Mitochondrial Dysfunction and Response After Stressing by Aluminum Nanoparticles (AlNPs) of *Helix sp*

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ABSTRACT

The enhanced uses of nanoparticles in our daily life have been augmented according to industrial development. Indeed, the nature and the properties of nanoparticles make them a good choice in many products from food enhancers, drugs, nanomaterials, medicine, electronics and cosmetics.

Our study is a trial on the effects of AlNPs (<10nm) on the mitochondrial status and possible dysfunction in detoxication organ of the snail *Helix* sp. and to assess the impact of these nanomaterials on swelling, permeability and respiration of hepatopancreatic mitochondria.

Obtained results presents an increased activity of many mitochondrial metabolization enzymes (mitGST, mitCAT, mitMDA, mitGPx) and a decrease in GSH level. Thus, an increase in mitochondrial swelling and permeability have been showed with a slow rate of respiration level according to the controls group.

Finally, our results suggested that nanoparticles of aluminum affect directly the mitochondrial function by the perturbation of general metabolic status of snails exposed to 5 and 10µg /g of NPs of Al each 2 days.

Keywords: AlNPs, *Helix sp*, mitCAT, mitGPx, mitGST, swelling, respiration, permeability.

INTRODUCTION

Synthetic nanoparticles are nanometric materials with a specific characterization which make them a choice in many industrial usage from food additives to drugs and very sophisticated medical products (Bigorgne, 2011). Alumina nanoparticles (AlNP) as many other nanoparticles are bioaccumulative substances by the passage through the biological barriers that make intoxication in organ systems including Nobel organs (Qinly *et al*., 2018).

However, the high usage of nanoparticles without knowing their environmental and health impacts. Indeed, these nanoparticles make a very diversified interaction with biological systems. Industrialization of nanoparticles as many other products leads to subproducts and wastes that we did not know their environmental impact (Triboulet, 2013).

Nanotechnology industries create a new notion of pollution (nanopollution) which we don't know any future impact on our community. Nanoparticles are synthetic or natural from volcans or forest fires, of course with nanoparticles manufactured by human (Baratli, 2015).

Because of their little size nanoparticles (NPs) could penetrate through B-B barrier and B-T barrier and through placenta to reach neonatal babies (Keelan, 2011; Zoroddu *et al*., 2014). Therefore, NPs with its very specific characterization in mass and physiochemical parameters can find way to reach many organs and induce harmful effects (Chen *et al*., 2008). Alumina nanoparticles (AlNPs) are used in many industrial domains, pharmacological industries and many other fields with a unique physicochemical propriety (Kagan *et al*., 2005).

Studies on nanoparticles and especially AlNPs have shown a high amount of passage through biological membranes and filters of many tissues and organs (Morsy *et al*., 2016b), leading to neurotoxicity (Oesterling *et al*., 2008) as well as pneumotoxicity (Li *et al*., 2016), liver (Shrivastava *et al*., 2014). However, no studies have addressed whether AlNP induces problems on terrestrial Mollusca and mitochondrial dysfunction.

MATERIAL AND METHODS

Experimental Animals and Treatments

Helix sp, known as the Garden Snail was used in our experiments, it is a pulmonary gastropod mollusk Stylommatophora belonging to the Helicidae family.

This snail was collected from Bekkaria in Tebessa region and cultivated in laboratory for15 days as adaptation period and 3 months as period of treatment, under the following conditions: photoperiods of 18/24 H light, temperature of 20 ± 2 ° C., hygrometry of 75% to 95%; Feeding to wheat flour (Gomot, 1997 ; Coeurdassier *et al*., 2001).

Helix are grouped in transparent perforated plastic boxes. Each box contains a wet sponge to maintain moisture and humidity.

Treatment of the animals was carried by injection with a micro-syringe. We used two doses 5µg/g/2d and

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10µ /g/2days, with a placebo injection to the controls with a physiological water.

After the treatment period, the snails are fasted for 48 hours in order to empty their digestive tract, the animals are then sacrificed by freezing at -20 °C. and then dissected to remove the digestive gland.

Extraction of mitochondria

All operations were carried on ice. cells were placed into buffer A containing 50 mM tris, 1 mM EGTA, 70 mM Sucrose, 210 mM Mannitol, pH 7.40 at +4°C. Then, the homogenate was centrifuged at 1300g for 3 min, 4 °C. The supernatant was centrifuged at 10,000g for 10 min, 4 °C to sediment mitochondria. Finally, the mitochondrial pellet was washed twice and then suspended in 50 mM Tris, 70 mM sucrose, 210 mM mannitol, pH 7.4 at +4 °C. Protein content was routinely assayed with a Bradford assay using bovine serum albumin as a standard (Salmi *et al*., 2017; Gasmi *et al*., 2018). Mitochondria were kept on ice and used within 4 h.

