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Evaluation of the Oxidative Stress Induced by Nanoparticle Manufactured (ZnO) in *Saccharomyces cerevisiae*

Nedjoud Grara
Laboratory of Cellular Toxicology, Department of Biology, Faculty of Sciences, Badji Mokhtar University, Annaba, 23000, B.P.12, Algeria
Email: grara120@yahoo.fr

Khaldi Fadila
Mohamed Echerif Messadia University, Department of Biology, Faculty of Natural Sciences and Life, BOX/1553, Souk Ahras 41000, Algeria
Laboratory for the Study and Research of Condensed States, Department of Physics, University Badji Mokhtar, BP 12, Annaba, 23000. Algeria

Manel Bouloudenine
Mohamed Echerif Messadia University, Department of Biology, Faculty of Natural Sciences and Life, BOX/1553,Souk Ahras 41000,Algeria

Naili Asma
Mohamed Echerif Messadia University, Department of Biology, Faculty of Natural Sciences and Life, BOX/1553,Souk Ahras 41000, Algeria

Messaadia Nouseiba
Mohamed Echerif Messadia University, Department of Biology, Faculty of Natural Sciences and Life, BOX/1553,Souk Ahras 41000, Algeria

***Abstract* – The aim of the study is to examine the impact of ZnO (manufactured nanoparticle) on physiological and biochemical parameters in a microorganism *Saccharomyces cerevisiae* after short time of treatment (180mn).The main results show that the presence of ZnO affects the growth of yeasts, on the metabolic level, ZnO causes a disturbance in the rate of total protein and carbohydrates. En regard, biomarkers we identified a disturbance glutathione and Catalase activity.**

Keywords – ZnO, Manufactured Nanoparticle, *Saccharomyces cerevisiae*,

I. INTRODUCTION

The presence of unnatural substances in the environment increases with their use by humans. Thus the development of nanotechnologies and their applications will almost certainly result in an increase in the concentration of nanoparticles in air, water and soil. They can therefore achieve ecosystems [1] High concentrations of fine particles (particle size less than 2.5 microns) have a short term impact on health especially in regard to respiratory and cardiovascular problems [2], exposure to high concentrations of fine particles can lead to a reduction in life expectancy [3].

The direct effects of the toxicity of nanoparticles on organisms are mainly due to their chemical composition and surface reactivity. They can also serve as vectors of other pollutants, by fixing of other pollutants on the surface increasing or decreasing the bioavailability of other toxic or interact with proteins such as enzymes [1] .Among the microorganisms most commonly used by man as a major model of the eukaryotic cell in cellular and molecular biology: *Saccharomyces cerevisiae*, it is mainly used in the production of knowledge metabolite and biomass. yeasts are now used in the fields of medical research and biotechnology and also generally like other eukaryotes and Gram (-) aerobic yeast *Saccharomyces cerevisiae* has a non-enzymatic antioxidant, glutathione (γ-L-glutamyl-L-cystinyl glycine), which can a from participating in the antioxidant defense as a source of reducing equivalents, and secondly to serve as a cofactor for certain enzymes involved in protection against oxidative stress [4].

The objective of this work is to investigate the use of the yeast *Saccharomyces cerevisiae* to characterize the presence and toxicity of pollutants, a manufactured nanoparticle ZnO after a short treatment.

II. MATERIALS AND METHODS

Biological material

The biological material used is a mushroom unicellular eukaryote *Saccharomyces cerevisiae*, it is cultivated in the culture medium glucose yeast extract (20g glucose, 5g yeast extract and 1000 ml of distilled water, pH 5.6) [5] .

Chemical material:

The chemical material used is manufactured from a nanoparticle (ZnO) prepared by the method of co-precipitation [6], different dilutions of ZnO is the following concentrations: 0.25 mM, 1 mM and 2.5 mM and are prepared to go from a stock solution of 10 mM [7]- [8]-[9] .

