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# Electron Transport Chain Regulation Under Cadmium Stress in Mitochondria Isolated from Yeast Cells (*Saccharomyces Cerevisiae*).

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## ABSTRACT

Cadmium is an extremely toxic pollutant of the environment. It has many toxic effects in several organisms and its toxicity is exerted by the oxidative damages that it induces at the cellular level. Its long half-life (15 to 30 years) allows it to accumulate in many organs and tissues. Mitochondria are the main targets of cadmium. Cellular mode of action of cadmium on the functioning of the Mitochondrial Electronic Transport Chain (METC) remains unclear.

The present study is carried out to examine the mechanism of cadmium Cd-induced toxicity (500 µM) in isolated yeast mitochondria and the possible potential role of aspirin (500 µM) under (Cd2+) toxicity. We evaluated the effects of Cd on mitochondrial function such as Respiratory Electron Chain (state 3 and 4), Respiratory Control Ratio (RCR), Transmembrane Electrical Potential (ΔΨ), ATP levels, ROMs generation, Antioxidant enzyme Catalase (CAT), MalonyDialdehyde (MDA/lipid peroxidation) level and finally test the effect of aspirin on these changes. Cd affects the oxygen consumption of mitochondria respiration chain by a decrease in the rate of ADP phosphorylation (inhibition of State 3). Strong Stimulation of oxygen consumption in state 4 is observed. A decrease in (RCR) value observed is due to the disturbances observed in oxidations rates of states 3 and 4.

These results suggest that mitochondrial damage observed, resulting in the uncoupling of the oxidative phosphorylation, depression in ATP synthesis level, reduction of RCR and dissipation of transmembrane electrical potential (ΔΨ) which is the fundamental parameter of the oxidative phosphorylation via the mitochondrial complex V. We also observed an increase in antioxidant

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enzyme (CAT) and level of stress metabolites such as malondialdehyde (MDA), and ROMs in Cd-treated organelles. Addition of aspirin to mitochondrial suspension attenuated Cd negative effect on these parameters. Evaluation of the energetic regulation of the COx and AOX patways under Cd treatment was carried out. The results showed that Cd induced a strong inhibition of the COx pathway and the addition of aspirin seems to maintain this effect. Aspirin could form a complex with Cd, capable of generating strong resistance in yeast mitochondria to induced toxicity.

**Keyword:** Cadmium, *S. cerevisiae*, Mitochondria, Mitochondrial Electronic Transport Chain, COx, AOX, Uncoupling, Aspirin.

## Introduction

Heavy metals are the most toxic components that affect aquatic and terrestrial organisms, particularly due to the significant increase in their environmental concentrations (Ernst,1980; Hafner *and al.*,1990; Rahman and Singh, 2019). The similar physico-chemical properties of heavy metals and their long biological half-life (Hilmy *and al.*,1985), give them similar characteristics that allow them to be easily absorbed by living organisms, where they accumulate in large quantities in many tissues (Forster and Wittmann,1979). Their incorporation is at the origin of various toxicity both at the cellular level and at the sub-cellular level by affecting the vital metabolic pathways such as the energy metabolism of the mitochondria (Woolhouse,1983).
Among the most dangerous heavy metals, cadmium, due to its unique high affinity for the free electron pairs of the SH groups, constitutes one of the most toxic (Maghsoudi *and al.*, 2021; Branca *and al.*, 2020).

Excess cadmium is responsible for numerous toxic effects for plants, animals and humans: generation of free radicals, growth retardation, induction and/or inhibition of enzymes, etc. (Rao, 1986; Kumar and Sharma, 2019). It can also inhibit the growth of many microorganisms including yeasts (Sbartai, 2005; Guelti *and al.*, 2003). The disturbances induced by Cd lead to unfavorable consequences at the cellular level (Zaoui and Djebar, 2011). Mitochondria being the most sensitive targets (Miccadei *and al.*, 1993). Many studies have evaluated the effect of Cd on oxygen consumption as well as on the redox state of electron transporters in rat liver

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mitochondria. According to Miccadei *and al.,* (1993) cadmium is responsible for an inhibition of uncoupler-stimulated oxidation on various NADH-related substrates as well as that of succinate. Experiments on specific segments of the respiratory chain have shown that cadmium does not inhibit the flow of electrons through cytochrome oxidase (COx), but would rather be responsible for an alteration of the flow of electrons through the ubiquinone-b-complex-cytochrome c (Castiglione and Bianchi, 1989; Alexandre and Lehninger, 1984).

At the same time, another xenobiotic has been shown, according to several studies, to induce effects on the mitochondrial respiratory chain, namely acetylsalicylic acid, more commonly known by the trade name "aspirin". Aspirin is a nonsteroidal anti-inflammatory widely used as an analgesic, antipyretic and antiplatelet agent (Tanasescu *and al.,* 2000; Laborier *and al.*, 2021). Numerous studies stipulate that prolonged use of aspirin in humans constitutes a protective factor in the occurrence of certain cancers as well as protection against the development of colon cancer and other cancers of the digestive system (Shi *and al.*, 1999; Thun *and al*., 1991; Kune *and al*., 1988). Animal experimentation has revealed that aspirin is able to inhibit tumors of the breast (McConnick *and al*., 1985), colon, stomach, liver and skin Guirguis-Blake *and al*., 2022; Suman *and al.,* 2021). Despite numerous investigations, the mechanism of action of aspirin remains unknown. Recent studies have shown that due to its chemical structure similar to that of salicylic acid, aspirin can protect cells by scavenging the hydroxyl radical (•OH-) (Wang and Walsh, 1995). Other studies show the antioxidant activity of aspirin against reactive oxygen species (oxygen free radicals and their metabolites) involved in many pathological processes (Shi *and al*., 1999; Djebar *and al*., 2021). In many plant species and micro-organisms, the mitochondria have two pathways (the called COx pathway: Cyt. c Oxidase and the called AOX pathway: Alternative Oxidase (Rich, 1978; Sankar *and al.*, 2022). Under normal conditions, the COx pathway is the most predominant pathway, however under stress (drought, MTE, salinity), the AOX pathway is strongly induced. A common factor in these situations is an inhibition of the cytochrome pathway (Purvis and Shewfelt,1993).