Mitochondrial respiration and swelling assay

According to the method of Krystal *et al*. (1996), we carried out the estimation of the mitochondrial permeability based on the rate of traverse of Ca^{++} followed by an increase in mitochondrial size detected at 540nm wavelength for 3 minutes and each 30 seconds. Respiration was estimated using an Oxygraph (Hansatech) according to the method described by Rouabhi *et al*. (2006a; 2006b; 2009).

Assessment methods

The activity of catalase was determined in the hepatopancreas colorimetric according to (Cakmak and Horst,1991), The speed of disappearance of H_2O_2 is monitored by observing the rate of decrease in absorbance at 240 nm. The measurement of glutathione S-transferase activity (GST) was determined according to the method of Habig *et al*. (1974), following the formation of 1-glutathione-2,4-dinitrobenzene resulting from conjugation between substrate (1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione. Reduced glutathione was determined spectrophotometrically, according to the method of Weckbecker and Cory. MDA can be detected by a colorimetric reaction with thiobarbituric acid (TBA). The MDA is assayed according to the method of Esterbauer *et al*. (1992). The enzyme activity of glutathione peroxidase was evaluated by the method of Flohe and Günzler (1984) using H2O2 as substrate.

Aluminum nanoparticles

Nanoparticles are prepared in the laboratory of physics and nanoparticles, after characterization of AlNPs, all particles are under 10nm (<10nm)

Statistical analysis

All results data were analyzed with statistical software (minitab 20.0 and Microsoft excel 19.0), ANOVA one (means ± SDV), all results are compared with the Student's t test.

RESULTS

RESULTS

The results are expressed by the mean ± (standard deviation) of n experiments. The differences are considered: significant when P≤0.05, very highly significant when P ≤ 0.001, highly significant when P ≤0.01 (Fig.4-9) .

