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Protective Effects of Orange Juice Extract on Chromium-Induced Oxidative Damage in Saccharomyces Cerevisiae

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Abstract: *Chromium is a highly toxic non-essential metal which is used in the production of steel and other alloys, in metal finishes and leather tanning. Chromium exposure has been linked to inhibition of growth or metabolic activity and may arise due to enhanced generation of reactive oxygen species (ROS) and oxidative damage in metal-treated micro-organisms. The yeast Saccharomyces cerevisiae provides an attractive alternative system for elucidating the mechanism(s) of metal toxicity. In this study, seven groups were created. i: Control group, ii: K2Cr2O7 group, iii: 5 mM K2Cr2O7+ orange juice (OJ) group, iv: 10 mM K2Cr2O7 + OJ group, v: 15 mM K2Cr2O7 + OJ group, vi: 20 mM K2Cr2O7 + OJ group, vii: 25 mM K2Cr2O7 + OJ group. After sterilization, fruit juice (20%) and K2Cr2O7 were added at different concentrations to Saccharomyces cerevisiae (S. cerevisiae) cultures which were developed at 30°C for 1h, 3h, 5h and 24 hours (overnight). S. cerevisiae cell growth was analyzed by spectrophotometer, total protein alterations was identified by SDS-PAGE electrophoresis and measured with biuret method. As a results; cell growth increased in OJ groups to which OJ was taken in comparison to the positive control (K2Cr2O7) group at different growing times (1, 3, 5 and 24 hours) ($p<0,05$). As a result OJ has a protective effect for reduce the oxidative damage and raised cell growing and improve protein synthesis in S. cerevisiae culture.*

Keywords: *Gene product, Orange juice extract, oxidative damage, SDS-PAGE, S. cerevisiae.*

I. Introduction

The yeast *Saccharomyces cerevisiae* is one of the most popularly used eukaryotic model systems for studying a wide range of biological phenomena at both the molecular and cellular level, including cell cycle regulation, DNA repair, signal transduction and cell polarity control. In recent years, fruit extracts known to be rich in antioxidants, minerals and vitamins are being used on yeasts in many studies [1-4]. Chromium which is a transition element is very toxic for plants and microorganisms. Chromium which has oxidative effects is used widely in the industry and is one of the major pollutants while also having carcinogenic effects [3]. Oxidative damage can be completely or partially removed thanks to various foods with strong antioxidant effects [5-9]. Orange juice has disinfectant effect against microorganisms as well as antioxidant effects on the cellular level. Thus, it is used against some diseases and oxidative damages. Orange is very rich in terms of vitamin C and thus it has antioxidant properties. Orange juice has an acidity of around 5 % and thus its pH value varies between 2-3. [10,11]. Many studies carried out put forth that different fruit content increases cellular development in yeasts, improves protein synthesis and displays protective features against oxidative stress [12, 13, 14]. In this study, seven different groups were formed to examine the oxidative damage caused by $K_2Cr_2O_7$ oxidant material on *Saccharomyces cerevisiae* and the effects of orange juice on cellular development in yeasts and protein expression have been examined.

II. Material And Methods

2.1 Research groups

Seven groups were composed. After sterilization, fruit juice (15%) and $K_2Cr_2O_7$ were added different concentration to *Saccharomyces cerevisiae* (*S. cerevisiae*) cultures and the cultures were improved at 30^0C for 1h, 3h, 5h and 24 hours (overnight). occurrence media of *S. cerevisiae*: For the developed and reproduce of yeast, YEPD (for 50 mL 1.75 g yeast extract, 1.6 g trypton, 1.5 g glucose) besides, for the growth and reproduce of *S. cerevisiae*, orange juices was added and improved. After sterilization, samples were incubated for 1h, 3h, 5h, 72 h (overnight, h: hour) at 30°C [7].