Measured parameters:

Monitoring the growth of yeast by measuring the optical density at 620nm tale is a white culture medium without yeast[9], extraction of metabolites was performed according to the method of Shibko et *al.*[10] with which the proteins were quantified by the method of Bradford [11], the determination of carbohydrates is performed according to the method of Duchateau & Florkin [12], glutathione is estimated by a method Weckberker and Cory[13] and Catalase activity is directly followed by a method of Regoli & Principato [14].

Statistical analysis

The results are shown as mean ± standard error, the results are compared by the nonparametric Kruskal-Wallis, the software MINITAB Version 14.0, the significance level chosen is $p < 0.05$ [15] .

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III. RESULTS

Effect of ZnO on the growth of Saccharomyces cerevisiae:

Figure (1) shows the evolution of yeast growth over time, we note that in controls, growth tends to increase with the time it reaches its maximum at the 120mn (minutes) and gives a plateau. In contrast, in the treated concentrations (0.25, 1, 2.5 mM), we observe a decrease in the growth of a dose-dependent manner compared to controls. Statistical analysis shows that there is no significant difference between the control and the treated concentrations (0.25mM, 1 mM and 2.5 mM).

Effect of ZnO on biochemical parameters in Saccharomyces cerevisiae:

Effect of ZnO on the rate of total protein:

From Figure (2), we find that in the Treaties, the rate of total protein tends to decrease in a dose-dependent. Statistical analysis reveals that there is no significant difference between the Total protein levels in control and treated with different concentrations of ZnO (0.25mM, 1 mM and 2.5 mM).

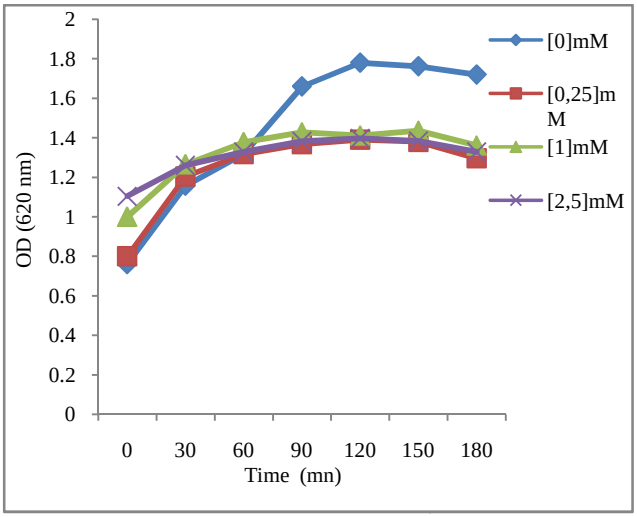

Fig.1. Effect of ZnO on the growth kinetics of *Saccharomyces cerevisiae* (after 180 mn).

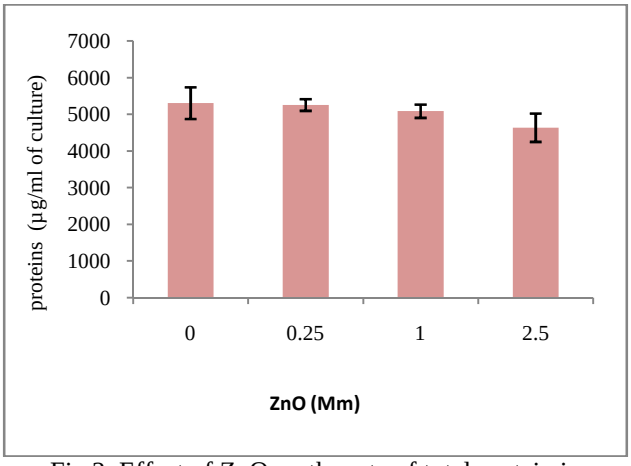

Fig.2. Effect of ZnO on the rate of total protein in *Saccharomyces cerevisiae* (after180mn).

Effect of ZnO on the concentrations of total carbohydrates:

From Figure (3) shows that the rate of total carbohydrate tends to increase in a dose-dependent manner to those treated with different concentrations (0.25, 1, 2.5 mM). Statistical analysis shows that recorded a significant difference among treated (0.25mm). Also note a significant difference for those treated with the concentration (1 mM) and a highly significant difference for those treated by the concentration (2.5 mM) compared with controls always.