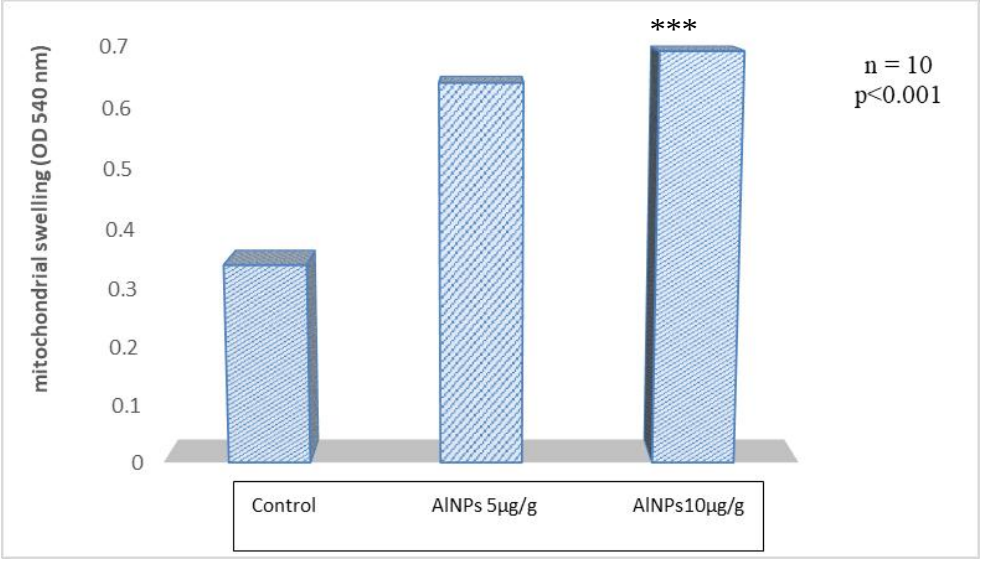

Figure 1. Effect of AlNPs on *Helix sp* mitochondrial swelling at OD540nm

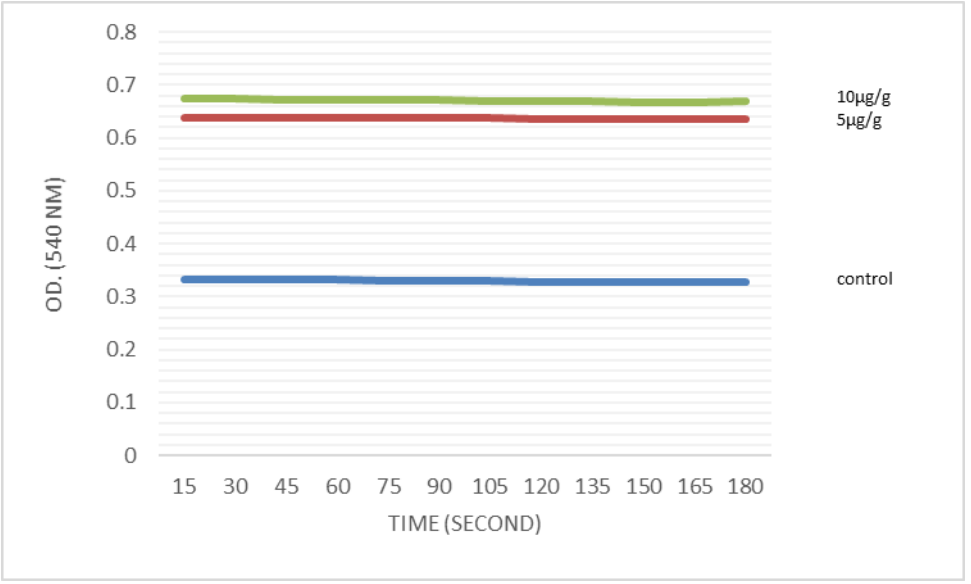

Figure 2. Effect of AlNPs on mitochondrial permeability at OD_{540}nm after 90 days

