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Saccharomyces cerevisiae as a model for the evaluation of dihydropyridine calcium antagonist effects

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Abstract

In this work, we are interested to using *Saccharomyces cerevisiae* as eukaryotic model and an alternative one to animal testing for bringing out the effect of nifedipine, a dihydropiridine L-type calcium channel blocker. Thus, a culture of *Saccharomyces cerevisiae* was treated with different concentrations of this xenobiotic (0.01, 0.05, 0.5, 1 mM) during 3 hours. After that we measured the levels of some stress biomarkers (GSH, GST and peroxidases activities). The results obtained show a significant/high significant increase in all studied biomarkers following treatment with a dose-response manner. These data highlight an oxidative stress induced by the presence of the calcium antagonist, and expressed by a stimulation of the oxidative enzymes activities

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Introduction

The understanding of the cellular function and the different biological processes are the basis of all scientific research, including drug research, this last one which also based on the knowledge about the mechanism of action of novel therapeutic agents and their biological targets. Between the several stages of drug research, the evaluation of the toxicity has an important place and can be a reason for drug attrition (figure 1).

Thereby the determination of drug targets and evaluate their toxicity are a very challenging issue (Guengerich and MacDonald, 2007). In the order to discover, screening, evaluate and develop this molecules, the choice of experimental model is crucial, requiring a multifactor's consideration. Among model organisms *Saccharomyces cerevisiae* represents an excellent cellular model (for discovering and studying drugs) due to the fact that it is an eukaryotic organism so its cellular organization was highly similar to higher eukaryotic cells at both macromolecules and organelles levels, but also for the simplicity of their system and the ease of access. In addition to its genome is entirely sequenced and whose genes are easily manipulated, deleted, replaced, which make it a perfect genetic model (Oswald, 2006 / Dolinski and Botstein, 2006 / Akiyoshi *et al.,* 2009 / Wu *et al.,* 2010). At the same time, the response is easier to read, and when extrapolated to humans, we can have a better understanding of molecular mechanisms of toxicity (Braconi *et al.,* 2006)

The usefulness of this eukaryotic organisms in drug assess has been already demonstrated (Buschini *et al.,* 2003 ; Lee *et al.,* 2005 ; Sturgeon *et al.,* 2006 ; Menacho-Márquez and Murguía, 2007; Guiffant, 2008 ; Stepanov *et al.,* 2008 ; Bouillet *et al.,* 2012 ; Matuo *et al,* 2012/ Cherait and Djebar, 2013).

In this study, we used this multiple uses species, as well in the industrial manufacturing, than as an excellent eukaryotic model to assess the effects of nifedipine, a calcium antagonist belonging to dihydropyridines family widely used for the treatment of cardiovascular diseases (Parreira *et al,* 2003 ; Poole-wilson *et al,* 2006 ; Cao *et al.*, 2010). Nifedipine inhibits selectively and at very low concentrations, the entry of calcium ions at the voltage-gated L-type channels (Mc Donough, 2004; Triggle *et al,* 2006; Valentin *et al*, 2009; Mc Donough and Bean, 2010). It is known that in human cell there are several subtypes of calcium channels voltage-dependants classified electrophysiologically into five classes on the basis of Ca^{2+} currents called L (Long lasting), N (Neither T nor L), T (transient), R (resistant) and P/ Q (P for the Purkinje cells of the cerebellum, where it found and Q for alphabetic order after P) (Catterall, 2000; Yamakage and Namiki, 2002, Le Chevoir, 2008). Only the L-type calcium channels are sensitive to the dihydropyridines. It is also known that *Saccharomyces cerevisiae* have a voltage-gated calcium-channel homologue Cch1, pharmacologically similar to L-type calcium channels (Walker, 1998 ; De Souza Pereira *et al.,* 2001; 2003; Teng *et al.,* 2008). Thereby the influx of calcium inside *Saccharomyces cerevisiae* can be regulated by these drugs.

Materials and methods

Biological material

The biological material chosen for our investigation is a fungus unicellular eukaryotic, the yeast *Saccharomyces cerevisiae,* an optimal eukaryotic model system to study toxic effects and mammalian biological responses upon exposure to exogenous and endogenous perturbations.

