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OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*) ETHANOLIC EXTRACT PROTECTS YEAST CELLS FROM HYPER- AND HYPO-GLYCEMIC STRESS

Miroslava Požgajová[1], Marek Kovár[2], Alica Navrátilová[3], Veronika Fialková[1], Zuzana Chlebová[1]*

Address(es): Dr. Ing. Miroslava Požgajová,
[1] AgroBioTech Research Center, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976 Nitra, Slovakia, miroslava.pozgajova@uniag.sk.
[2] Institute of Plant and Environmental Science, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976 Nitra, Slovakia, marek.kovar@uniag.sk.
[3] Institute of Nutrition and Genomics, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976 Nitra, Slovakia, alica.navratilova@uniag.sk.

*Corresponding author: miroslava.pozgajova@uniag.sk

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ABSTRACT

Glucose is the primary source of carbon and energy. Cells possess a sophisticated mechanism for sensing glucose and responding to it appropriately. However, hyperglycemia represents the predominant risk factor for the development of diabetes. On the other hand, nutrient starvation, including glucose, may lead to the development of malnutrition. Noteworthy, glucose restriction has been associated with significantly delayed aging. Thus, controlled glucose intake is vital for healthy living. The presented study shows the effect of the oyster mushroom *Pleurotus ostreatus* ethanolic extract on fission yeast *Schizosaccharomyces pombe* undergoing glucose-derived stress.
Fruiting bodies of *P. ostreatus* were used for ethanolic extraction and its anti-hyper- and hypo-glycemic stress activity was investigated. Compared were cells treated with ethanolic extract of *P. ostreatus* (1 or 2%) with untreated control. Different glucose concentrations in growth media were used to analyze its impact on growth intensity, generation time (gt), metabolic activity, ROS generation, MDA content, and cell death and apoptosis of *S. pombe* cells.
Glucose deprivation or its over-supplementation resulted in growth retardation and prolonged cell doubling time (gt) which was significantly adjusted by the oyster mushroom extract. Interestingly, oyster mushroom extract reduced excessive ROS and formed MDA that were produced as a consequence of glycemic stress. Unexpectedly, the extract was able to increase the metabolic activity of cells supplemented with high glucose content and thereby protected them from death.
Taken together, our study suggests that growth media supplementation with the ethanolic extract of *P. ostreatus* protects *S. pombe* cells from glucose-mediated stress through alleviation of oxidative stress and enhancement of metabolic activity.

Keywords: *Pleurotus ostreatus*, *Schizosaccharomyces pombe*, glucose, stress, ROS, MDA, metabolic activity