The question is how cadmium acts on these two pathways and what role of aspirin could play in this process?. A third element have drawn our attention, it is the Cd-ROMs-Aspirin relationship in the regulation of these two pathways (Minagawa *and al*., 1992; Minakami *and*

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*al.*,1989; Chen *and al*., 2020). Therefore, the effect of cadmium and the role of aspirin on the regulation of the mitochondrial electron transport chain (COx and AOX) has been studied.

## Materials and methods

### Isolation of mitochondria:

The isolation of highly pure mitochondria with a good structural and functional integrity from the yeast cell suspension was carried out according to the method of Pan *and al.*, 2018. After having isolated them using an isolation buffer (KCl 180 mM, 10 mM KH2PO4 (pH 7.2)), the cells described were incubated in dithiothreitol buffer, in a first step and then activated with zymolyase, in a second step. The cells were washed and then potted in ice-cold SEM buffer. Mitochondria were separated by differential centrifugation. Using Bradford's test, protein concentration was obtained and mitochondria were aliquoted in 1 mg aliquots.

### Proteins Assay

The determination of mitochondrial proteins is carried out according to the method of Bradford (1976). The absorbance is measured with a spectrophotometer (Jenway 6505) at the wavelength 596 nm. The standard protein used is BSA.

### Treatment of mitochondria by cadmium

Mitochondria were treated with 0.5 mM of CdCl2 for 5 minutes at 25°C in standard incubation medium containing 150 mM mannitol, 100 mM KCl and 10 mM MOPS, pH 7.3, in the absence or presence of 0.5 mM Aspirinbefore the addition of succinate.

### Mitochondrial respiration and membrane potential activities:

Determinations of oxidation rates in phosphorylation state 3 (Vox3) and non-phosphorylation state 4 (Vox4) are measured in parallel and simultaneously with potential membrane $\Delta\psi$ at 25°C using Clark electrode oxygen (Hansatech unit, UK), connected to a computer and a TPP+ (TetraPhenylPhosphonium) sensitive electrode (Duthie *and al*., 1991; Davy de Virville *and al.*, 1994). The reaction mixture with a volume of 2 ml contains 0.3M of mannitol, 10mM of phosphate buffer (KH2PO4/K2HPO4) and (pH7.2); 5mM of MgCl2, 10mM of KCl and 1g.l−1 of BSA. Initiation of respiratory state 3 is obtained by adding 5 mM of succinate and a small

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quantity of ADP (30 mM). Respiratory state 4 is obtained after total consumption of the added ADP (ADP phosphorylated into ATP). The mitochondrial membrane potential (Δψ) was monitored by evaluating transmembrane distribution of the (TPP+), TPP+ uptake was measured from the decreased TPP+ concentration in the medium. At the end of each trace, the membrane potential is completely reduced by adding valinomycin (100 ng/mg of MP protein), (Djebar 1988).

**Catalase (CAT) activity**

Catalase activity is determinated according to Cakmak and Horst (1991). The reaction mixture contained 100µl enzyme extract, 50µl H2O2 (300mM) and 3 ml of 50mM KH2PO4 (pH 7.2). The measurement of the disappearance of H2O2 by catalase in the reaction mixture is carried out by spectrophotometry (Jenway 6505) at 240 nm.

**Estimation of ROMs content:**

ROMs content is measured by using (FRAS Evolvo System srl-Parma) with diagnostic kits (Pasquini *and al*., 2008). The assay was adapted to our working conditions and biological model, *S. cerevisiae.*

**Measurement of MalonDiAldehyde (MDA)**

The assay of malondialdehyde is carried out according to the method of Draper *and al*., (1990). This colorimetric method involves the reaction of malondialdehyde (MDA) (product of lipid peroxidation) with thiobarbituric acid (TBA), giving a red color-pink measured by spectrophotometry at 532 nm (Aguilar Diaz De Leon and Borges, 2020). Result was expressed in µmol/mg proteins.

**ATPase activity**

ATPase activity is determined by measure of ATP by bioluminescence using the reaction catalyzed by the luciferin-luciferase association (Bomsel, 1973). The reaction is triggered by adding 1 mM of ATP. The assay is done using a luminometer (Pico-ATP) and the light energy is transformed into an electrical signal proportional to the amount of ATP present. Standard solutions of determined concentrations of ATP are used.

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### Determination of GSH level

The level of total SH compound (GSH) was determined with according to Wechbeker *and al.*, (1988). GSH was assayed by adding 2 ml of 0.5 Mm 5, 5'-dithio-bis -2-nitro benzoic acid, (DTNB) in 0.2 M phosphate buffer, pH 8.0 to appropriate volume of treated mitochondria suspension. The reaction of GSH with DTNB forms a yellow colored complex. The monitoring of the reaction is carried out after 2 min at a wavelength of 412 nm.

## Results

### Cadmium and aspirin effects on mitochondrial oxidation :

Mitochondria isolated from yeast cells (*S. cerevisiae*) show good functional properties. Addition of succinate followed by ADP induces high oxygen consumption observed through oxidation rates at state 3 (Fig. 1).

