ISSN: 2577 - 8005

Research Article

Medical & Clinical Research

Invitro Cytotoxicity and Glucose Uptake Activity of Mushroom Pleurotus Eous in Methanol Extract Using L-6 Cell Line

K Shoba[1] and S Krishnakumari[2*]

[1]Department of Biochemistry, New Prince ShriBhavani Arts and Science College, Chennai-600100 Tamilnadu, India

[2]Department of Biochemistry, Kongunadu Arts and Science College (Autonomous), Coimbatore-641029, Tamilnadu, India

[*]Corresponding author
Dr. S. Krishnakumari, Department of Biochemistry, Kongunadu Arts and Science College (Autonomous), Coimbatore-641029, Tamilnadu, India.
E-mail: shoba_ajj@rediffmail.com

Submitted: 04 Dec 2018; **Accepted**: 12 Dec 2018; **Published**: 07 Jan 2019

Citation: *K Shoba and S Krishnakumari. (2019). Invitro Cytotoxicity and Glucose Uptake Activity of Mushroom Pleurotus Eous in Methanol Extract Using L-6 Cell Line. Med.Clin.Res, 4(1), 1-3.*

Abstract

The aim of the present study is to evaluate the cytotoxicity and glucose uptake activity of methanolic extract of pleurotuseous mushroom using L6 cell lines. An MTT assay is a colorimetric assay based on assessing the cell metabolic activityThe results showed that the extracts did not confer any cytotoxicity. The effects were observed in treated cells up to 6.25 mg/ml extract as measured with MTT. Also the mushroom extracts shows better glucose uptake potential. The results were compared with Insulin a standard antidiabetic drug. Insulin enhance the glucose uptake upto 97.8%. The L6 cell lines enhance the glucose uptake by 57.4% at 65µg concentration.

Introduction

Mushrooms are an assemblage of fleshy macroscopic fungi [1, 2]. They possess a distinctive fruiting body that could be hypogeous or epigeous, large enough to be seen by naked eyes and to be picked by hands [3]. Mushrooms have been treasured all through the globe as food and medicine for thousands of years. In countries, such as China, India, Japan and Korea, medicinal mushrooms have a long history of use in traditional folk medicine for treatment of various diseases [4, 5]. Medicinal mushrooms are used as both nutritional and therapeutic foods.

Mushrooms also contain important micronutrients (vitamins) and non nutrients (phenolics), that contribute to antioxidant property which can be valuable as a dietary supplement in favor of the patients suffering from a majority of disease conditions like Alzheimer's disease, atherosclerosis, cancer, diabetes mellitus [6-10]. Mushrooms are known to contain compounds which help in proper functioning of the liver [25], pancreas and other endocrinal glands, thereby promoting formation of insulin and related hormones which ensure healthy metabolic functioning [11-13]. Diabetes is metabolic disease usually caused by a combination of hereditary and environmental factors, which result in hyperglycemia and other classical symptoms, especially polyuria, polydipsia, and polyphagia. Eventually, hyperglycemia leads to serious damage in blood vessels and the nerves as well as blurred vision and irritability. According to the World Health Organization (WHO), the number of people with diabetes will be doubled with in less than 30 years. Around 30 million people were characterized as diabetic in 1985 worldwide compared to around 171 million cases in 2010 and almost 377 million estimated in 2030. Drugs as well as medicinal food additives are necessary in advanced cases [14,15]. Quantification of cell viability and proliferation form the fundamental for numerous in vitro assays in response to external factors. An MTT assay is a colorimetric assay based on assessing the cell metabolic activity. L6cells represent a good model for glucose uptake because they have been used extensively to elucidate the mechanisms of glucose uptake in muscle. Hence the present study was aimed to screen the cytotoxicity of the methanolic extract of the mushroom pleurotuseous by MTT assay and to evaluate their glucose uptake in skeletal muscle cells (L-6 cell line).

Materials and Methods

Mushroom Extraction

The fresh fruiting bodies of the mushroom were shade dried and powdered in a mixer grinder. Extract of mushroom were prepared by using methanol solvents. Dried powder was weighed carefully and used for methanol extract preparation through soxhlet apparatus at respective temperature. The supernatant collected is used for further use.

Determination of Cytotoxicity in L-6 cell line

L6 (Rat muscle) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India.The assessment of cytotoxicity of pleurotuseousmushroom on L6 cells was done by the reduction of 3-(4,5- dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) to formazan. Cells were seeded in a 96-well plate (1x 10 cells/well), and left to attach on to the medium for 48-72hrs before being exposed to Pleurotus Eous Mushroom, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 hrs, add various concentrations of the samples in 0.1% DMSO for 24hrs at 5 % CO_2 incubator and View the images. Cells were incubated with MTT (20µl, 5mg/mL) in phosphate- buffered saline solution was added. After 4hrs incubation, 1ml of DMSO was added. Viable cells were determined by the absorbance at 540nm.

Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The effect of the samples on the proliferation of L6 cells was expressed as the % cell viability, using the following formula:

% cell viability = A540 of treated cells / A540 of control cells × 100%

Glucose uptake activity assay (L6 cell line)

L6 cells grown in 12-well plates were washed twice with serum-free MEM and incubated with 0.5 ml of the same medium at 37°C for 2 h. The cells were washed three times with KRP buffer and incubated with 0.9 ml KRP buffer at 37°C for 30 min. The sample concentrations (70, 140µg) were then added to the wells and incubated at 37°C for 20 min. Glucose uptake was initiated by the addition of 0.1 ml KRP buffer containing 0.037 mM/l 2-deoxy-D-[3H] glucose and 0.001 mmol/l glucose. After 15 min, the assay was terminated by washing the cells three times with cold PBS. The cells were lysed with 0.7 ml of 1% Triton X-l00 at 37ºC for 20 min. take the OD value at 417 nm.

Glucose uptake increase rate (%) = (As/A0) ×100.

Here, AS is the glucose uptake of samples of different concentrations and Aois the glucose uptake of control.

Table1: In vitro cytotoxic effect of pleurotuseous using MTT Assay

S.No	Concentration µg/ ml	Absorbance 540nm	% cell Viability
1	200	0.12	8.6
2	100	0.53	38.4
3	50	0.77	55.7
4	25	0.96	69.5
5	12.5	1.13	81.8
6	6.25	1.22	88.4
7	Control cells	1.38	100

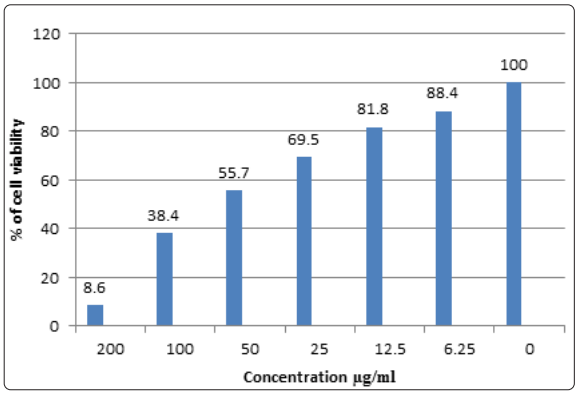

Figure 1: Cell viability assay on L 6 cell line of pleurotuseous mushroom

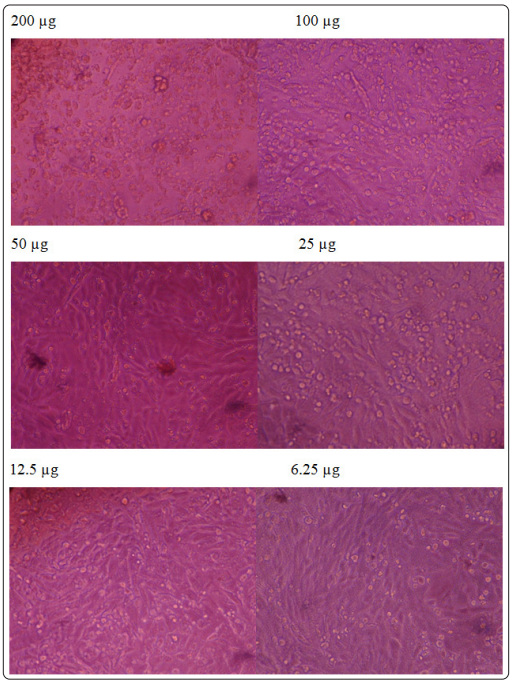

Figure 2: Images showing the effects of pleurotus eous mushroom on different con.c using L6 cell lines

L6 Control Cells

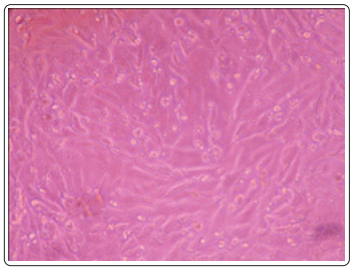

Table 2: in vitro glucose uptake studies in L6 cell line

Sample	% of glucose uptake assay			
Concentration(µg) Methanol extract	Control	Sample	Insulin	% of Glucose uptake
65 µg	-	0.81	-	57.4
32.5 µg	-	0.90	-	63.8
Insulin (1 µg)	-	-	1.38	97.8
control	1.41	-	-	100

Results and Discussion

The MTT assay based on the reduction of MTT (yellow colored) and other tetrazolium dyes depends upon cellular metabolic activities due to NAD (P) H-dependent cellular oxidoreductase enzymes (Figure 1). The healthy and rapidly growing cells exhibit high rates of MTT reduction to formazan while the dead or inactive cells fail to do so. The final product of MTT reduction is a purple color formazan that can be easily dissolved in DMSO. Viability in the MTT assay is connected with the quantification of formazan at 540 nm which is linearly associated with the enzyme activity and indirectly the number of viable cells. High purple color intensity

denotes higher cell viability while the decrease in purple color intensity signifies the reduced cell number and thus cytotoxicity of the given substance [16].