INTRODUCTION

Glucose is the dominant metabolic substrate which also serves as a signaling molecule as it is involved in the regulation of various physiological and pathological processes. It is the fundamental carbon and energy source for most cell types, including unicellular microorganisms or higher eukaryotic cells. For mammals, maintenance of constant glucose level in the bloodstream is crucial as the impaired regulation of blood glucose concentrations leads to severe diseases such as diabetes (**Kim *et al.*, 2013**). Glucose is not able to freely diffuse through the cytoplasmic membrane, thou it is taken up by the cells and metabolized to drive various energy-consuming cellular processes required for cell viability. In addition, glucose regulates the expression of genes predominantly those that encode proteins of the glycolytic and respiratory pathways, stress response, utilization of glucose or alternative carbon sources, or those that influence cell growth (**Yilmazer *et al.*, 2021**). Manipulating the glucose concentration in the diet or growth media has multiple implications (**Tarhan & Çakır, 2021**). Elevated glucose shortened lifespan in *Caenorhabditis elegans* through downregulation of AMPK, FOXO, and glyoxalase, while in *Saccharomyces cerevisiae* reduction of glucose levels from 2% to 0.1% induced transient cell cycle delay followed by cell adaptation to low-glucose and prolonged yeast lifespan up to 50% (**Lee *et al.*, 2009; Saitoh & Yanagida, 2014; Jiang *et al.*, 2000**). However, an increase in the glucose concentration resulted in superoxide ion production, leading to DNA replication stress and growth arrest or apoptosis of *S. cerevisiae* cells (**Ruckenstuhl *et al.*, 2010**). Notably, exposure of stationary yeast cultures to pure glucose solution, in the absence of additional nutrients, led to cell death (**Granot & Snyder, 1991**). A variety of substances with medicinal properties have been used to regulate the impact of hypo- and hyper-glycemic stress. Mushrooms, owing not only to their nutritional but also to their medicinal value, have been used for thousands of years. Modern research confirms that the therapeutic effect of traditionally used species as fungal polysaccharides is associated with a positive effect on human health (**Vamanu, 2013; Hamad *et al.*, 2022**). In general, mushrooms are low in calories, fats, or sugars, and high in vegetable proteins, complex polysaccharides, phenols, vitamins, and minerals which predestinate them for a valuable healthy food. The major bioactive compounds of fungi, known as mycochemicals, mainly consist of polysaccharides (β-glucans), peptides, lectins, polyphenols, polyketides, proteins, terpenoids, and enzymes (**Carrasco-González *et al.*, 2017; Jayakumar *et al.*, 2009**). Oyster mushroom *Pleurotus ostreatus* is known to possess medicinal properties as it contains higher concentrations of cystine, methionine, and aspartic acid compared to other edible mushrooms. Moreover, lovastatin and its analogs that are highly abundant in oyster mushrooms are associated with its anti-hypercholesterolemic effect and hypoglycemic potential. Potent ROS scavenging abilities triggering inhibition of lipid peroxidation activities of oyster mushroom have also been described (**Ravi *et al.*, 2013; Asrafuzzaman *et al.*, 2018**). Although the acute and chronic hypoglycemic potential of the oyster mushroom has already been investigated in an animal model system, we were interested in its ability to regulate hypo- and hyper-glycemic stress in a single-celled model organism the fission yeast (*Schizosaccharomyces pombe*). *S. pombe* is a well-established unicellular eukaryotic model system for studies of cell-cycle regulation and checkpoint pathways (**D'Urso & Nurse, 1995**). Moreover, it has been used for studies focused on oxidative stress response (**Majumdar *et al.*, 2012; Navrátilová *et al.*, 2021**) and glucose metabolism (**Hoffman, 2005; Toyoda *et al.*, 2021**).
In this study, to investigate the effect of oyster mushroom on hypo- and hyper-glycemic stress, we used *S. pombe* as a model organism and exposed it to different glucose concentrations ranging from zero to 20%. Ethanol extract of dried fruiting bodies of *P. ostreatus* was applied to growth media and its effect on cell response to various glucose concentrations was investigated.

MATERIAL AND METHODS

Yeast cultivation and growth conditions

Schizosaccharomyces pombe strain *SP72 h+ ade6-M210 ura4-D18 leu 1-32* was used for all experiments. Cells were cultured in the complete YES liquid medium containing 0.5% yeast extract, 3% glucose, and amino acids as described by **Kovár *et al.* (2022).** Cells were grown under aerobic conditions at 30 °C, under 150 rpm shaking to obtain yeast culture in an exponential growth phase. The optimum growth temperature for *S. pombe* cells is 30 °C, thus this temperature was used throughout the experiments. Glucose-restrictive conditions were achieved by the use of media containing either 0 or 0.1% glucose, while for high glucose conditions growth medium contained 6, 10, and 20% glucose. If not stated differently, cells for the overnight (o/n) culture were seeded in Becher glass to 10 mL liquid YES medium for 14 hours, next day, the culture was diluted to OD_{600} = 0.3 and used for the analyses in four biological replicates.

Ethanol extract of oyster mushroom

Samples of *Pleurotus ostreatus* were obtained from university sources as described in the study of Golian et al. (**2022**). Oyster mushroom strain used throughout the study was chosen according to its β-glucan content that ranged from 46-56% and was assessed as high β -glucan content (Golian et al. 2022). Fruiting bodies were dried and homogenized to smooth powder of which 0.5 g was filled to 10 mL ethanol (96%). Extraction at laboratory temperature was performed for 24 hours under rotating conditions, followed by centrifugation at 980 x g for 10 min. The obtained extract was stored at 4°C.

Analyses of glucan content in *S. pombe* cells treated with *P. ostreatus* extract

Cells from the overnight culture were treated with 2% ethanolic extract of *P. ostreatus* or 2% ethanol as the respective control, for 1 hour at 30°C and 150 rpm shaking, while untreated cells were used as the formal control of the analysis. Afterwards, samples were washed by centrifugation of 2200 x g for 1 minute in deionized water and dried at 50°C for 48 hours. Dried pellets were used for glucan content analyses. The analytical kit β-Glucan Assay Kit (Yeast and Mushroom) with the product code: K-YBGL from the manufacturer Megazyme (Megazyme, Bray, Co. Wicklow, Ireland) was used to determine the β-glucan content in the samples. The methodology for determining β-glucan content, all devices used during the analyses and calculations of β-glucan values are described in more detail in the publication by Golian *et al*. (**2022**).