Chemical material

Nifedipine ($C_{17}H_{18}N_2O_6$), inhibits selectively the transmembrane calcium by blocking the L-type calcium channels. It was obtained from national control laboratory of pharmaceutical products LNCPP (Algeria) and dissolved in acetone and further diluted in distilled water with 1% final concentration of acetone.

Treatment

A culture of *Saccharomyces cerevisiae* was isolated in a culture medium (0.25 g / L glucose, 10 g yeast extract / L, 25 mL of glycerol and 940ml of distilled water) (Pol, 1996) and treated by four concentrations of nifedipine (0.01mM, 0.05mM, 0.5mM and finally 1mM) during 3 hours.

Measurement of Biochemical and Enzymatic Parameters

After treatment of cells yeast, we measured the production of some stress biomarkers (GSH, GST and peroxidases activities).

Thus, a culture of *Saccharomyces cerevisiae* was stopped on exponential growth phase by a centrifugation at 1500 × g for 10 min. Afterwards the cells yeast was sonicated with 50 mM potassium phosphate buffer (pH 7.5) on the ice tray. The homogenate was centrifuged at 10000 × g for 20 min at 4°C and the supernatant which is the enzyme extract was stored at -80C until use.

Measurement of Glutathione (GSH)

The dosage of glutathione was quantified according to the colorimetric method of Weckberker and Cory, 1988.

The method involved oxidation of GSH by the sulfhydryl reagent 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5′-thio-2-nitrobenzoic acid (TNB), measurable at 412 nm. The concentration glutathione is expressed in µM of GSH per mg of proteins.

Measurement of Glutathione S-transferase

The Glutathione *S*-transferase (GST), an important group of enzymes, are involved in the detoxication system to protect cells against xenobiotics.

The assay is based on the conjugation of the glutathione thiol group's to the CDNB (1-Chloro-2,4-dinitrobenzene) substrate, in the presence of glutathione. The absorbance was determined at 340 nm. (Habig *et al.,* 1974)

Measurement of Peroxidases Activities

The peroxidase is a key enzyme of the antioxidant network that converts hydrogen peroxide to water, as the catalase, but in the presence of a specific substrate.

To following the peroxidases activities, we prepared an assay mixture containing 50 mmol/L phosphate buffer (pH 7.5), 20 mM guaiacol or ascorbate for respectively gaiacol-peroxidase and ascorbate peroxidase assays, 40 mM H2O2 and 0.01 ml of enzyme extract. The reaction was initiated by adding H_2O_2 and the absorbance change was monitored by UV/Vis spectrometer at 470 nm for gaiacol-peroxidases and 290 nm for ascorbate-peroxidases. The enzymatic activities have been expressed using µM /mg protein.(Fielding and Hall, 1978)

Statistical Analysis

The analysis of variance with two controlled factors is used to estimate the differences reported for the different studied parameters.

The data are represented by the mean more or less the standard deviation (m ± s).

Differences were considered significant when *p < 0.05; very significant when **p < 0.01; and very high significant when ***p < 0.001.

This test is performed using the analysis software statistical processing of data: Minitab version 16.

Results

GSH levels

Figure 05 illustrates the effects of the various concentrations of nifedipine on the GSH rate, one of the most frequently used indicators of stress biomarkers preventing damage to important cellular components caused by reactive oxygen species and free radicals (Pompella *et al.,* 2003).

This figure shows that the treated cells by nifedipine present a rather significant/ high significant decrease

in GSH level. Indeed, the level of GSH decreased from 2, 632± 0,204 µM/mg prot in controls to 1,451± 0,250 µM/mg prot in cells treated by 1mM concentration of our molecule. (to 1.326 ± 0.310 in cells treated by 0.5mM nifedipine).

