Protective effect of *Bacopa monnieri* (L.) Pennell leaf extract on H_2O_2 induced cell death in *Saccharomyces cerevisiae*

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ABSTRACT

The present study was conducted to study the effect of the methanolic extract of *Bacopa monnieri* leaves on the extent of cell death under conditions of oxidative stress. The cells used were *Saccharomyces cerevisiae* cells and the oxidant used to induce oxidative stress was H_2O_2. The cells were subjected to oxidative stress and the effect of the extract in counteracting this stress was assessed by analyzing characteristic apoptotic events. The results obtained clearly indicated that the exposure to H_2O_2 resulted in a steep rise in the number of *S. cerevisiae* cells undergoing apoptosis. *Bacopa monnieri* leaf extract, by itself, did not cause an increase in the extent of apoptosis. When co-administered along with H_2O_2, the plant extract resulted in a markedly decreased number of apoptotic cells. Thus, it is evident that the methanolic extract of *Bacopa monnieri* leaves protects the yeast cells from oxidative stress-induced death.

Keywords: Oxidative stress, H_2O_2, *Bacopa monnieri*, *Saccharomyces cerevisiae*, Apoptotic events

INTRODUCTION

Oxidative stress is described generally as a condition, under which increased production of free radicals, ROS and oxidant-related reactions result in damage to cells (Chung *et al*. 2007). Oxidative stress damage various cellular macromolecules which include DNA molecules, proteins and lipids. This damage may result in many diseases, including diabetes mellitus, atherosclerosis, myocardial infarction, arthritis, anemia, asthma, inflammation, neurodegenerative diseases and carcinogenesis (Oyedemi et al, 2010). Natural antioxidants neutralize the harmful effects of excessive formation of ROS and its supplementation is advised for cancer, cardiovascular diseases and various other pathologies. Antioxidant find importance in the treatment of diverse metabolic disorders linked to free radicals, such as diabetes, cancer, atherosclerosis and neurodegeneration (Eshwarappa et al, 2015). Thus, the search for crude drugs of plant origin with antioxidant activity has become a central focus of research. In accordance with this, the present study was focused on analysing the antioxidant activity of the candidate plant *Bacopa monnieri*. *Bacopa monnieri* (L.), Pennell, commonly known as "Brahmi" is a member of the Family Scrophulariaceae (Anonymous, 1997). It possesses hepatoprotective, antiulcer, bronchovasodilatory, anti-inflammatory (Channa et al, 2006) and anti-helicopylori properties (Goel et al, 2003). The present study was an attempt to study the effect of *B. monnieri* leaf extract on H_2O_2 induced oxidative stress using *in vitro* model namely *Saccharomyces cerevisiae* cells.

MATERIALS AND METHODS

Fresh leaves of *B. monnieri* were collected and homogenized (1g in 10ml of methanol), centrifuged at 2000rpm for 5 min and dried at 60°C protected from light. The residue was weighed and dissolved in dimethyl sulfoxide to obtain the desired concentration (20mg/20µl).

Yeast cells were inoculated in the YPD medium on the penultimate day of each assay and the flask was incubated in a temperature controlled orbital shaker at 30°C overnight. The medium was separated by centrifuging at 1000g for 15 min. The cells collected in the pellet were washed twice with saline and then resuspended in saline. Aliquots containing 10^6 cells were incubated for one hour at 30°C with or without H_2O_2 (200µM) and the methanolic leaf extract (20mg) of *B. monnieri*. A smear was made from the treated cells and used for various staining techniques, whereas the cells in suspension were used to determine the viability.

The treatment groups set up for the study were

- Untreated *S. cerevisiae* cells

- H_2O_2 treated (positive control) *S. cerevisiae* cells
- Methanolic extract of *B. monnieri* leaves treated cells
- H_2O_2 + methanolic extract of *B. monnieri* leaves treated cells

The extent of cytotoxicity and survival in the oxidant induced cells both in the presence and the absence of the leaf extract were determined by the MTT dye reduction assay (Igarashi and Miyazawa, 2001) and SRB assay (Skehan et al, 1990) respectively. The morphological changes in the cells were followed by Giemsa staining (Chih et al, 2001) and nuclear changes by PI staining (Sarker et al, 2000). The nuclear changes in apoptotic cells, with minor modifications (EtBr staining) was studied as per the method proposed by Mercille and Massie (1994). Apoptotic cells were detected with DAPI staining technique (Rashmi et al, 2003). All the parameters studied were analysed statistically and the values were expressed as mean ± Standard Deviation. One way ANOVA with $P<0.05$ was considered significant and, one way ANOVA followed by post-hoc Fischer analysis was done to test the levels of statistical significance.