Methanol extract of pleurotuseous mushroom toxicity was tested in vitro in L6- cell following MTT as described in the materials and methods. Extract concentrations that kept at least 90% cell viability were considered as safe. (Table 1 & Figure 1) The results showed that the extracts did not confer any cytotoxicity. The effects were observed in treated cells up to 6.25 mg/ml extract as measured with MTT.Viable cells were determined by the absorbance at 540nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. IC 50 value of Methanol extract is 65 µg. L6 cell lines are used to determine the glucose uptake activity of Methanol extract of pleurotuseous mushroom and the results are presented in Table 2. The glucose utilization in L6 cell lines showed that the gymnemic acid fractions of Gymnemasylvestre were found to be prominent over control. The L6 cell lines enhance the glucose uptake by 57.4% at 65 µg/ml concentration. These results were compared with insulin which were used as the standard antidiabetic drugs. Insulin at a concentration of 1IU/ml at a concentration of 97.8µg/ml were found to enhance the glucose uptake over control. Hence, it can be concluded that the Methanol extract of pleurotuseous mushroom is found to be nontoxic and safe and also may be effective in glucose uptake.

Conclusion

Here experimental studies of Methanol extract of pleurotuseous mushroom exhibited considerable antidiabetic activity and low cytotoxicity on L6 cell lines. Also the mushroom extracts shows better glucose uptake potential.The study found that the invitro results paved the way for in vivo studies.

Conflict of Interests

There is no conflict of interests regarding the publication of this paper.

References

1. Lindequist U, Niedermeyer TH, Julich WD (2005) the pharmacological potential of mushrooms.Evid Based Complement Alternat Med 2: 285-299.
2. Badole SL, Patel NM, Thakurdesai PA, Bodhankar SL (2008) Interaction of Aqueous Extract of Pleurotuspulmonarius (Fr.) Quel-Champ. With Glyburide in Alloxan Induced Diabetic Mice. Evid Based Complement Alternat Med 5: 159-164.
3. Chang ST, Miles PG (1992) Mushrooms biology-a new discipline. Mycologist 6: 64-65.
4. Silva DD, Rapior S, Hyde KD, Bahkali AH (2012) Medicinal mushrooms in prevention and control of diabetes mellitus. Fungal Diversity 56: 1-29.
5. Lee KH, Morris-Natschke SL, Yang X, Huang R, Zhou T, et al. (2012) Recent progress of research on medicinal mushrooms, foods, and other herbal products used in traditional Chinese medicine. J Tradit Complement Med 2: 84-95.
6. Rai M, Acharya K (2012) Evaluation of antioxidant and nitric oxide synthase activation properties of Volvariellavolvacea. Int J Pharmacy Pharmaceutical Sci 4: 460-463.
7. Acharya K, Rai M (2013) Proximate composition, free radical scavenging and NOS activation properties of a wild edible mushroom. Int J Pharmacy Pharmaceutical Sci 5: 67-72.
8. Chatterjee S, Datta R, Dey A, Pradhan P, Acharya K (2012) In vivo Hepatoprotective Activity of Ethanolic Extract of Russulaalbonigraagainst Carbon Tetrachloride-Induced Hepatotoxicity in Mice.Res J Pharmacy Technol 5: 1034 -1038.
9. Rai M, Acharya K (2012) Proximate composition, frees radical scavenging and NOS activation properties of Ramariaaurea. Res J Pharmacy Technol 5: 1421-1427.
10. Chatterjee S, Biswas G, Basu SK, Acharya K (2011) Antineoplastic effect of mushrooms: a review. Aust J Crop Sci 5: 904-911.
11. Wasser SP, Weis AL (1999) Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives. Int J Med Mushr 1: 31-62.
12. Zhang HN, Lin ZB (2004) Hypoglycemic effect of Ganodermalucidumpolysaccharides. ActaPharmacol Sin, 25: 191-195.
13. Chen J, Mao D, Yong Y, Li J, Wei H, et al. (2012) Hepatoprotective and hypolipidemic effects of water-soluble polysaccharidic extract of Pleurotuseryngii. Food Chem 130: 687-694.
14. Wild S, Roglic G, Green A, Sicree R, King H (2004) Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 27: 1047-1053.
15. Kadan S, Saad B, Sasson Y, Zaid H (2013) In vitro evaluations of cytotoxicity of eight antidiabetic medicinal plants and their effect on GLUT4 translocation. Evidence Based Complementary and Alternative Medicine 2013: 549345.
16. Berridge MV, Herst PM, Tan AS (2005) Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. Biotechnol Annu Rev 11: 127-152.