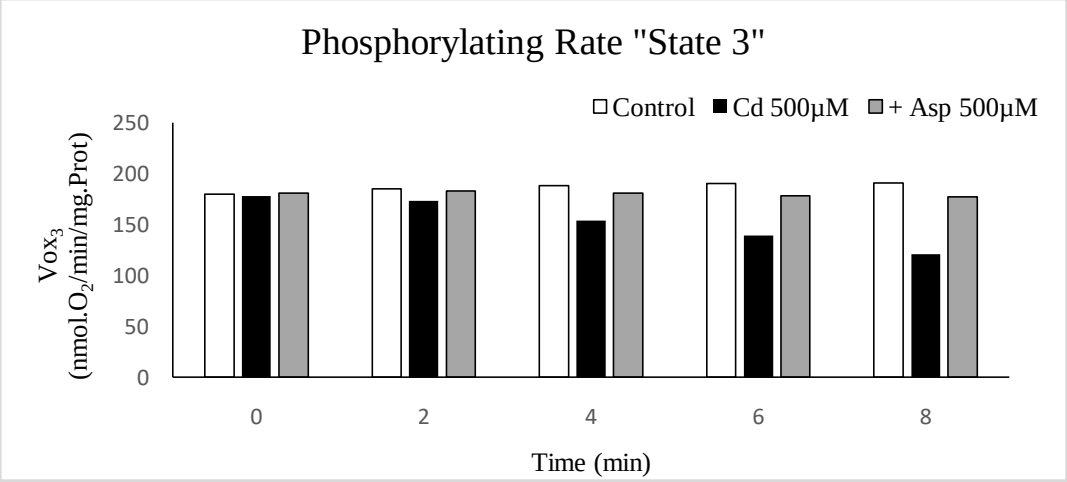


**Figure 1.** Effect of cadmium and aspirin on oxidative rate state 3 in isolated yeast cells mitochondria.

In the control mitochondria, there is a slight increase in these speeds, which range from 180 nmol.O2/min/mg Prot. at 0 minutes at 191 nmol.O2/min/mg Prot. at 8 minutes (Fig.1). Exposure to Cd (500µM) resulted in rapid and strong inhibition of ADP-stimulated respiration (35% compared to controls) from 178 nmol.O2/min/mg Prot. at 0 minutes at 121 nmol.O2/min/mg Prot. at 8 minutes. The mitochondria treated with aspirin (500 µM) showed a respiratory rate identical to that of control mitochondria (Fig. 1).

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The oxidation rates in state 4, after total consumption of ADP (phosphorylation of ADP into ATP), show oxygen consumption by the control mitochondria (60 nmol.O2/min/mg Prot. at time 0 min) to decrease to 54 nmol.O2/min/mg Prot. after 8 min of reaction), (Fig. 2).

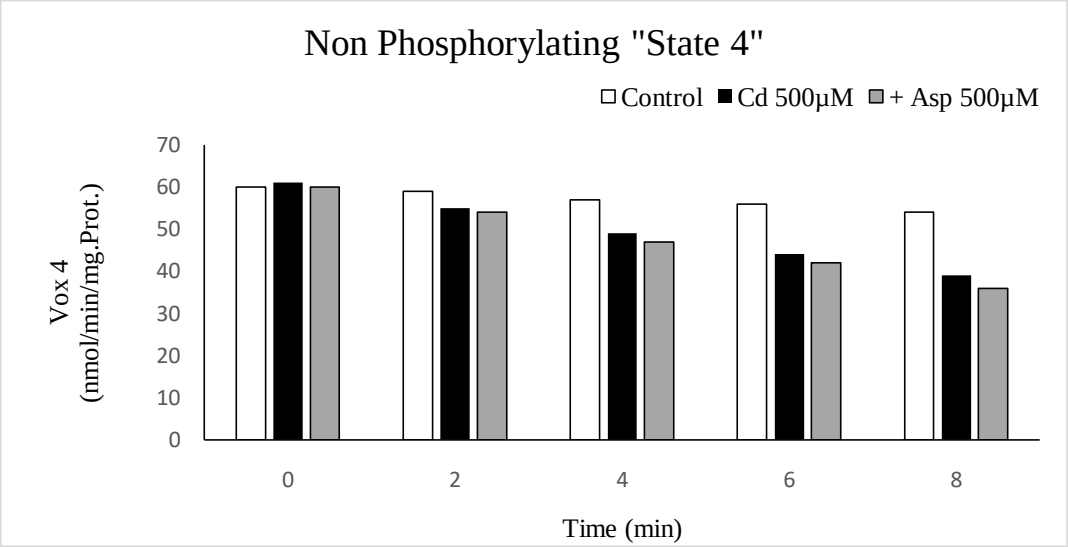


**Figure 2.** Effect of cadmium and aspirin on oxidative rate state 4 in isolated yeast cells mitochondria.

Exposure to cadmium (500µM) significantly affects the oxidation rates in state 4 (61 nmol.O2/min/mg Prot. at time 0 min to 39 nmol.O2/min/mg Prot. after 8 min, a reduction of nearly 40%). These values are 30% lower than those obtained in control mitochondria. This effect could reflect a movement of ions which could affect the permeability of the mitochondrial membrane and thus induce a dysfunction of the mitochondrial respiratory chain causing a disturbance of cellular energy. At the same time, in the presence of 500 µM of aspirin, the values of the oxidation rates at state 4 recorded are close to those obtained in the control mitochondria (60 nmol.O2/min/mg Prot. at 0 min at 36 nmol. O2/min/mg Prot. after 8 min). This seems to indicate that the observed disturbance of the oxidation rate induced by cadmium in state 4 seems insensitive to the presence of aspirin (Fig. 2).

