Journal of
Babol University of Medical Sciences
JBUMS
e-ISSN: 2251-7170 p-ISSN: 1561-4107

Investigating the Neurotoxicity Caused by Tricyclazole and Thiophanate Methyl in Wistar Rats

Z. Harsini (DVM)[1], S. M. Hosseini (PhD)[*1], F. Pourabdolhossein (PhD)[2]

1.Department of Pathology, Faculty of Veterinary Medicine, Babol Branch, Islamic Azad University, Babol, I.R.Iran.

2.Department of Physiology, School of Medicine, Babol University of Medical Sciences, Babol, I.R.Iran.

Article Type

Research Paper

Received:
Oct 20th 2022
Revised:
Dec 10th 2022
Accepted:
Dec 14th 2022

ABSTRACT

Background and Objective: Along with the steady growth of the population, the widespread use of systemic fungicides, which leads to increased productivity and higher yield of food products, has been given a lot of attention. Therefore, considering the cytotoxic effects of systemic fungicides tricyclazole and thiophanate methyl, the present study was conducted with the aim of investigating the neurotoxicity caused by the use of fungicides tricyclazole (TCZ) and thiophanate methyl (TM) in Wistar rats.

Methods: In this experimental study, 32 male Wistar rats were randomly divided into 4 groups of 8 including: control group, groups receiving pesticide mixtures orally at doses of (A) TM 664 + TCZ 25, (B) TM 498 + TCZ 19 and (C) TM 332 + TCZ 13 (mg/kg body weight) and brain tissue sampling was done after 28 days. Nissl and hematoxylin-eosin staining were used for qualitative assessment of pathological lesions and quantitative counting of brain cells.

Findings: In the histopathological examinations of the groups that received toxins, it was observed that the neurons became necrotic, and the increase of microglia cells in the hippocampus and cerebral cortex was also observed. The results of cell counting indicated the lowest number of neurons in group A in the cerebral cortex (171.40±4.88), CA1 (152.80±5.99), CA2,3 (127.90±8.36) and CA4 (59.20±3.86), which showed a significant decrease compared to the control group ($p<0.05$).

Conclusion: The results of the study showed that the mixture of tricyclazole and thiophanate methyl caused damage to brain neurons in the cerebral cortex and different areas of the hippocampus and subsequently caused a decrease in the number of neurons in these areas; Of course, the amount of damage was directly related to increase in the dose.

Keywords: *Tricyclazole, Thiophanate Methyl, Hippocampus, Neurons, Histopathology*.

Cite this article: Harsini Z, Hosseini SM, Pourabdolhossein F. Investigating the Neurotoxicity Caused by Tricyclazole and Thiophanate Methyl in Wistar Rats. *Journal of Babol University of Medical Sciences*. 2023; 25(1): 108-15.

***Corresponding Author: S. M. Hosseini (PhD)**
Address: Department of Pathology, Faculty of Veterinary Medicine, Babol Branch, Islamic Azad University, Babol, I.R.Iran.
Tel: +98 (11) 32415000. **E-mail:** dr_hosseini2323@yahoo.com

Introduction

In developed countries, the use of pesticides is one of the ways to increase the production of fruits, vegetables and grains. Pesticides have long played an important role in agriculture by protecting crops and improving productivity. Pesticides are often divided into classes of insecticides (insects), herbicides (weeds), fungicides (fungi, mold) and rodenticides (rodents) based on the target species they affect, and within each class, there are several subclasses with different chemical and toxicological characteristics. The type or chemical composition of pesticides that a person or population is exposed to determines the degree of involvement of body organs. Pesticides are capable of causing acute, subacute and chronic toxic effects in body organs (1).

Various studies have shown that one of the systems that can be affected by the acute and chronic effects of pesticides is the nervous system, which manifests itself in the form of severe signs and symptoms in acute exposure to high doses, or with weaker effects in chronic exposure to low doses, and can lead to changes in various functions related to this system and increase the possibility of neurodegenerative diseases (2). Neurotoxicity is defined as any adverse effect on the central or peripheral nervous system caused by chemical, biological or physical agents, which can be due to a number of inherent characteristics of this system such as dependence on aerobic metabolism, the presence of axonal transport or neurotransmission process (3).

The mixture of fungicides from one chemical group causes pathogen resistance against them, while the mixture of fungicides from different chemical groups increases the effect and range of disease control in plants (4). The mixture of tricyclazole and methyl thiophanate fungicide is widely used in agriculture to eliminate blast disease, which is considered as one of the most important fungal diseases of rice (5).