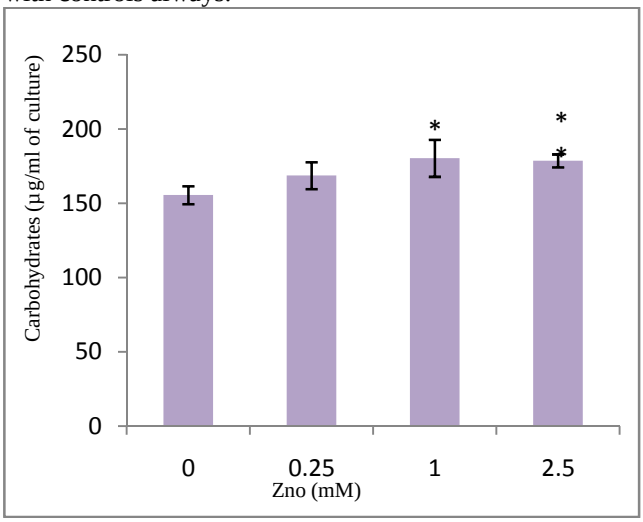

Fig.3. Effect ZnO on the rate of total carbohydrate in *Saccharomyces cerevisiae* (after 180mn).

Effect of ZnO on the glutathione (GSH):

Figure (04) illustrates the variations of GSH after treatment (180mn), we find that the presence of xenobiotic GSH levels tended to decrease for those treated by different concentrations.L statistical analysis shows that 'there is no significant difference for those treated with different concentrations (0.25 mM, 1 mM and 2.5 mM).

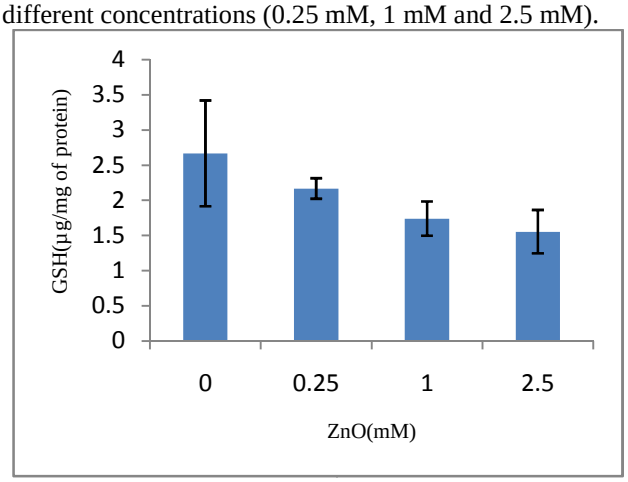

Fig.4. Effect of ZnO on GSH levels in *Saccharomyces cerevisiae* (after 180mn)

Effect of ZnO on Catalase activity:

Figure (5) represents the variation of the activity Catalase (after 180 mn of treatment) in *Saccharomyces cerevisiae*. Our results show that the presence of xenobiotic Catalase activity tends to increase in a dose-

dependent and very highly significant for those treated with concentrations (0.25mm and 1 mM). Then there is a very highly significant decrease for those treated the concentration (2.5 mM).

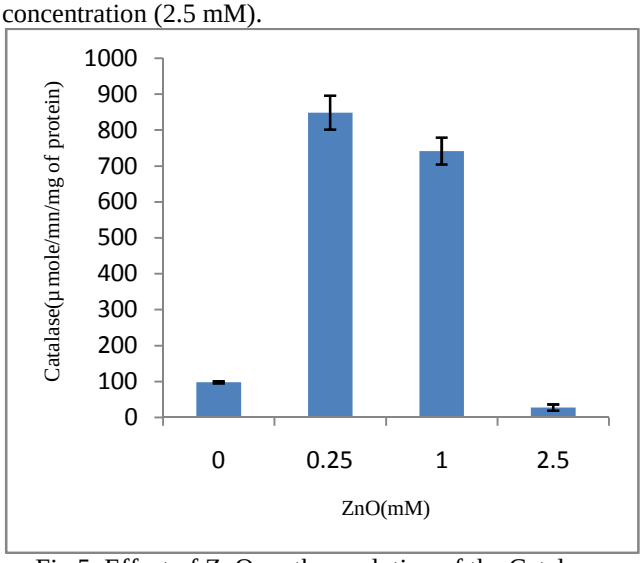

Fig.5. Effect of ZnO on the evolution of the Catalase activity in *Saccharomyces cerevisiae* (after 180 mn).

