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ORIGINAL ARTICLE

Impact of Imidacloprid on the Mitochondrial Function of *Wistar* Rats' Nervous System

Sarra Zouaoui, Rachid Rouabhi*

Laboratory of Toxicology and Ecosystems Pathologies, Applied Biology Department, Echahid Cheikh Larbi Tebessi University, Tebessa, 12000, Algeria

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KEYWORDS

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ABSTRACT: Pesticides are chemicals used to struggle against harmful pests; they could affect any compartment after getting into nature. Most pesticides are made up of only a selective molecule. When compared to bulk materials like powders, plates, and sheets, pesticides have a relatively large specter-to-volume ratio because of their extremely potent chemical makeup. Because of this characteristic, pesticides might have unexpected chemical and physical characteristics because they interact with the molecules in the environment and compartment. The present assessment aims to test the neurotoxicity induced by a pesticide of Imidacloprid at 1.252 mM kg^{-1} per day in the *Wistar* rat's brain. After gavage to the rats for 3 months in laboratory conditions of the groups (light/dark; humidity), tests on nervous system enzymes figured that pesticide caused a significant enhancement of inter-mitochondrial metabolites amount (proteins, fats, and carbohydrates) and mitochondrial enzyme activity (GST and SOD); decreased amount of mitochondrial GSH; an enhancement of mit-CAT and mit-GPx activity; a rise in MDA level. Mitochondrial functions of the treated Rat's brains showed a notable rise in mitochondrial swelling and permeability. Rather, there was a statistically significant drop in the amount of oxygen consumed by mitochondrial respiration. All results confirm that the pesticide caused a dose-dependent response.

INTRODUCTION

Pesticides are present in all of our environmental compartments, air, soil, fruits, and food [1]. However, due to their linking qualities, which make them extremely bioactive and possibly toxic to exposed species, toxicovigilance concerns have been increased about the massive usage of pesticides [2]. Scientists claimed that a little amount of pesticide (<10 ng) comports as gases that can easily reach all tissues disturbing the general cell metabolism [3].

The lipophile and hydrophile properties of pesticides, determine the ability to pass through epithelial and endothelial barriers into the lymph and blood to be transported by the bloodstream and lymph stream to various organs and tissues, such as the brain, heart, liver, kidneys, spleen, bone marrow, and nervous system, are major factors in determining their toxicity [4].

On the whole, considering the wide use of pesticides in various daily life and extant theories about the destructive effects of these matters, this work was conducted to learn the impact induced by a pesticide of Imidacloprid on the nervous system mitochondrial function of *Wistar* rats.

Imidacloprid, a global standard among neonicotinoid pesticides, exemplifies its ubiquitous use and widespread adoption. Pesticides, which include neonicotinoids, play an important role in the chemical pantheon, which is

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*Corresponding author: r_rouabhi@univ-tebessa.dz (R. Rouabhi)
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used in a variety of farming domains [3]. However, their excessive employment disrupts the delicate balance of environmental and human well-being. Neonicotinoids, which have neuro-active characteristics similar to nicotine, are the most effective neurotoxic insecticides in modern pest management. They pervade the worldwide market, exerting control over agricultural landscapes, and are used to protect crops from the ravages of insect pests [4].

MATERIALS AND METHODS

Animals

This study was carried out at the laboratory of toxicology and ecosystem pathologies, Echahid Cheikh Larbi tebessi, Tebessa University on male *Wistar* rats, provided by Pasteur institute of Algier. Rats are weighed about 240–260 g. Animals were grouped into control groups and treated groups. Rats have direct access to water and aliment. The temperature was maintained between 23 °C, and 25 °C, and acceptable humidity with photoperiod 12/12 h.

Chemicals

In this work, we used Imidacloprid as a neonicotinoid pesticide provided by Ain El-Baida agriculture products seller.

Isolation of the brain

After receiving treatment for three months, the rats were sacrificed by decapitation following deep ether anesthesia. The brains were swiftly removed and divided into two sections, the first of which was kept at -80 °C to isolate the mitochondria, and calculating the parameters of matrix oxidative stress (GSH, GST, CAT, SOD, GPx, and MDA). The second section was used fresh to complete the assays on mitochondrial integrity and functions.