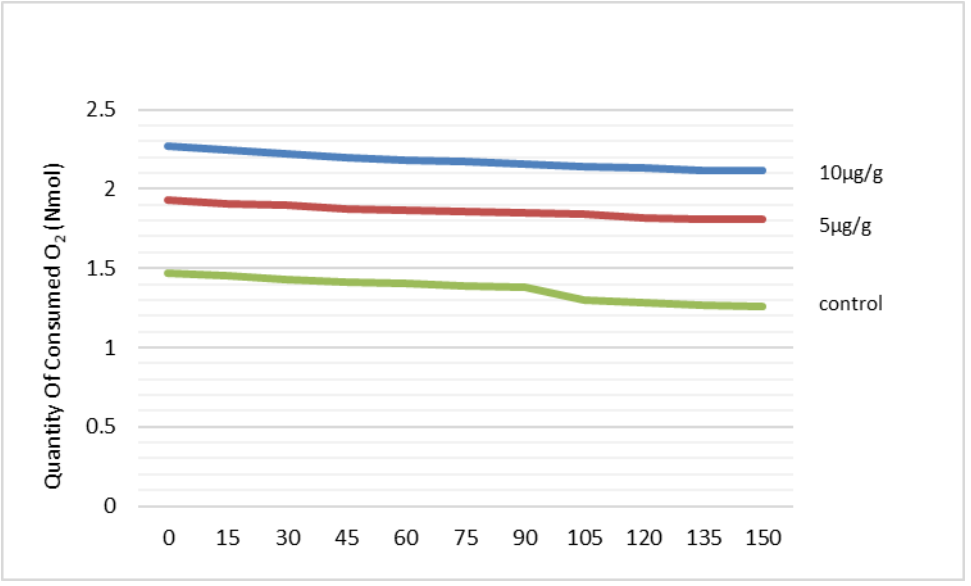

Figure 3. Effect of AlNPs on mitochondrial respiration (nmoles) after 90 days

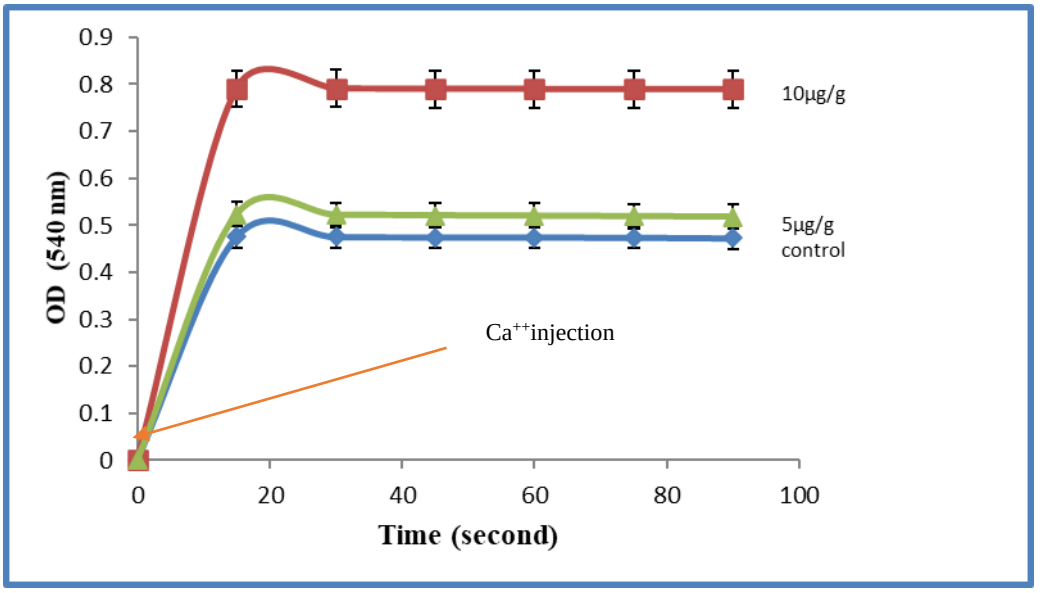

Figure 4. Effect of Alumina nanoparticles on hepatopancreatic mitochondrial permeability at OD=540nm after 90 days Treatment

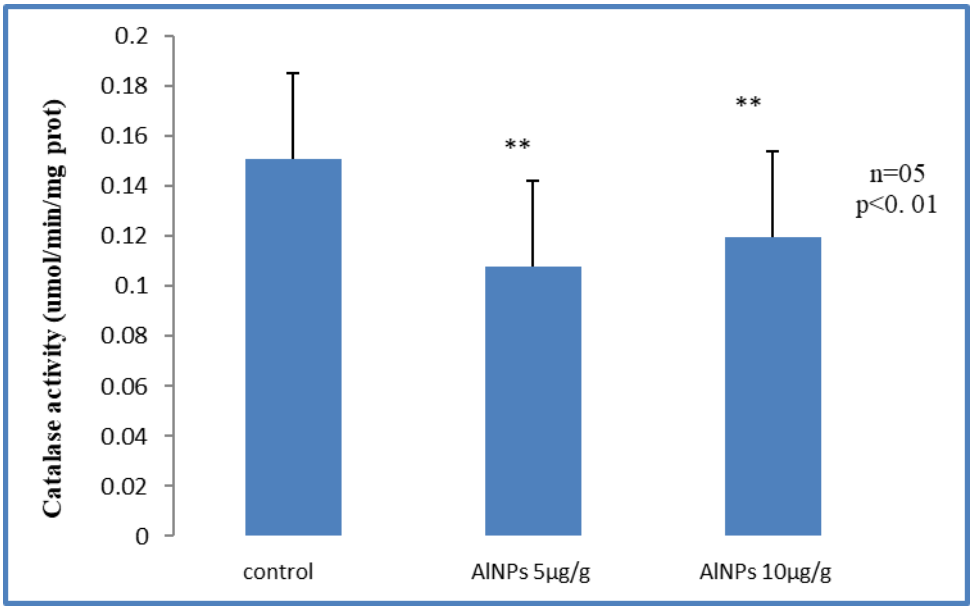

Figure 5. Effect of AlNPs on the variation of mitCAT (µM/mg) after 90 days

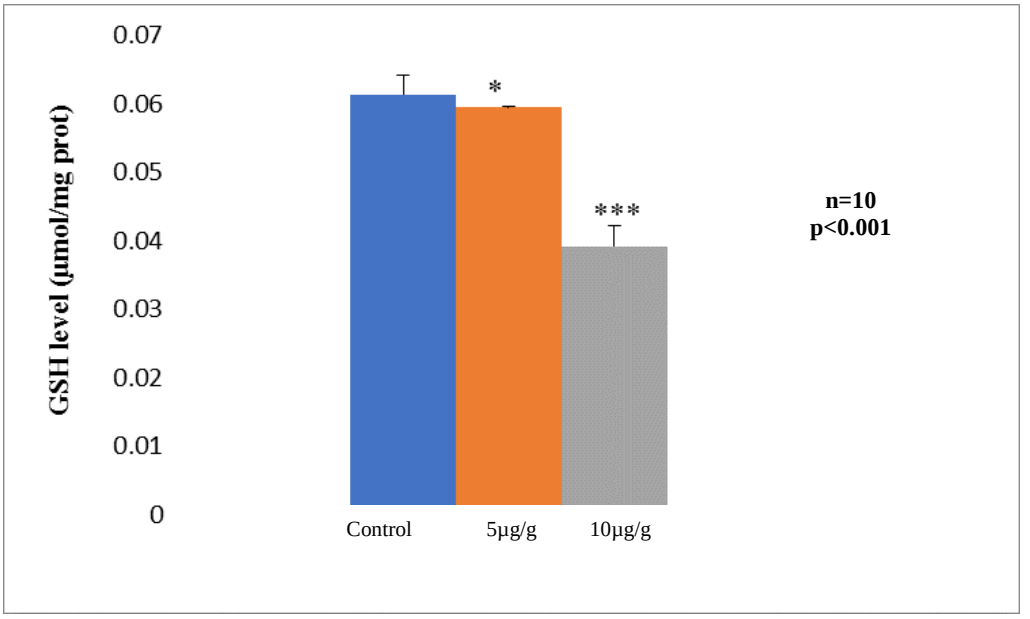

Figure 6. Variation of hepatopancreatic mitGSH (µM/mg prot.) in the control and treated snails