Tricyclazole is a systemic fungicide from the triazole family that can remain in the soil for more than 11 months. Due to its stability and accumulation in the soil, it provides long-term protection of rice during the entire growth process. Therefore, the potential environmental risk of tricyclazole is significant and it can be absorbed by the skin and mucous membranes immediately after exposure (6). The rate of absorption of tricyclazole in mammals is very high and its oral bioavailability is more than 90%. It is extensively metabolized and excreted through urine, feces and bile (7). Experimental studies have shown that long-term exposure to tricyclazole increases the destruction of hemoglobin and decreases its synthesis, resulting in chronic anemia (8). It also causes damage to the liver by inhibiting cytochrome CYP450 and cholesterol synthesis, and increases DNA damage by activating anti-apoptotic growth arrest and induction of genes (6).

Methyl thiophanate is a systemic fungicide with extensive internal absorption that has the ability to prevent and control agricultural crop diseases. The fungicidal mechanism of methyl thiophanate is such that by converting to methyl-2-benzimidazolecarbamate (MBC), it can cause the destruction of fungi in animal and plant tissues as well as in water (9). The toxicity of this fungicide can be attributed to oxidative damage to the red blood cell membrane (10), increased catecholamine levels and reduced synthesis and release of corticosterone (11), changes in the structural and functional integrity of proteins associated with plasma membranes (12).

As the studies show that the use of systemic fungicides leads to lesions of the nervous system, and on the other hand, the use of these compounds as the strongest response to biological threats in food sources is undeniable, and based on our investigations in authentic scientific sources, no study has been conducted regarding nervous system injuries including brain tissue changes after using the mixture of thiophanate methyl and tricyclazole. Therefore, the present study was conducted with the aim of investigating the neurotoxicity caused by the consumption of tricyclazole and methyl thiophanate in Wistar rats.

Methods

With the code of ethics approved in the Islamic Azad University, Babol branch (IR.IAU.BABOL.REC.1400.122) and compliance with ethical guidelines, this experimental study was conducted on 32 Wistar male albino rats with a mean weight of 200±20 grams (6-8 weeks). After random sampling, the rats were divided into four groups of eight, including one control group (ctrl) and three experimental groups receiving the mixtures of thiophanate methyl and tricyclazole (TM + TCZ) and were kept in the animal house at a temperature of 23±5 °C, 55% humidity and 12 hours light/dark cycle with free access to food and water. The study was started after one week so that the animals would adapt to the environmental conditions. The control group was given only water without pesticide mixture, and the other three groups were given pesticide mixtures in three doses of (A) TM 664 mg/kg + TCZ 25 mg/kg, (B) TM 498 mg/kg + TCZ 19 mg/kg and (C) TM 332 mg/kg + TCZ 13 mg/kg prepared in corn solvent and adjusted to body weight. The mixture of mentioned pesticides was administered orally and weekly for 28 days (13). Food and water consumption were controlled manually every day.

Histopathological examination: on the 28th day of the study, 70 mg/kg of ketamine and 20 mg/kg of xylazine were used intraperitoneally to induce anesthesia, and then the rats were sampled for histological examination and the resulting samples were immediately placed in formalin solution 10% and they were then dehydrated by ethanol and separated in paraffin, and using a microtome machine (Leitz 1512), sections were made in the coronal planes with a thickness of 8 micrometers (in the bregma region, 2.5 to 4.5 mm from hippocampus) and placed on a slide and common laboratory protocols were considered to examine the morphology and identify the basic structure of healthy neurons from destroyed neurons in the brain tissue, and the slides were stained with hematoxylin-eosin and Nissl using Cresyl Violet (14).

In order to perform quantitative studies and count cells after Nissl staining, at least 4 consecutive sections at a distance of 100 µm from 4 regions of the cerebral cortex, CA1, CA2, CA3, CA4 Dentate Gyrus of the hippocampus were selected and examined by light microscopy (Olympus CX31) with 40x magnification, and the cells of these areas were counted 120 square mm in the cortex, 32 mm in the CA1-4 areas, and 42 mm in the DG of the hippocampus. Images were recorded by a microscope and the neurons of these areas were measured using Capture software (15, 16).

In order to analyze the results obtained from the histological examinations, SPSS version 26, one-way ANOVA and Tukey's multiple comparison test were used, and $p \leq 0.05$ was considered significant.