2.2 Orange juice extract and $K_2Cr_2O_7$ Chemical

Fruits (From center county of Mohammadia) were washed under water circulation. The juice was obtained in a FMC extractor, FS BR 1 model (Brasil) and filtered. After pasteurization, the natural juice extract was added in to S. cerevisiae media cultures and inserted 20% (v/v) ratio in at the duplicating for 30^0C. $K_2Cr_2O_7$ was added in $K_2Cr_2O_7$ and OJ+ $K_2Cr_2O_7$ groups.

2.3 Cell concentration results

In these measurements, culture samples that were grown at 30^0C for 1, 3, 5 hours and overnight (24 hours) have been analyzed. The calculation has been conducted using a spectrophotometer at 600nm (OD_{600}).

2.4 SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis)

The samples of *Saccharomyces cerevisiae* cultures were maked for SDS-PAGE after which they were charged to sample loading wells to be subject to electrical current and after this process the gels were dyed, their images were taken and the intergroup protein bandings were used as data in the study [15].

2.5 Protein concentration measurements

The total proteins content were evaluated according to biuret method. Protein standards at different concentrations were obtained using bovin serum albumin (BSA) protein. Hence, the total protein amount in *Saccharomyces cerevisiae* groups corresponding to this standard value was computed. Calculation has been realized using a spectrophotometer at 540nm (OD_{540})

2.6 Statistical Analysis

For statistical analysis the SPSS 20.0 software was used. The comparison between experimental groups and the control group was made using one way Anova Post Hoc Hochberg and Games-Howell test. Statistically significant differences among groups have been indicated as $p<0.05$ and the statistically non-significant differences have been indicated as $p>0.05$. Standard deviations were indicated as ±.

III. Results And Discussion

The present investigation throws light on the antioxidant capacity of orange juice extract on *Saccharomyces cerevisiae* growth and total protein content. The results obtained from this study will be an important reference for future outcomes. When the results put forth in Table 1 and Fig.1 are examined, it can be observed that there are statistically significant ($p<0.05$) differences among groups at different development times. It can be seen that orange juice added to the cultures protects cellular development against the negative effects of chromium and indeed in some cases increased it. When the biuret protein results given in Table 2 and Fig.2 are examined, we can state that orange juice triggers protein synthesis in yeasts. It is observed that the protein density is greater in yeast groups with added orange juice in comparison with the positive control group. When the SDS-PAGE supernatant and pellet total protein bands in Fig.3 and Fig.4 are examined, it can be observed that orange juice increases protein band intensity at a statistically significant rate. We had obtained similar results in our previous studies with fruit juices such as pomegranate juice, apple juice and cherry on *S. cerevisiae*. It was determined during the study we carried out using pomegranate juice that the negative effects of the hydrogen peroxide radical are eliminated by pomegranate juice and that pomegranate juice increases cellular development in yeasts while at the same time providing a protective effect against oxidative damage. It was also determined that pomegranate juice has a protective role against H_2O_2 damage in yeasts even above normal development temperatures (60^0C) [7]. It was also determined in the study in which we examined the protective effects of grapefruit juice against the negative effects of chromium in yeasts that grapefruit juice has positive effects on cellular development in *S. cerevisiae* and that it decreases chromium damage [13]. Auesukaree et.al. (2015) put forth that *Moringa oleifera* leaves have a cellular development providing effect against cadmium mediated oxidative stress in *S. cerevisiae* thus stating that some plant, vegetable and fruit contents have a protective effect against cellular stress in yeasts [16]. Vicario et.al. (2015) carried out a study examining the chemical and cellular effects of orange juice on *S. cerevisiae* in which they indicated that vitamin C rich orange has many therapeutic effects and that it provides an antioxidant cellular defense against oxidative stress in yeasts while also putting forth based on HPLC and other chemical analyses results that fruits with high vitamin C content have significant protective effects in yeasts [17]. Farcasanu et.al. (2014) carried out a study examining the effect of blueberry extract against cadmium effect in *S. cerevisiae* in which they used many biochemical parameters and emphasized the protective role of blueberry extract against cadmium toxicity in yeast cells while also putting forth that it increases cellular viability and development [18].

