Inhibición del estrés oxidativo inducido con peróxido de hidrógeno en *Saccharomyces cerevissiae* (evaluación de un extracto acuoso de *Camellia sinensis)*

Correspondencia: Instituto de Medicina Tropical - Facultad de Medicina - Universidad Central de Venezuela.

Consignado el 14 de Mayo del 2021 a la Revista Vitae Academia Biomédica Digital.

PALABRAS CLAVE: compuestos fenólicos, antioxidante, modelo biológico, peróxido de hidrogeno.

INHIBITION OF HYDROGEN PEROXIDE INDUCED OXIDATIVE STRESS IN SACCHAROMYCES CEREVISSIAE (EVALUATION OF AN EXTRACT OF CAMELLIA SINENSIS)

SUMMARY

The research aimed to evaluate the antioxidant effect of an organic aqueous extract of green tea (Camellia sinensis), by inducing an oxidative stress with hydrogen peroxide (H2O2) to Saccharomyces cerevissiae. Materials and methods: Green tea leaves were used to obtain the aqueous extract. Prior to the evaluation of the extract, the concentration of phenolic compounds was determined by the Folin-Ciocalteu method. For the evaluation of the antioxidant capacity, the biological model of S. cerevissiae was used. Results: The concentration of total phenolic compounds in the extract was 216.23±2.13 mg of GAE / g PM. In the evaluation of the antioxidant activity, a marked inhibitory effect of the oxidative stress in the presence of H2O2 was observed. Conclusion: the high content of bioactive compounds present in C. sinensis, represent an alternative in the treatment and prevention of diseases linked to a high expression of reactive oxygen species.

KEY WORDS: Phenolic compounds, Antioxidant, Biological model, Hydrogen peroxide

INTRODUCTION

Tea (*Camellia sinensis)* is a popular, socially accepted, safe and healthy beverage that is widely consumed around the world. Tea is beneficial to prevent and cure a variety of diseases related to oxidative stress (1-3). The health benefits ascribed to the consumption of teas are thought to be associated with their high content of bioactive ingredients such as polyphenols. The latter are secondary plant metabolites and include the subclasses of flavonoids, flavones, flavonols, flavanols, isoflavones, flavanones and anthocyanidins (4).The major active flavonoids in green tea are epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) (5,6).

The structural features of green tea catechins that significantly contribute to their antioxidant action are the presence/absence of the galloylmoiety and the number and positions of the hydroxyl groups on the rings. The latter determine their ability to interact with biological matter through hydrogen bonding, or electron and hydrogen transfer processes within their antioxidant activities. In fact, the antioxidant mechanism implies hydrogen atom transfer or single electron transfer reactions, or both (7).

The objective of this research was to evaluate, the effect antioxidant in *Saccharomyces cerevissiae*, of aqueous extract organic green tea artisanal (China).

MATERIALS AND METHODS

Origin of plant material (PM)

Non-commercial organic green tea from China, stored in its package until analysis.

Sample preparation for extraction

2 grams of plant material were weighed. This was poured into a 400 mL Beaker, to which 200 mL of distilled water previously heated to the boiling point was added. The sample was slightly stirred for 4 min and filtered using Whatman No. 4 paper (8).