Results

In the histopathology of the hippocampus and cerebral cortex using hematoxylin-eosin and Nissl staining in the control group, the neurons showed clear spherical nuclei and cytoplasm, which indicated the normality of the morphological and histological characteristics and there was no sign of cellular and degenerative changes. There was nothing special in the whole tissue. Certain changes were observed in the groups receiving mixed toxins, including the shrinking of the neuron cell body, eosinophilic cytoplasm, pyknosis of the nucleus, necrosis of neurons, and changes in the size of pyramidal neurons in the hippocampus and cerebral cortex. Nevertheless, gliosis was also observed in the cerebral cortex and hippocampus in all groups (Figure 1).

Exposure to the mixture of tricyclazole and methyl thiophanate toxins in all three groups significantly led to a decrease in the number of neurons in the cerebral cortex and different areas of the hippocampus (CA1, CA2, CA3 and CA4) compared to the control group (Table 1). However, in the number of DG neurons in group A (175.20±5.16), B (177.20±5.23), C (180.80±6.74) compared to the control group

(190.00±2.92), no significant difference was observed. The findings showed the highest reduction in group A compared to other groups ($p<0.05$).

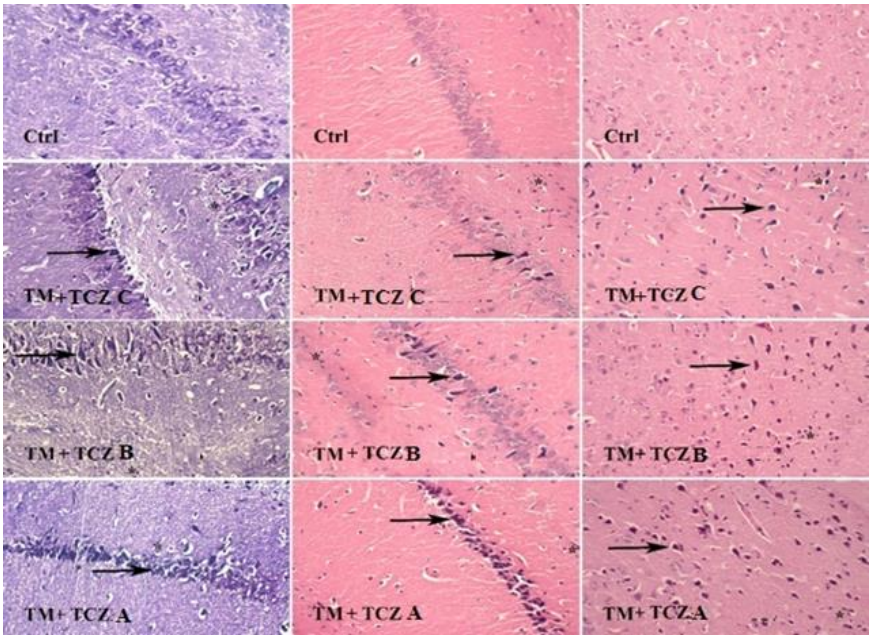

Figure 1. Brain tissue and hippocampus. **Normal tissue conditions in the control group, necrosis (arrow to the right), gliosis (star), 40x magnification. The left column is Nissl staining of the hippocampus, the central column is hematoxylin and eosin staining of the hippocampus, the right column is the hematoxylin and eosin staining of the cerebral cortex.**

Table 1. Comparison of the number of neurons in the cerebral cortex and hippocampus between the groups receiving the combined poison and the control group

Groups	Cortex Mean±SD	CA1 Mean±SD	CA2,3 Mean±SD	CA4 Mean±SD	DG Mean±SD
Control	201.50±2.62[a]	203.30±2.82[a]	182.95±5.69[a]	80.00±2.54[a]	190.00±2.92[a]
Methyl Thiophanate + Tricyclazole (A)	171.40±4.88[b]	152.80±5.99[c]	127.90±8.36[c]	59.20±3.86[b]	175.20±5.16[a]
Methyl Thiophanate + Tricyclazole (B)	179.40±4.88[b]	165.90±8.34[b,c]	147.00±8.50[b,c]	65.30±3.26[b]	177.20±5.23[a]
Methyl Thiophanate + Tricyclazole (C)	182.80±6.58[a,b]	180.60±8.43[a,b]	161.00±5.98[a,b]	71.60±4.34[a,b]	180.80±6.74[a]

The letters show the mean values of each group and the difference of letters in one column indicates significant difference compared to others (Tukey's test, one way ANOVA, $p<0.05$).