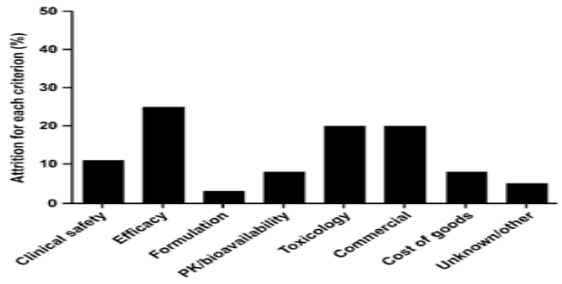

Fig. 1. Reason for drug attrition in the year 2000 (Kola *et al.,* 2004).

The Figure 06 show a dose-dependent increase of GST activity on the cells yeast treated with different nifedipine concentrations compared to controls. The GST increase from 1,264 ± 0,314 µM/mg prot in the control to 8,061 ± 0,808 µM/mg prot in the yeast cells treated with the strongest nifedipine concentration.

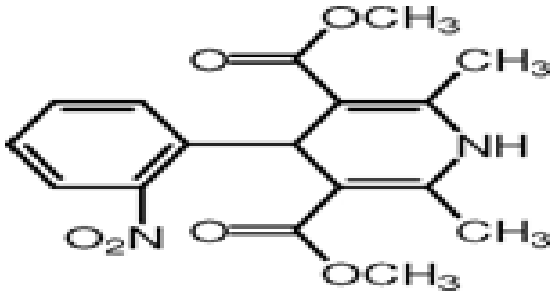

Fig. 2. Nifedipine structure**.**

This enzyme plays an important role on the detoxification system and catalyzes the conjugation of the reduced form of GSH to xenobiotic substrates such as drugs for the purpose of detoxification.

Peroxidases Activities

The effect of the calcium antagonist in peroxidases activities on *S. cerevisiae* is pictured in fig 6, and show an increase of ascorbate-peroxidase and gaiacol-peroxidase activities due to the presence of our molecule.

Discussion

The results obtained in this study shown that the administration of nifedipine led to a decrease the levels of reduced glutathione (GSH) and increase the activities of glutathione-S-transferase (GST), gaiacol-peroxidases (GPx) and ascorbate-peroxidases (APx) which may be indicate a generation of oxidative stress by this drug.

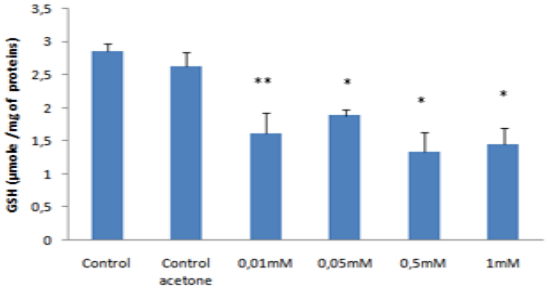

Fig. 3. Evolution of GSH level as a function of different Nifédipine concentrations on Saccharomyces cerevisiae

The decrease in GSH level is a consequence of the increase on its utilization by the antioxidant enzymes glutathione-S-transferase (as is shown in our results) and glutathione peroxidase. This result is in agreement with those found by (Vitcheva *et al.,* 2009) on male wistar rats and tends to show an oxidative stress caused by the nifedipine presence. A GSH will be conjugate with free radical and ROS, this conjugate reaction gives a radical thiol and during which the GSH is oxidized to glutathione disulphide (GSSG), a chain reaction ensues leading to the conversion superoxyde anions by the superoxide dismutase (SOD) into hydrogen peroxide (H_2O_2) which itself will be converts into water (H_2O) by the peroxidases enzymes. Therefore, GSH acts as a cofactor in the removal of toxic radicals. During oxidative stress GSH level declines and GSSG level increases (Ray *et al.,* 2005 / Ray *et al.,* 2012).

Literature data shown that Nifedipine induce cytochrome P 450 (Kastelova *et al.,* 2000; Drocourt *et al.,* 2001) which can be responsible of reactive oxygen species (ROS) formation and may explain the GSH depletion and antioxidants enzymes stimulation.