RESULTS AND DISCUSSION

The extent of survival of *S. cerevisiae* cells subjected to oxidative stress in the presence and the absence of *B. monnieri* leaf extract was assessed by MTT and SRB assays. The per cent viability was quantified and the results obtained are represented in Figs.1 and 2 respectively. MTT is considered to be a reliable assay to determine the extent of cell viability. Upon exposure to H_2O_2, there was a drastic reduction in the number of viable cells. The administration of the methanolic extract of *B. monnieri* leaves effectively counteracted the effect of H_2O_2 and the number of viable cells was brought back almost to the control values. This trend was observed both in the MTT and in the SRB assay. The cytotoxicity of the methanolic extract of latex of *Euphorbia antiquorum* in *S. cerevisiae* cells increased in a dose dependent manner (Sumathi et al, 2011) as determined by MTT and SRB assays.

The most observable morphological changes that characterize apoptosis are cell shrinkage and membrane blebbing. These changes were observed and quantified in the yeast cells subjected to oxidative stress in the presence and the absence of the leaf extracts, using phase contrast microscopy and the results are presented in Table 1. H_2O_2 exposure caused a steep rise in the number of cells undergoing apoptosis (Plate 1). The plant extract, by itself, did not cause an increase in the extent of apoptosis. When administered along with H_2O_2, the plant extract resulted in a markedly decreased number of apoptotic cells. The effect of the plant extract *Rhinacanthus nasutus* on oxidative stress-induced apoptosis in *S. cerevisiae* cells was studied (Nirmaladevi 2008).

Nuclear changes, like nuclear fragmentation and marginalization, are the signs of apoptosis in cells. Cells undergoing apoptosis become increasingly permeable to propidium iodide (PI), which is too large a molecule to enter live and active cells. Therefore, PI staining is taken as an index of the extent of apoptosis in the cells (England *et al*. 2004). The number of yeast cells exhibiting apoptosis-associated PI staining in the presence or absence of H_2O_2 and/or the leaf extract of *B. monnieri* is presented in Table 2. The exposure to the oxidant caused a very high number of yeast cells to become permeable to PI, indicating oxidation-induced apoptosis (Plate 2), which decreased sharply with the administration of the methanolic extract of *B. monnieri* leaves.

Ethidium bromide, which is an intercalating agent, can be used to visualize the changes that occur in the nucleus during apoptosis (Cury-Boaventura et al, 2004). The induction of apoptosis in *S. cerevisiae* cells in the presence and the absence of *B. monnieri* leaf extract were quantified by EtBr staining (Table 3). It is clear that oxidative stress caused a significant increase ($P<0.05$) in the number of cells undergoing apoptosis. This cytotoxic action was effectively counteracted by the co-administration of the methanolic extract of *B. monnieri* leaves (Plate 3).

Nuclear changes that occur during apoptosis can be observed by fluorescent staining using DAPI (Plate 4). The number of cells that exhibited DAPI stained apoptotic nuclear changes in *S. cerevisiae* exposed to H_2O_2 and / or *B. monnieri* extract is presented in Table 4. As deducible from the values, H_2O_2 caused a significant ($P<0.05$) proportion of the cells to commit to apoptosis, as evident by their increased permeability to DAPI. The methanolic extract of *B. monnieri* leaves was highly efficient in combating this oxidative-stress induced cell death. The methanolic extract of *Zea mays* leaves exhibited maximum protection for yeast cells subjected to oxidative stress, which was evident from the reduced apoptotic events determined by EtBr, PI and DAPI staining (Balasubramanian and Padma 2013).

SUMMARY AND CONCLUSION

In conclusion, the results obtained clearly indicated that the exposure to H_2O_2 resulted in a steep rise in the number of cells undergoing apoptosis. *B. monnieri* leaf extract, by itself, did not cause an increase in the extent of apoptosis. When co-administered along with H_2O_2, the plant extract resulted in a markedly decreased number of apoptotic cells. Thus, it is evident that the leaf extract protects the yeast cells from oxidative stress-induced death.