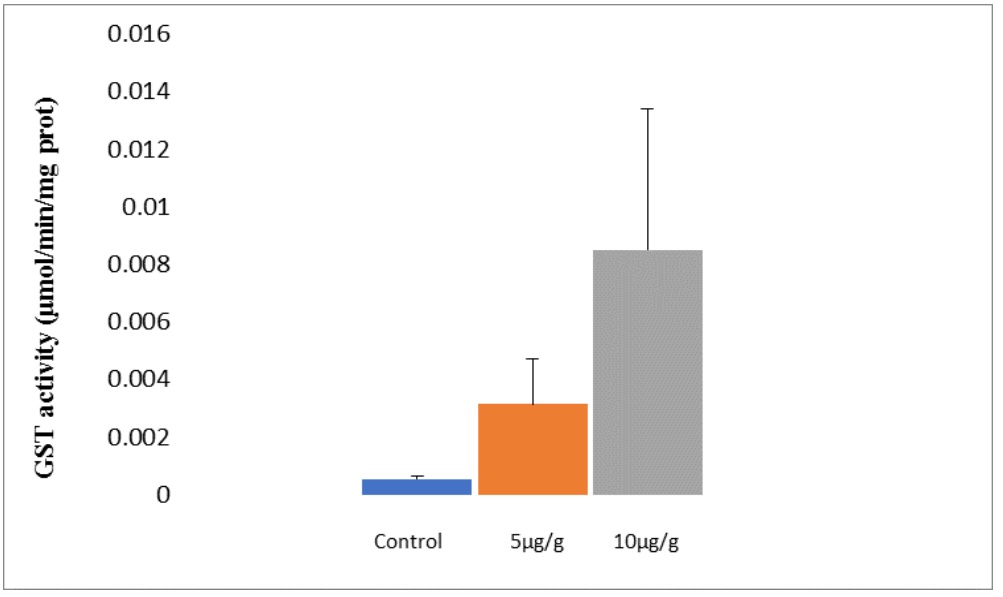

Figure 7. Effect of AlNPs on the variation of mitGST (µmol/min/mg prot.)

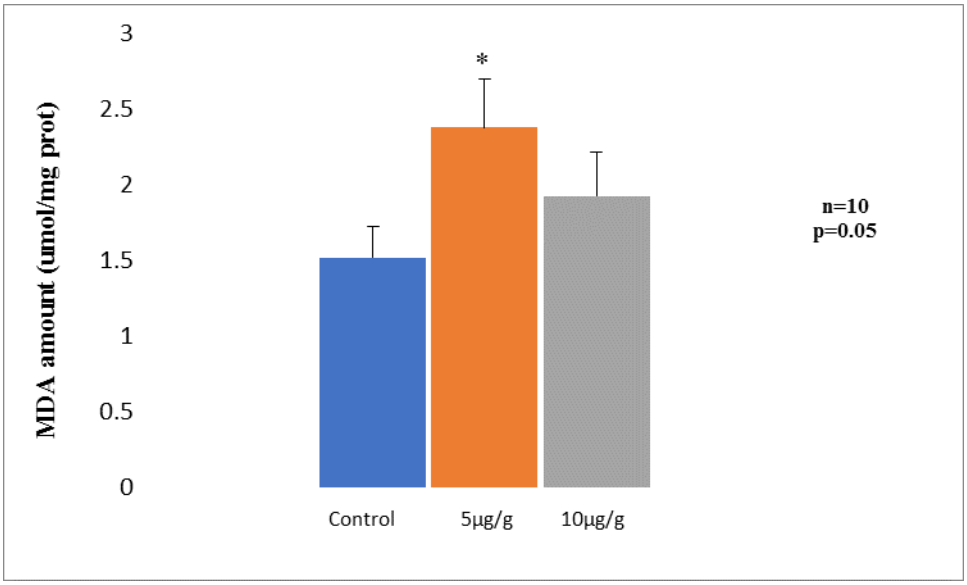

Figure 8. Variation of MDA level (µM/mg prot) in *Helix* after 90 days of treatment

