Bactris Setosa Mart. (Tucum-do-cerrado) Aqueous Extract Increases Growth and Viability in Saccharomyces Cerevisiae BY4741 and YAP1Δ Under Stress Caused by Menadione and Hydrogen Peroxide

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Research Article

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Abstract

Yeast cells from *Saccharomyces cerevisiae* can increase endogenous antioxidant response when stressed to prevent cell death. YAP1 is a transcription factor responsible to activate genes that encoding antioxidant enzymes such as superoxide dismutase and catalase and can be an important key to protect these cells. Tucum-do-cerrado (*Bactris setosa* Mart.) is a Brazilian fruit rich in polyphenols and bioactive compounds mainly found in the peel. This study investigated cell growth and viability using *S. cerevisiae* wild type and yap1Δ strains exposed to tucum-do-cerrado peel aqueous extract and hydrogen peroxide (H_2O_2) and menadione induced oxidative stress.

Yeast cells from BY4741 and yap1Δ were exposed to different concentrations of tucum extract, menadione and hydrogen peroxide separated and together in mixed groups for 20h and measured for growth curve. For colony survival yeast cells were exposed to these compounds for 72h in ágar plates and colonies were counted. Results showed that aqueous extract of tucum-do-cerrado was capable to recover BY4741 density of cells stressed with both menadione and H_2O_2 but not for yap1Δ strain. Besides, higher concentrations of the extract demonstrated a delay in cell growth. Colony survival showed that the exposition to tucum extract resulted in colony recover in BY4741 yeast cells but not for mutant yap1Δ strains which maintained low viability even with high extract concentration. In conclusion, despite *S. cerevisiae* antioxidant response to menadione and H_2O_2 is different, the protection afforded by tucum extract in H_2O_2 stressed cells, is probably through an YAP1 pathway.

1. Introduction

Saccharomyces cerevisiae antioxidant response is carried out through several mechanisms, including metaloproteins, which protect the cells from iron and copper toxicity, and the product of the CUP1 gene, which protects cells against menadione-induced stress. *S. cerevisiae* also has cytoplasmic membranes with high content of saturated fatty acids, that are more resistant to lipid peroxidation, and vitamin C, both of which may provide an efficient protection from stress[1]. Yeast cells have both, enzymatic and non-enzymatic antioxidant systems[2]. The main enzymes are superoxide dismutase (SOD), catalase and peroxidases, such as cytochrome c peroxidase and glutathione peroxidase (GPX). The non-enzymatic defense system includes scavenger molecules such as glutathione[3].

Another key mechanism used by yeast against oxidative stress is regulation of the endogenous antioxidant system by transcription factors. While in animal cells this mechanism mainly involves the transcription factor Nrf-2 (*NF-E2-related factor 2*), that activates genes that encoding antioxidant enzymes[4], *S. cerevisiae* has the transcription factor YAP1 (*Yeast Activator Protein 1*) that plays similar role when cells face oxidative stress situations. Yeast cells have sensors that detect low levels of hydrogen peroxide and activate the thioredoxin and glutathione pathways, which activate YAP1, this process is known as antioxidant and redox signaling[5]. YAP1 activation occurs by its nuclear export regulation and this process is mainly mediated by Chromosomal Maintenance 1 (Crm1), also known as Exportin 1, which exports YAP1 to the cytoplasm in normal conditions[6]. However, under stress

conditions both cysteine-rich domains located in N and C terminal parts of the YAP1 protein constitute disulfides between the N-terminal Cys303 and the C-terminal Cys598, mainly in response to oxidation caused by hydrogen peroxide (H_2O_2). As a result of this structural change, interaction between YAP1 and Cmr1 is inhibited, which causes the nuclear accumulation of YAP1 and its consequent activation[7].

A decrease in cellular growth can be used by yeast as a physiological response to prevent oxidative stress caused by molecules such as H_2O_2 and menadione[8]. It has been shown that menadione increases H_2O_2 production and it is responsible for the generation and accumulation of reactive oxygen species (ROS), that affect the morphology and viability of yeast cells[9,10].

