Evaluation of Protective Effect of *Pleurotus ostreatus* Against Insulin Producing RINm5F Cells

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Abstract

Determination of antidiabetic activity by cell line method is one of the foremost techniques compared to in vivo animal model. It is fruitful in determining the mechanism of drugs. The RINm5F cells are considered to produce insulin and it is one of most widely used insulin-secreting cell lines. In the present study, we assessed the defensive property of *Pleurotus ostreatus* against RINm5F cells. The apoptosis of RINm5F cells were determined by providing the cell with ethanol extract of *P. ostreatus* in presence of streptozotocin (STZ). The STZ treated group significantly increases the apoptotic population compared to the normal group. The extract treated RINm5F cells manifested the decline in apoptotic population resembled to STZ treated RIN cells. The present study scientifically supported the protective effects of the *P. ostreatus* against the oxidative damage induced by STZ.

1 Introduction

Diabetes is a perplexing metabolic disorder which is distinguished by a disruption in the homeostasis amidst the restraint of glucose levels and insulin sensitivity¹. The diabetes mellitus has been perceived as a prospering world-wide prevailing by many health promotion groups, including the World Health Organization (WHO)². It is assessed that by 2030 more than 552 million people will be pretended from the diabetes. Diabetes is a known chief peril factor for cardiovascular disease, metabolic syndrome, dyslipidemia, and end-stage renal disease³. The insulin is concealed by pancreatic β -cells, and it is present in pancreatic islets of Langerhans. Insulin is a vital hormone with a prominent role in the expansion and evolvement of tissues and the restraint of glucose homeostasis⁴. The free radical produce by oxidative stress is conception to be a serious imperil factor in the inception and succession of diabetes. Free radical exerts toxic effects on β -cell, resulting in β -cell dysfunction and eventually apoptosis. This leads to decline in β -cell and/or total β -cell mass, and consequently causing insulin deficiency. Moreover, evidence from both human disease and mouse models have shown that apoptosis is the main form of β -cell death in diabetes mellitus⁵. The restorative advents intended to detention β -cell apoptosis by modifying the equilibrium between offending and defending mechanism(s), may be of relevance in the management of diabetes.

In comparison with other tissues, pancreatic β -cells show an ignoble enzymatic antioxidant defence status. Overexpression of the antioxidant enzymes escorted insulin-producing cells against chemical sources of oxygen free radicals⁶. Mushrooms accumulate a wide variety of secondary metabolites including phenolic compounds. Phenolics are one of the major groups of non-essential dietary components that have been associated with the inhibition of atherosclerosis, cancer and management of diabetes.

Today molecular biology techniques furnish the prospect to engineer new pancreatic β -cell lines that occupy numerous characteristics of normal insulin-secreting cells. The RINm5F cells are considered to produce insulin and it is one of most widely used insulin-secreting cell lines. The RINm5F cell contains mainly insulin and small amounts of glucagon and somatostatin⁷. Therapeutic approaches designed to arrest β -cell apoptosis by modifying the equilibrium between offending and defending mechanism(s), may be of significance in the management of type 2 diabetes. In the present study, we evaluated the protective effect of *P. ostreatus* against RINm5F cells.

2 Materials and Method

2.1 Cell culture

A rat insulinoma cell line (RINm5F) was procured from NCL, Pune and maintained at 37°C under a humidified, 5% CO₂ atmosphere in

RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 units/ml of penicillin, and 100 µg/ml of streptomycin, and the medium was changed every two days. Cells from passages 20–60 were used.

2.2 Plant material

The *P. ostreatus* was collected from the different area of Bastar and Sarjuga district. The collected material was authenticated by Dr. A.P. Singh, Principal Scientist, Department of Agronomy College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). The material was dried under shade, powdered mechanically and stored in air tight container.

2.3 Preparation of extracts

The powder of the *P. ostreatus* was extracted successively with petroleum ether, ethyl acetate and ethanol by cold maceration method. The extract was filtered and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely, and later dried in a desiccator. The ethanol extract of *P. ostreatus* was kept in air tight container for further study.

2.4 Measurement of apoptosis

The degree of apoptosis was determined using Propidium iodide (PI) stain which intercalates into double stranded nucleic acids that can be excited by 488 nm lasers. The cells, subjected to treatment were lysed using a lysis buffer containing Tris HCl (100 mM), NaCl (154 mM), CaCl₂ (1 mM), MgCl₂ (0.5 mM) and 0.1% NP 40. Propidium iodide (50 µg/ml) was added to the lysing solution to stain the cells. RNase (40 µg/ml) was also added, as PI is known to intercalate with RNA. The lysing solution containing the cells was then incubated in the dark at room temperature for 30 minutes and the fluorescent intensity was measured using a flow cytometer⁸.

