

DÉBORA LUANA RIBEIRO PESSOA  
(ORGANIZADORA)

**Atena**  
Editora  
Ano 2020

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# FARMÁCIA NA ATENÇÃO E ASSISTÊNCIA À SAÚDE

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**Edição de Arte:** Luiza Alves Batista  
**Revisão:** Os Autores  
**Organizadora:** Débora Luana Ribeiro Pessoa

**Dados Internacionais de Catalogação na Publicação (CIP)**

F233 Farmácia na atenção e assistência à saúde 2 /  
Organizadora Débora Luana Ribeiro Pessoa. – Ponta  
Grossa - PR: Atena, 2020.

Formato: PDF  
Requisitos de sistema: Adobe Acrobat Reader  
Modo de acesso: World Wide Web  
Inclui bibliografia  
ISBN 978-65-5706-673-7  
DOI 10.22533/at.ed.737201512

1. Farmácia. 2. Saúde. I. Pessoa, Débora Luana Ribeiro  
(Organizadora). II. Título.

CDD 615

Elaborado por Bibliotecária Janaina Ramos – CRB-8/9166

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## APRESENTAÇÃO

A coleção “Farmácia na Atenção e Assistência à Saúde” é uma obra que tem como foco principal a apresentação de trabalhos científicos diversos que compõe seus capítulos, relacionados às Ciências Farmacêuticas. O volume abordará de forma categorizada e interdisciplinar trabalhos, pesquisas, relatos de casos e/ou revisões que transitam nas diversas áreas de atuação do profissional Farmacêutico.

O objetivo central foi apresentar de forma sistematizada e objetivo estudos desenvolvidos em diversas instituições de ensino e pesquisa do país. Em todos esses trabalhos a linha condutora foi o aspecto relacionado à atenção e assistência farmacêutica, farmácia clínica, produtos naturais, fitoterapia e áreas correlatas. Estudos com este perfil são de extrema relevância, especialmente para a definição de políticas públicas de saúde e a implementação de medidas preventivas na atenção à saúde.

Temas diversos e interessantes são, deste modo, discutidos aqui com a proposta de fundamentar o conhecimento de acadêmicos, mestres e todos aqueles que de alguma forma se interessam pelas Ciências Farmacêuticas, pois apresenta material que demonstre estratégias, abordagens e experiências com dados de regiões específicas do país, o que é muito relevante, assim como abordar temas atuais e de interesse direto da sociedade.

Deste modo a obra “Farmácia na Atenção e Assistência à Saúde” apresenta uma teoria bem fundamentada nos resultados obtidos pelos pesquisadores que, de forma qualificada desenvolveram seus trabalhos que aqui serão apresentados de maneira concisa e didática. Sabemos o quão importante é a divulgação científica, por isso evidenciamos também a estrutura da Atena Editora capaz de oferecer uma plataforma consolidada e confiável para estes pesquisadores exporem e divulguem seus resultados.