The content of glucans of *S. pombe* cells incubated with *P. ostreatus* ethanolic extract or ethanol, as a control, remained unchanged (Table 2).

Table 2 Content of glucans in *S. pombe* cells treated with *P. ostreatus* extract

Sample	total glucan (mg)	alpha glucan (mg)	Total glucan (% w/w)	Alpha glucan (% w/w)	Beta-glucan (% w/w)
Untreated cells	90.85	100.90	16.7456	0.1749	16.5707
2 % Et-OH	90.18	100.11	16.2676	0.1541	16.1135
2 % EXT Oyster mush.	91.37	103.56	16.3180	0.1470	16.1711
Megazyme control*	90.77	100.58	50.4394	0.7043	49.7351

Determination of growth intensity

Cells were grown overnight (o/n), next day washed in YES without glucose, diluted to OD_{600} = 0.3, and supplemented with glucose (Centralchem, Bratislava, Slovakia) to final concentration 0, 0.1, 3, 6, 10, and 20%. Yeast cells treated with 1 or 2% oyster mushroom ethanolic extract (extract) was referred as experimental group, control group contained 2 % ethanol in the growth medium. Samples were incubated at 30 °C and 150 rpm in 24 well plates. Optical density at 600 nm was measured every 3 hours within 9 hours of incubation and after 24 hours of incubation by Glomax Multi Detection System (Promega Corporation, Madison, WI, USA). The growth intensity, relative growth rate (RGR), and generation time (gt) are calculated as described by **Požgajová *et al.* (2020)**.

Cell extract preparation

Yeast samples from the o/n culture were washed with YES without glucose, diluted to OD_{600} = 0.3, and supplemented with the appropriate amount of glucose. In addition, 2% oyster mushroom ethanolic extract was added to the experimental group or 2% ethanol was added to the control group of cells and incubated for 1 hour. Afterwards, cells were centrifuged at 7850 x g for 90 s, washed and resuspended in PBS (pH 7.0). Cell homogenization was performed by sonication by the use of Digital Sonifier 450, Branson Ultrasonics Corp, Danbury, CT, USA. Sonication at 3× 30 s intervals (repeated 1s pulses followed by 1 sec pause) and power 80 W on ice caused rupture of cells. After 5 min centrifugation at 14000 x g and 4 °C, the obtained whole cell extract was used for further experiments.

Metabolic activity

The metabolic activity of yeast was determined according to **Kovár *et al.*, 2022**. Briefly, cells prepared in the same way as for the cell extract preparation were centrifugated 90 s at 10000 x g and washed with PBS (pH 7.0). Cells resuspended in 1 mL of 0.5% 2,3,5-triphenyl tetrazolium chloride (TTC) were incubated at 30 °C for 20 h without access to light. Red formazan was extracted by 1 mL of ethanol:acetone (2:1) solution shortly before cells were lysed by sonication. Metabolic activity was then calculated as relative units (r.u.) of absorbance (measured at 485nm) per mg protein.

Malondialdehyde (MDA) content

MDA content, the lipid peroxidation marker, was determined as previously described (**Kovár *et al.*, 2022**). Briefly, the TBA solution was mixed with the supernatant of each cell extract, boiled at 95 °C for 30 min, cooled rapidly on ice, and centrifuged at 7850 x g for one minute. MDA content (nmol.μg^{-1} protein) was calculated from the absorbance value detected at 532 and 600 nm, with the use of molar absorption coefficient 153 $mM^{-1}.cm^{-1}$.

Bradford assay

Protein concentration in each sample was determined according to Bradford **(1976)** at 600 nm using bovine serum albumin (Sigma-Aldrich, St. Louis, MO) as standard.

ROS generation

ROS formation was determined in the control and extract-treated yeast cells incubated with indicated glucose concentrations for 1 hour with OD_{600} adjusted to 1. Procedure was performed as previously described by **Kovár *et al.* (2022)**.