Tucum-do-cerrado (*Bactris setosa* Mart.) is a Brazilian fruit and is known for its antioxidant properties *in vitro* and *in vivo*[11,12]. The fruit has bioactive compounds such as vitamin C, quercertin, catechin, peonidin, anthocyanins and flavonoids, mainly found in the peel, that also showed major antioxidant activity *in vivo* and *in vitro* when compared to the pulp[11–16]. It is not known if these compounds can influence yeast cell growth and the YAP1 pathway. Therefore, the aim of this study was to evaluate cell growth and viability using a *S. cerevisiae* strain that is unable to express YAP1 and the correspondent wild type strain exposure to tucum-do-cerrado peel aqueous extract and H_2O_2 and menadione induced oxidative stress.

2. Material And Methods

2.1 Extract of tucum-do-cerrado

Fruits were obtained from a local merchant from “Fazenda Grama”, Teresópolis de Goiás – GO/Brazil. Fruits were washed with distilled water and peel was manually removed and lyophilized. For preparation of the crude aqueous extract, 1g of pulverized peel was mixed with 10 ml of distilled water. The contents were shaken for 16 h at 4ºCand filtered through a 0.22 µm filter. The aqueous extract was stored in a -20 ºC freezer until use.

2.2 Yeast strains

S. cerevisiae cells wild type BY4741 (*MATa his3 leu2 met15 ura3*) and mutant yap1Δ (wich doesn’t contain the yap1 transcription factor) were used for growth and survival experiments. Strains were provided by Dr. Marcos Dias Pereira from Chemistry Institute of Federal University of Rio de Janeiro, Brazil.

2.3 Cell culture and growth

S. cerevisiae cells were maintained in Yeast extract, Peptone, Dextrose (YPD) medium composed by 1% yeast extract, 2% peptone, 2% dextrose and 2% ágar at 4ºC. Yeast growth were performed in YPD liquid medium at 28ºC and 200 rpm. Growth evaluation for further experiments was performed by optical density (OD_{600}) at 600 nm.

2.4 Yeast dry weight curve

Inoculum for each yeast strain (BY4741 and *yap1Δ)* were prepared using 50ml of YPD medium and 1 colony each strain. After 18h incubation at 28ºC and 200 rpm, 2ml of inoculum was transferred to 2ml tubes previously weighed. Samples were centrifuged at 10.000 rpm for 5 min at room temperature. The supernatant was discarded and yeast cells were heated at 60ºC until reach stable weight. For number of cells (mg/ml)

$$stable\ weight - inicial\ weight = \frac{final\ weight}{diluition} x\ 1.000$$

were used. Diluitions of 20, 25, 50 and 100 were made for each strains and absorbance readed in spectrophotometer at 600 nm. Angular coefficient was calculated and used to adjust number of cells to be used on further analysis.

2.5 Growth curve

One inoculum for each yeast strain (BY4741 and *yap1Δ)* were prepared using 10ml of YPD medium and 1 colony of each strain. After 18h incubation at 28ºC and 200 rpm, absorbance was at 600 nm and cells were adjusted to 40 µg/mL[17] in a final volume of 1ml for every experimental group. Two control groups were made, one just with yeast cells and medium and one added with 1% (v/v) ethanol. Aqueous extract of tucum were added in several concentrations (10, 25, 50, 100 and 150 µg/ml), menadione (5 and 15 µM) and hydrogen peroxide (0.5 and 1 mM) to form other experimental groups. These compounds were tested separated and together in mixed groups. Cells were plated at 96 well plates and growth measured each 30min during 20h at Synergy HTX Multi-Mode Microplate Reader 600nm. Data was collected on Gen5 software.

2.6 Cell Viability

Inoculum (BY4741 and *yap1Δ)* were prepared using 10ml of YPD medium and 1 colony for each strain. After 18h incubation at 28ºC and 200 rpm, absorbance was measured at 600 nm and cells were adjusted to 40 µg/mL[17] in to a 5ml final volume for every experimental group. Aqueous extract of tucum were added at 50 and 150µg/ml concentrations, menadione at 5µM and hydrogen peroxide at 0.5mM to form other experimental groups. Next, groups were incubated for 24h at 28ºC and 200 rpm and after this time cells were washed with PBS buffer x1, diluted in YPD medium at 10^0, 10^{-1}, 10^{-2} e 10^{-3} and 10µl plated in YPD medium solid 2% ágar (w/v) for each group. Plates were incubated in bacteriological incubator for 72h at 28ºC. After this time colonies were counted and 10^{-3} dilution was used for analysis.