IV. Figures and Tables

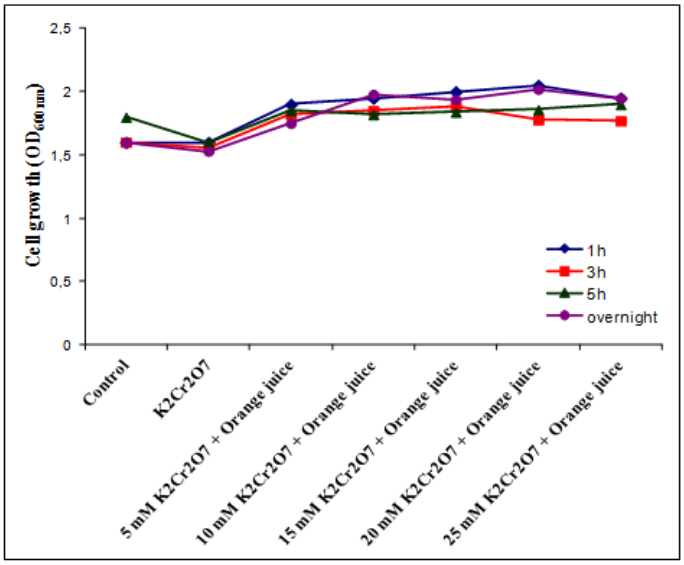

Figure1. The growing of Saccharomyces cerevisiae in orange juice at different hours

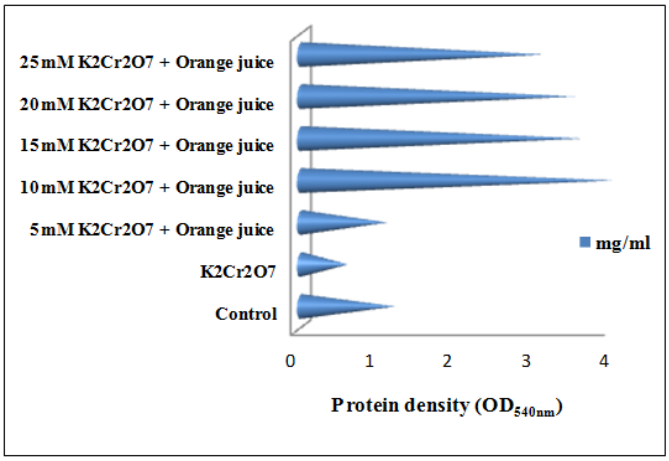

Figure2. Protein densities of among the groups

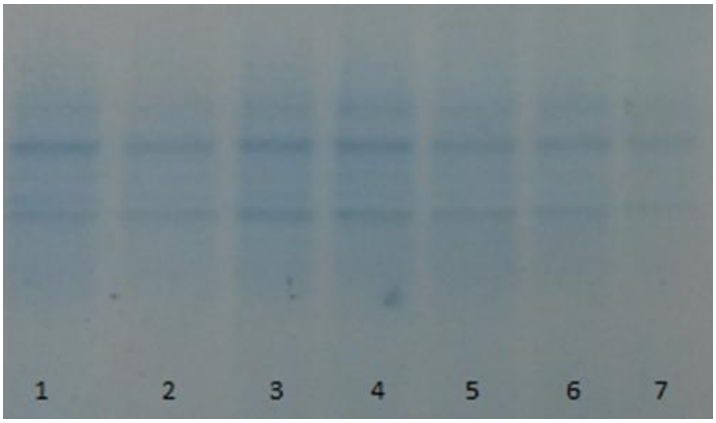

Figure 3. SDS-PAGE supernatant total protein bands profiles for development at 30°C. Lanes, 1: Control; 2: K2Cr2O7; 3: 5 mM K2Cr2O7 + OJ; 4: 10 mM K2Cr2O7+ OJ; 5: 15 mM K2Cr2O7 + OJ; 6: 20 mM K2Cr2O7 + OJ; 7: 25 mM K2Cr2O7 + OJ