Determination of apoptosis and necrosis

After 1 h of incubation with indicated glucose concentrations with and without extract treatment, the evaluation of the yeast cells apoptosis and necrosis was performed according to **Kovár *et al.* (2022)**.

Statistical analysis

The Statistica software version 10 (StatSoft Inc., Tulsa, OK, USA) was used for statistical evaluation. Results obtained from the performed experiments are presented as the mean ± S.D. (standard deviation). Lavene's and Cochran's tests were used to evaluate the homogeneity and normal distribution of the experimental data. One- and two-way ANOVA tests (Duncan test and Fisher's LSD post hoc test) were used to evaluate the statistical significance of the obtained differences and were set up to $p < 0.05$ *, 0.01**, and 0.001 ***.

RESULTS AND DISCUSSION

Growth of cells under hypo- and hyper-glycemic stress

Standard growth conditions of *S. pombe* cells that involve a nutritionally rich medium containing 3% glucose with the optimal temperature of 30°C under aerobic conditions ensure exponential growth of the yeast. Glucose restriction decreases the growth rate of cells as 0.1% of glucose in the medium causes a marked reduction of the growth intensity, while a glucose-free medium hinders cell growth almost completely. A high glucose environment is also responsible for the delay in the growth intensity (Figure 1). Similarly, high glucose was reported to cause proliferation defects of human endothelial cells or gingival fibroblasts (**Chen *et al.*, 2007; Buranasin *et al.*, 2018**). Limited glucose conditions are associated with the delay in the cell cycle and glucose fasting results in ATP drop, metabolic deprivation, and quick loss of viability (**Pluskal *et al.*, 2011**). Ethanolic extract of *P. ostreatus* significantly improved the reduced growth of cells upon hyperglycemic conditions, while reduced growth caused by hypoglycemic

conditions was only slightly influenced by oyster mushroom extract and did not reach statistical significance.

This suggests the protective effect of oyster mushroom ethanolic extract against glucose-mediated disorders of cell proliferation. As glucose is involved in the regulation of gene transcription, variations in the glucose level are likely to cause changes in the gene transcription that result in a delay in cell division or quiescence. As the extract of *P. ostreatus* was shown to suppress the proliferation of breast and colon cancer cell lines through induction of tumor suppressor genes expression (**Jedinak & Sliva, 2008**), we assume that the addition of *P. ostreatus* ethanolic extract may, to some extent, revert glucose-altered gene expression leading to improvement of the division.