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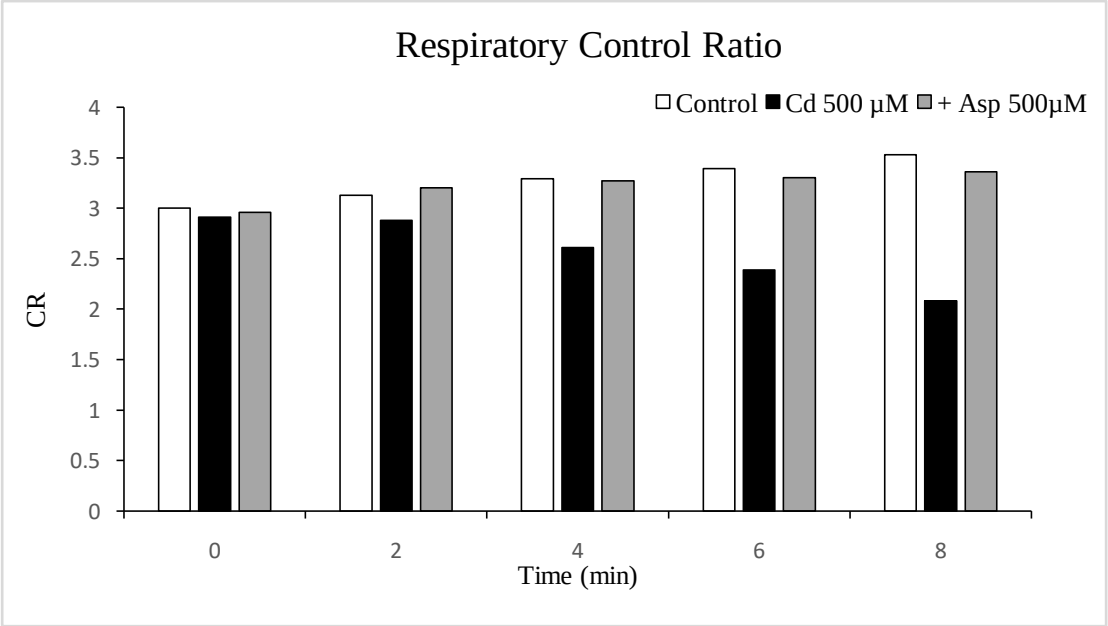


**Figure 3**. Effect of cadmium and aspirin on Respiratory Control «RCR» in isolated yeast cells mitochondria.

The ratios of RCR respiratory controls calculated following the effects of cadmium treatments on the mitochondria isolated from *S. cerevisiae* cells (Fig. 3) decrease following the decrease in oxidation rates in state 3 and 4. At the same time, the evolution oxidation rates in state 3 and 4 of mitochondria treated with cadmium in the presence of aspirin demonstrates a clear increase in the RCR obtained. Thus the strong decrease in RCR observed following treatment with 500 µM of Cd is strongly reduced in the presence of 500 µM aspirin. The RCR obtained in the presence of aspirin are very close to those of the control mitochondria. Thus, the reports of RCR respiratory controls which reflect the state of coupling of the mitochondrial respiratory chain show a strong disturbance of the functioning of the oxidophosphorylating activity generated by exposure to cadmium. The presence of aspirin seems to correct this disturbance and consequently restores the energetic functioning of the mitochondrial respiratory chain.

## Cadmium and aspirin effects on Transmembrane Electrical Potential of Mitochondria (ΔΨ):

The transmembrane electrical potential (ΔΨ) in mitochondria plays a decisive role in maintaining the functional balance of the oxidation of respiratory substrates and the phosphorylation of ADP into ATP at the level of the mitochondrial respiratory chain. In order to examine the action of

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cadmium and aspirin, we treated mitochondria isolated from yeast cells with a protonophore, CCCP (Tab. 1).

**Table 1 :** Effects of cadmium and aspirin on Transmembrane Electrical Potentiel ΔΨ in yeast cells mitochondria.

| ΔΨ (mV) | (Time (min) (+ Cd 500 µM) | | | | | | |
|---|---|---|---|---|---|---|---|
| | + Succ. (20 mM)0 | 1 | 2 | 3 | 4 | 5 | |
| **- Asp (500µM)** | 232 | 182 | 129 | 78 | 76 | 75 | 75 |
| **+ Asp (500 µM)** | 230 | 186 | 176 | 152 | 138 | 120 | 98 |

Studies on succinate energized mitochondria were performed using 500 µM Cd. Table 1 shows that the ΔΨ of control mitochondria is 230 mV. Exposure to cadmium immediately induces a strong dissipation of ΔΨ which reaches 60% after 5 minutes of exposure. The ΔΨ dissipation is partially inhibited by addition of aspirin (50%).

**Cadmium and aspirin effects on ATPase activity of Yeast cells Mitochondria:**

Figure 4 shows the effects of 500 µM cadmium on the mitochondrial ATP activity.

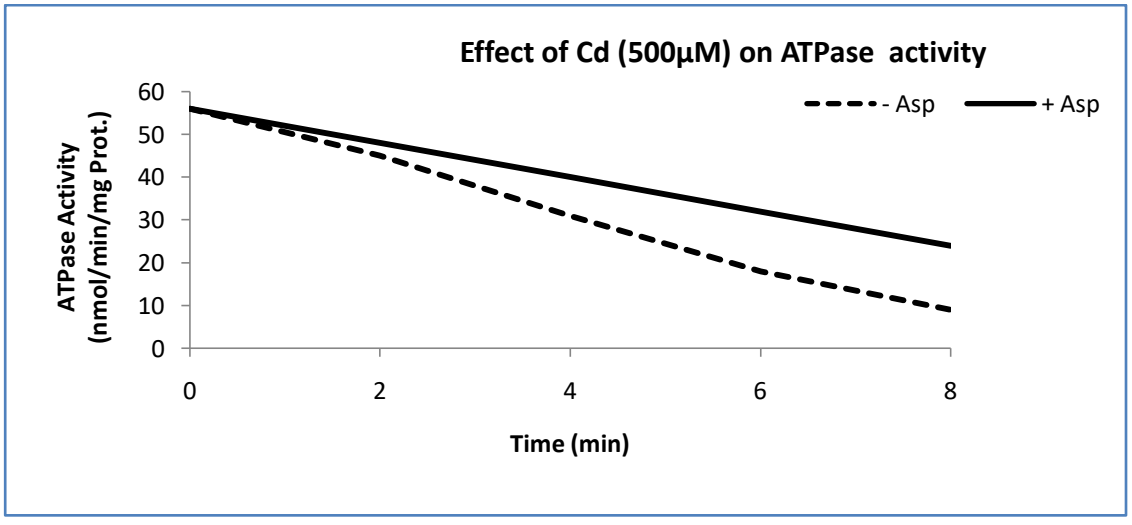


**Figure 4**. Effect of cadmium and aspirin on ATPase activity in isolated yeast cells mitochondria.

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A significant inhibition of ATPase activity is observed with time (85%), reflecting a significant drop in ATP synthesis. However, the addition of 500µM cspirin to assay medium causes a strong slowing down of this inhibition by 40%.