Débora Luana Ribeiro Pessoa

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# CAPÍTULO 1

## FLAVONOIDS AND GLUTATHIONE AS PROTECTIVE AGENTS FOR LEAD ACETATE TOXICITY IN

### *Saccharomyces cerevisiae*

Data de aceite: 01/12/2020

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**ABSTRACT:** Lead is considered a toxic heavy metal that is involved in generating oxidative stress, increasing reactive species of oxygen (ROS). Previous investigations have reported that natural antioxidants, sources of reduced glutathione (GSH) and flavonoids, such as rutin and quercetin, presents protective capacity against heavy metals. In this sense, we investigated here the effect of flavonoids and GSH against lead acetate-induced cytotoxic on *Saccharomyces cerevisiae* and the interaction of this metal with Fenton reaction. Cells of *S. cerevisiae* were exposed to lead acetate at 1mM. To evaluate the protective and reverse action of GSH against lead acetate, cells were treated with a curve of GSH concentrations (0.012, 0.025 and 0.050 mM). After, GSH concentration was associated with lead acetate. While to analyze rutin and quercetin protective action, cells were treated at 0.025 mM of these flavonoids in association to lead acetate. Also, the interaction of lead acetate with Fenton reaction was evaluated using H<sub>2</sub>O<sub>2</sub> 10 mM. Our findings revealed that GSH and quercetin did not avoid lead acetate damage. However, rutin increased cellular survival in cells exposed to lead acetate. Furthermore, lead acetate did not increase H<sub>2</sub>O<sub>2</sub> toxicity, indicating that the toxic mechanisms of this metal are not involved with Fenton reaction. Rutin could be target to development of new therapies against toxic heavy metal, decreasing the toxic effects of this metal.

**KEYWORDS:** Antimicrobials, Cytotoxicity, Enzyme, Toxicity, Yeasts.

## FLAVONÓIDES E GLUTATIONA COMO AGENTES PROTETORES CONTRA A TOXICIDADE DO ACETATO DE CHUMBO EM *Saccharomyces cerevisiae*

**RESUMO:** O chumbo é considerado um metal pesado tóxico que está envolvido na geração de estresse oxidativo, aumentando as espécies reativas de oxigênio (ROS). Investigações anteriores relataram que antioxidantes naturais, fontes de glutathione reduzida (GSH) e flavonóides, como rutina e quercetina, apresentam capacidade protetora contra metais pesados. Nesse sentido, investigamos aqui o efeito de flavonóides e GSH contra citotoxicidade induzida por acetato de chumbo em *Saccharomyces cerevisiae* e a interação deste metal com a reação de Fenton. As células de *S. cerevisiae* foram expostas a acetato de chumbo a 1 mM. Para avaliar a ação protetora e reversa do GSH contra o acetato de chumbo, as células foram tratadas com uma curva de concentração de GSH (0,012, 0,025 e 0,050 mM). Posteriormente, a concentração de GSH foi associada ao acetato de chumbo. Enquanto para analisar a ação protetora da rutina e da quercetina, as células foram tratadas com 0,025 mM desses flavonóides em associação ao acetato de chumbo. Além disso, a interação do acetato de chumbo com a reação de Fenton foi avaliada usando  $H_2O_2$  10 mM. Nossos resultados revelaram que o GSH e a quercetina não evitaram danos ao acetato de chumbo. No entanto, a rutina aumentou a sobrevivência celular em células expostas ao acetato de chumbo. Além disso, o acetato de chumbo não aumentou a toxicidade do  $H_2O_2$ , indicando que os mecanismos tóxicos desse metal não estão envolvidos na reação de Fenton. A rutina pode ser alvo de desenvolvimento de novas terapias contra metais pesados tóxicos, diminuindo os efeitos tóxicos desse metal.

**PALAVRAS-CHAVE:** Antimicrobianos, Citotoxicidade, Enzima, Toxicidade, Leveduras.

### 1 | INTRODUCTION

Lead is a nonessential element used to fabrication of many materials. This metal is used in different industries such as automobiles, paint, ceramics, and plastics (1). However, lead is considered a toxic heavy metal that can generate soil and air contamination. Also, lead exposure can occur in occupational activity since industrial workers have contact with this metal, for instance in smelting operations in furnaces, ship breaking, and construction works involving demolition and renovation (2).

Lead exposure can generate many disorders, such as biochemistry, physiological and behavior alterations. This metal can interact and modify several biological molecules, such DNA, RNA, proteins, and lipids (3). Moreover, this metal in high concentrations and during a long exposure can cause injuries and disturbers in many parts of the organism, such as central nervous system, blood, kidney, and in reproductive organs (4).

Further, lead presents high affinity for sulfhydryls (SH) group found in proteins. This association can inhibit or inactivate the synthesis of enzymes as well as it is able to decrease the enzyme interaction with some essential metals important for antioxidant enzyme activities (5). In this sense, lead intoxication can induce DNA damage as well as can inactive enzymes that are responsible for genetic repair (6).