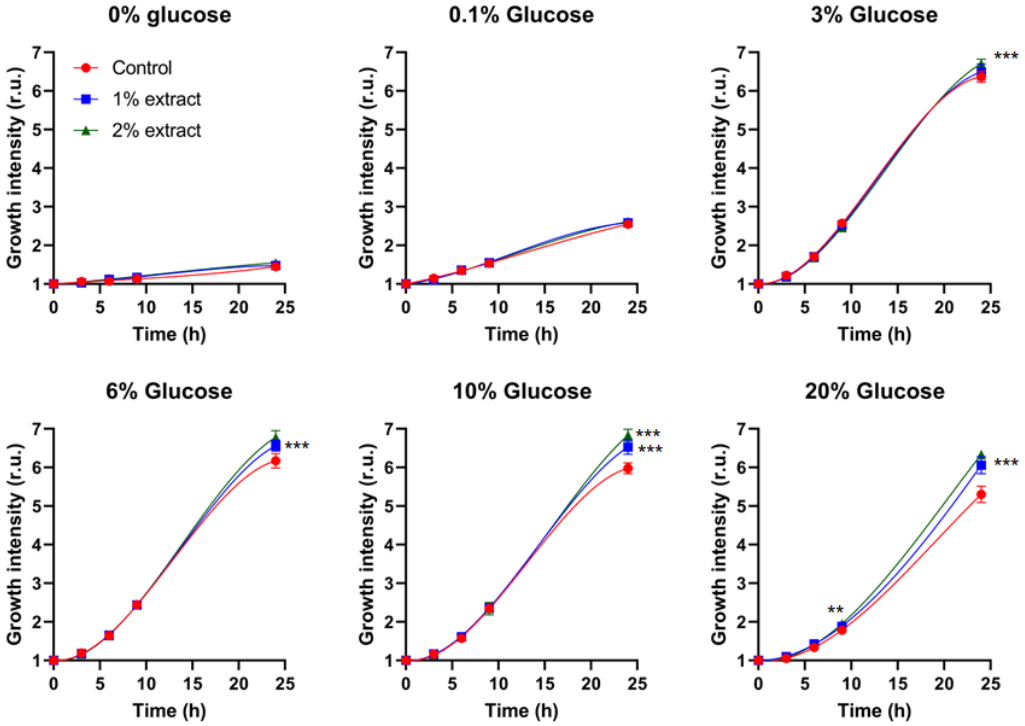

Figure 1 Growth intensity of *S. pombe* cells

Cells were incubated with indicated glucose concentrations for 3, 6, 9, and 24 hours with and without ethanolic extract of oyster mushroom. Growth intensity was calculated from cell density measurements at 600 nm. Each point indicates a mean value with a standard deviation (SD) of 8 biological replicates obtained from two individual experiments. Statistical differences are indicated as $p < 0.001$ ***.

Growth intensity largely depends on generation time which reflects the time required for cell doubling. Indeed, stress caused by low or high glucose concentration in the growth media resulted in the increase of cell doubling time, while the addition of 1 or 2% oyster mushroom extract decreased generation time significantly (Figure 2A). Relative growth rate (RGR) that represents cell gain every three hours of incubation is also influenced by glucose concentration. Low and high concentrations (10 and 20%) of glucose decrease gain of cells each third hour compared to the control. Cell treatment with ethanolic extract of *P. ostreatus* caused a slight but significant increase in reduced RGR (Figure 2B). High and low glucose conditions markedly affect the proliferation of most cells (**Horsophonphong *et al.*, 2020; Pluskal *et al.*, 2011**) due to variable glucose-mediated responses including alterations in gene expression, metabolic processes, or antioxidant status of the cell. This suggests that the positive effect of *P. ostreatus* extract on cell proliferation results from its collateral impact on the regulation of different essential cellular processes.

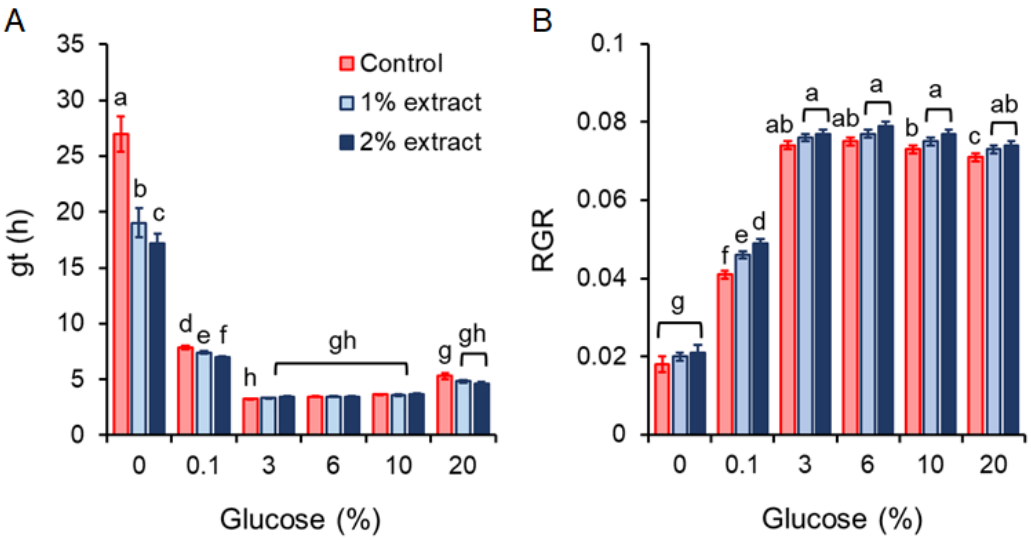

Figure 2 Generation time (gt) and relative growth rate (RGR) of cells exposed to low or high glucose

(A) Time required for cell doubling is represented as generation time and differs depending on the glucose concentration in the media. Addition of the *P. ostreatus* ethanolic extract significantly decreases generation time that is increased due to glucose restriction, while high glucose concentration affected gt only slightly. Depicted is gt after 9 hours of incubation as it represents intensive exponential vegetative growth of yeast cells. (B) Relative growth rate defines cell gain every third hour of incubation, the gain of cells between 6 and 9 hours of incubation is depicted. Low glucose conditions caused marked reduction of the cell growth that was improved by ethanolic extract addition. High glucose conditions led to slight, but significant reduction of the cell mass gain under 10 and 20% glucose in the media which increased by the addition of 1 and 2% *P. ostreatus* ethanolic extract. Individual bars represent mean ± SD of two independent experiments, each comprised of 4 biological replicates.. Statistical analysis was performed by Duncan's post-hoc test and different letters above bars indicate statistical significance at $p < 0.05$ level of significance.