**Cadmium and aspirin effects on Malondialdehyde content of Mitochondria (MDA):**

The measurement of MDA content in mitochondria isolated from *S. cerevisiae*was time-dependent.

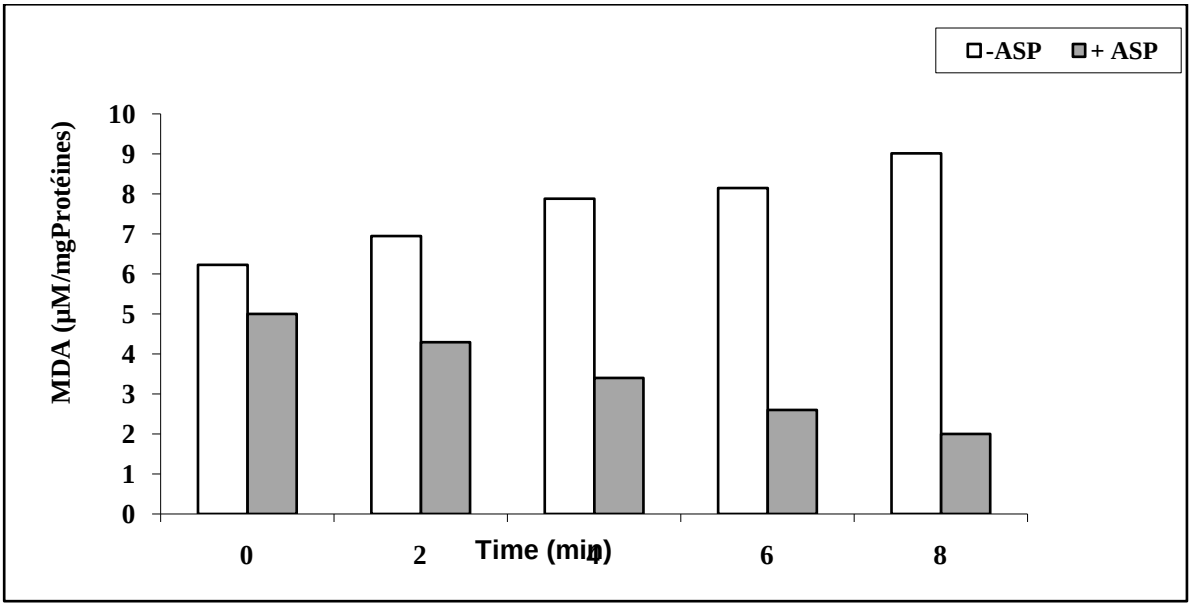


**Figure 5**. Effect of cadmium and aspirin on MDA content in isolated yeast cells mitochondria.

Cd treatment at 500 µM increased MDA content increased at different time compared with the controls (t:0min), (Fig.5), and after 8 min of reaction the highest level of MDA was obtained (9.02). Thus, treatment with cadmium causes a strong stimulation of MDA levels which reaches 35%. This reflects stress at the level of the mitochondrial respiratory chain, which is one of the main production sites of Reactive Oxygen Species (ROS) at the cellular level. Nevertheless, there is a drop in the MDA level in the presence of aspirin (500 µM), showing a decrease in lipid peroxidation at the mitochondrial level.

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**Cadmium and aspirin effects on Alternative Oxidase of Mitochondria (AOX):**

In plant mitochondria, alternative oxidase (AOX) is one of the terminal oxidases of the mitochondrial respiratory chain.

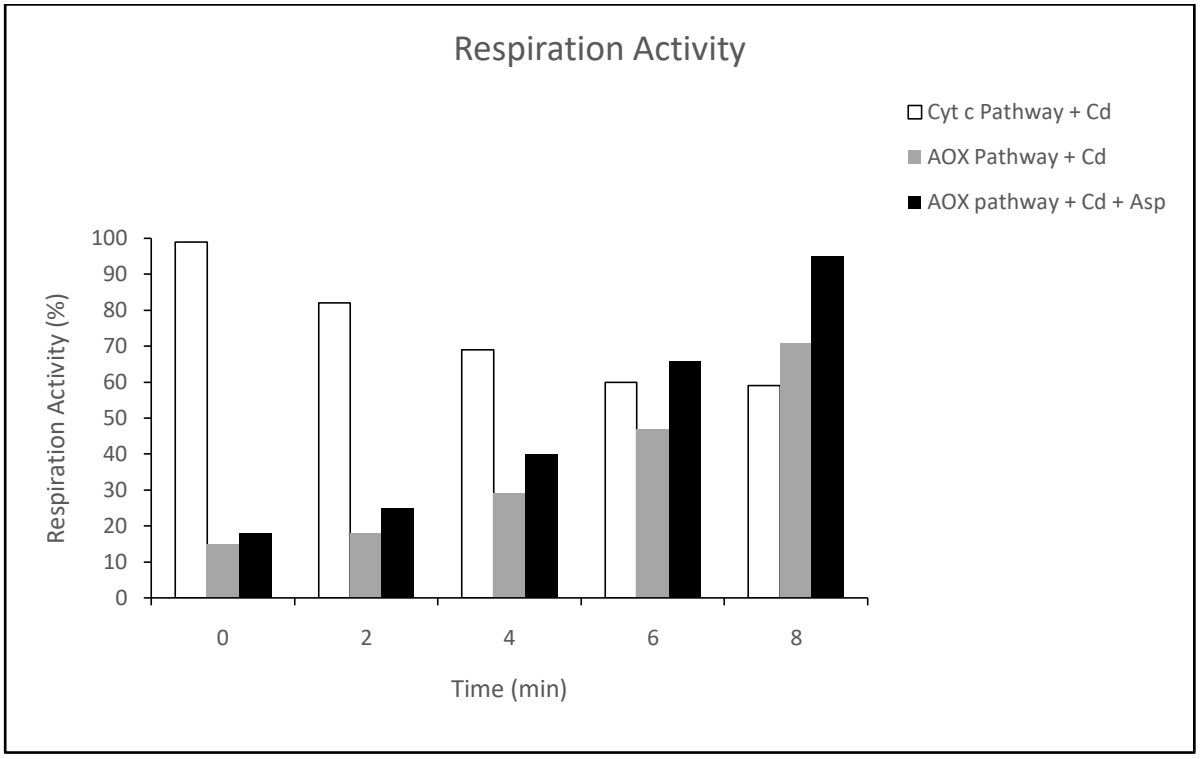


**Figure 6**. Effect of cadmium and aspirin on AOX activity in isolated yeast cells mitochondria. Cadmium concentration (500µM), aspirin concentration (500µM).