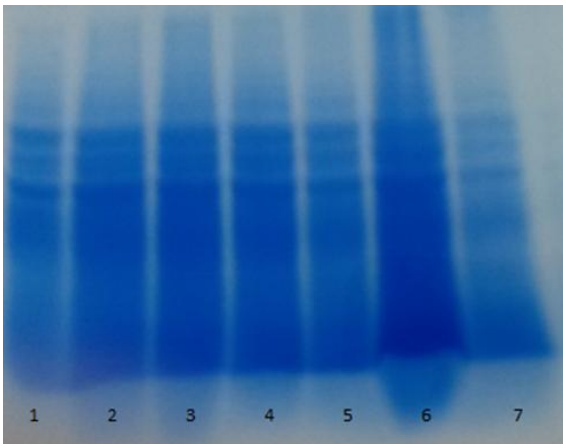

Figure4. SDS-PAGE pellet total protein bands profiles for development at 30°C. Lanes, 1: Control; 2: K2Cr2O7; 3: 5 mM K2Cr2O7 + OJ; 4: 10 mM K2Cr2O7+ OJ; 5: 15 mM K2Cr2O7 + OJ; 6: 20 mM K2Cr2O7 + OJ; 7: 25 mM K2Cr2O7 + OJ

Table1. The growing of *Saccharomyces cerevisiae* in orange juice extract at different hours.

OD_{600} 30 ^{0}C	**1h**	**3h**	**5h**	**Overnight**
Control	$1,59 \pm 0.002^a$	$1,60 \pm 0.002^b$	$1,83 \pm 0.002^b$	$1,59 \pm 0.002^b$
$K_2Cr_2O_7$	$1,60 \pm 0.002^a$	$1,56 \pm 0.002^a$	$1,67 \pm 0.002^a$	$1,50 \pm 0.002^a$
5 mM $K_2Cr_2O_7$ + orange juice	$1,88 \pm 0.002^b$	$1,83 \pm 0.002^e$	$1,90 \pm 0.002^c$	$1,75 \pm 0.002^c$
10 mM $K_2Cr_2O_7$ + orange juice	$2,01 \pm 0.002^d$	$1,85 \pm 0.002^f$	$1,83 \pm 0.002^b$	$2,04 \pm 0.002^f$
15 mM $K_2Cr_2O_7$ + orange juice	$2,04 \pm 0.002^e$	$1,87 \pm 0.002^g$	$1,90 \pm 0.002^c$	$1,98 \pm 0.002^d$
20 mM $K_2Cr_2O_7$ + orange juice	$2,08 \pm 0.002^f$	$1,77 \pm 0.002^d$	$1,89 \pm 0.002^c$	$2,08 \pm 0.002^g$
25 mM $K_2Cr_2O_7$ + orange juice	$1,98 \pm 0.002^c$	$1,69 \pm 0.002^c$	$1,90 \pm 0.002^c$	$2,00 \pm 0.002^e$

** a, b, c, d, e, f, g; among the groups which bearing of different letter are significant ($p < 0.05$)
Anova Post Hoc Hochberg and Games-Howell Test

Table2. Biüret protein density

OD_{600} at 30 ^{0}C	**mg/ml**
Control	1
$K_2Cr_2O_7$	0.6
5 mM $K_2Cr_2O_7$ + orange juice	1
10 mM $K_2Cr_2O_7$ + orange juice	4
15 mM $K_2Cr_2O_7$ + orange juice	3,5
20 mM $K_2Cr_2O_7$ + orange juice	3,5
25 mM $K_2Cr_2O_7$ + orange juice	3

V. Conclusion

These results indicate the antioxidant capacity of orange juice thus making us think that it can have similar effects on humans like its effects on *Saccharomyces cerevisiae*. So, we are of the opinion that similar results can be obtained for humans when fruits and their juices are consumed regularly.

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