Mitochondrial preparation

The whole mitochondrial fractions are extracted from the various brain regions using a slightly modified version of the procedure outlined in [5]. In summary, after washing the tissues in cold respiration buffer (pH 7.4, 50 mM Tris-HCl, 250 mM sucrose, 1 mM d-Ethyl Diamine Acetic Acid (EDTA), BSA 0.2 %), they were chopped, homogenized, and centrifuged at 3500 g for 10 minutes. The pellet was then re-centrifuged under the same conditions. After combining the supernatants from the two centrifugations, they were centrifuged for 20 minutes at 15000 g. After repeatedly washing the resulting pellet under the same conditions with PB buffer (50 mM tris-HCl, 250 mM sucrose) with a pH of 7.4, the mitochondrial pellets were suspended in 300 µl of PB buffer and refrigerated at -20 °C until they were needed. To rupture mitochondria, repetitive homogenization followed by freezing and defrosting was used to create mitochondrial matrix from suspensions of mitochondria. The supernatant was thought to be the source of mitochondrial GSH, GPx, GST, CAT, SOD, and MDA after centrifugation at 10,000 g for 10 min.

Mitochondrial biomarkers assessment

CAT, GST, and GPx activity

Trials are conducted on the activity of glutathione-s-transferase (GST), catalase (CAT), and guaiacol peroxidase (GPx) following the methods of [6 - 8] respectively; Protein estimation was measured following the method of [9] using BSA as a control standard.

GSH and MDA level

Reduced glutathione (GSH) level was assessed using [10] protocol. The level of malondialdehyde (MDA) of brain mitochondria was assessed using [11] protocol (high pick at 530 nm).

Superoxide dismutase (SOD) assessment

Using the method of [12], brain mitochondria superoxide dismutase (SOD) was measured at the wavelength of 560 nm. Briefly, A combination of 2 ml of the reactive medium (sodium cyanide 10^{-2} M, NBT solution at 1.76 × 10^{-4} M, EDTA 66 mmol, methionine 10^{-2} M, riboflavin 2 µmol, pH 7.8) was combined with 50 µl of matrix fraction. This combination was exposed to a 15 W lamp for 30 minutes to cause riboflavin to photo-react. The color turned blue when NBT was reduced to formozan.

Fresh mitochondrial function assessment

We estimated the mitochondrial permeability using the approach of [13], which involves measuring the rate at which Ca^{++} traverses the mitochondria and then looking for an increase in the size of the mitochondria at 540 nm wavelength every 30 seconds for three minutes. An Oxygraph (Hansatech) was used to measure respiration following the technique of [14, 15].

Statistical Study

All analyses were conducted with MINITAB (version 20) software. Microsoft Office Excel 2019 was used to design all diagrams and tables. The findings are given as the mean ± SEM of six rats per group. The P criterion of significance was $0.05 \geq p \geq 0.01$, very significant at $0.01 \geq p \geq 0.001$, and very highly significant at $p < 0.001$; more than 0.05 the differences are not significant (ns).

RESULTS AND DISCUSSION

Imidacloprid exposure and impacts on brain mitochondria of rats at (1.252 mM kg^{-1} per day of body weight; orally), showed very important results that reflect the unsafety of this pesticide as a xenobiotic on the human’s nervous system.

Neonicotinoid effects are realized using some biochemical parameters [16, 17]. Imidacloprid was shown as a toxic substance to mammals (Table 1). It was listed as one of the dangerous compounds that the Agency for Toxic Compounds and Disease Registry prioritized [18]. The GSH levels were also decreased significantly in the imidacloprid-treated rats. GSH is necessary for the survival of cells. It is found in most cells that hydrophily conjugate xenobiotics, and it is most likely the most significant defense mechanism against free radical scavenging and the prevention of xenobiotics' electrophilic assault on biological macromolecules [19]. The decreasing of these parameters is due to the enzymes decreasing, it is important to understand that mitochondria are now under oxidative stress.

Table 1. Mitochondrial brain parameters of rats after subchronic treatment with imidacloprid for 90 days.

Parameters	**Proteins (mg)**	**Carbohydrates (µg)**	**Lipids (µg)**
Control	1.609 ± 0.0275	11. 528±0.5681	213.007±1.227
Imidacloprid	1.541 ± 0.02597**	9.451±0.5439**	165.433±1.235***

mean ± SE .t-test was used for multiple comparisons. ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05.

Study of antioxidant Enzymatic and non-enzymatic parameters

Detoxification enzyme assessment was directly done in vivo and the results are shown in (Table 2), where a very important perturbation was marked according to the control.

Table 2. (GSH. GPx. MDA. SOD. GST and CAT) variations during 90 days of Imidacloprid at the dose of 1.252 mM kg^{-1} per day of body weight.