This metal can generate toxicity by inducing oxidative stress through overproduction of reactive oxygen species (ROS) by interference with heme synthetic pathway and indirectly via depletion of the cellular antioxidants (7). Lead is responsible to increase ROS levels, such as superoxide ( $O_2^{\cdot-}$ ) and hydrogen peroxide ( $H_2O_2$ ) (8). Moreover, high concentrations of  $H_2O_2$  can interact with iron molecules and to generate Fenton reaction. This reaction is responsible to produce hydroxyl radical ( $OH^{\cdot}$ ), that has high affinity to DNA and this interaction can cause genetic mutations, corroborating to development of many diseases, such as cancer. However, whether lead can interact directly in Fenton reaction is yet unclear.

Exogenous and endogenous systems are responsible to neutralize ROS overproduction for maintaining the organism homeostasis. Endogenous system is compound by enzymes, such as dismutase superoxide (SOD), catalase (CAT) and glutathione peroxidase (GPX) (9). SOD is responsible to dismutase  $O_2^{\cdot-}$  into molecular oxygen ( $O_2$ ) and  $H_2O_2$ , while CAT and GPX neutralize  $H_2O_2$ . CAT is involved to catalyze  $H_2O_2$  into  $O_2$  and water ( $H_2O$ ). GPX neutralize  $H_2O_2$  by oxidation of reduced glutathione (GSH) in  $H_2O$ . While, exogenous system is compound by antioxidants that are present in the diet, they are found in fruits and vegetables for instance (10). Many natural products are sources of GSH that participate in the antioxidant endogenous system. Also, rutin and quercetin are examples of exogenous antioxidants that are considered natural flavonoids which present notable antioxidant activity (11).

Rutin, widely found in vegetables and fruits, was first revealed in 19<sup>th</sup> century in buckwheat (12). This molecule presents many pharmacological actions, such as antiallergic (13), anti-inflammatory, antitumour, antibacterial, antiviral, and antiprotozoal properties (14). Moreover, Guo et al (15) reported that rutin shows high scavenging activity and antioxidant capacity since it presents antiplatelet, antiviral and antihypertensive properties and strengthening the capillaries of blood vessels as wells. Also, a previous study reported that rutin has a dose-response effect in inhibiting low-density lipoprotein (LDL) peroxidation (16) and it presents antioxidant activity in Fenton reaction (17).

Rutin has been investigated to avoid heavy metal toxicity. Mirani et al. (18) reported that this molecule presents notable activity against cadmium, an extremely toxic metal. The findings revealed that high dose treatment of rutin (80 mg kg<sup>-1</sup> b.wt.) decreased lipid peroxidation and restored the amount of GSH significantly. Also, the results suggested that rutin normalized the antioxidants enzymes activity SOD, CAT and GST. These findings suggested that rutin present protection action against cadmium-induced oxidative damage in the liver of mice.

Moreover, quercetin, another member of flavonoids family, is frequently found in apples, onions, and green tea. This bioactive molecule has been commonly study by its functional proprieties and health benefits, since it is considered a remarkable antioxidant dietary (19). Quercetin presents important functional activity by its antioxidant and free radical

scavenging capacity, such as inhibition of xanthine oxidase and lipoxygenase, enzymes associated to inflammation, atherosclerosis, cancer and ageing process. Further, recent studies have suggested that quercetin presents *in vitro* effects against cardiovascular diseases, since this molecule is able to act in systemic and coronary vasodilatation and antiaggregant to reduce blood pressure, oxidative status and end-organ damage (20).

Previous studies also reported the protective activity of quercetin against heavy metals. Bu et al. (21) reported that quercetin presents antioxidant capacity against cadmium-induced oxidative toxicity on germ cells in male mice. Also, Wang et al (22) suggested that this molecule presents a protective effect against cadmium-induced renal uric acid transport system alteration and lipoperoxidation in rats. In addition, Park et al (23) reported that quercetin has a protection action against toxicity of copper on *Fusarium culmorum*.