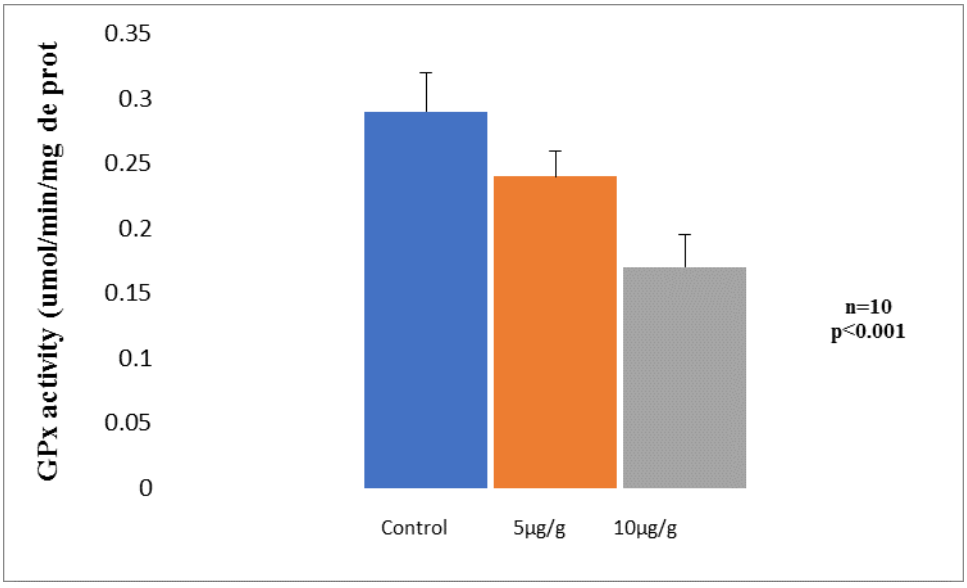

Figure 9. Effect of AlNPs treatments on the variation of mitGPx (µM/min/mg prot)

DISCUSSION

Mitochondria are a microscopic membrane-bound cell organelles that generate most of the chemical energy needed to power the cell's biochemical reactions: respiration, oxidative phosphorylation.

The impact of nanoparticles especially AlNPs on general metabolism and health including respiration metabolism is little known, experiences in this way demonstrated a decrease in cellular respiration in the presence of synthetic chemicals by the impact on electron transmission through respiratory queue. Sbartai *et al*. (2009) and Benbouzide *et al*. (2012) demonstrated that there is an inhibition of respiration after stressing by Bifenazate and Phosphoramidate. *Euglena gracilis* mitochondria have been inhibited strongly by chromium (Cr) (Jasso-Chavez *et al*., 2010). Boulassel *et al*. (2014) also have presented a result on the amount of succinate in isolated mitochondria.

All these works are in accordance with our findings indeed, the mitochondrial respiration of Helix was inhibited in dose dependent manner, this is due perhaps by the perturbation in normal electron transmission

through mitochondrial layers that cause the death of cells confirming the role of free radical in this phenomenon, and the high level of swelling and permeability.

These two last impacts are the main cause of some NPs on specific receptors including the PTPM. The main consequence of the opening of PTPMs penetration out of the cytochrome c that induces the stimulation of caspase enzymes (Taib *et al*., 2017). Chagra *et al*. (2009) have been shown that the treatment of mitochondria with Cd affect directly the swelling and the permeability, that is the case in our results with AlNPs.

According Ivanina *et al*. (2008) and Garceau *et al*. (2010), Aluminum may affect the enzymes involved in respiration, by binding with Thiol group's enzymes. More sensitive complex through all respiratory chain to NPs are II and III (Wang *et al*., 2004).

The response of hepatopancreas to nanoparticles of aluminum was assessed with the control of oxidative stress enzymes that enter in detoxification process such as Catalase and Glutathione-S-transferase but in mitochondrial matrix of course with following the level of GSH and MDA. Our results showed more and more impacts in dose-dependent manner, indeed, a decrease in mitCAT activity an increase in mitGST activity followed by the decrease in general GSH level. The lipid peroxidation was assessed by the quantification of Malondialdehyde (MDA), which showed an increase which accounts for lipid peroxidation. All these parameters play a great role in cellular equilibrium and defense against any ROS attacks (Uday *et al*., 1990; Van Acker and Bast, 1993). Our results are in concordance with the results of Canesi *et al*. (2010b) that found an increase in the activity of detoxication enzymes of digestive gland of the mold exposed 24h to NP-TiO_2.

Conclusion

In conclusion, our trials was focused on the impact of aluminum nanoparticles on mitochondria by assessing the swelling, permeability, enzymes perturbation and we have demonstrated that these nanoparticles could pass through biological barriers and affect many receptors and vital process of rats; these perturbations also affect the mitochondria which is the most important cellular constituent that provoke after an apoptosis or necrosis to exposed cells.

