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Original Article

EVALUATION OF ANTIOXIDANT ACTIVITY OF MAGNOLOL IN *SACCHAROMYCES CEREVISIAE*

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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 (H_2O_2) concentration. At 1.5 mmol concentration of H_2O_2, we observed 50 % of cell survivability in CFU and spot assay results. The sensitivity of *S. cerevisiae* to H_2O_2 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′-dichlorodihydrofluoresceindiacetate (H_2DCFDA) 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 H_2O_2 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 H_2O_2 induced oxidant mediated cell death.

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

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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, anticancer, 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 investigate 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) (MATα *his3*Δ1: *leu2Δ:met15Δ: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 H_2O_2 in *S. cerevisiae*

Spot assay

Exponentially growing (OD_{600}= 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 H_2O_2. After incubating at 30 °C for 3 d, plates were imaged using SYNGENE gel doc, USA.

CFU assay

Exponentially growing (OD_{600} = 0.5) cells were treated with different concentrations of H_2O_2. 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 H_2O_2 [5].

CFU assay

Exponentially growing yeast cells were treated with or without magnolol for 1 h and then exposed to H_2O_2 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 H_2O_2 for 1 h. Cells were

washed with sorbitol buffer (pH 7.4) and incubated with H_2DCFDA (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 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, short pulses of 1 min were used with 1 min intervals on ice. Extracts were centrifuged at 6000 rpm for 3 min, and the supernatant was incubated in a boiling water bath for 15 min. After cooling, the malonialdehyde (MDA) content was measured at 532 nm. Protein content was estimated using Bradford's method [6]. The levels of LPO were expressed as µM of MDA/mg protein [7].

CAT assay

The treatment and cell pelleting were similar as explained above. The cell extracts were prepared by suspending the cells in lysis buffer (50 mmol Tris, 150 mmol NaCl, 50 mmol EDTA, and 0.2 mmol phenylmethylsulfonyl fluoride pH 7.2) with glass beads, vortexed 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 H_2O_2 at 240 nm. The CAT activity was expressed as nmol/mg of protein [8].

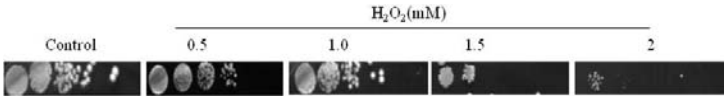
Statistical analysis

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.

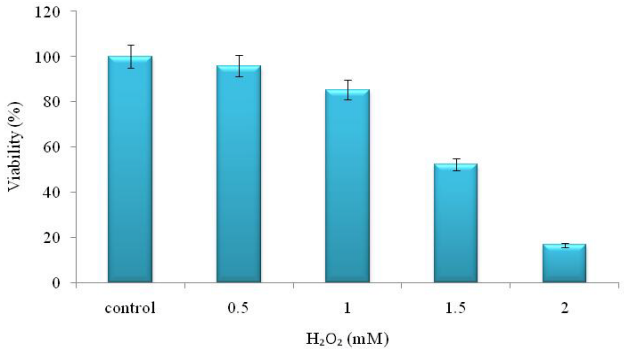
RESULTS

Optimization of H_2O_2 concentration to study the antioxidant activity of magnolol

We exposed *S. cerevisiae* cells to a different concentration of H_2O_2. Spot assay results (fig. 1A) showed that the sensitivity of cells increased with increase in H_2O_2 concentration. Similar result was also observed in CFU assay where 50 % of survivability was recorded at 1.5 mmol H_2O_2 concentration. Therefore, we fixed the concentration at which fifty percent of the cell would survive on H_2O_2 treatment to study the antioxidant activity of magnolol.



A.



B.

Fig. 1: Effect of H_2O_2-induced cell death on *S. cerevisiae* cells A) Spot assay B) Colony forming unit assay. Data are mean±SD of three independent experiments

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 H_2O_2 treatment resulted in decreased cell survivability when compared to control. In contrast pre-treatment with magnolol protected the cells from H_2O_2 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 H_2O_2. Yeast cells grown to the exponential phase and pre-treated with or without magnolol for 1 h and then exposed to H_2O_2 (1.5 mmol) for 1 h. The analysis of cell survivability in fig. 2B showed that the treatment with H_2O_2 showed decreased (50 %) survivability, whereas magnolol treatment showed increased (75 %) survivability compared to control.

Magnolol scavenge ROS in *S. cerevisiae* cells

H_2DCFDA staining was carried out to measure whether the accumulation of ROS induced by H_2O_2 and scavenged by magnolol. The fluorescent microscopic images showed that the increases in the number of green fluorescent cells in H_2O_2 treated culture were decreased when cells pre-treated with magnolol followed by H_2O_2 (fig. 3A). The results obtained by the spectrofluorometer (fig. 3B) showed more fluorescent intensity in H_2O_2 treated cells, whereas cells pre-incubated with magnolol showed a 1.2 fold decrease in fluorescence intensity compared to H_2O_2 alone treated cells.

Magnolol reduce LPO in *S. cerevisiae*

Exposure of yeast cells to H_2O_2 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 H_2O_2 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 H_2O_2 treated and control (fig. 5). This result indicated magnolol induce CAT activity in addition to scavenging ROS directly to protect cells from oxidative stress.

