EVALUATION OF ANTIOXIDANT ACTIVITY OF MAGNOLOL IN SACCHAROMYCES CEREVISIAE

SUBASRI SUBRAMANIYAN, MADHU DYAVAIAH*
Department of Biochemistry and Molecular Biology, Pondicherry University, Pondicherry 605014
Email: madhud14@yahoo.co.in

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ABSTRACT

Objective: To investigate the antioxidant activity of magnolol in Saccharomyces cerevisiae (S. cerevisiae) model.

Methods: Antioxidant activity of magnolol was demonstrated in S. cerevisiae using spot assay, colony forming unit (CFU) assay, detection of reactive oxygen species (ROS) by fluorescent microscopy and spectrofluorometer, lipid peroxidation (LPO) and catalase (CAT) assay.

Results: A dose-dependent increase in sensitivity of S. cerevisiae was observed with increase in hydrogen peroxide (H2O2) concentration. At 1.5 mmol concentration of H2O2, we observed 50 % of cell survivability in CFU and spot assay results. The sensitivity of S. cerevisiae to H2O2 was protected by magnolol treatment. In spot assay, magnolol pre-treatment showed the similar growth pattern as that of control and in CFU assay 75 % of survivability was observed. Fluorescence microscopic images and fluorescence intensity levels using 2’,7’-dichlorodihydrofluorescein diacetate (H2DCFDA) showed less number of fluorescence cells and 1.2 fold decrease in fluorescence intensity in magnolol pre-treated cells. The cellular protection in the cells pre-treated with magnolol followed by H2O2 was correlated with the decreased LPO and increase in CAT activity was recorded.

Conclusion: The result of the present study demonstrates that magnolol protects S. cerevisiae cells from H2O2 induced oxidant mediated cell death.

Keywords: Oxidative stress, ROS, Polyphenol, Magnolol, Antioxidant

INTRODUCTION

ROS mediated oxidative stress plays a significant role in human health and disease management. ROS are non-stable and highly reactive molecules produced as a by-product of cellular metabolism. These ROS cause damages to macromolecules like protein, lipid, and DNA. Alteration in these molecules are associated with many diseases such as cancer, neurodegenerative disease, cardiovascular disease, rheumatoid arthritis, and aging. Antioxidants produced naturally in the body helps to combat against these ROS. But in diseased condition these antioxidants are not sufficient to detoxify free radicals[1]. Plant phytochemicals possess antioxidant properties play a significant role as therapeutic agent to overcome the oxidative stress induced by ROS. Plant derived phytochemicals such as polyphenols, alkaloids flavanoids, terpenoids, and saponins are gaining much importance in many diseases such as cardiovascular diseases, neurodegenerative diseases and cancers.[2]. Polyphenols are the most abundant antioxidant phytochemical present in fruits, vegetables, red wine and legumes, coffee, and tea. These polyphenols act either by activating the antioxidant enzymes or by scavenging the free radicals. Magnolol is an active polyphenolic compound isolated from Magnolia officinalis (M. officinalis) a Chinese plant[3]. It possess various medicinal properties includes antioxidant, antiinflammatory and antimicrobial without any toxicity[4]. Antioxidant property of magnolol is due its chemical structural features, biphenyl structure substituted with an allyl group. In spite of extensive studies on the antioxidant property of magnolol in vitro, the molecular mechanism how it works in vivo remains unclear. Therefore, we investigated the antioxidant activity of magnolol in S. cerevisiae model. Yeast S. cerevisiae is the first eukaryotic organism whose genome has been completely sequenced. About 30 % of the human homolog genes were conserved in yeast. In this study, we demonstrated antioxidant activity of magnolol in S. cerevisiae model.

MATERIALS AND METHODS

The yeast S. cerevisiae (BY4741) (MATa his3Δ1: leu2Δmet15Δ:ura3Δ), was donated by T. Begley, CNSE, SUNY, Albany, USA. Yeast strain was routinely grown in YPD medium containing 2 % (w/v) bacteriological peptone, 1 % yeast extract, 2 % glucose. Magnolol was purchased from Sigma-Aldrich, USA. All other chemicals were purchased from Himedia, India.

Optimization of growth inhibitory concentration of H2O2 in S. cerevisiae

Spot assay

Exponentially growing (OD600 = 0.5) S. cerevisiae cells were 10-fold serially diluted for five times and spotted on YPD plates containing with or without different concentration of H2O2. After incubating at 30 °C for 3 d, plates were imaged using SYNGENE gel doc, USA.

CFU assay

Exponentially growing (OD600 = 0.5) cells were treated with different concentrations of H2O2. After 1 h of incubation in a shaker incubator at 30 °C, cells were serially diluted and spread on to YPD plates. After 3 d of incubation at 30 °C, the colonies were counted and the results were expressed as percentage of survival in relation to 100 % growth in untreated control from the average standard deviation of three independent experiments.