REFERENCES

Anonymous, (1997). Indian medicinal plants: a sector study. Occasional paper No. 54. Export-Import Bank of India, Quest Publications, Bombay, India.

Balasubramaniam, K., & Padma P. R. (2013) *Zea mays* leaf extracts protect *Saccharomyces cerevisiae* cell against oxidative stress-induced cell death, J. Acute Med., 3**,** 83-92.

Channa, S., Dar, A., Anjum, S., Yaqoob, M., & Rahman A. (2006) Antiinflammatory activity of *Bacopa monniera* in rodents, J. Ethnopharmacol., 104, 286-289.

Chih, H. W., Chill, H. F., Tang, K.S., Chang, F. R., & Wu Y. C. (2001) Bullatacin, a potent antitumour annona ceousacetogenin, inhibits proliferation of human hepatocarcinoma cell line 2.2.15 by apoptosis induction, Life Sci., 69, 1321-1331.

Chung, S. W., Chung, H. Y., Toriba, A., Kameda, T., Tang, N., Kizu, R., & Hayakawa K. (2007) An environmental quinoid polycyclic aromatic hydrocarbon, acenaphthenequinone, modulates cyclooxygenase2 expression through reactive oxygen species generation and nuclear factor kappa b activation in A549 cells, Toxicol. Sci., 95**,** 348–355.

Cury-Boaventura, M. F., Pompeia, C., & Curi R., (2004) Comparative toxicity of oleic acid and linoleic acid on Jurkat cells, Clin Nutr., 2, 721-732.

England, K., O`Driscoll, C., & Cotter T.G. (2004) Carbonylation of glycolytic proteins is a key response to drug-induced oxidative stress and apoptosis, Cell Death Differ., 11, 252-260.

Eshwarappa, R. S. B., Iyer, S., Subaramaihha, S. R., Richard, S. A., & Dhananjaya, B. L. (2015) Antioxidant activities of *Ficus glomerata* (moraceae) leaf gall extracts, Pharmacogn Res., **7**, 114.

Goel, R. K., Sairam, K., Babu, M. D., Tavares, I. A., & Raman A. (2003) *In vitro* evaluation of *Bacopa monniera* on anti-helicobacter pylori activity and accumulation of prostaglandins, Phytomed., 10, 523-527.

Igarashi, M., & Miyazawa T. (2001) The growth inhibitory effect of conjugated linoleic acid on a hepatoma cell line Hep G2, is induced by a change in fatty acid in cells, Biochem. Biophys. Acta / Molecular and Cell Biology of Lipids, 1530, 162-171.

Mercille, S., & Massie B. (1994) Induction of apoptosis in nutrient - deprived cultures of hybridoma and myeloma cells, Biotech. Bioengin., 44, 1140-1154.

Nirmaladevi R. (2008) Antioxidant responses evoked by *Rhinacanthus nasutus* leaf extracts in oxidatively stressed *in vitro* systems, A thesis submitted to Avinashilingam University for Women, Coimbatore in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry.

Oyedemi, S. O., Bradley, G., & Afolayan A. J. (2010) *In -vitro* and *-vivo* antioxidant activities of aqueous extract of *Strychnos henningsii Gilg*, Afr. J. Pharm. Pharmacol., 4, 70-78.

Rashmi, R., Kumar, S. T. R., & Karunagaran D. (2003) Human colon cancer cells differ in their sensitivity to curcumin-induced apoptosis and heat shock protects them by inhibiting the release of apoptosis-inducing factor and caspases, FEBS Lett., 538, 19-24.

Sarker, K. P., Obara, S., Nakata, M., Kitajima, I., & Maruyama, I. (2000) Anandmide induces apoptosis of PC-12 cells: involvement of superoxide and caspase-3, FEBS Lett., 472, 39-44.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D. et al. (1990) New colorimetirc cytotoxicity assay for anticancer drug screening, J. Natl. Cancer Inst, 82, 1107-1112.

Sumathi ,S., Malathy, N., Dharani, B., Sivaprabha, J., Hamsa, D., Radha, P., & Padma P. R. (2011) Cytotoxic studies of latex of *Euphorbia antiquorum* in *in vitro* models, J. Med. Plant Res., 5, 4715-4720.