2.7 Statistical Analysis

Results are expressed as mean ± SEM. Samples were compared using one-way analysis of variance (ANOVA) and the post hoc test of *Bonferroni*. For statistics significance, *P*-value < 0.05 was used. Analysis

were made by software GraphPad Prism v6. For yeast dry weight curve software Microsoft Excel was used.

3. Results

The yeast cell growth coefficient found in dry weight curve and used to calculate cell concentration for both yeast strains (BY4741 and yap1Δ) was 0.5 (*not shown*). Both yeast strains were exposed to stress with menadione and H_2O_2 in varied concentrations, as showed in Fig. 1, BY4741 strain stress were significative ($p < 0.05$) when exposed to H_2O_2 and decrease cell concentration in both strains were dose dependent demonstrating the potential to slow cell growth in addition to reduction of cell density after exposition time. All concentrations for both menadione and H_2O_2 had a significant impact ($p < 0.05$) on yap1Δ strain cells. Ethanol (1%) did not show potential to reduce growth. These results were used to select concentrations to further analysis.

When exposed to different concentration of tucum-do-cerrado extract (Fig. 2) cells from BY4741 and yap1Δ were not significatively affected in cell density. In higher concentrations (100 and 150 µg/ml) was observed a delay in cell growth. Results for menadione (15µM) and H_2O_2 (0.5mM) induced stress show that cells with all tucum extract concentrations (10, 25, 50, 100 and 150 µg/ml) managed to recover cell density at the end (Fig. 3). Unlike wild type *S.cerevisiae* cells, yap1Δ strain had a significative ($p < 0.05$) decrease in cell density for both stressors but did not recover when exposed to tucum extract in any tested concentrations (Fig. 3).

Colony count results showed first that cell viability on BY4741 and yap1Δ were significatively reduced ($p < 0.05$) at 15µM menadione and 0.5mM H_2O_2 (Fig. 4) corroborating the result found in growth curves. When exposed to 50 and 150µg/ml of tucum extract, BY4741 yeast viability was not affected but for yap1Δ strain all concentrations of tucum extract significantly reduced colony viability (Fig. 4). However, the lowest concentration of tucum indicated a major effect in reducing mutant colony cells viability.

Tucum extract showed in a dose-dependent way a protection effect for menadione 15µM in wild type and mutant yeast colony viability (Fig. 4). In a different way, exposition to 5.0mM H_2O_2 resulted in colony viability recover in BY4741 yeast cells but did not showed the same result for mutant yap1Δ strains which maintained low viability even with high tucum extract concentration (Fig. 4).

4. Discussion

In our study, *S. cerevisiae* cells were treated with different compounds, including ethanol, the vehicle used to dissolve menadione. This was done because ethanol can reduce yeast cell viability of yeasts when in high concentrations[18]. Yeast cells BY4741 and yap1Δ, in the presence of ethanol, grew in a similar way as cells in the presence of YPD medium only. Therefore, we can exclude any ethanol effect in yeasts treated with menadione.

Previous studies showed that wild type (BY4743 and BY4741) *S. cerevisiae* have their growth reduced with 1to 4 mM H_2O_2 and 150μM to 0.75mM menadione[8,19]. Hydrogen peroxide can reduce BY4741 yeast colony formation as well, at 2.5mM concentration[20]. We found that 0.5mM H_2O_2 and 15μM menadione caused a significative reduction in cell grown in both wild (BY4741) and mutant (yap1Δ) *S. cerevisiae*, which indicates that the BY4741 strain is sensitive to lower concentrations of H_2O_2 than the concentrations investigated[8,20,21]. The yap1Δ strain was not investigated in this same context by other authors. The observed increased deleterious effects of the oxidants, H_2O_2 and menadione, on yap1Δ, when compared with its wild type counterpart BY4741, demonstrates the important role of this transcription factor for antioxidant protection.

