^{c}	15.62 ± 1.21^{b}

[#]Values are expressed as mean ± SD, n=6. Values within the same row not sharing common superscript letters (a-d) differ significantly at P<0.05 by DMRT. Units: AST, ALT, LDH-µ mol of pyruvate liberated/min/L or mg protein; ALP-µ mol of phenol liberated/min/L or mg protein.

3.4. Effect of *A. bisporus* extracts on marker enzymes

Biomarker enzymes such as of AST, ALT, ALP and LDH were used in the evaluation of hepatic disorders [14]. As shown in Table 1, the activities of AST, ALT, ALP and LDH were found to be significantly (P<0.05) increased in serum and decreased in liver when compared to normal control rats. Administration of ABEE (200 mg/kg b.wt) and ABME (200 mg/kg b.wt) to diabetic rats resulted in significant restoration

of all the enzymes to near normal in serum and liver. An increase in the activities of serum AST, ALT, ALP and LDH in alloxan-induced diabetic rats substantiated the hepatic damage [15]. Diabetes causes cell damage by altering the cell membrane architecture which results in enhanced activities of marker enzymes in diabetic rats.

3.5. Effect of *A. bisporus* extracts on lipid profile

Hyperlipidemia is a known complication of diabetes mellitus [16] and coexists with hyperglycemia and is characterized by increased levels of cholesterol, triglycerides and phospholipids [17]. Table 2 presents the levels of cholesterol, triglycerides and phospholipids in serum and liver of control and experimental rats. Serum and liver of diabetic rats showed significant elevation ($P<0.05$) in the levels of cholesterol, triglycerides and phospholipids, when compared with normal rats. Oral administration of ABEE (200 mg/kg b.wt) and ABME (200 mg/kg b.wt) to diabetic rats significantly reversed all these changes to near normal levels. This hypolipidemic effect may be due to an increase in insulin secretion that ultimately led to a decrease in the synthesis of cholesterol and fatty acid [18].

Table 2: Effect of *A. bisporus* extracts on the levels of lipids in serum and liver of normal and alloxan induced diabetic rats.

Parameters	Normal control	Alloxan (150 mg/kg)	ABEE (200 mg/kg) + Alloxan	ABME (200 mg/kg) + Alloxan
Serum				
TC (mg/dl)	84.40 ± 1.65^{a}	161.62 ± 2.28^{d}	89.14 ± 1.67^{b}	92.3 ± 2.04^{c}
TG (mg/dl)	43.08 ± 2.72^{a}	140.69 ± 3.14^{c}	58.71 ± 2.35^{b}	58.93 ± 1.91^{b}
PL (mg/dl)	102.03 ± 2.01^{a}	183.04 ± 3.17^{d}	106.18 ± 1.59^{b}	112.15 ± 1.04^{c}
Liver				
TC (mg/g)	3.01 ± 0.09^{a}	6.43 ± 1.16^{c}	3.95 ± 0.12^{b}	4.16 ± 0.19^{b}
TG (mg/g)	2.94 ± 0.17^{a}	4.39 ± 0.11^{d}	3.23 ± 0.01^{c}	3.08 ± 0.16^{b}
PL (mg/g)	7.21 ± 0.73^{a}	11.51 ± 1.56^{b}	7.83 ± 0.29^{a}	8.02 ± 0.21^{a}

[#]Values are expressed as mean ± SD, n=6. Values within the same row not sharing common superscript letters (a-d) differ significantly at $P<0.05$ by DMRT.

4. CONCLUSION

In conclusion, the administration of ABEE (200 mg/kg b.wt) and ABME (200 mg/kg b.wt) to alloxan-induced diabetic rats possessed hypoglycemic effect and restored the activities of marker enzymes and other biochemical substances involved in the glucose metabolism. Even though both the extracts showed good hypoglycemic activity, ABEE exhibited better activity than ABME. These findings suggest that *A. bisporus* extracts has complimentary potency to develop an antihyperglycemic agent for the treatment of diabetes mellitus.