In this sense, since lead can increase oxidative stress, deregulate the normal organism functional and corroborate to development of many disorders, investigations that reveal new therapies to reduce the lead toxicity effect, mainly based on antioxidants supplements, are very important. Therefore, the aim of the present study was evaluated the protection effect of GSH, rutin and quercetin against lead acetate-induced toxicity on *Saccharomyces cerevisiae* as well as we evaluated whether lead acetate could potentiate Fenton reaction in the same model.

## 2 | METHODS

### 2.1 Yeast lineage

A haploid lineage was used in this study, *S. cerevisiae* XV 185-14C (MATa ade 2-1, arg 4-17, his 1-7, lys 1-1, trp 1-1, trp 5-48, hom 3-10), kindly given by Dr. R.C. Von Borstel, of the Genetic Department, University of Alberta, Canada.

### 2.2 Culture medium and solutions

The initial cell growth was induced in complete liquid medium Yeast Extract-Peptone-Dextrose (YEPD) composed of 2% of glucose (E. Merck), 1% of extract of yeast (E. Merck), and 2% of peptone (Gibco BRL). Number of cell viability was evaluated in YEPD solid medium containing 2% of bacto agar (E. Merck). Cells were rinsed, resuspended, and diluted in 0.9% of NaCl saline solution (Reagem). Cells were treated with lead acetate (E. Merck) at 1 mM, H<sub>2</sub>O<sub>2</sub> (E. Merck) at 10 mM, rutin (E. Merck) at 0.025 mM, quercetin (E. Merck) at 0.025 mM and reduced glutathione (GSH) (Biochemical) at 0.012, 0.025 and 0.050 mM concentrations. All the solutions were performed using sterile distilled water, immediately before used.

### 2.3 Cell growth conditions

Cell cultures were used in the stationary growth phase. This phase was obtained from cells of a line stored in refrigerator in 10 ML of liquid medium YEPD and incubated for 24 hours at 28°C. After that, cells were rinsed with 0.9% of NaCl and counted in a Neubauer chamber.

### 2.4 Evaluate parameters of lead and glutathione interaction

First all,  $2 \times 10^7$  cells/mL were treated with 0.012, 0.025 and, 0.050 mM of GSH to evaluate the cellular viability and to determinate the higher noncytotoxic GSH concentration. After, cells were treated with the better concentration of GSH found in the previous curve in association with lead acetate at 2 mM in a solution previously prepared. Also, we evaluated whether GSH could be able to prevent and/or reverse toxicity induced by lead acetate exposure, using isolated solutions. To analyze prevent effects of GSH, cells were first treat with GSH and after exposed to lead acetate. While, to evaluate reverse action of GSH, cells were exposed to lead acetate and after GSH. Cells only treat with 0.9% NaCl were used as negative control. Also, two positive controls were performed, cells treated only with GSH and just with lead acetate. Treated cells were incubated at 28°C for 21 hours with shaking. After the treatment incubation, cells were diluted, seeded in Petri plates, and incubated at 28°C for 48 hours. Cell viability was measured by counting of colonies formed in Petri plates.

### 2.5 Evaluation of rutin and quercetin antioxidant capacity against lead toxicity

The antioxidant capacity of flavonoids, rutin and quercetin, against lead acetate-induced toxicity was analyzed using  $2 \times 10^7$  cells/mL of *S. cerevisiae* exposed to these agents. Cells were treated with rutin and quercetin at 0.025 mM concentration and after exposed to lead acetate at 2 mM. Treated cells were incubated at 28°C for 21 hours with shaking. After the treatment incubation, cells were diluted, seeded in Petri plates, and incubated at 28°C for 48 hours. Cell viability was measured by counting of colonies formed in the Petri plates.