The respiratory activity of mitochondria isolated from *S. cerevisiae* and treated with 500 µM cadmium decreases by approximately 40% with time. This reduction concerns the classic respiratory patway (Cyt C pathway, COx). At the same time, treatment with cadmium stimulates the alternative patway (AOX pathway, AOX) by almost 80%. The addition of aspirin 500 µM significantly increases this activity to reach 95%. Thus cadmium and aspirin at 500 µM cause a strong stimulation of alternative respiratory activity (AOX) in mitochondria isolated from *S. cerevisiae.*

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**Cadmium and aspirin effects on Catalase Activity of Mitochondria (CAT):**

The evaluation of the activity of the enzyme catalase revealed an increase in the latter in the treated with 500 µM of cadmium.

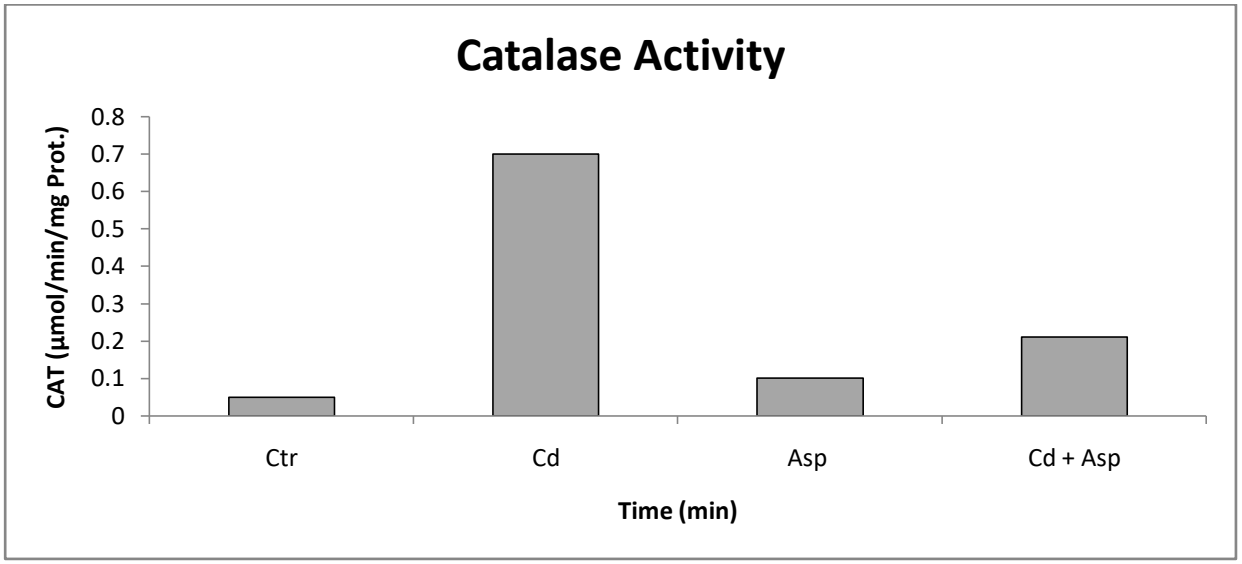


**Figure 7**. Catalase activity in mitochondria isolated from yeast cells and treated with cadmium 500 µM in presence and absence of aspirin 500 µM.

Compared to controls, Cd increased CAT activities 14-fold at 500 µM and 2-fold in the presence of 500 µM Asp (Fig. 7). Significant changes in CAT activities were found upon Cd treatment in the presence of 500 µM of Asp.

**Cadmium and aspirin effects on Reactive Oxygen Metabolites generation (ROMs):**

The results are shown in Table 2 (Tab.2).

**Table 2 :**ROMs level in mitochondria isolated from *S. cerevisiae* and treated with cadmium in presence or absence of aspirin.

| **Cd (µM)** | **ROMs (U/CARR)** | |
|---|---|---|
| | **- Asp.** | **+ Asp.** |
| **0** | 08 | 09 |
| **100** | 12 | 10 |

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| | | |
|---|---|---|
| **200** | 18 | 12 |
| **300** | 26 | 13 |
| **400** | 44 | 16 |
| **500** | 69 | 20 |

In mitochondria treated with cadmium (500 µM)an increase in total ROMs was observed (Tab. 2). The level peak was observed at the highest concentration of Cd. This production was significant and increased with the concentration in particular for the highest concentration compared to the controls. This increase is of the order of 88% for the highest concentrations (500 µM). Addition of aspirin at 500 µM drastically reduced the production of ROMs by approximately 30 compared to Cd treated mitochondria.