Parameters	**Mit GSH µmol mg^{-1}**	**Mit GPx µmol min^{-1} mg^{-1}**	**Mit GST µmol min^{-1} mg^{-1}**	**Mit CAT µmol min^{-1} mg^{-1}**	**Mit MDA nmol mg^{-1}**	**Mit SOD U mg^{-1}**
Control	0.000381 ± 9.8837E-06	1.05806 ± 0.03435197	0.004508 ± 0.000737068	0.000312 ± 8.09321E-06	2.110885 ± 0.213383	16.0141 ± 0.2462043
Imidacloprid	0.00056 ± 2.79E-05 ***	0.41418 ± 0.0162 ***	0.008075 ± 0.000433 ***	0.00054 ± 2.241E-05 ***	4.528575 ± 0.28759 ***	12.415 ± 0.87229**

Mean ± SE .t-test was used for multiple comparisons. ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05

Mitochondrial stress parameters testing

The injection of imidacloprid may have resulted in significant ROS generation in the brain mitochondria (Table 3), which may have increased malondialdehyde due to greater lipid peroxidation and produced this variation in the rate of different antioxidants [20].

A decrease in CAT and GPx activities causes the mitochondrion to create radical OH° and produce more H_2O_2 [21]. Therefore, the overproduction of hydrogen peroxide and the subsequent drop in GSH following xenobiotic intoxication are the primary causes of the reduction in GPx activity [22, 23].

Therefore, environmental pollutants can directly target the mitochondria, causing the production of reactive oxygen species (ROS). These ROS can then mediate other oxido-reduction reactions and further induce the depletion of antioxidant defenses, potentially promoting mitochondrial damage and the depletion of anti-oxidant molecular systems in the brain cell [24]. The growing buildup of ROS, which directly damages brain mitochondria [25, 26].

Table 3. Mitochondrial functionality parameters after 90 days of exposure.

Parameters	Swelling (Optic density)	O_2 consumption	Permeability (delta OD/delta time)
Control	17.141 ±0.0034	4.33 ± 1.02****	0.16 ±0.01***
Imidacloprid	0.156 ±0.0001	1.16 ± 0.51****	0.27±0.02****

Mean ± SE .t-test was used for multiple comparisons. ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05.

Imidacloprid may interact with the enzymes of thiol groups, hence affecting the enzymes involved in respiration [27, 28]. Complex II and III are the most susceptible complexes to pesticides over the whole respiratory chain [29].

Furthermore, there is a connection between membrane potential collapse and mitochondrial malfunction, which is often caused by an excess of $O2^-$ [30; 31]. A critical measure of mitochondrial respiratory activity, has shown a decline in the rate at which it consumes oxygen and, therefore, in the effectiveness of its operation.

CONCLUSIONS

We investigate the toxic effects of the neonicotinoid pesticide imidacloprid on mitochondria by measuring swelling, permeability, and perturbation of enzymes. We have also shown that sub-chronic exposure to imidacloprid can cause abnormalities and oxidative stress in all areas of the adult male rats' brains.

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Conflict of interests

Authors declared that there is no conflict of interests

REFERENCES

1. Kong B., Seog J.H., Graham L.M.,Lee S.P., 2011. Experimental considerations on the cytotoxicity of pesticide s. Nanomedicine. 6, 929–941.

2. Cronholm P., Karlsson HL., Hedberg J., Lowe TA., Winnberg L., Elihn K., Wallinder IO., Moller L., 2013. Intracellular uptake and toxicity of Ag and CuO pesticide s: a comparison between pesticide sands their corresponding metal ions. Small. 9, 970–982.

3. Vishwakarma V., Samal S.S., Manoharan N. 2010. Safety and risk associated with pesticide s-a review. Journal of Minerals and Materials Characterization and Engineering. 9(5), 455.

4. Dukhin A., Swasey S., Thommes M., 2013. A method for pore size and porosity analysis of porous materials using electroacoustics and high frequency conductivity. Colloids and Surfaces. 437, 127-132.

5. Clayton D.A., Doda J.N. and Freiberg E.C., 1974. The absence of a pyrimidine dimer repair mechanism in mammalian mitochondria. Proc. Natl Acad Sci USA. 71, 2777–2781.

6. Habig W.H., Pabst M.J., Jakoby W.B., 1974. Glutathione-S-transferase the first step in mercapturic acid formation. Journal of Biology and Chemistry. 249, 7130–9.