Secondary plant metabolism compounds have been extensively investigated for their antioxidant potencial. Curcumin, when used at the concentrations of 50 and 150μg/ml, delays *S. cerevisiae* cell growth, without reducing cell density, after 20 hours of exposure[22]. Pomegranate juice (100μl/ml) increases yeast cell density after 72 hours, but also delay the cell cycle[23]. According to our findings, concentrations of 100 and 150μg/ml of tucum-do-cerrado aqueous extract had similar effects on BY4741 and yap1Δ yeast strains, indicating that some tucum-do-cerrado compounds may delay cell growth progression without causing cell death. A propolis alcoholic extract was shown to be nontoxic to wild type *S. cerevisiae* at the concentrations of 50 and 100μg/ml which is similar to the concentrations we used in our study. Such concentrations were toxic to the mutant yap1Δ strain while had no effect on the wild type yeast strain.[24].

Some foods rich in polyphenols have been shown to maintain normal cell growth even when cells are stressed with H_2O_2 or menadione[19,22,23]. In the same experimental condition, tucum extract was effective on improve cell growth on wild *S. cerevisiae* in concentrations such as 10, 25 and 50μg/ml. The higher concentrations (100 and 150μg/ml) also improved cell growth but delayed cell growth progression and maintained cell density slight lower than control group. Moreover, tucum extract was not able to increase cell growth on mutant stressed cells from *S. cerevisiae*, suggesting that YAP1 gene expression may be involved in protection mechanism on this strain.

Table 1 is a summary of the main results obtained in this study. In the experiments made in liquid medium, tucum extract showed no protection from the action of both, H_2O_2 and menadione in the mutant yeast strain, but restored growth to the level in the absence of the oxidants in the wild type strain. Regarding the results in solid medium, the tucum extract showed partial protection from the action of menadione in the mutant and in the wild type strain. Protection against H_2O_2 is non-existent in the mutant and partial in the wild type. This indicates that tucum protection against H_2O_2 is dependent on YAP1, but not in the case of menadione.

Table 1
Effects of tucum-do-cerrado extract on growth and cell viability of wild type (BY4741) and mutant (yap1Δ) *Saccaromyces cerevisiae* strains.

Medium	**Liquid**				**Solid**			
Tucum(µg)	1 mMH_2O_2		15 uMMD		1 mM H_2O_2		15 uMMD	
	BY4741	yap1Δ	BY4741	yap1Δ	BY4741	yap1Δ	BY4741	yap1Δ
50	+++	0	+++	0	n.d.	n.d.	n.d.	n.d.
100	+++*	0	+++*	0	++	0	++	+
150	+++*	0	+++*	0	+	0	+++	+++

0, no effecton restoration of growth or cell viability; +, some protective effect; ++, medium protective effect; +++, restoration of growth or cell viability to the level in the absence of the oxidant; * indicates a delay in growth; n.d., not determined. All data compared to the control samples corresponding to yeast cells growth in YPD medium alone.

Saccharomyces cerevisiae has different responses to stress caused by menadione and H_2O_2[25,26]. The antioxidant response of these cells is adapted to react to different levels of hydrogen peroxide by activating expression of genes coding for antioxidant enzymes such as SOD1 and SOD2 that are targeted by YAP1[5,27]. Menadione can increase superoxide and H_2O_2 concentrations[28] in the mutant strain, therefore the protection afforded by tucum extract in menadione stressed cells, is probably partially through an YAP1 independent pathway.

Declarations

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Author contributions:

Study concept and design: Élida Geralda Campos, Renata Cristina da Silva; acquisition of data: Renata Cristina da Silva; analysis and interpretation of data: Renata Cristina da Silva and Élida Geralda Campos; drafting of the manuscript: Renata Cristina da Silva and Élida Geralda Campos; statistical analysis: Renata Cristina da Silva; obtained funding: Renata Cristina da Silva; contributed reagents/materials/analysis tools: Marcos Dias Pereira; study supervision: Élida Geralda Campos. Authors contributed equally to this work.

Conflict of Interest Statement:

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial or non-financial interest in the subject matter or materials described in this manuscript.

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Ethics declaration:

This study had no ethical approval required. Additionally, each of the authors confirms that this manuscript has not been previously published and is not under consideration by any other journal.

Data availability:

All data generated or analysed during this study are included in this manuscript.

Declarations of interest:

none.