Table 1. Effect of *B. monnieri* leaf extract on the morphological changes in *S. cerevisiae* cells subjected to oxidative stress (Giemsa staining).

Sample	No. of apoptotic cells / 100 cells		Apoptotic ratio	
	Control	H_2O_2 treated	Control	H_2O_2 treated
No extract	4 ± 2	85 ± 2[a]	0.04	5.66
Methanol extract	2 ± 2	24 ± 1[abc]	0.02	0.32

The values are mean ± SD of triplicates
a - Statistically significant (P<0.05) compared to untreated control
b - Statistically significant (P<0.05) compared to oxidant treated group
c - Statistically significant (P<0.05) compared to plant extract treated group

Table 2: Effect of *B. monnieri* leaf extract on the nuclear changes in *S. cerevisiae* cells subjected to oxidative stress (PI staining).

Sample	No. of apoptotic cells / 100 cells		Apoptotic ratio	
	Control	H_2O_2 treated	Control	H_2O_2 treated
No extract	7 ± 2	81 ± 3[a]	0.08	4.26
Methanolic extract	2 ± 2[a]	30 ± 3[abc]	0.02	0.43

The values are mean ± SD of triplicates
a - Statistically significant (P<0.05) compared to untreated control
b - Statistically significant (P<0.05) compared to oxidant treated group
c - Statistically significant (P<0.05) compared to plant extract treated group

Table 3: Effect of *B. monnieri* leaf extract on the nuclear changes in *S. cerevisiae* cells subjected to oxidative stress (EtBr staining).

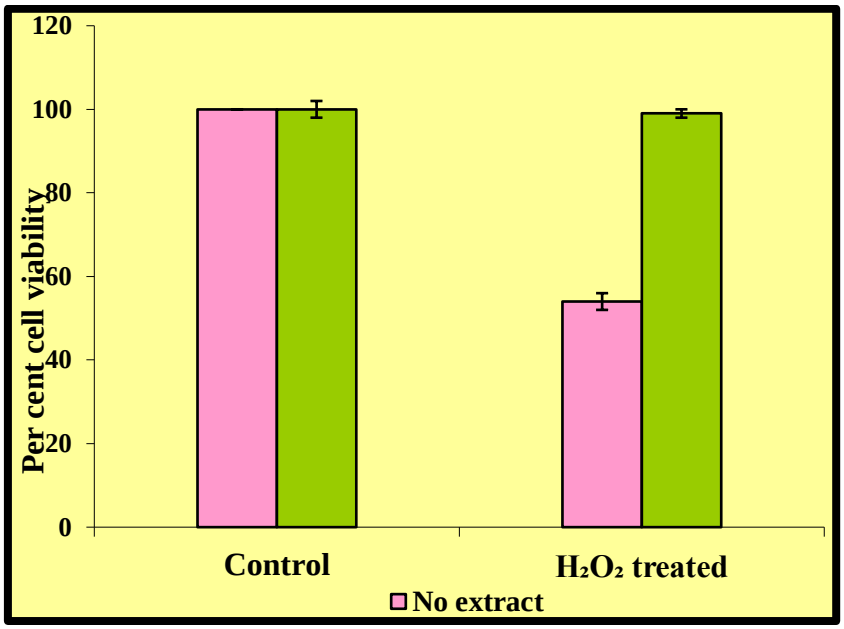
Sample	No. of apoptotic cells / 100 cells		Apoptotic ratio	
	Control	H_2O_2 treated	Control	H_2O_2 treated
No extract	7 ± 3	86 ± 2[a]	0.08	6.14
Methanol extract	6 ± 2[a]	10 ± 2[bc]	0.06	0.11

The values are mean ± SD of triplicates
a - Statistically significant (P<0.05) compared to untreated control
b - Statistically significant (P<0.05) compared to oxidant treated group
c - Statistically significant (P<0.05) compared to plant extract treated group

Table 4: Effect of *B. monnieri* leaf extract on the nuclear changes in *S. cerevisiae* cells subjected to oxidative stress (DAPI staining).