### 2.6 Analyses of lead acetate interaction with Fenton reaction

To verify whether lead acetate could interact with Fenton reaction,  $2 \times 10^7$  cells/mL of *S. cerevisiae* were exposed to a solution previously prepared contained lead acetate at 2 mM and  $H_2O_2$  at 0.2 mM. Moreover, additional tests were performed using isolated solutions of lead acetate at 2 mM and  $H_2O_2$  at 0.2 mM. First, cells were exposed to lead acetate and after cells were treat with  $H_2O_2$ . Also, we tested the inverse, first cells were treated with  $H_2O_2$  and after cells are exposed to lead acetate. Treated cells were incubated at 28°C for 21 hours with shaking. After the treatment incubation, cells were diluted, seeded in Petri plates, and incubated at 28°C for 48 hours. Cell viability was measured by counting of colonies formed in the Petri plates.

## 2.7 Statistical analyses

First all, data were transformed to percentages against a negative control group that was considered as 100% of survival. Results were statistically analyzed using two-way ANOVA analysis of variance followed by a Tukey post hoc test, using Graphpad prism software, version 5.0 (Graphpad Prism software, 2015; San Diego, CA, USA). Results of  $p \leq 0.05$  were considered significant. The experimental assays were repeated four times at least.

## 3 | RESULTS AND DISCUSSION

### 3.8 Evaluation of reduced glutathione and lead acetate interaction

Previously, a curve of GSH concentrations was performed on *S. cerevisiae* cells to evaluate the higher noncytotoxic GSH concentration to it be used in all the experiments in association to lead acetate (Figure 1). Results revealed that the higher noncytotoxic GSH concentrate was determined at 0.025 mM.

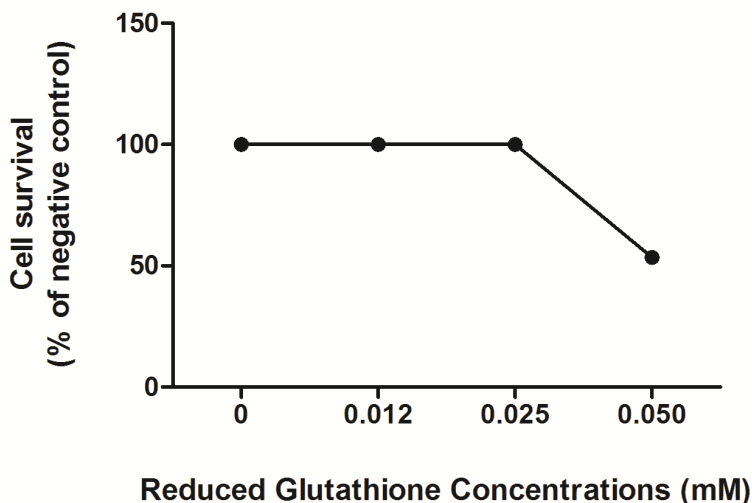


Figure 1- Survival values of *Saccharomyces cerevisiae* yeast cells treated with different concentrations of reduced glutathione.

After the determination of the better concentration of GSH, we tested here whether this molecule could revert and/or prevent the lead acetate-induced toxicity (Figure 2). The results suggest that lead acetate control (only cells treated with lead acetate) drastically decreased cell survival when compared to negative control (only cells) ( $p \leq 0.0005$ ).



Unfortunately, our findings indicate that cells exposed to lead acetate with and without GSH showed no significant statistical difference in the survival rates. In this sense, our results indicate that GSH is not able to protect toxicity induced by lead acetate even though when this active molecule is added before lead acetate exposure. Not even, GSH presented effect of reverse lead acetate toxicity, when cells were first exposed to lead acetate and after treated with GSH. Moreover, the solution composed of GSH and lead acetate previously prepared also showed no significant statistical difference.