ROS generation and MDA content in *S. pombe* cells undergoing hypo- and hyper-glycemic stress

To determine if a low or high glucose environment causes oxidative stress to the cell, ROS generation and MDA content were determined in yeast cells treated with different glucose concentrations. Additionally, the effect of *P. ostreatus* ethanolic extract was also evaluated. The formation of ROS was glucose concentration dependent. Interestingly, the ethanolic extract of *P. ostreatus* significantly reduced ROS generation under all glucose conditions (Figure 3A). MDA content as an indicator of oxidative stress revealed that high glucose conditions (10 and 20%) led to oxidative stress of *S. pombe* cells. Oyster mushroom extract protected yeast cells from oxidative stress as it significantly decreased raised MDA content (Figure 3B). Glucose accumulation in mutated yeast cells that are unable to metabolize glucose or display increased glucose uptake is associated with the decrease in life span of such cells that tightly correlates with an increased ROS production and the decrease in oxidative stress resistance (**Roux *et al*., 2009**). On the other hand, calories restriction by reduction of glucose levels in the yeast culture prolongs the life span and, similar to our data, leads to a reduction of oxidative stress and decreased ROS production (**Wei *et al*., 2008**). Moreover, according to **Palabiyik *et al*.** (**2012**) repression of glucose-mediated oxidative stress of *S. pombe* cells is predominantly associated with the glucose sensing/signaling processes rather than glucose metabolism and availability. Therefore, we assume that the positive effect of the *P. ostreatus* extract against glucose-induced oxidative stress is due to its influence on processes regulating glucose signaling rather than glucose sequestration. This is supported by the study of **Asrafuzzaman *et al.*** (**2018**) who revealed that oyster mushroom treatment results in the repression of hyperglycemia through the increase of p-AMPK levels and expression of GLUT4 in the muscle and adipose tissue of diabetic rats.

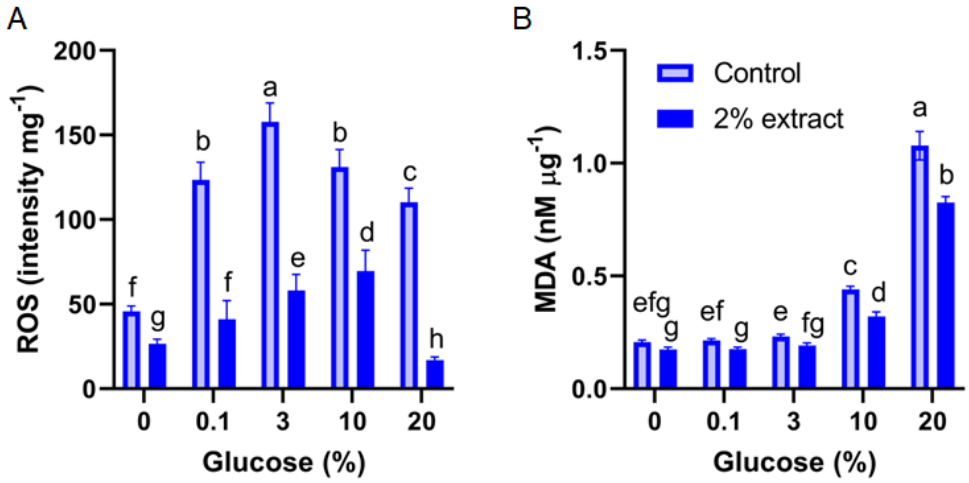

Figure 3 Determination of glucose-mediated oxidative stress
(A) ROS formation was analyzed by the use of H_2DCFDA which is converted to highly fluorescent DCF in the presence of ROS. Changes in the levels of fluorescence were detected and ROS formation was expressed in mg of protein after 1 hour of exposure. Ethanolic extract of *P. ostreatus* (2%) protected cells from the excessive ROS under all glucose conditions. (B) Malondialdehyde (MDA) is the end product of lipid peroxidation and is used as a marker of oxidative stress. Conditions of high glucose (10 and 20%) resulted in increased oxidative stress that was significantly reduced by *P. ostreatus* ethanolic extract. Individual bars represent the mean ± SD of 4 individual samples (biological replicates). Statistical analysis was performed by Duncan's post-hoc test and different letters above bars indicate statistical significance at 0.05 level of significance.