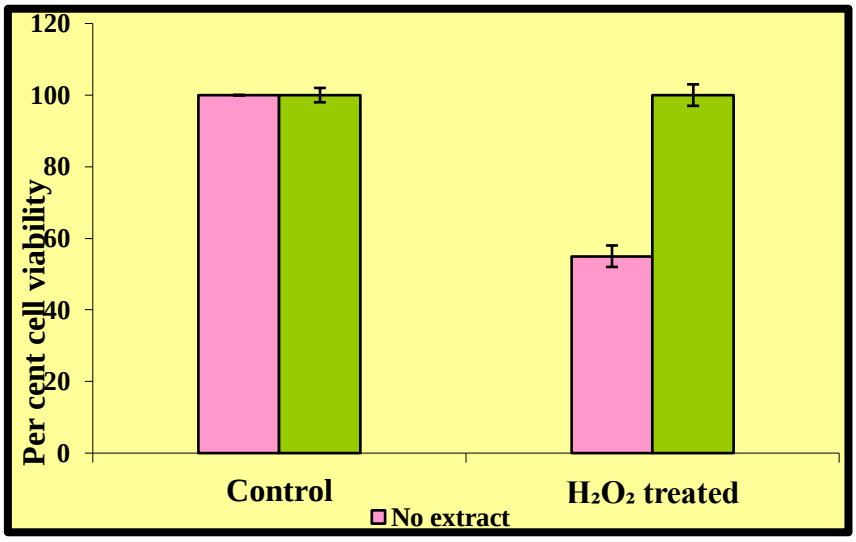
Sample	No. of apoptotic cells / 100 cells		Apoptotic ratio	
	Control	H_2O_2 treated	Control	H_2O_2 treated
No extract	8 ± 2	76 ± 3[a]	0.09	3.17
Methanol extract	6 ± 2	14 ± 3[abc]	0.06	0.16

The values are mean ± SD of triplicates
a - Statistically significant (P<0.05) compared to untreated control
b - Statistically significant (P<0.05) compared to oxidant treated group
c - Statistically significant (P<0.05) compared to plant extract treated group



The values of the negative (untreated) control group were fixed as 100% viability and the per cent viabilities in the other groups were calculated relative to this.

Fig. 1. Effect of *B. monnieri* leaf extract on the viability of *S. cerevisiae* cells subjected to oxidative stress as determined by MTT assay.



The values of the negative (untreated) control group were fixed as 100% viability and the per cent viabilities in the other groups were calculated relative to this.

Fig. 2. Effect of *B. monnieri* leaf extract on the viability of *S. cerevisiae* cells subjected to oxidative stress as determined by SRB assay.

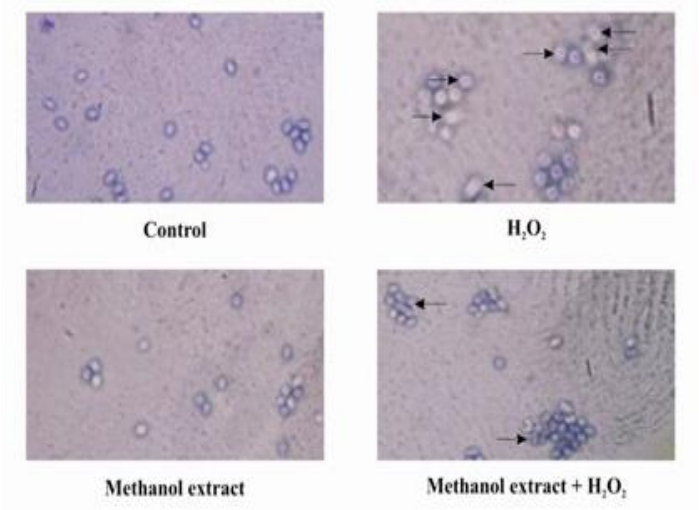


Plate 1: *S. cerevisiae* cells stained with Giemsa (Oxidant-H_2O_2).