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Figures

Figure 1

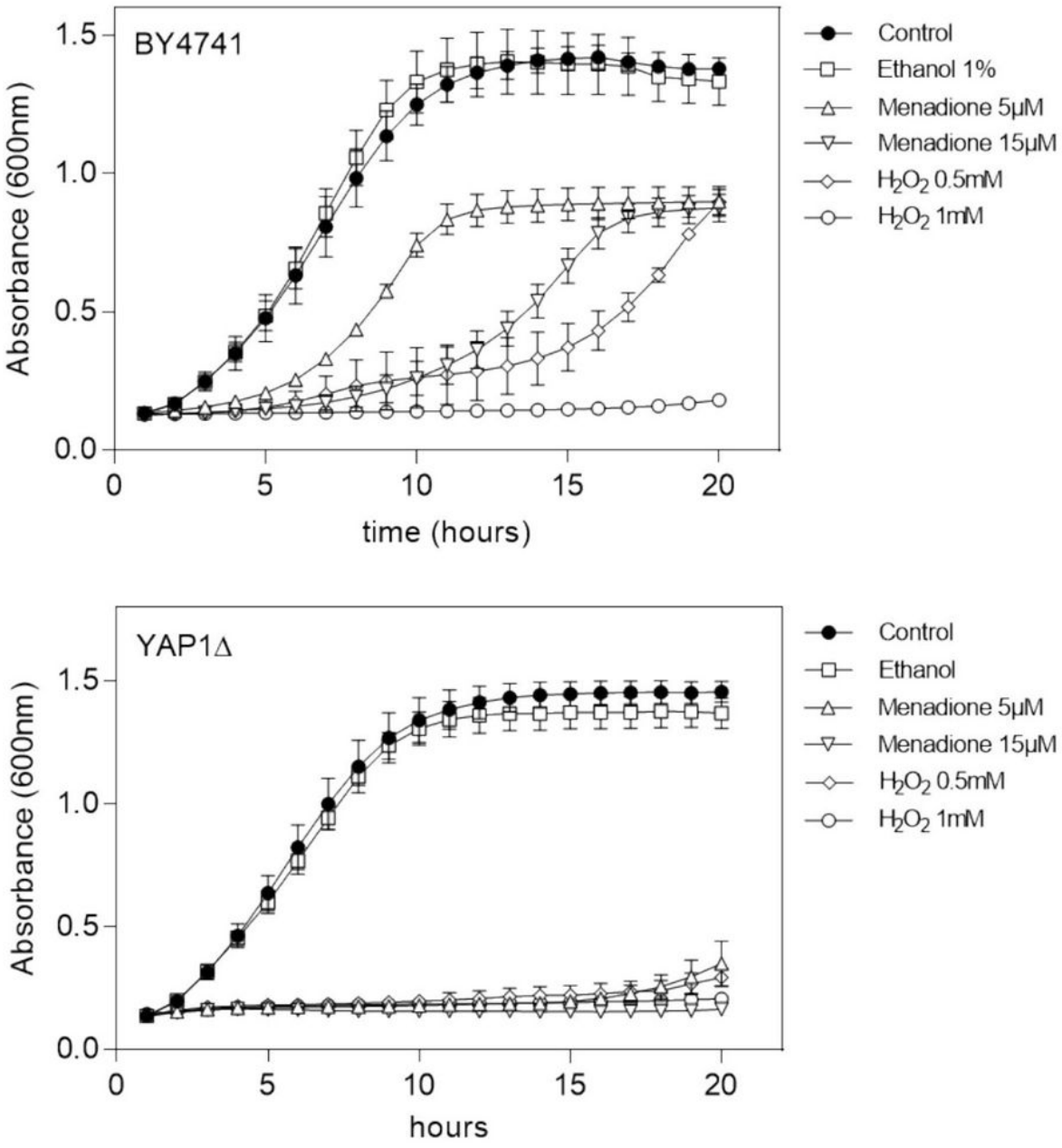

Figure 1

Growth curves of S. cerevisiae strains (BY4741 and yap1Δ) exposed to ethanol 1%, menadione (5µM and 15µM) and H2O2 (0.5mM and 1mM). Error bars indicate the standard error of the mean (n=3) of three different experiments.