Discussion

In the present study, the most pathological changes were observed after receiving the systemic fungicide mixture of tricyclazole and thiophanate methyl at doses of (664 mg/kg+25 mg/kg) in the cerebral cortex and hippocampus. The smallest mitochondrial disorders in brain cells occur due to the high dependence of these cells to energy and due to a weak antioxidant system that leads to the loss of neurons, irreversible changes

and increased vulnerability of the nervous system following the use of pesticides (17, 18). Therefore, it can be argued that the severity of damage to brain cells depends on the dosage of fungicide compounds. Since the first responses in the central nervous system occur after the damage of chemical compounds to the brain, the migration of inflammatory cells and microglia to the damaged area (19), it was seen in the present study that the mixture of fungicides thiophanate methyl and tricyclazole leads to gliosis in hippocampus and cerebral cortex. Several evidences, like other studies, showed that the use of systemic fungicides leads to an increase in glial cells in the cerebral cortex (20, 21).

In the present study, it was seen that the mixture of systemic fungicides led to a decrease in the number of brain neurons. Also, in a study conducted by Lafon et al. with the aim of investigating exposure to fungicide residues and beta-amyloid accumulation in a laboratory rat model of Alzheimer's disease, it was shown that the mixture of fungicides can increase the accumulation of microglia cells as well as decrease and degeneration of brain neurons, especially in the hippocampus region (22). The study conducted by Regueiro et al. also showed that the use of the fungicides Kresoxim-methyl, Cyazofamid and Pyraclostrobin through the reduction of ATP and the increase of cytosolic calcium led to an increase in the death of neurons, followed by a decrease in the number of cells (23), and these studies are consistent with the present study.

Since the mixture of two fungicides tricyclazole and thiophanate methyl was used in this study, the results of the histology of the cerebral cortex and hippocampus showed that exposure to the mixture of these toxins in different doses leads to pathological changes including necrosis and morphological changes of cells. Of course, this damage has a direct relationship with increasing the dose, and the present study is consistent with the study conducted by Abd El-Moneim Ibrahim et al. among 89 male Wistar rats with the aim of investigating brain lesions caused by the mixture of two pesticides, chlorpyrifos and cypermethrin, which led to the degeneration and necrosis of pyramidal neurons in the brain tissue (24).

Toxins of the triazole family lead to inhibition of cytochrome CYP450 and cholesterol synthesis, increase in serum triglyceride and lactate levels, production of free radicals, irreversible disturbances in metabolic regulations and increase in the number of cells through the activation of anti-apoptotic processes and finally neuropathological lesions in the peripheral and central nervous system and for this reason, these fungicides are classified under genetic and cellular toxins (6, 25, 26). Pathological changes such as chromatolysis and necrosis of neurons as well as neurodegeneration have been seen in the fungicide Penconazole from the triazole family (27) and the study of Hamdi et al. showed damage to brain neurons, increased cell death following lipid peroxidation and DNA fragmentation after the use of systemic fungicide Epoxiconazole from the triazole family (28). Furthermore, studies showed that methyl thiophanate affects biological cellular activities such as ATP synthesis, signaling, regulation of biosynthetic and catabolic reactions, changes in macromolecules such as proteins, lipids and DNA, reduction of membrane fluidity, inactivation of membrane-bound enzymes such as ATPase, loss of essential fatty acids and the transfer of metabolites and ions (10, 12) and eventually these disorders lead to pathological lesions in cells, including brain neurons (29). Neurological lesions in the cerebral cortex and hippocampus are one of the pathological lesions that have been studied and approved by Ebedy et al. with the aim of investigating behavioral changes following the use of Carbendazim as one of the metabolites of methyl thiophanate among 60 Wistar male rats (30).

According to the findings, it can be concluded that the mixture of fungicides in high doses leads to an increase in neurotoxicity, followed by severe tissue damage.

Conflict of interest: The authors declare that there is no conflict of interest.

Acknowledgment

We hereby acknowledge the efforts of the colleagues at the Clinical Research Development Unit of Ayatollah Rouhani Medical Center, Mrs. Mina Galeshi and other colleagues who helped us in conducting this study.