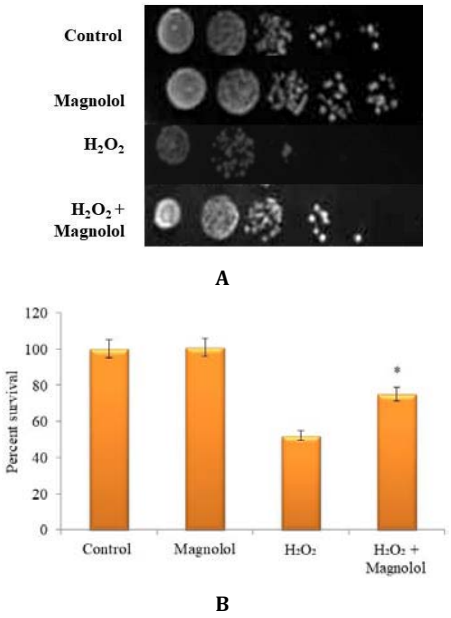


Fig. 2: Effect of magnolol on the viability of *S. cerevisiae* cells. A) Spot assay B) Colony forming unit assay, data are mean±SD. of three independent experiments. * *p*<0.05

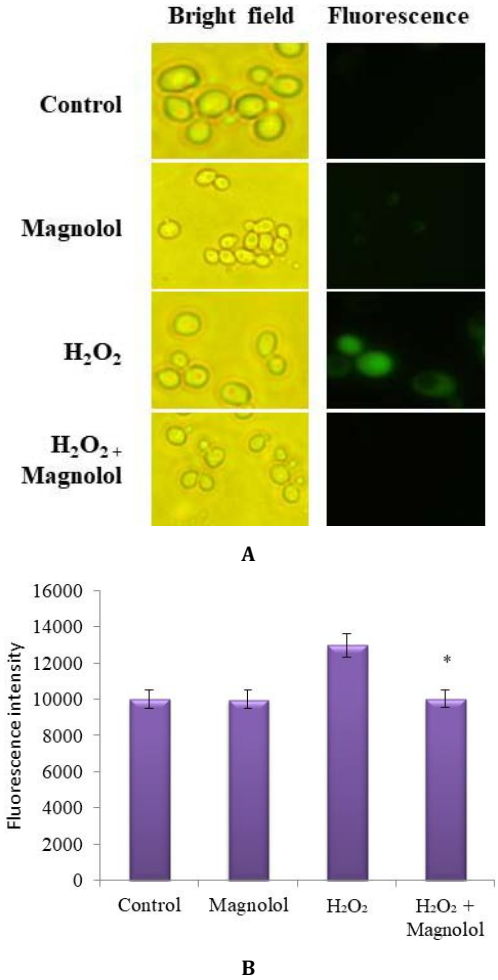


Fig. 3: Detection of ROS A) Fluorescent microscopy B) Spectrofluorometer Data are mean±SD. of three independent experiments. * *p*<0.05

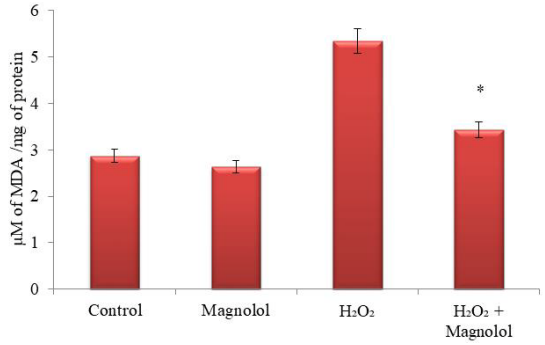


Fig. 4: Effect of magnolol on lipid peroxidation, data are mean±SD. of three independent experiments. * *p*<0.05

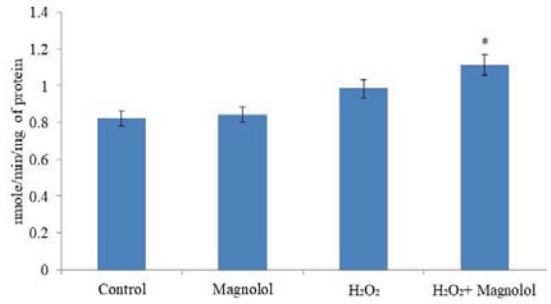


Fig. 5: Effect of magnolol on catalase activity, data are mean±SD of three independent experiments. * *p*<0.05

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 H_2O_2 treatment. H_2O_2 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 H_2O_2 sensitivity assay showed that 1.5 mmol H_2O_2 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 H_2O_2 induced sensitivity and showed better cell survivability compared to H_2O_2 alone treated cells (fig. 2A and B) Magnolol pre-treatment reduces the ROS positive cells in H_2O_2 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. 3A 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_2O_2 resistance accompanied with decrease of ROS in *S. cerevisiae* was demonstrated using various antioxidants such as propolis [15], lisosan 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_2O_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_2O_2-Hydrogen peroxide, ROS-Reactive oxygen species, H_2DCFDA-2′,7′-Dichlorodihydrofluorescein diacetate, DCF-2′,7′-dichlorofluorescein, TBA-Thiobarbituric acid, TCA-Tricarboxylic acid, Hcl-Hydrochloric acid, CAT-Catalase, SOD–Superoxide dismutase, LPO–Lipid peroxidation, MDA–malondialdehyde, 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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