Paradoxically, another study rapport that nifedipine increase the GSH rate by modulation of biliary GSH and GSSG/conjugate efflux in rat liver due to the stimulation of the GSH secretion (Yang and Hill, 2001) which is in disagreement with our found. When the work of (Gaafa ***and al.***, 2011) demonstrates that nifedipine doesn't influence the GSH rate.

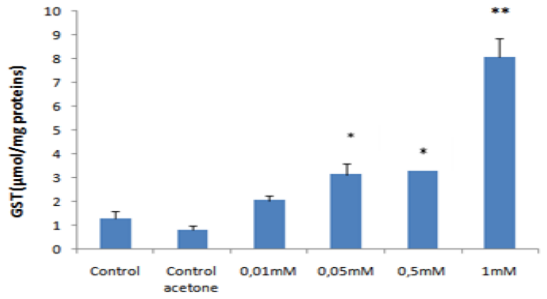

Fig. 4. Evolution of glutathione S-transferase activity as a function of different nifédipine concentrations on *Saccharomyces cerevisiae*.

When the increase of APx and GPx indicate that nifedipine caused an occurrence of oxidative stress translated by the outbreak of detoxification system. Our results are in desagreement with the work of (Wang *et al.,* 2011) on the plants peroxidase, who show that nifedipine affected the activity of peroxidase because the plant defense systems rely in part on a finely regulated cross-talk between calcium and H_2O_2 and the nifedipine as L-type calcium channels blocker inhibit the expression of the calcium gradient, thereby this would cause the decrease of peroxidases activities.

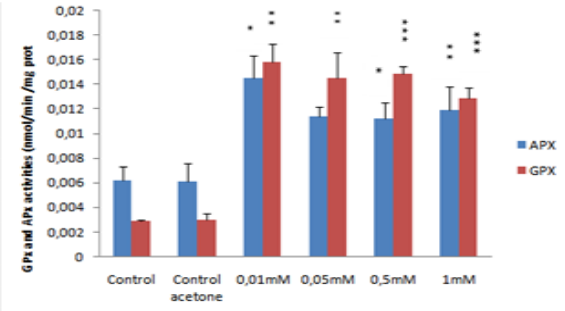

Fig. 5. Evolution of Peroxidases activities (GPx: gaiacol-peroxidases and APx: ascorbate-peroxidases) as a function of different nifédipine concentrations on *Saccharomyces cerevisiae*

In the other side, nifedipine is known for its antioxidant properties (Mak *et al.,* 2002 / Berkels *et al.,* 2005/ Yamagishi *et al.,* 2006*)*. This antioxidant action is due to fact that nifedipine indirectly regulates the expression and activities of antioxidant enzymes such as superoxide dismutase (Fukuo *et al.,* 2002 ; Passacquale *et al.,* 2008) these one catalyse the dismutation of superoxide ($O_2^{\cdot}$) into oxygen (O_2) and hydrogen peroxide (H_2O_2). This will cause the activation of the peroxidases enzymes such as catalase, glutathione peroxidase, gaiacol-peroxidase and ascorbate-peroxidase and this is confirmed by the increase of those activities in our study and even an increase in the catalase activity in our precedent study (Cherait and Djebar, 2013) which will converts hydrogen peroxide into water.

So we can conclude that nifedipine in the presence of stress induced by another molecules such as in the case of cyclo-sporine induced nephrotoxicity (Chander and Chopra, 2005) or renal tubular toxicity caused by gentamicin (Jin Li *et al., 2009)* or even the cocaine toxicity (Vitcheva *et al.,* 2011) will act as a protective and antioxidant agent due to its inhibitory effects of stress and ROS increases (Mamczarz *et al., 1998 /* Rojas-Rivera *et al., 2009)* but in the absence of another stress causes this drug will be the oxidative stress source as in the case of our studies. This hypothesis needs further investigations that are an object of future studies.

Furthermore, we notice that the yeast *Saccharomyces cerevisiae*, is well-established and convenient eukaryote model chiefly for toxicology studies but also for understanding the mechanisms of drug action and the cellular response interfering in drugs treatment. Nevertheless, *Saccharomyces cerevisiae* cannot completely substitute the mammalian cells and animal models especially for the complexity of genetic interactions in these models.

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