## Discussion

The potentially harmful effect of Cd on mitochondria has been reported by several *in vivo* studies (Stacey *and al*.,1980; Rao and Gardner,1986; Muller and Stacey,1988) and on cells (Jarvisalo *and al*.,1980;Polykretis *and al*.,2019) as well as isolated mitochondria (Carmen *and al*.,1986; Luo *and al.*,2021). Some studies report that theses subcellular organelles are the preferred targets of Cd cytotoxicity (Martel,1990; Jamall and Sprowls,1987).Our study focuses on the one hand on the effects of exposure of Mitochondria isolated from *S. cerevisiae* to 500 µM of cadmium and on the other hand on the protective role of aspirin on cadmium-induced cytotoxicity. The observations reported in this paper show that cadmium is a potent disruptive (dysfunction of the cellular energy transduction) of the Electronic Transport Mitochondria Chain (ETMC) of mitochondria isolated from *S. cerevisiae*. At first, Cadmium effects on the rate of Oxidation in state 3 and 4 was measured. The results obtained show that upon treatment with Cd (500 µM), a significant decrease in rate oxidation in state 3 was observed in the mitochondria isolated from *S. cerevisiae*. This is in agreement with previous studies on isolated mitochondria from Zea mays and alveolar macrophage (Miller *and al*., 1973; Mustafa and Cross 1971). According to many studies that have investigated the effects of Cd on ETMC Complex II (Liu and Liun, 1990; Fariss, 1991; Jay *and al.*, 1991; Korotkov *and al.*, 1998; Belyaeva *and al.*, 2011; Casalino *and al*., 2002; Wang *and al.*, 2004; Modi and Katyare, 2009), the decrease in succinate oxidation

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(Vox3), in mitochondria is due to the interaction of Cd with the SDH complex 2 (Succinate Dehydrogenase of Cytochrome C Oxidase: COxII) complex and does not directly affect the cytochromes of METC (Mitochondrial Electron Transport Chain). These data support the hypothesis that the SDH (COxII) complex is the primary target site for cadmium-mediated respiration inhibition (Branca *and al.*,2020). At the same time, the oxidation rates in state 4 which reflects proton leakage was also affected by exposure to Cd. The Respiratory Control Ratio (RCR), which is the measure of mitochondrial coupling significantly decreased as a result of the observed changes rates oxidation in states 3 and 4 following the exposure to Cd. In contrast, recent studies (Wang *and al*., 2004), showed that Cd binds weakly to the UQ site, causing a change in its structural conformation which would trigger a partial inhibition of electron transfer to the cyt. b. This will lead to an accumulation of UQs (SemiUQ) at the level of complex 3. The instability of UQs will generate an electron transfer towards 02 resulting in the formation of ROMs (wang *and al*., 2004 ; Michell,1976 ; Hansford *and al.*,1997).The addition of aspirin reduces the measured inhibition of oxidation rates in states 3 and 4 and improves the obtained RCR values. (Cannino, 2009; Djebar *and al.*, 2020).

The possible mechanisms of inhibition of energy regeneration capacity are related to the decrease in $\Delta\psi$. Our results indicate that exposure to Cd induced a significant reduction of the mitochondrial membrane potential. A similar Cd effect has been reported with isolated mitochondria from rat kidney (Floridi *and al.*,1983; Miccadei, 1993). It is well-known that the mitochondrial transmembrane electrical potential depends upon the H+ gradient, product by the METC localized on the inner mitochondrial membrane (Mattie and Freedman,2001). Therefore, a reduction of proton gradient $\Delta\mu H+$, can occur via an alteration in the activity of the ETMC after exposure to Cd. This was confirmed by Rao*and al* (1983) who reported the inhibition of METC (succinic dehydrogenase, COxII), in the liver, kidney, testis and lungs of rats after administration of Cd. Protection provided by aspirin against cadmium-induced toxicity was observed in this study. These observations are similar to those of Gao *and al.,* (2022) in which toxicity induced in endothelial cells by the Fenton catalyst iron was attenuated by pre-treatment with aspirin ($\Delta\psi$).The bioluminescence analysis (Fig. 4) shows that cadmium decrease the ATP synthesis in mitochondria isolated from *S. cerevisiae*. It is established that cadmium might increase the proton leak using two possible ways : 1. Cadmium-induced leak (increase in the passive permeability of the Mitochondrial Inner Membrane to protons), 2. Cadmium-induced slip (decrease in the H+/O

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stoichiometry of the proton-pumping complexes of the ETMC) (Waisberg *and al*.,2003). Cadmium provokes the inhibition of the respiration rate and consequently decreases ΔΨ, which causes a secondary decrease in the rate of phosphorylation. These two correlated effects of cadmium cause a severe inhibition of ATP synthesis in mitochondria isolated from *S. cerevisiae.* Investigations on respiration rates of mitochondria (Green and Reed,1998; Rikans and Yaman,2000) suggest that interference with energy-conserving reactions contributes to the toxicity of heavy metals and direct inhibition of ATP-synthesizing reactions. This leads a disturbance of mitochondrial bioenergetics which constitute the primary effects of heavy metals and uncouple mitochondria (Huettenbrenner *and al.*,2003; Cannino *and al.*,2009; Green and Reed,1998). In contrast, the addition of aspirin led to the lowered ATP activity inhibition. Thus, our study showed that the addition of aspirin to the mitochondrial suspension of *S. cerevisiae* treated with 500 µM of Cd attenuated to a large extent the harmful effects caused by the imposed stress (Cd), to the mitochondrial metabolism.

In the present study, the oxidative damage was evaluated by the level of formation of MDA, an end product of lipid peroxidation, to determine whether cadmium exposure causes oxidative stress. The formation of MDA was increased in mitochondria exposed to Cd compared to control. This confirm some of others studies which reported an increase in lipid peroxidation under the influence of Cd (Desagher and Martinou, 2000; Lee *and al*.,2004). Lipid peroxidation is known to cause membrane injury by inactivation of membrane enzymes and receptors, (Luqman and Rizvi, 2006). This effect causes the dysfunction of mitochondria. Aspirin treatment significantly reduced the stimulatory effect of cadmium on malondialdehyde (MDA). The effects of aspirin in mitochondria could be explained by a possible hypothesis that aspirin interferes with several unrelated pathways that are yet to be characterized and aspirin is only capable of reversing the antioxidant balance of the organelle (Zhang *and al.*,2021).