Figure 2

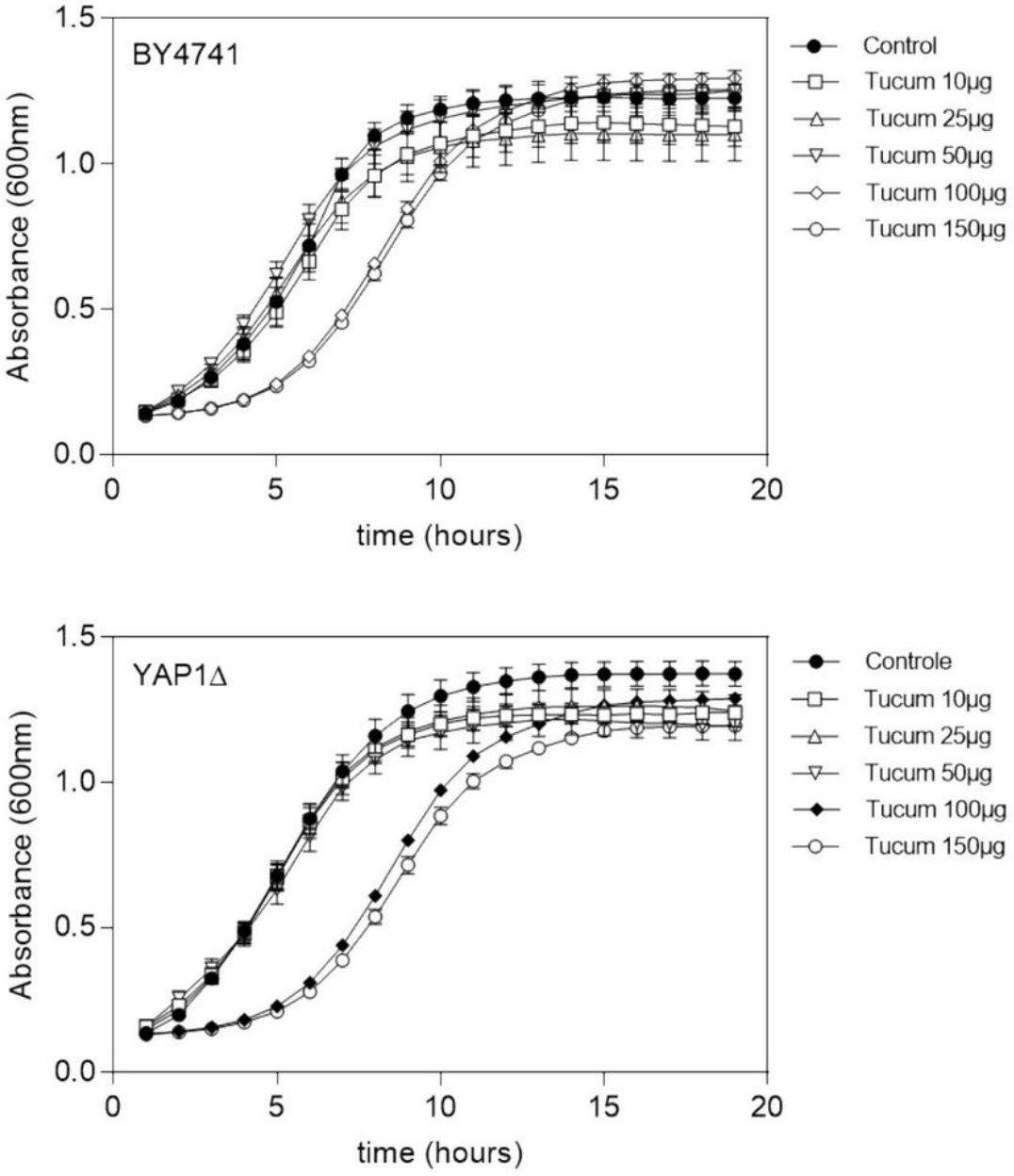

Figure 2

Effect of different concentrations of tucum's aqueous extract (10, 25, 50, 100 and 150µg/ml) in S. cerevisiae strains (BY4741 and yap1Δ) growth curves. Error bars indicate the standard error of the mean (n=3) of three different experiments.