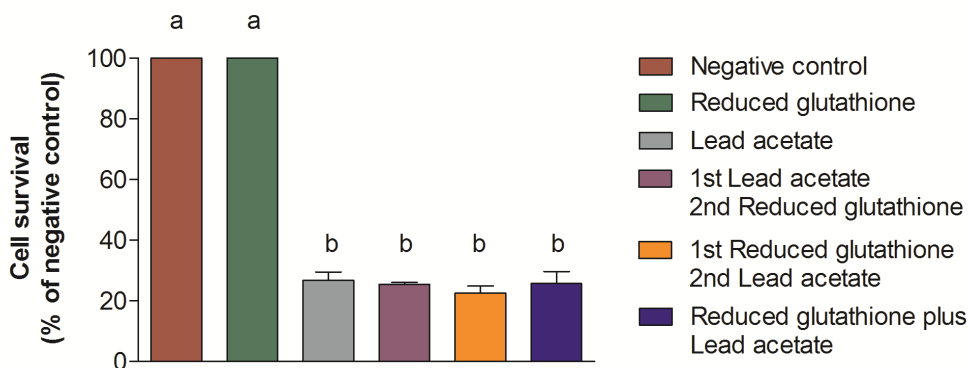


Figure 2- Survival values of *Saccharomyces cerevisiae* yeast cells treated with lead acetate (1 mM) and reduced glutathione (0.025 mM) in different conditions. Different letters represent significant statistical difference by Tukey post hoc test set at  $p \leq 0.05$ .  $n=4$ . Effect of flavonoids against lead acetate toxicity.

We investigated the antioxidant effect of flavonoids, rutin and quercetin, against lead acetate toxicity on *S. cerevisiae* (Figure 3). Our results suggest that rutin and quercetin controls (cells treated with these molecules) did not show significant statistical difference when compared to negative control (only cells and medium). On the other hand, lead acetate control (cells treated with lead acetate) decreased cell survival in almost 70% when compared to negative control (only cells and medium) ( $p \leq 0.0005$ ).

Moreover, our findings revealed that rutin is able to protect cells of lead acetate damage. In this sense, the results suggest that cells treated with rutin and lead acetate presented significant higher levels of survival ( $p \leq 0.05$ ) than cells treated only with lead acetate. However, quercetin did not show the same protective action that rutin. Treated cells with quercetin and lead acetate presented the same survival rates, no significant statistical difference was found in this treatment.

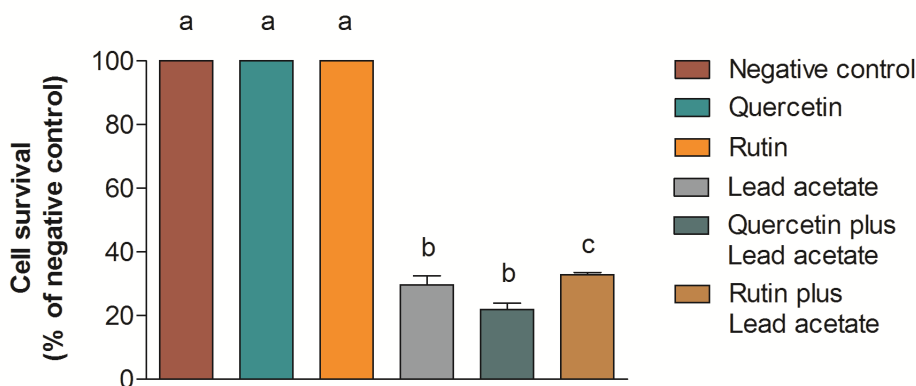


Figure 3-Survival values of *Saccharomyces cerevisiae* yeast cells treated with lead acetate (1 mM), quercetin (0.025 mM) and rutin (0.025 mM). Different letters represent significant statistical difference by Tukey post hoc test set at  $\leq 0.05$ .

To analyze whether lead acetate could interact with Fenton reaction, cells of *S. cerevisiae* were treated with lead acetate and  $H_2O_2$  (Figure 4). Findings indicate that the positive controls, only cells treated with lead acetate and cells just exposed to  $H_2O_2$ , presented lower survival levels when compared to negative control (only cells and medium) ( $p \leq 0.0005$ ).