Metabolic activity of cells under different glucose conditions and the effect of *P. ostreatus* extract

The functionality of mitochondria is represented by metabolic activity of cells as mitochondria of viable and metabolically active cells reduce TTC to red colored formazan detectable at 485 nm. Changes in glucose concentrations negatively affected the metabolic activity of *S. pombe* cells, as both low and high concentrations of glucose led to a decrease of formazan formation revealing that metabolic activity is largely dependent not only on low but also high glucose concentration. Cell treatments with *P. ostreatus* ethanolic extract (2%) improved metabolic activity of cells incubated with 10% glucose, and control cells (3% glucose). The extract was, however, not able to improve the metabolic activity of cells grown on zero glucose and low glucose conditions and also cells with very high glucose (20%) suggesting that such conditions are detrimental for the cell (Figure 4). Reduction of tetrazolium salts to colored formazan products by metabolically active cells, a widely used method for assessment of cell viability, varies in the dependence of cell types, growth conditions, nutrition availability, and culture age. In agreement with our data, a decrease in D-glucose level in the culture medium is typically accompanied by a decrease in tetrazolium reduction (**Vistica *et al*., 1991**). Rat primary cardiomyocytes and yeast cells surrounded by high glucose levels display increased glucose metabolism, and increased metabolic intensity of trehalose, and glycerol, however, hyperglycemia was also associated with the increase in cell death (**Xu & Fang, 2021; Xie *et al*., 2022**) supporting our findings that high glucose stress negatively affects cell metabolic activity resulting in compromised vitality.

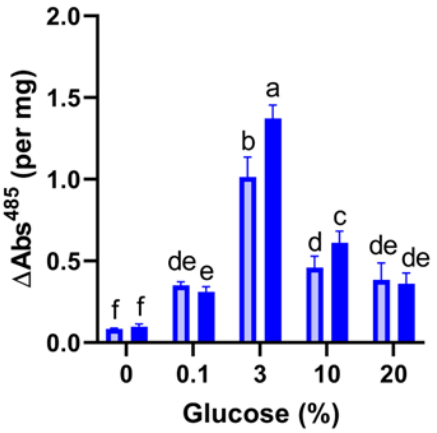

Figure 4 Determination of cell metabolic activity
Cell incubation with low or high glucose concentration for 1 hour decreases its metabolic activity compared to the control (3% glucose). Cell treatment with *P. ostreatus* ethanolic extract (2%) was able to improve the metabolic activity of cells incubated with 10% glucose and, interestingly, control cells incubated with 3% glucose. However, decreased metabolic activity of cells incubated under zero, 0.1 and 20% glucose conditions was not significantly affected by the addition of *P. ostreatus* ethanolic extract. Bars in the graph represent mean ± SD of 4 individual samples. Statistical analysis was performed by Duncan's post-hoc test and statistical significance is indicated as different letters above bars at $p < 0.05$ level of significance.

Cell death induced by variable glucose concentrations

Glucose deprivation of *S. pombe* cells resulted in the slight increase of dead cells caused by apoptosis as 3% of microscopically evaluated yeast cells showed apoptotic markers. On the other hand, high glucose conditions caused increase in