IV. DISCUSSION

Numerous studies have shown that the toxicity of metal nanoparticles involves a dissolution of nanoparticles and therefore chemical toxicity associated with ions released or the toxicity of a metal nanoparticle ZnO is always compared to ions Zn^{2+}[1]-[7] and the generation of reactive oxygen species (ROS) as the major mechanism involved in the cellular toxicity of nanopaticules [16]-[17].

In this study, we set ourselves the aim to highlight the effect of a xenobiotic (metal nanoparticle ZnO) newly synthesized on a mushroom unicellular eukaryote *Sacchromyces cerevisiae*, a microorganism most exploited in the industrial world unicellular eukaryote and a fungus used in academic research [6]-[3]. We opted initially for a rapid cytotoxicity test to classify the toxicity of these molecules (metal nanoparticle based ZnO) tested through the evolution of the growth curve of yeast culture. Our results showed a slight decrease in the growth of yeast treated with all concentrations tested, tested the xenobiotic is toxic to yeast *Sacchromyces cerevisiae*, this toxicity is manifested primarily by a slight inhibition of cell growth. This brings us to confirm the influx of xenobiotics in the inner cells, despite the presence of the cell membrane forms a barrier against the massive entry of xenobiotics but remains permeable [18]. Our results are in agreement with the work of Angelique [1], which brought to light a cytotoxic effect was observed in *E. coli MG1655* and was connected to a penetration nanopaticules metals (TiO_2) through the bacterial cell wall. Our results also agree with the work of Sondi and Salopek-Sondi [19] showed that disruption of the membrane is the cause of toxicity. Indeed, it has been shown that silver nanoparticles adhere to the wall, causing the formation of pits and permeable the membrane, thus leading to expulsion from the cytosol of the bacterium.

Because of their surface reactivity, nanoparticles are capable of inducing the production of reactive oxygen species (ROS) and cause oxidative stress to the cell [20]-[21].

Assuming that any type of chemical stress causes oxidative damage can occur, causing oxidative stress and significantly affects the cell and its function. During oxidative stress, ROS not detoxified by the antioxidant system will oxidize macromolecules,such as lipids, proteins, sugars and nucleic acids, disrupting their chemical structures and altering their biological functions [22]. Moreover, the excess general or localized zinc is responsible for its toxic effects [23]. Zn can move from an antioxidant role to a role peroxidizing originally indirect formation of free radicals [22].Our results show a disturbance in the rate of total protein under the effect of chemical stress in yeast treated, Our results show a non-significant decrease in the rate of total protein in yeast treated with different concentrations of ZnO nanoparticles after (180mn), this decrease is explained by an inhibition of the mechanisms involved in detoxification [24]-[25].

Our results are in agreement with those of Grara et *al.*, [24] and Nzengue [25] which showed that short exposures appear to inhibit antioxidant enzymes such as stress in contrast, prolonged exposure results in increased activities of some antioxidant enzymes.

The decrease in total protein levels observed at the end of treatment is explained biochemically by several mechanisms including the formation of lipoproteins, which are used to repair damaged cells and tissues or by direct use by cells for energy needs these stress conditions [26]. These results are in agreement with the work of (Padmaja and Rao [27], which showed a decrease in protein level in the digestive gland of the snail fresh water, *Bellamyadis similis* after exposure to pesticides (carbamates).