Further, our results show no significant statistical difference between cells treated with lead acetate and  $H_2O_2$  in none of conditions and exposure order of the stressor agent. In this sense, our findings revealed that lead acetate did not increase  $H_2O_2$  toxicity, and indirectly this metal did not interact to Fenton reaction.

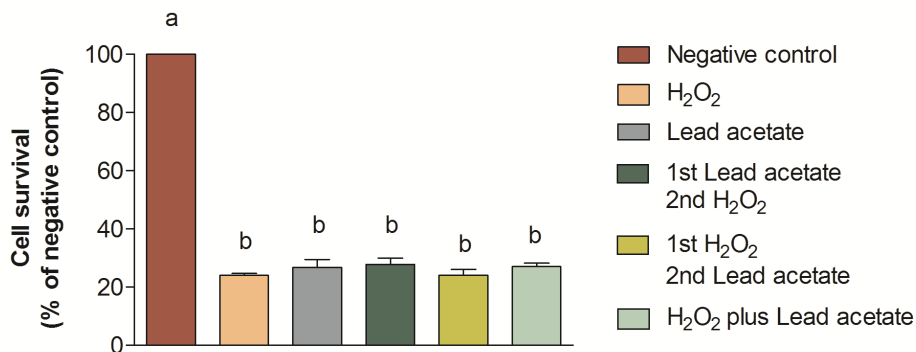


Figure 4- Survival values of *Saccharomyces cerevisiae* yeast cells treated with lead acetate (1 mM) and  $H_2O_2$  (10 mM) in different conditions. Different letters represent significant statistical difference by Tukey post hoc test set at  $\leq 0.05$ .

The present study investigated the interaction of lead acetate in Fenton reaction as well as the antioxidant capacity of flavonoids, rutin and quercetin, and GSH against lead acetate-induced toxicity on cells of *S. cerevisiae*. Our findings revealed that GSH and quercetin did not avoid lead acetate damage. However, rutin increased cell survival of cells exposed to lead acetate. Furthermore, lead acetate did not increase  $H_2O_2$  toxicity, indicating that the toxic mechanisms of this metal are not involved with Fenton reaction (Figure 5).