Antioxidant activity of magnolol

Spot assay

Exponentially growing yeast cells were treated with or without magnolol 20µM for 1 h and then spotted on to YPD plate containing with or without 1.5 mmol H2O2 [5].

CFU assay

Exponentially growing yeast cells were treated with or without magnolol for 1 h and then exposed to H2O2 for 1 h. Subsequently, cells were serially diluted and plated onto YPD plate and incubated for 3 d [5].

Detection of ROS level

Exponentially growing yeast cells were treated with or without magnolol for 1 h and then exposed to H2O2 for 1 h. Cells were
DCFDA staining was carried out to measure whether the treated and control (fig. 5). This result indicated and re-suspended in a solution containing 0.375 g TBA (thiobarbituric acid)-15 % TCA (tricarboxylic acid)-0.25 N Hcl (hydrochloric acid). The cell suspension was vortexed in presence of glass beads for 3 min, and washed with sorbitol buffer (pH 7.4) and incubated with H₂DCFDA (20 µM) for 30 min in dark at room temperature. Immediately after incubation, cells were harvested by centrifugation (5000 rpm for 5 min. at 4 °C), and washed thrice with PBS (phosphate buffered saline). The cells were observed under fluorescent microscope and fluorescence intensity was measured using a spectrofluorometer with an excitation wavelength of 485 nm and an emission wavelength of 525 nm at fluorescent microplate reader (Molecular Devices, USA) [5].

**LPO assay**

Yeast culture was centrifuged, the pellet was washed with PBS twice and pre-treated with or without magnolol for 1 h and then exposed for 10-15 cycles (30 s each), followed by 30 s of cooling. The mixture was then centrifuged and the supernatant was taken for CAT assay. CAT activity was determined spectrophotometrically by monitoring the disappearance of H₂O₂ at 240 nm. The CAT activity was expressed as nmol/mg of protein [8].

**Statistical analysis**

Results are reported as means±SD of three independent experiments. The values were analyzed by the one way anova (ANOVA). Values of p<0.05 were considered to be statistically significant.

**RESULTS**

**Optimization of H₂O₂ concentration to study the antioxidant activity of magnolol**

We exposed *S. cerevisiae* cells to a different concentration of H₂O₂. Spot assay results (fig. 1A) showed that the sensitivity of cells increased with increase in H₂O₂ concentration. Similar result was also observed in CFU assay where 50 % of survivability was recorded at 1.5 mmol H₂O₂ concentration. Therefore, we fixed the concentration at which fifty percent of the cell would survive on H₂O₂ treatment to study the antioxidant activity of magnolol.

**Antioxidant activity of magnolol**

The antioxidant activity of magnolol was tested in *S. cerevisiae* using spot and CFU assay. In the spot assay, control and magnolol alone treated followed the same growth pattern, whereas H₂O₂ treatment resulted in decreased cell survivability when compared to control. In contrast pre-treatment with magnolol protected the cells from H₂O₂ stress, and the spots appeared similar to that of control (fig. 2A).

CFU assay was performed to calculate the percentage protection conferred by magnolol on the survival of yeast cells against oxidative stress induced by H₂O₂. Yeast cells grown to the exponential phase and pre-treated with or without magnolol for 1 h and then exposed to H₂O₂ (1.5 mmol) for 1 h. The analysis of cell survivability in fig. 2B showed that the treatment with H₂O₂ showed decreased (50 %) survivability, whereas magnolol treatment showed increased (75 %) survivability compared to control.

**Magnolol scavenge ROS in *S. cerevisiae* cells**

H₂DCFDA staining was carried out to measure whether the accumulation of ROS induced by H₂O₂ and scavenged by magnolol. The fluorescent microscopic images showed that the increases in the number of green fluorescent cells in H₂O₂ treated culture were decreased when cells pre-treated with magnolol followed by H₂O₂ (fig. 3A). The results obtained by the spectrofluorometer (fig. 3B) showed more fluorescent intensity in H₂O₂ treated cells, whereas cells pre-incubated with magnolol showed a 1.2 fold decrease in fluorescence intensity compared to H₂O₂ alone treated cells.

**Magnolol reduce LPO in *S. cerevisiae***

Exposure of yeast cells to H₂O₂ showed increased LPO compared to control. In contrast, LPO was decreased significantly in the cells pre-treated with magnolol (fig. 4). The result indicated magnolol decreased the constitutive levels of lipid damages and protects cells from H₂O₂-induced oxidative stress.