AOX acts as a regulatory pathway in the highly coupled and regulated electron transport process in mitochondria, thereby providing and maintaining essential metabolic homeostasis by directly reducing oxygen to water. In *S. cerevisiae* isolated mitochondria, a decrease in respiration activity (COx pathway) occurs after the exposure of the organelles to cadmium (500 µM). This decrease in respiratory activity reaches a maximum (40%) at 8 min of reaction. At the same time, exposure to cadmium significantly stimulates respiratory activity (AOX pathway) by 80% and the addition of aspirin seems to maintain or even slightly increase this activity (82%). It is

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confirmed that AOX was found to modulate oxidative challenge due to cadmium exposure (Keunen *and al.*,2016). The effect of aspirin in response to cadmium exposure may have stabilized homeostasis balance by suppressing mitochondrial dysfunction. This study suggests that AOX activity may be (indirectly?) influenced by aspirin, an important component in many biotic stress responses.

The exposure of *S. cerevisiae* to cadmium increases the catalase (CAT) activity, an essential enzyme in the detoxification mechanisms, which catalyzes the conversion of H2O2 to molecular water and oxygen (Zeriri *and al.*, 2012). This observation is confirmed in our study using mitochondria isolated from *S. cerevisiae*. It can be explained by the activation of an antioxidant mechanism to prevent the accumulation of ROMs (Cherait and Djebar, 2013). Significant increase in CAT activity (90%) after cadmium exposure compared with controls is due to disrupt of the metabolic balance between the production/scavenging of ROMs, (Morakinyo *and al.*,2011).Catalase activities from *S. cerevisiae* mitochondria treated with cadmium after addition of aspirin were substantially inhibited (70%).

In order to examine the effects of cadmium on the synthesis of ROMs, we have measured the ROMs produced in the presence of cadmium and then to find out what would be the effect of aspirin on this synthesis. The production of ROMs does not necessarily lead to oxidative stress in the case where the latter contributes in increasing the defense processes, (Poletta *and al*., 2016). In this context, Reactive Oxygen Metabolites (ROMs) were quantified. We found that cadmium could directly increase levels of reactive oxygen metabolites (ROMs) by 8 times particularly at high concentration of cadmium (500 µM). This result is in agreement with Atailia (2016) who observed a strong production of ROMs on the hemolymph of the pulverized propeller of snails exposed to chronic metal pollution and Khen *and al.*,(2017) on the toxicity of the TiO2 microparticles on the bioindicator model snail *Helix aspersa*. Aspirin treatment demonstrated a reduction in mitochondrial Reactive Oxygen Metabolites (ROMs) production. Aspirin would then be at the origin of the recovery of redox homeostasis which would constitute the target of the improvement of the alterations generated by the oxidative stress at the level of the mitochondrial functions which are at the origin of the improvement of energy metabolism in these organelles.

Finally, AOX seems to play a key role in the protection against abiotic stress (MTE: Metallic Trace Elements, Cd) in plants and many other microorganisms (Casalino *and al.*,2002). In some studies Cd-treated organisms maintained partial energy coupling but displayed a rapid early fall

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in Complex 4 activity followed a progressively decreasing Complex II (Toury *and al*., 1985). In our study the primary target of action of the Cd on COx is the complex II (COxII) whose inhibition blocks electron transfer and electron accumulation, resulting in ROMs production (Branca *and al.*,2020).The crucial role of Complex II in ROMs generation was reported by Quinlan *and al.*, (2012). In some others study, mitochondria isolated from animal liver and heart, showed that even though only Complex III was demonstrated responsible for ROMs production (Wang *and al*., 2004).

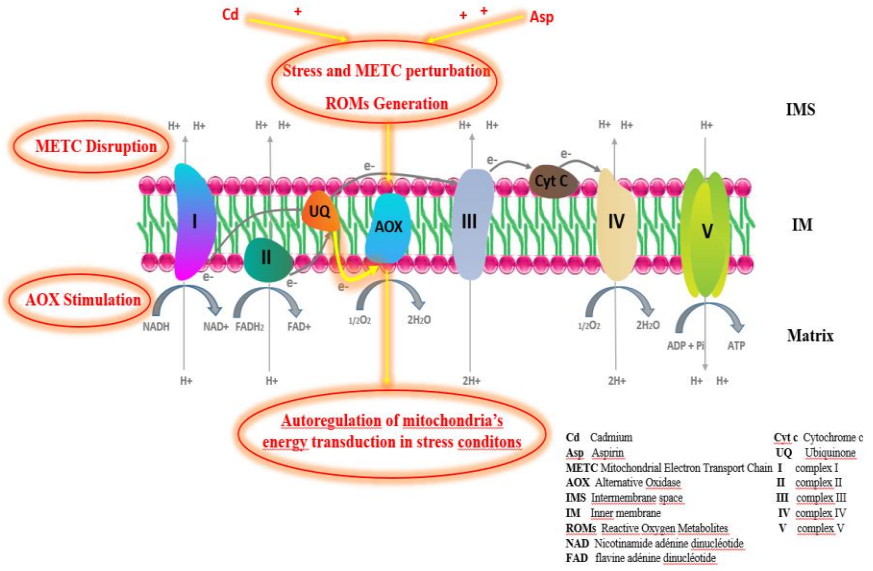


**Figure 8**. Schematic representation for METC regulation under stress (Cd).

Under stress (Cd) due METC complex dysfunction, (ΔΨ decrease, ATP synthesis reduction, disruption of MDA level, CAT inhibition and ROMs generation/accumulation) there is leakage of electrons from the METC complexes as ROMs causing AOX stimulation which generates passage through the METC near the UQ pool, thereby directly reducing O2 to H2O.

## Conclusion

In conclusion it appears that Cd acts mainly on mitochondrial compartment by inducing oxidative cellular stress, modulating METC homeostasis and lipid peroxidation, altering membrane structure and ultimately causing apoptosis, as summarized in Fig. 1. Hypothesis and the addition of aspirin further stimulates AOX activity. The present work allows us to suggest

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that during the first Cd-Induction followed by the second aspirin-stimulation by AOX, the Reactive Oxygen Metabolites (ROMs) produced could act as second messengers which are major in the maintenance of mitochondrial energy homeostasis which is essential for the survival of microorganisms.

## Conflict of interests

The authors declare no conflicts of interest.

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