Figure 3

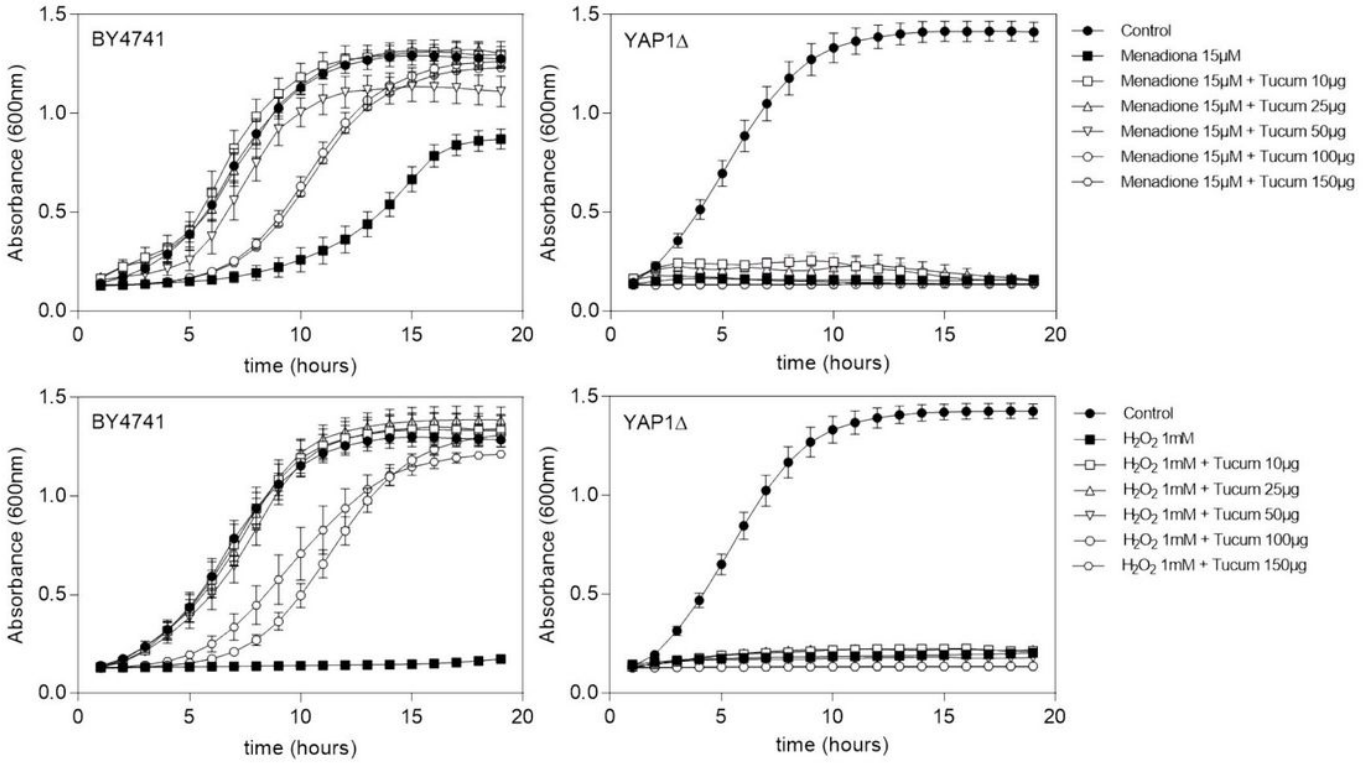

Figure 3

Effect on growth curves of different concentrations of tucum's aqueous extract in S. cerevisiae strains (BY4741 and yap1Δ) only with YPD medium (C) or exposed to menadione (15μM) (M), H2O2 (1mM) (H), tucum extract 50μg/ml (MT1) or 150μg/ml (MT2) and menadione (15μM), tucum extract 50μg/ml (HT1) or 150μg/ml (HT2) and 1mM of H2O2. Error bars indicate the standard error of the mean (n=3) of three different experiments.