5. REFERENCES

1. Guk CC, Conie C, Harris MI. Mortality in adults with and without diabetes in a national cohort of the US population. Diab Care 1998; 21: 1138-1145.
2. Sicree R, Shaw J, Zimmet P. Diabetes and impaired glucose tolerance. In: Gan D (ed) Diabetes atlas, 3rd edn. International Diabetes Federation, Brussels, 2006; 15-103.
3. Wu QP, Xiao C, Yang XB, Zhang J. Hypoglycemic effects of components extracted from edible and medicinal fungi and their mechanisms of action. Acta Edulis Fungi 2009; 16: 80-86.
4. Palanisamy UD, Ling LT, Manaharan, T, Appleton D. Rapid isolation of geranin from *Nephelium lappaceum rind* waste and its anti-hyperglycemic activity. Food Chem 2011; 127: 21-27.
5. Ye M, Qiu T, Peng Wei, Chen, WX, Ye, YW, Lin YR. Purification, characterization and hypoglycemic activity of extracellular polysaccharides from *Lachnum calyculiforme*. J Carb Polym 2011; 86: 285-290.
6. Chandra R, Pandey VN, Singh HB. Extract of white button mushroom (*Agaricus bisporus*) for bio-medicinal molecules. CIB Tech J Pharm Sci 2012; 1: 9.
7. Jagadish LK, Venkata Krishnan V, Shenbhagaraman R, Kaviyarasan V. Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J. E.Lange) Imbach before and after boiling. Afr J Biotechnol 2009; 8(4): 654-661.
8. Sasaki T, Matsy S, Sonae A. Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation. Rinshokagaku 1972; 1: 346-353.
9. Morales MA, Jabbagy AJ, Tarenzi HP. Mutations affecting accumulation of glycogen. Neurospora Newsletter 1973; 20: 24-25.
10. Gayathri M, Kannabiran K. Antidiabetic and ameliorative potential of *Ficus bengalensis* bark extract in streptozotocin induced diabetic rats. Ind J Clin Biochem 2008; 23: 394-400.
11. Saravanan G, Ponmurugan P, Senthil Kumar GP, Rajarajan T. Modulatory effect of S-allylcysteine on glucose metabolism in streptozotocin induced diabetic rats. J Func Foods 2009; 1: 336-340.
12. Latha RCR, Daisy P. Insulin secretagogue, antihyperlipidemic and other protective effects of gallic acid isolated from *Terminalia bellerica Roxb* in streptozotocin induced diabetic rats. J Chem Biol Interact 2011; 189: 112-118.
13. Stalmans W, Cadefau J, Wera S, Bollen M. New insight in to the liver glycogen metabolism by glucose. J Biochem Soc Transact 1997; 25: 19-25.
14. Ohaeri OC. Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats. J Biosci Rep 2001; 21: 19-24.
15. Navarro CM, Montilla PM, Martin A, Jimenez J, Utrilla PM. Free radicals scavenger and antihepatotoxic activity of *Rosmarinus tomentosus*. J Planta Medica 1993; 59: 312-314.
16. Shew WH, Jeng CY, Lee WJ, Lin SY, Pei D, Chen YT. Simvastatin treatment in postprandial hypertriglyceridemia in type 2 diabetes mellitus patients

with combined hyperlipidemia. J Metab 2001; 50: 355-359.
17. Bagdade JD, Helve E, Taskinen MR. Effect of continuous insulin infusion therapy lipoprotein surface and core lipid composition in IDDM. J Metab 1991; 40: 445-449.
18. Bopanna KN, Kannan J, Sushma G, Balaraman R, Rathod SP. Antidiabetic and antihyperlipaemic effects of neem seed kernel powder on alloxan diabetic rabbits. Ind J Pharmacol 1997; 29: 162-167.

Conflict of Interest: None

Source of Funding: Nil