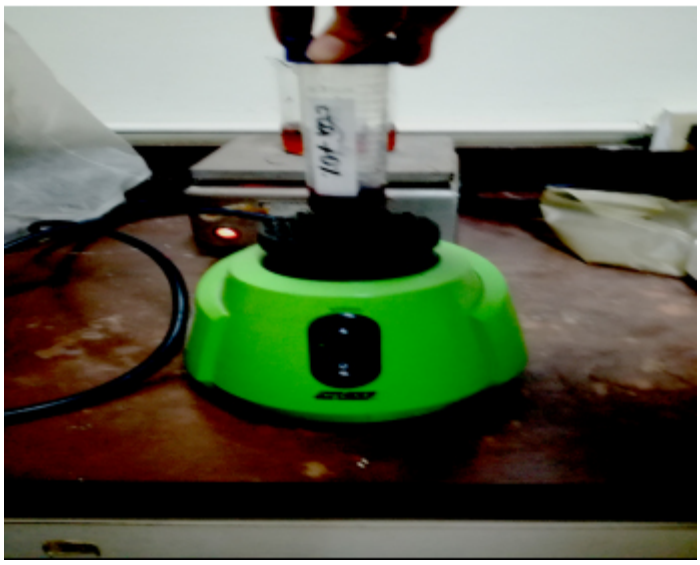

Figure 1. Aqueous extract of *Camellia sinensis*. Agitation prior to evaluation

Determination of total phenolics

For the determination of total phenolics, 50 µL were mixed with 250 µL of the Folin-Ciocalteu 1 N reagent (Analytical grade, Merck). It was left to stand for 8 minutes and then 750 µL of 20% Na2CO3 and 950 µL of distilled water were added. Was incubated for 30 min at room temperature and the absorbance was read on a Genesis 20 UV/VIS spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). A calibration curve for Gallic Acid (Sigma-Aldrich, Germany) was prepared with concentrations of 50, 100, 200, 300, 400, 500 and 1000 ppm. The results were expressed in mg of Gallic Acid Equivalents (GAE) / g of PM (9).

Biological effects in cell model (*S. cerevisiae*-oxidative stress with H2O2)

This essay seeks to evaluate the ability of the compounds present in the extracts to promote the growth of yeast when subjected to oxidative stress with hydrogen peroxide, for which the following experimental sequence was carried out (10): The positive control with ascorbic acid, the negative without antioxidant and a blank only with yeast and culture medium. Before starting the absorbance measurements, *S. cerevisiae* was inoculated for 24 h under agitation at 37 ° C with 8 mL of YPD culture medium (peptone 2% w / v, glucose 2% w / v), 25 µL of yeast stock (solution of *S. cerevisiae* with a cell concentration of 5.3 x 10^5 cells / mL), 100 µL of ascorbic acid and 80 µL of the extract of the samples. In control negative and white were inoculated only 8 mL of YPD and 25 µL of the yeast stock. After this incubation time of the yeast under the different conditions the absorbance was measured at 600 nm, this was approximately 0.240 and corresponded to the starting point of latency period of *S. cerevisiae*. Then 160 were added µL of 0.8 mM H2O2 to the positive control, negative and the extract to be evaluated at different concentrations. It was incubated at 37 ° C under stirring for 8 h and readings were taken every 30 min. Before each reading they waved the glass tubes for 10 s in vortex to ensure the homogeneity of the cells in the culture medium, then 100 µL of each test was taken and then taken to plates 96 wells orbital shaking was performed before each reading 20 s and the absorbance readings were taken at 600 nm.

Statistical analysis

Analyses were done in triplicate, and the results were expressed as means ± standard deviation (SD). Results of antioxidant activity were each subjected to analysis of variance (ANOVA).

RESULTS

The results obtained show that the pure (490 µg/mL, 216.23±2.13 mg of GAE / g PM) and diluted extract was able to inhibit the oxidative stress in *S. cerevisiae*, with significant difference ($p< 0.05$) in relation to the positive control of Vitamin C. The latency period was similar in both the pure, diluted and positive control extracts, with an exponential growth of *S. cerevisiae* in the pure extract from 90 min (Fig 2).

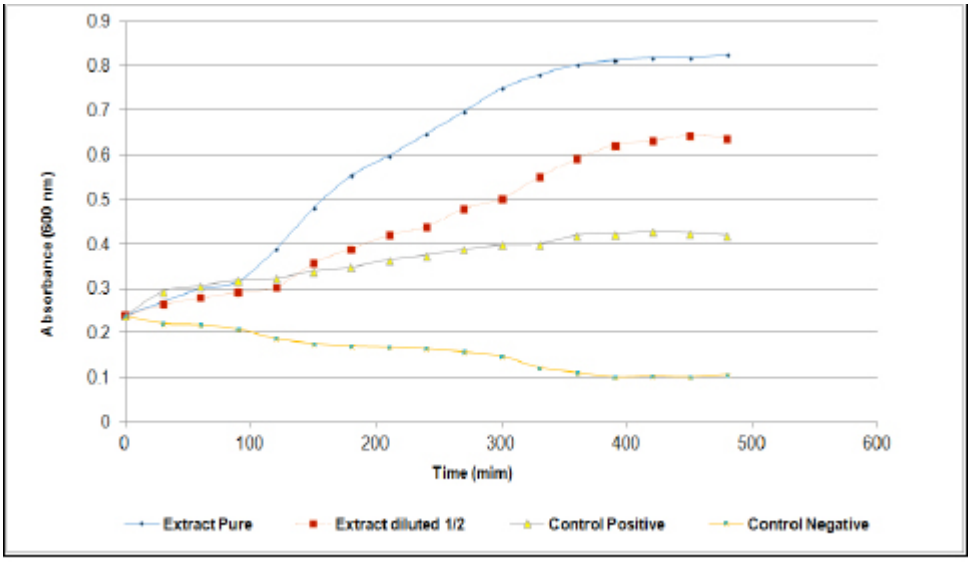

Figure 2. Evaluation of the antioxidant capacity of the pure extract and diluted 1/2 of *C. sinenesis*