cell death by necrosis. Strikingly, ethanolic extract of *P. ostreatus* considerably protected cells from death caused by variabilities in glucose concentrations in the growth media (Figure 5A and B). In our study we have investigated early cell response to low and high glucose as the incubation time was set up, in accordance with the data showing ROS elevation and increased oxidative stress, to 1 hour. Cells depleted from glucose showed markers of apoptosis although only 3% of cells underwent apoptosis showing that the majority of cells survived awaiting better conditions for their growth. This is in agreement with the study of **Xie *et al.* (2022)** showing that glucose deprivation induces cell quiescence rather than death. On the other hand, high glucose conditions (100 mM) have been reported to induce primary necrosis in exponentially growing yeast cells (**Valiakhmetov *et al.*, 2019**). In consistence, our data indicate that *S. pombe* cells growing under nutritionally rich conditions exposed to high glucose conditions are more susceptible to necrotic cell death although only less than 3% of cells underwent necrosis. However, as cell type or differences in the experimental setup bring variable results of cell responses to hyperglycemic conditions (**Kageyama *et al.*, 2011; Kang *et al.*, 2020**), we assume that longer incubation period of the model organism under the glucose-stressed conditions or concomitant restriction in the nutrients in growth media could modify a portion of death cells either by apoptosis or necrosis. Nevertheless, *P. ostreatus* ethanolic extract significantly protected *S. pombe* cells from glucose stress suggesting its protective effect against hypo- or hyper-glycemic stress. Interestingly, similarly to *P. ostreatus* ethanolic extract (2%), other components such as emodin or quercetin, having biologically active properties, have been shown to possess protective effect against cellular disorders caused by hyperglycemia (**Ozyel *et al.*, 2021; Gao *et al.*, 2015**).

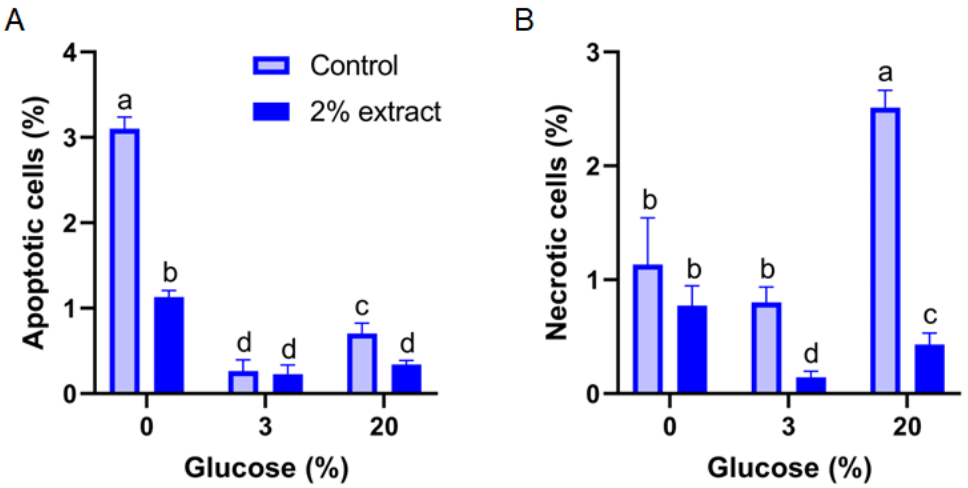

Figure 5 Cell death by apoptosis or necrosis
Cell incubation with zero glucose induces increase in the apoptotic cell death (A), while high glucose conditions lead to necrotic events (B). Ethanolic extract of *P. ostreatus* is capable to protect *S. pombe* cells from death under conditions of zero and high (20%) glucose. Individual bars represent mean ± SD of 4 experiments each representing percent of 100 evaluated cells. Statistical analysis was performed by Duncan's post-hoc test and different letters above bars indicate statistical significance at 0.05 level of significance.

CONCLUSION

Glucose plays a dominant role in the regulation of various physiological and pathological processes, hence changes in its concentration in the diet or growth media has multiple implications. In the presented study, we have evaluated the significance of variations in glucose concentration in the growth media for growth, metabolic activity, oxidative stress, and death of the yeast *Schizosaccharomyces pombe*. Oyster mushroom *Pleurotus ostreatus* is known for its health-protective and medicinal properties and is used as an important nutritional supplement. The effect of the ethanolic extract of *P. ostreatus* was thus analyzed in response to hypo- and hyper-glucose challenging conditions. Glucose restriction affected cell growth as it diminished the metabolic activity of the cell and increased cell death by apoptosis. High glucose conditions caused delay in cell growth and oxidative stress resulting in the increase of necrotic cell death. Ethanolic extract of *P. ostreatus* showed protective ability against glucose-mediated cellular disorders. Our data suggest that the extract of *P. ostreatus* might protect the organism from negative conditions caused by variations in glucose levels in the diet or growth media due to its collateral impact on the regulation of different essential cellular processes. To the best of our knowledge, this is the first study showing that the ethanolic extract of oyster mushroom alleviates hypo- and hyper-glycemic stress of *S. pombe* cells, thus the results of our study might serve as the basis for subsequent investigation.

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