3 Results

3.1 Effect on apoptosis

The RINm5F cells were treated the ethanol extract of *P. ostreatus* in presence of STZ. The fluorescent intensity was measured using a FAC Scan after treating with PI staining. Table 1 revealed that STZ treated group significantly increases the apoptotic population compared to the normal group. The extract treated RINm5F cells manifested the decline in apoptotic population resembled to STZ treated RIN cells. The extract offered significant protection at 25 µg/ml, while the Glibenclamide at a dose of 1 µg/ml provoked noteworthy protection of RINm5F cells against STZ induced damage.

Table 1 Effect of extract on apoptosis induced by STZ in RINm5F cells

Group	% Cells Apoptosis
Cell control (Normal)	10.12±0.12
Cell + STZ	28.36±0.52 ^a
Cells + STZ + Extract (2.5 µg/ml)	19.72±0.39
Cells + STZ + Extract (5 µg/ml)	17.63±0.42
Cells + STZ + Extract (10 µg/ml)	16.24±0.19
Cells + STZ + Extract (25 µg/ml)	14.59±0.23 [*]
Cells + STZ + Extract (50 µg/ml)	12.84±0.72 [*]
Cell + STZ + Glibenclamide (1 µg/ml)	13.29±0.54 [*]

All values are expressive of Mean ± SD, ^ap < 0.05 as compared to Untreated cells, ^{*}p < 0.05 as compared to STZ treated cells

5 Discussions

Diabetes mellitus is a chronic disease characterizes high blood-glucose levels resulting deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin^{9,10}.

The STZ is generally used to induce hyperglycemia in rats. Ramachandran *et al.* (2011) documented that STZ leads to alkylation of pancreatic deoxyribonucleic acid by penetrating to the β-cell via glucose transporter 2 and incites stimulation of poly (ADP ribosylation) that results in reduction of cellular nicotinamide adenine dinucleotide (NAD⁺) and adenosine triphosphate (ATP). Enhanced ATP dephosphorylation provides the substrate for xanthine oxidase. Consequently, production of free radicals leads to severe necrosis of pancreatic β-cells^{11,12}.

RINm5F cell line is insulin releasing pancreatic beta cell line extensively applied to check the antidiabetic activity of plants and β-cell dysfunction. The RINm5F are susceptible to free radicals causing to cell apoptosis, and resulting in reduction of insulin secretion. In present study, STZ treated RINm5F cell significantly increased the apoptosis of cell and decrease insulin level compared to untreated RINm5F cell i.e. normal group. The apoptosis of cell indicates the acute necrosis of pancreatic β-cells. It is documented that DNA fragmentation is a distinctive method of apoptotic cell death in different cell types, comprising β-cell lines.

The extracts treated RINm5F cell exhibited the decrease in apoptosis of cell compared to STZ treated cells. It indicates that the extracts containing antioxidant component, which neutralize the free radical produces by STZ, and maintains the cell integrity.

Moreover, the effect of extract on insulin secretion was also evaluated. It is reported that the damaged pancreatic beta cells lose their capacity to secrete insulin. The results of insulin secretion exhibits the decrease in insulin secretion of STZ treated RINm5F compared to normal group cells. It confirms the DNA of RINm5F were damage, and leading apoptosis of cells. The extract treated RINm5F cells enhanced the insulin secretion compared to STZ treated cell. This justifies the protective effects of extract against STZ free radicals. The antiapoptotic property of extract of *P. ostreatus* can help in protecting the β -cell mass and maintaining the insulin secretion of β -cell. Hence the *P. ostreatus* may produce beneficial effect against diabetes through their antiapoptotic actions.

6 Conclusion

In conclusion, the extracts of *P. ostreatus* exhibited reduction in apoptosis of RINm5F cells against STZ treated cells. The present study scientifically supported the protective effects of the *P. ostreatus* against the oxidative damage induced by STZ. This indicates that the possible mechanism for antidiabetes property of mushroom is due to its antiapoptotic action. Our finding suggests that *P. ostreatus* may be a potential therapeutic option for diabetes.

7 Acknowledgments

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