**Magnolol induces CAT activity**

We measured the CAT activity to test whether the induction of enzymatic defence contributes to the protective effect of magnolol or it protects cells from scavenging ROS. The results showed that CAT activity was increased in the cells pre-treated with magnolol when compared to H₂O₂ treated and control (fig. 5). This result indicated magnolol induce CAT activity in addition to scavenging ROS directly to protect cells from oxidative stress.
Fig. 2: Effect of magnolol on the viability of *S. cerevisiae* cells. A) Spot assay B) Colony forming unit assay, data are mean±SD. of three independent experiments. *p<0.05

Fig. 3: Detection of ROS A) Fluorescent microscopy B) Spectrofluorometer Data are mean±SD. of three independent experiments. *p<0.05

Fig. 4: Effect of magnolol on lipid peroxidation, data are mean±SD. of three independent experiments. *p<0.05

Fig. 5: Effect of magnolol on catalase activity, data are mean±SD of three independent experiments. *p<0.05

**DISCUSSION**

ROS are toxic molecules produced during cellular metabolism and also induced by environmental chemicals and radiation. They act as signalling molecules and also control processes such as cell growth and development. Oxidative stress increases the level of ROS which causes damage to protein, lipid, and DNA that leads to many pathological diseases. To overcome this stress, the biological system has the well-developed antioxidant machinery to scavenge the free radicals. Cells have two antioxidant systems (i) enzymatic antioxidants such as superoxide dismutase (SOD), CAT, glutathione peroxidase (GPX), glutathione reductase (GR) and (ii) non-enzymatic antioxidants like ascorbic acid (AA), reduced glutathione (GSH), and α-tocopherol. There will be always equilibrium between oxidant and antioxidant. When ROS exceeds the levels, then cells are said to be under oxidative stress. Plant-derived antioxidants are of great interest to mitigate the oxidative stress induced damages in the cells. Plants are the potential source which help in the management of many diseases through its antioxidant property [9]. In this study, magnolol, a bisphenol compound from *M. officinalis* has been employed to study its antioxidant property in yeast model organism against *H₂O₂* treatment. *H₂O₂* is the most toxic free radical generator during cellular metabolism. It generates OH via Fenton reaction which is highly reactive and damage macromolecules. The results from *H₂O₂* sensitivity assay showed that 1.5 mmol *H₂O₂* killed 50% of yeast cells as shown in CFU assay results (fig. 1B). In the antioxidant protection assay, magnolol pre-treatment rescued the cells from *H₂O₂* induced sensitivity and showed better cell survivability compared to *H₂O₂* alone treated cells (fig. 2A and B). Magnolol pre-treatment reduces the ROS positive cells in *H₂O₂* treated cultures. These results suggest that the ROS scavenging ability of magnolol protected the cells from oxidative-mediated cell death in *S. cerevisiae*. Magnolol possesses allyl group, that proved to be an effective hydroxyl radical and superoxide radical scavenger [10]. The protective effect of magnolol correlates with the decrease in intracellular oxidation (fig. 2A and B) and lipid oxidation (fig. 4). Polyphenolic compounds are preferentially incorporated into membrane lipid bilayers and act as hydrogen donors, trapping free radicals and inhibiting the formation of lipid radicals. The decrease in LPO has been associated with hepatoprotective activity [11].
Magnolol has been reported to be 1000 times more potent than α-tocopherol in reducing the LPO [4] and 340 times inhibiting MDA formation in rat hepatic mitochondria[12]. It protected the sperm motility by decreasing LPO [13]. It also reduced the ROS accumulation in acrolein-induced oxidative stress [14]. Increasing H$_2$O$_2$ resistance accompanied with decrease of ROS in S. cerevisiae was demonstrated using various antioxidants such as propolis [15], lisoan G [16], ginkgo biloba leaf [17], resveratrol [18] and quercetin [19]. The antioxidant enzyme level reflects the anti-oxidation status in yeast. A significant increase in CAT activity in S. cerevisiae cells pre-treated with magnolol (fig. 5), suggesting that magnolol induce CAT activity and that could provide antioxidant protection in the cells against oxidative stress. Studies have shown that magnolol treatment increased the CAT and SOD activity in the Kunming mice [20]. Our study also suggests using yeast as a model organism to study the antioxidant property of a natural compound in vivo.

CONCLUSION

Magnolol achieved excellent protection against H$_2$O$_2$ mediated oxidative stress which can be implicated in aging and aging associated diseases in human. Yeast can be used as a prominent model to study antioxidant property of a natural compound.

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ABBREVIATION

S. cerevisiae-Saccharomyces cerevisiae, CFU-Colony forming unit, H$_2$O$_2$-Hydrogen peroxide, ROS-Reactive oxygen species, H$_2$DCFDA-2’,7’-Dichlorodihydrofluorescein diacetate, DCF-2’,7’-dichlorofluorescein, TBA-Thiobarbituric acid, TCA-Trichloroacetic acid, HCl-Hydrochloric acid, CAT-Catalase, SOD-Superoxide dismutase, LPO-Lipid peroxidation, MDA-malonaldehyde, GPX- Glutathione peroxidase, GR-Glutathione reductase, AA-Ascorbic acid, GSH-Reduced glutathione, PBS-Phosphate buffered saline, M. officinalis-Magnolia officinalis

AUTHORS CONTRIBUTIONS

SS and MD have designed the study. SS performed the experiments. MD and SS drafted and corrected the manuscript.

CONFLICT OF INTERESTS

We declare that we have no conflict of interests

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