Our results show a disturbance in the rate of total carbohydrate under the effect of chemical stress in yeast *Sacchromyces cerevisiae* with a significant increase of total carbohydrate in yeast treated with different concentrations of ZnO after (180mn). In fact, carbohydrates are the primary sources and immediate energy in the stress condition, the carbohydrate reserves are depleted and the onset of glucose metabolism is increased to meet the energy demands of the biological model during stress [26]. Moreover disturbances observed rate of total carbohydrate in yeast *Sacchromyces cerevisiae* is explained by the depletion of energy requirements (carbohydrates), these results are consistent with those of EL-Wakil and Radwan [28], who suggested that the depletion of glycogen content in the tissue of the freshwater snail, *Bellamyadis similis*, exposed to Endosulfan, parathion methyl, and quinalphos of the Nuvan (pesticides) would result from the use of glycogen for energy generation, this demand is explained to the induction of hypoxia caused by pesticides.

In our work, we have demonstrated a decrease in a dose-dependent GSH in the presence of zinc oxide (ZnO) in yeast treated (after 180mn), this depletion can be explained by the direct binding of glutathione to metal

[29].In fact, many metals are characterized by a high affinity for thiol groups towards and form complexes with these chemical entities. This metal-glutathione interaction generally leads to the formation of thiyl radicals (-S º). Although these radicals are relatively stable and can interact with each other to form disulfide bridges not radical, they can also react with oxygen and generate reactive oxygen species [30].This highlights the role importance of glutathione in the Management of a metal stress[31],On the other hand the reactions of metals with glutathione results in either the formation of complexes [metal-GSH] or by oxidation of GSH [14] .The study of the Christie stable complexes with GSH are formed by Zn, Cd, Hg, Pb and Ni, these two reactions could explain the decrease of glutathione [32]-[33] ,According to Canesi and Viarengo [34] decreased glutathione content is mainly correlated with a decrease in the activity of the glutamyl cysteine synthetase, the latter involved in the biosynthesis of GSH. On the other hand the reduction of GSH can also be explained by the increase in the use of the latter with the GST in the conjugation reaction [35]- [36]. GSH scavenges reactive oxygen species because it reacts especially with the hydroxyl radical (OH) and O_2 [37].

On the other hand our results showed an increase in Catalase activity in yeast *Sacchromyces cerevisiae* treated ZnO, probably because of the intensification of the antioxydant activity to increased antioxidant activity in yeast cells, according to Halliwell and Gutteridge [32], increased oxidative stress enhances the activity of antioxidant enzymes in living beings. Indeed, Catalase activity consists of a transformation of the hydrogen peroxide (H_2O_2) in water and molecular oxygen (O_2). However, the production of hydrogen peroxide is induced by the presence of exogenous compounds to the body as is the case for metals [38] - [39], derived reactive oxygen can lead to oxidation of macromolecules (DNA, lipids and proteins) [40].SOD (super oxide dismutase) and Catalase play a role in protecting the body against oxidative stress damage [41], Catalase is therefore with (SOD) in the first line of defense against oxidative stress [42]-[43].

Our results are in agreement with those of Lu et *al.* [44] which showed an increased activity of catalase in two bacteria (*Escherichia coli K12* and *Stenotrophomona smaltophilia* WZ_2) after treatment with herbicides. Our results show a decrease in Catalase activity in yeast treated with concentration (2.5 mM after exposure 180mn). These results are in agreement with the work of Pandey et *al.*[45] revealed a Catalase activity decreased in the liver of the species *C. punctatus* exposed to endosulfan. Other studies confirm our results, those of Grara et *al.*[24] showed that inhibition of catalase activity in hepatopancreas and kidney of the snail *Helix aspersa* after exposure (7 days) by metal dust (Zn, Cu,...) . Nzengue [25] suggests that short exposures to Cd appear to inhibit antioxidant enzymes such as catalase stress by cons prolonged exposure results in increased activities of some antioxidant enzymes other hand, the increase of reactive species of oxygen (ROS) in the presence of cadmium is the displacement of the metals constituting the structure of the antioxidant enzymes.

V. CONCLUSION

This study shows the interest of the species used for the evaluation of the toxicity of ZnO in laboratory tests. It is clear that ZnO is the source of oxidative stress, which results in a slight inhibition of growth in the yeast *Saccharomyces cerevisiae* and disruption rate of total protein, carbohydrates, glutathione and activity Catalase. Thus, it is clear that the use of yeast is an essential tool for monitoring our environment.

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