REFERENCES

Acker, V., L.M.H. Koymans, A. Bast. 1993. Molecular pharmacological importance of vitamin E, structural aspects of NADP. Free radical. Biol. Med. 5:311-328.

Baratli, Y. 2015. Study of the toxicity of iron oxide nanoparticles (Fe3O4) in the rat, mitochondrial analyzes and oxidative stress. Doctoral thesis. University of Strasbourg and University of Carthage.

Benbouzid, H., H. Berrebbah, M. Berredjem, M.R. Djebar. 2012. Toxic effects of phosphoramidate on *Paramecuim sp*. with special emphasis on respiratory metabolism, growth, and generation time. Toxicological & Envoronmental Chemistery. DOI: 10.1080/02772248.p.6555-696.

Bigorgne, E. 2011. ecotoxicity of nanoparticles and by-products of alteration of titanium dioxide on the earthworm, eisenia fetida.) Doctoral thesis, Paul Verlaine-Metz University. P2.

Boulassel, A. 2014. Evaluation of the toxicity of two drugs: Paracetamol and Ibuprofen, on a cellular model: Paramecium tetraurelia.Test on the subcellular scale. Doctoral thesis. Universite Badji Mokhtar, Annaba.

Bradford, M. 1976. A rapid and sensitive method for the quantities of microgram quantities of protein utilizing the principle of protein binding. Anal Biochem. 72:248-254.

Cakmak, I. and W.J. Horst. 1991. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max). *Physiol. Plantarum.* 83:463-468.

Canesi, L., A. Viarengo, C. Leonzio, M. Filippelli and G. Gallo. 2010. Heavy metals and glutathione metabolism in mussel tissues. Aquat. Toxicol. 46:67-76.

Chagra, A., M. R Djebar, R. Rouabhi and H. Berrebbah. 2009. Cadmium Induced Changes in Metabolic Function of Mitochondrial Isolated from Potato Tissue (*Solanum tuberosum* L.). *American Journal of Biochemistry and Biotechnology* 5 (1):35-39.

Chen, L., R.A. Yokel, B. Hennig and M. Toborek. 2008. Manufactured aluminum oxide nanoparticles decrease expression of tight junction proteins in brain vasculature. J. Neuroimmune Pharmacol. 3:286–295. doi: 10.1007/s11481-008-9131-5

Coeurdassier, M., M. Saint-Denis, A. Gomot-de Vaufleury, D. Ribera and P.M. Badot. 2001. The garden snail (*Helix aspersa*) as bioindicator of organophosphorus exposure: effects of dimethoate on survival, growth and acetylcholinesterases activity. Environment. Toxicol. & Chem. 20:1951-1957.

Esterbaer, H., J. Gebicki, H. Puhl and G. Jungens. 1992. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Radic. Biol. Med. 13:341.

Flohe, L. and W.A. Gunzler. 1984. Assays of glutathione peroxidase. Methods Enzymol. 105: 114–121.

Garceau, N., N. Pichaud and P. Couture. 2010. Inhibition of goldfish mitochondrial metabolism by in vitro exposure to Cd, Cu and Ni. Aquatic Toxicology 98:107-112.

Gomot, A. 1997. Effets des métaux lourds sur le développement des escargots. Utilisation des escargots comme bio-indicateurs de pollution par les métaux lourds pour la préservation de la santé de l'homme. Bull. Acad. Natle. Méd. 181:59-75.

Habig, H., M.J. Pabs, and W.B. Jokoby. 1974. Glutathione-S-transferase: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249:7130–7139

Ivanina, A.V., E. Habinck, and I.M. Sokolova. 2008. Differential sensitivity to cadmium of key mitochondrial enzymes in the eastern oyster, *Crassostrea virginica gmelin* (*Bivalvia: Ostreidae*). Comparative Biochemistry and Physiology - C Toxicology and Pharmacology 148:72-79.

Jasso-Chavez et al., 2010. Toxic effects of Cr(VI) and Cr(III) on energy metabolism of heterotrophic *Euglena gracilis*. Aquat. Toxicol. 100:329–338.

Kagan, V. E., H. Bayir, and A.A. Shvedova. 2005. Nanomedicine and nanotoxicology: two sides of the same coin. J. Nanomed. 1:313–316. doi: 10.1016/j.nano.2005.10.003

Keelan, J.A. 2011. Nanotoxicology: nanoparticles versus the placenta. Nat. Nanotechnol. 6:263–264. doi: 10.1038/nnano.2011.65

Li, X., C. Zhang, X. Zhang, S. Wang, Q. Meng, S. Wu, et al. 2016. An acetyl-L-carnitine switch on mitochondrial dysfunction and rescue in the metabolomics study on aluminum oxide nanoparticles. Part. Fibre Toxicol. 13:4. doi: 10.1186/s12989-016-0115-y

McBride, H.M., M. Neuspiel, S. Wasiak. 2006. Mitochondria: More Than Just a Powerhouse. Current Biology 16:R551-R560.