DISCUSSION AND CONCLUSIONS

Experimental data indicate that tea polyphenols may offer indirect protection by activating endogenous defense systems (11). The observed effect is due to that the phenolics compounds, EC, ECG, EGC and EGCG, present in the extract organic green tea artisanal, can indirectly regulate the expression and activity of enzymes such as CAT, SOD and glutathione (12) (Fig. 2). Although it is not known with certainty what mechanisms *S. cerevisiae* carries out to promote its growth in an oxidative stress environment, it was evidenced that the compounds present in the extract are capable of stimulating the cells to follow their normal growth path, which is perhaps related to the ability of the extracts to activate or deactivate physiological mechanisms associated with the protection of the yeast or with simple reduction H2O2 (13, 14).

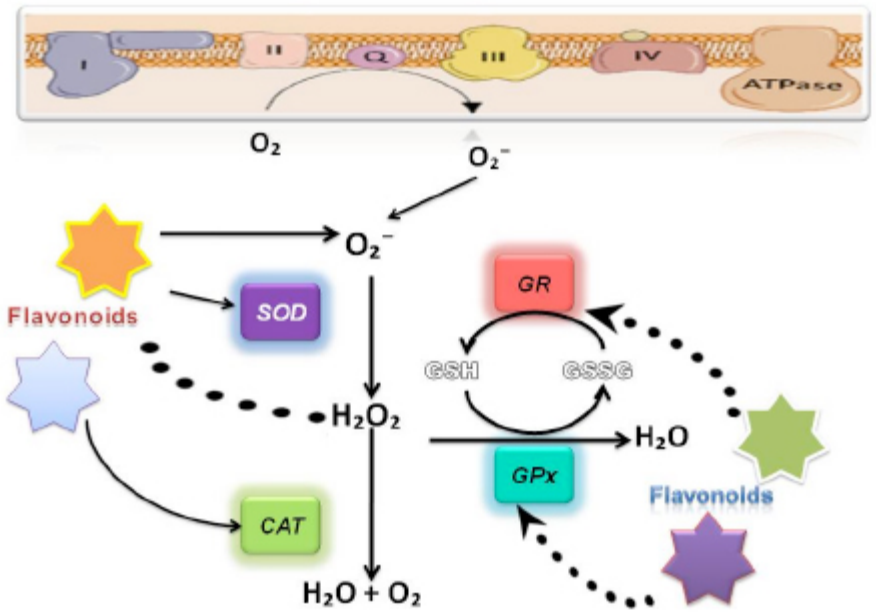

Figure 3. Effect on the enzyme system of phenolic compounds presentyes in *C. sinenesis*.

Previous studies have reported that different phenolic compounds (quercetin, resveratrol, and hesperidin), are able to protect yeast cells against damage caused by different stressing agents (14,15). The antioxidant effect of tea ingestion requires more evidence to unravel the mechanism of action and the ingredients involved. Despite there being no convincing evidence from long-term intervention studies in humans, tea flavanols are still considered to be the major candidates involved in the biological activity of teas. Possible mechanisms of action, such as the induction of an endogenous redox pathway or direct effects of polyphenol metabolites, should be elucidated so that the molecules responsible for the effect can isolated and clear-cut evidence can be obtained from long-term intervention studies(17).

Natural antioxidants are an interesting alternative in view of their variety of structures and chemical interactions, as well as the numerous biological activities they can perform. Intensive research activities are currently being carried out on plant antioxidants to meet this challenge (18). The *in vitro* and *in vivo* antioxidant activities of some natural products could be consistent in certain conditions. Overall, *in vitro* antioxidant activity and phenolic profiles of tea can provide

Conclusions

The extract of green tea showed a protective effect in the presence of an excess of H2O2 molecules, which favours or contributes to the metabolism of *S. cerevisiae*. The results in this research evidence that the consumption of green tea has beneficial effects on health and prevention of chronic pathologies, such as cardiovascular disease and cancer, in which oxidative stress plays a critical role.

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