7. Aebi. 1984. Catalase. In L. Packer (Ed), methods in enzymology, Academic press, Orlando. 105, 121-126.

8. Flohé L., Günzler W.A. 1984. Assays of glutathione peroxidase. Methods Enzymol.105, 114-21.

9. Bradford M., 1976. A rapid and sensitive method for the quantities of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochemistry. 72, 24854.

10. Weckbercker G., Cory J.G., 1988. Ribonucleotide reductase activity and growth of Glutathione-depended mouse Leukemia L1210 cells in vitro. Cancer Letter. 40, 257-264. PMID: 3289734.

11. Warso M.A., Lands W.E., 1983. Lipid peroxidation in relation to prostacy clin and thromboxane physiology and pathophysiology. Br Med Bull. 277-280.

12. Beauchamp C., Fridovich I., 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Biochem. 44, 276-287.

13. Kristal B.S., Park B.K., Yu B.P., 1996. 4-Hydroxynonénal est un puissant inducteur de la transition de perméabilité mitochondriale. Biol Chem. 6033–6038.

14. Rouabhi R., Gasmi S., Boussekine S., Kebieche M., 2015. Hepatic Oxidative Stress Induced by Zn and Opposite Effect of Se in *Oryctolagus cuniculus*. J Environ Anal Toxicol. 289.

15. Henine S., Rouabhi R., Gasmi S., Amrouche A., Abide A., Salmi A., 2016. Oxidative stress status, caspase-3, stromal enzymes and mitochondrial respiration and swelling of Paramecium caudatum in responding to the toxicity of Fe_3O_4 pesticide s, Environ Health Sci. 8, 161.

16. Suchail S., Guez D., Belzunees L.P., 2009. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in Apis mellifera. Environ Toxicol Chem. 20, 2482-2486.

17. Kowalczyk E., Kopff A., Kedziora J., Blaszczyk J., Kopff M., Niedworok J., Fijalkowski P. 2004. Pol J Environ Stud. 13(1), 41–43.

18. ATSDR (Agency for Toxic substances and Disease Registry). 2007, Fed. Regist. 12178–12179. [PubMed] [Google Scholar].

19. Al-Attar A.M., 2011 Antioxidant effect of vitamin E treatment on some heavy metals-induced renal and testicular injuries in male mice. Saudi J Biol Sci .18, 63–72.

20. Silva M.H., Gammon D., 2009. An assessment of the developmental, reproductive and neurotoxicity of endo sulfan. Birth Defects Res Dev Reprod Toxicol. 86, 1–28.

21. Cory-Slechta D., Thiruchelvam M., Ek R., Bk B., Brooks A., 2005. Developmental pesticide exposures and the Parkinson's disease phenotype. Birth Defects Res A Clin Mol Teratol. 73, 136–139.

22. Gasmi S., Rouabhi R., Kebieche M., Salmi A., Boussekine S., Toualbia N., Taib C., Henine S., Bouteraa Z., Djabri B. 2016. Neurotoxicity of acetamiprid in male albino rats and the opposite effect of quercetin. Biotechnol Ind J. 12(7), 113.

23. Zouaoui S., Rouabhi R., Bouzenzana S., Menaceur F., Gasmi S., Benaicha B., 2023. Assessment of two steroid hormones Progestin and estrogen on cellular model Paramecium: oxidative stress, Mitochondrial swelling and kinetic. Researchsquare. https://doi.org/10.21203/rs.3.rs-3742249/v1

24. Ali C., Réda D.M., Rachid R., Houria B., 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

25. Shi H., Hudson L.G., Liu K.J., 2004. Oxidative stress and apoptosis in metal ion induced carcinogenesis. Free Radic Biol Med. 37, 582–593.

26. Assefa Z., Van Laethem A., Garmyn M., Agostinis P., 2005. Ultraviolet radiation induced apoptosis in keratinocytes: on the role of cytosolic factors. Biochim Biophys Acta. 1755, 90–106

27. Ivanina A.V., Habinck E., and Sokolova I.M., 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.

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

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

30. Morris G., Berk M., 2015. Many roads to mitochondria dysfunction in neuro immune and neuropsychiatric disorders. BMC Med. 13, 68–75.

31. Rachid R., Djebar-Berrebbah H., Djebar M.R., 2008. Growth, chitin and respiratory metabolism of Tetrahymena pyriformis exposed to the insecticide Novaluron. Am Euras J Agric Environ Sci. 3, 873-881.