Morsy, G.M., K.S. El-Ala, and A.A. Ali. 2016. Studies on fate and toxicity of nanoalumina in male albino rats: lethality, bioaccumulation and genotoxicity. Toxicol. Ind. Health. 32:344–359. doi: 10.1177/0748233713498449

Oesterling, E., N. Chopra, V. Gavalas, X. Arzuaga, E.J. Lim, R. Sultana, et al. 2008. Alumina nanoparticles induce expression of endothelial cell adhesion molecules. Toxicol. Lett. 178 :160–166. doi: 10.1016/j.toxlet.2008.03.011

Phuong, ND. 2005. Thèse de doctorat, université Paris VI.

Qinli, Z., D. Yong, H. Kaihong, L. Huan, G. Fuping, J.M. Taylor, W. Xiaohong, D. Jeremy and N. Qiao, 2018. Exposure to Alumina Nanoparticles in Female Mice During Pregnancy Induces Neurodevelopmental Toxicity in the Offspring. Front. Pharmacol. 20. doi.org/10.3389/fphar.2018.00253

Rouabhi, R., H. Djebar and M.R. Djebar 2006a. Toxicity evaluation of flucycloxuron and diflubenzuron on the cellular model, *Paramecium sp*. African Journal of Biotechnology. 5(1): 045-048.

Rouabhi, R., H. Djebar, M.R. Djebar. 2009. Toxic Effects of Combined Molecule from Novaluron and Diflubenzuron on *Paramecium caudatum*. Am-Euras. J. Toxicol. Sci. 1(2):74-80.

Rouabhi, R., H. Djebar-Berrebbah and M.R. Djebar. 2006b. Toxic Effect of a Pesticide, Diflubenzuron on Freshwater Microinvertebrate (*Tetrahymena pyriformis*). Chinese Journal of Applied and Environmental Biology. 12(4):514-517.

Salmi, A., R. Rouabhi, N. Toualbia, Ch. Taib, H. Chenikher, S. Gasmi and S. Boussekine. 2017. Subcellular study of the cadmium selenide nanoparticles (CdSe-NPs) toxicity on a terrestrial bioindicator model Helix aspersa. Toxicol. Environ. Health Sci . 9: 135–140.

Sbartai, I., H. Berrebbah, R. Rouabhi, H. Sbartai, G. Smagghe and M.R. Djebar. 2009. Behavior of Paramecium sp. treated with bifenazate with special emphasis on respiratory metabolism, protein and generation time. American-Eurasian J. Toxicol. Sci.1:13-18.

Shrivastava, R., S. Raza, A. Yadav, P. Kushwaha, and S.J. Flora. 2014. Effects of sub-acute exposure to TiO2, ZnO and Al2O3 nanoparticles on oxidative stress and histological changes in mouse liver and brain. Drug Chem. Toxicol. 37:336–347. doi: 10.3109/01480545.2013.866134

Taib, C., R. Rouabhi, S. Gasmi, L. Bakhouche, G. Zarai, N. Toualbia, A. Salmi, S. Henine, et al. 2017. Toxicity of Fe3O4 nanoparticles on oxidative stress status, stromal enzymes and mitochondrial respiration and swelling of Oryctolagus cuniculus brain cortex. Toxicology and Environmental Health Sciences. 8(5):349-355. Doi: 10.1007/s13530-016-0296-7

Triboulet, S. 2013. Study of the effects of two types of metallic nanoparticles on murine macrophages by a proteomic approach. Doctoral thesis. Université de Grenoble. P 25.

Wang, Y., J. Fang, S.S. Leonard and K.M.K. Rao. 2004. Cadmium inhibits the electron transfer chain and induces reactive oxygen species. Free Radical Biology and Medicine 36:1434-1443

Weckbker, G. and JG. Cory. 1988. Ribonucleotide reductase activity and growth of Glutathoine depleted mouse leukemia L1210 cells in vitro. Cancer Lett. 40:257-264.

Zoroddu, M.A., S. Medici, A. Ledda, V.M. Nurchi, J.I. Lachowicz, and M. Peana. 2014. Toxicity of nanoparticles. Curr. Med. Chem. 21:3837–3853. doi: 10.2174/0929867321666140601162314