Figure 4

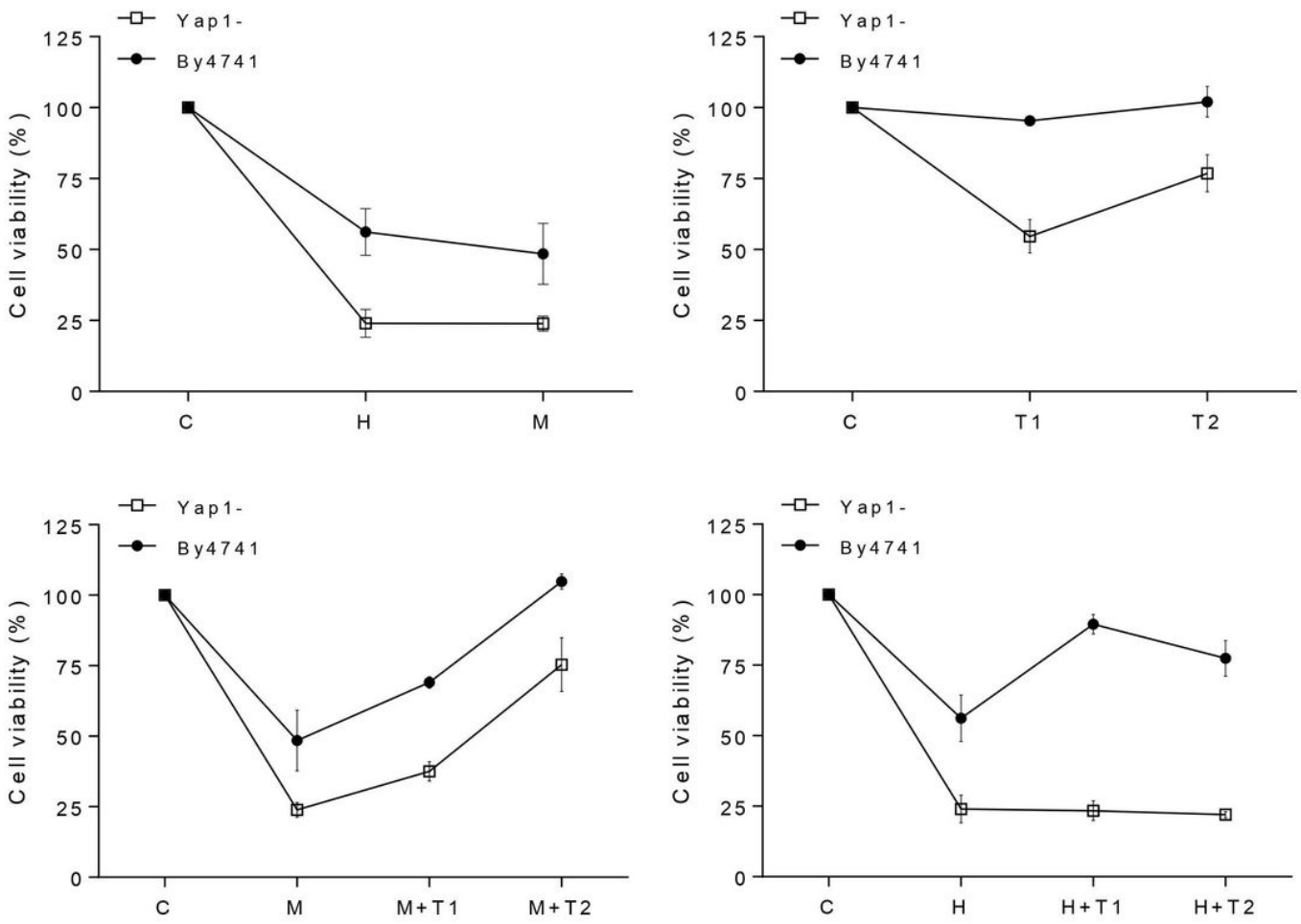

Figure 4

Colony viability of S. cerevisiae strains BY4741 and yap1Δ only with YPD medium (C) or exposed to menadione (5μM) (M), H2O2 (0.5mM) (H), 50μg/ml (T1) or 150μg/ml (T2) of tucum's extract, pretreated with 50μg/ml (MT1) or 150μg/ml (MT2) of tucum extract before menadione induced stress and with 50μg/ml (HT1) or 150μg/ml (HT2) of tucum extract before H2O2 induced stress. Error bars indicate the standard error of the mean (n=3) of three different experiments.