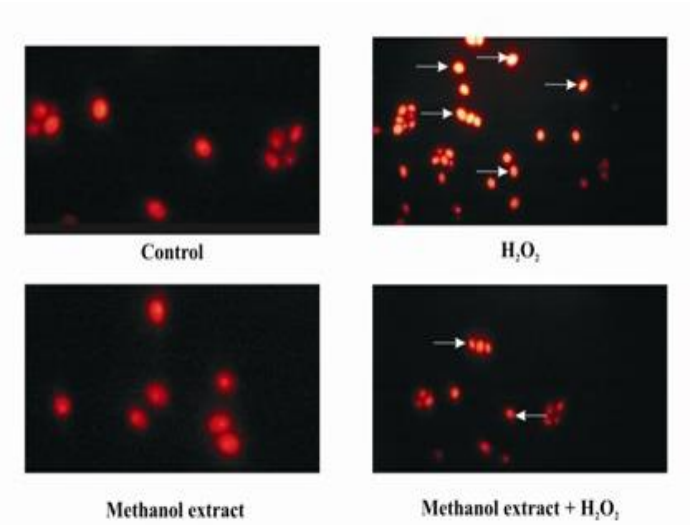


Plate 2: *S. cerevisiae* cells stained with PI (Oxidant-H_2O_2).

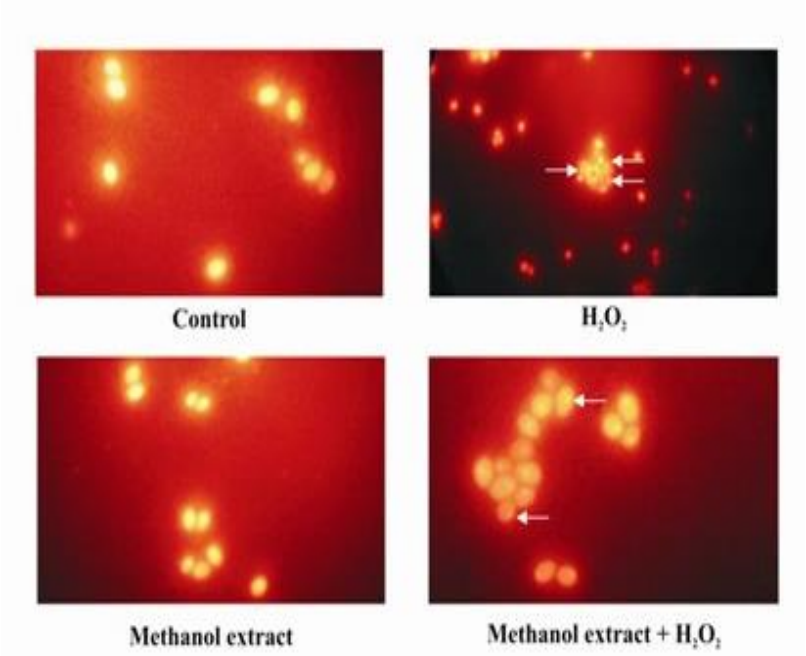


Plate 3: *S. cerevisiae* cells stained with EtBr (Oxidant-H_2O_2).

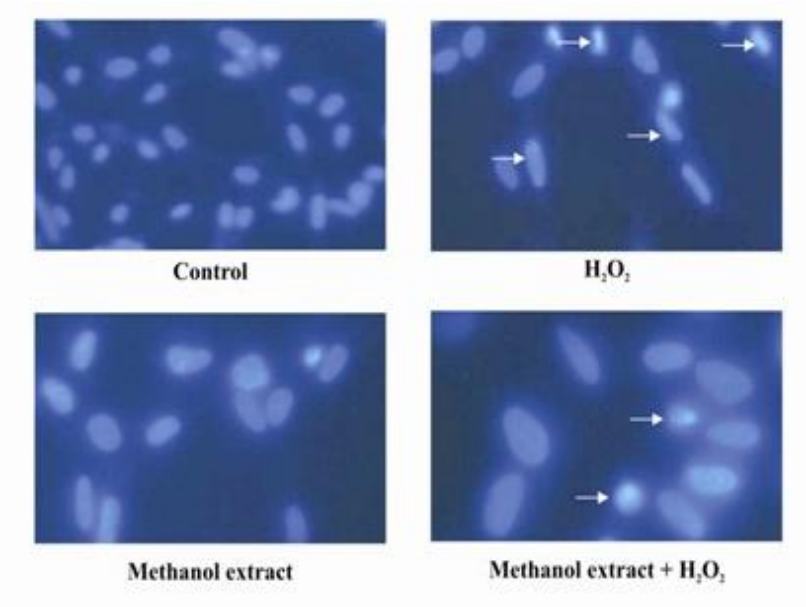


Plate 4: *S. cerevisiae* cells stained with DAPI (Oxidant-H_2O_2).