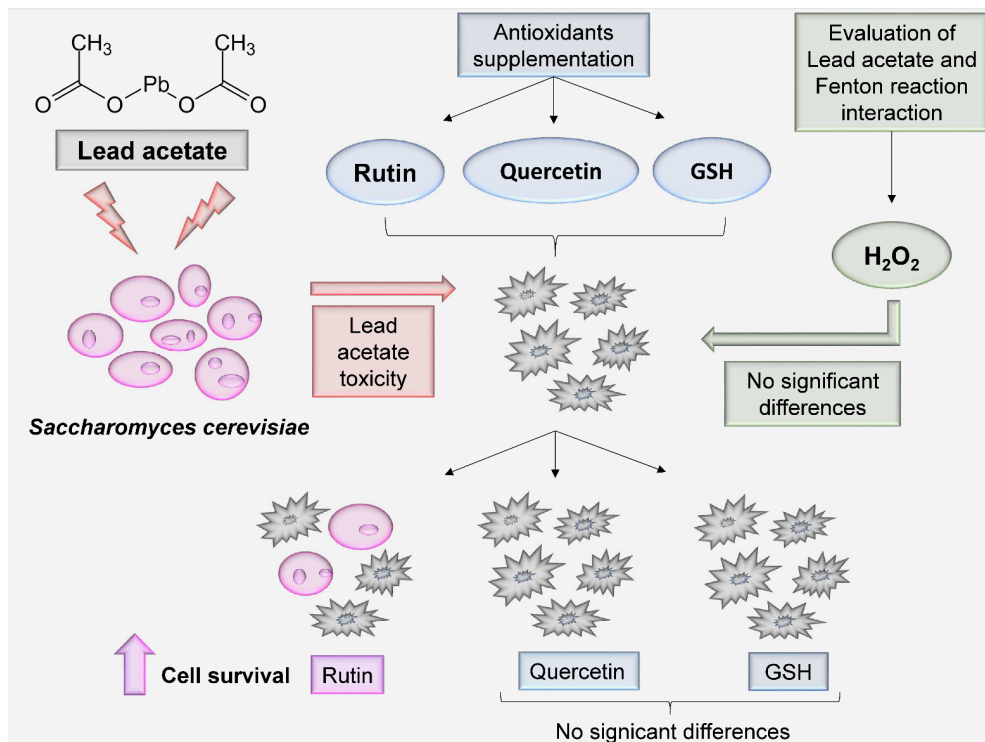


Figure 5- Lead acetate caused severe toxic effects on *Saccharomyces cerevisiae* yeast cells decreasing cell survival. Reduced glutathione (GSH) and quercetin did not avoid lead acetate damage. However, rutin increased cell survival of cells exposed to lead acetate. Furthermore, lead acetate did not increase  $H_2O_2$  toxicity, indicating that the toxic mechanisms of this metal are not involved with Fenton reaction.

In the present investigation, our results suggest that GSH did not present protective and reverse action against lead acetate toxicity. However, previous studies reported that this notable antioxidant molecule can neutralize toxicity effects of others toxic heavy metals. Xu et al (24) investigated the GSH interaction with lead acetate and cadmium on *Phanerochaete chrysosporium*, a genus of crust fungi. They reported that GSH has an excellent role in cadmium chelation and protection against the toxicity of this metal, while this effect was not found between GSH and lead acetate. Moreover, Srivastava et al (25)

suggested that GSH is able to chelate copper and decrease this heavy metal toxicity on *Hydrilla verticillate*. Despite flavonoids are considered important ROS scavengers (26), in our investigation yeast cells treated with quercetin did not present protective action against lead acetate toxicity. However, our findings indicate that rutin showed a significant protective action against this toxic heavy metal, increasing cell survival.

Although quercetin did not present protective effects in our study against lead acetate, Bu et al (21) suggested that this bioactive molecule showed antioxidant protective action against cadmium. This toxic heavy metal induced oxidative toxicity on germ cells in male mice. This damage was repaired through antioxidant activity of quercetin by decreasing of renal uric acid transport system alteration and lipoperoxidation caused. Also, a previous study reported that quercetin is able to avoid copper toxicity on *Fusarium culmorum* (23).

On the other hand, our results revealed that rutin presented protective action by increasing cell survival. A previous investigation performed by Mirani et al (18) corroborated with our findings. They reported that rutin presented protective action against cadmium-induced damage on mice liver by decreasing lipid peroxidation, restoring the amount of GSH and normalizing antioxidants enzymes activity (SOD, CAT and GST).

Moreover, based on our findings, we suggest that lead acetate did not interfere in Fenton reaction. However, other toxic heavy metals have been associated to this reaction. In this sense, Liu et al (27) reported that cadmium is involved in endogenous production of iron-dependent OH<sup>•</sup> as a mechanism of cadmium-induced oxidative stress.

## 4 | CONCLUSIONS

In conclusion, we described here the effect of rutin, quercetin and GSH against lead acetate-induced cytotoxicity on *S. cerevisiae* and the interaction of this metal with Fenton reaction. The present investigation revealed that rutin is a potential antioxidant flavonoid that is involved in decreasing lead acetate-induced toxicity. On the other hand, GSH and quercetin did not present protective action against lead acetate damage and this metal seems not to be involved in Fenton reaction. Therefore, despite *in vitro* study limitations, rutin could be a target for development of new therapies against toxic heavy metal, using *in vivo* models to confirm these findings.

## 5 | CONFLICTS OF INTEREST

There are no conflicts to declare.

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