References

1.Lippmann M. Asbestos and other mineral and vitreous fibers. In: Lippmann M, Leikauf GD, editors. Environmental toxicants: human exposures and their health effects, 4th ed. Wiley; 2020. p. 389-453.
2.Hassanen EI, Hussien AM, Hassan NH, Ibrahim MA, Mehanna S. A Comprehensive Study on the Mechanistic Way of Hexaflumuron and Hymexazol Induced Neurobehavioral Toxicity in Rats. Neurochem Res. 2022;47(10):3051-62.
3.Costa LG, Giordano G, Guizzetti M, Vitalone A. Neurotoxicity of pesticides: a brief review. Front Biosci. 2008;13(4):1240-9.
4.Carisse O. Fungicides. IntechOpen; 2010. p. 74-69.
5.Padasht Dehkaei F, Khosravi V, Dodabeinajad E, Pourfarhan H, Dariush S. Efficacy of a mixture fungicide tricyclazole + thiophanate methyl in comparison with some commonly used fungicides in controlling of rice blast disease in northern Iran. Cereal Res. 2012;2(4):317-28. [In Persian]
6.Rowshanaie T, Sadoughi M, Fattahi E. The Effects of Tricyclazole on HepaticEnzyme Changes and Tissue Damage in the Fetus of Laboratory Mice. J Babol Univ Med Sci. 2015;17(7):51-7. [In Persian]
7.Corvaro M, Gollapudi BB, Mehta J. A critical Assessment of the Genotoxicity Profile of the Fungicide Tricyclazole. Environ Mol Mutagen. 2020;61(3):300-15.
8.NathPandit D, Rani U, Sardana M. Tricyclazole Induced Changes in Certain Haematological Indices of Channapunctatus (Bloch) as Diagnostic Biomarkers of Stress Response. Int J Pharm Bio Sci. 2021;12(1):b97-103.
9.Jia K, Cheng B, Huang L, Xiao J, Bai Z, Liao X, et al. Thiophanate-methyl induces severe hepatotoxicity in zebrafish. Chemosphere. 2020;248:125941.
10.Ben Amara I, Ben Saad H, Cherif B, Elwej A, Lassoued S, Kallel C, et al. Methyl-thiophanate increases reactive oxygen species production and induces genotoxicity in rat peripheral blood. Toxicol Mech Methods. 2014;24(9):679-87.
11.Capaldo A, Gay F, De Falco M, Virgilio F, Valiante S, Laforgia V, et al. The newt Triturus carnifex as a model for monitoring the ecotoxic impact of the fungicide thiophanate methyl: adverse effects on the adrenal gland. Comp Biochem Physiol C Toxicol Pharmacol. 2006;143(1):86-93.
12.Feki A, Saad HB, Jaballi I, Magne C, Boudawara O, Zeghal KM, et al. Methyl thiophanate-induced toxicity in liver and kidney of adult rats: a biochemical, molecular and histopathological approach. Cell Mol Biol (Noisy-le-grand). 2017;63(2):20-8.
13.do Amaral LA, Farias Pereira Subtil Cavalcante AC, da Silva Fleming de Almeida T, Marques Romeiro Santos M, Candeloro Portugal L, Suzuki Dos Santos B, et al. Acute and subacute (28 days) oral toxicity studies of tucum almond oil (Bactris Setosa Mart.) in mice. Drug Chem Toxicol. 2022;45(4):1754-60.
14.Suvarna SK, Layton C, Bancroft JD. Bancroft's Theory and Practice of Histological Techniques, 8th ed. Elsevier; 2019. p. 126-37.
15.Farokhcheh M, Hejazian L, Akbarnejad Z, Pourabdolhossein F, Hosseini SM, Mehraei TM, et al. Geraniol improved memory impairment and neurotoxicity induced by zinc oxide nanoparticles in male wistar rats through its antioxidant effect. Life Sci. 2021;282:119823.
16.Nobakht M, Hoseini SM, Mortazavi P, Sohrabi I, Esmailzade B, Rahbar Rooshandel N, et al. Neuropathological changes in brain cortex and hippocampus in a rat model of Alzheimer's disease. Iran Biomed J. 2011;15(1-2):51-8.
17.Ghasemnejad-Berenji M, Nemati M, Pourheydar B, Gholizadeh S, Karimipour M, Mohebbi I, et al. Neurological effects of long-term exposure to low doses of pesticides mixtures in male rats: Biochemical, histological, and neurobehavioral evaluations. Chemosphere. 2021;264(Pt 2):128464.

18.Aloizou AM, Siokas V, Vogiatzi C, Peristeri E, Docea AO, Petrakis D, et al. Pesticides, cognitive functions and dementia: A review. Toxicol Lett. 2020;326:31-51.
19.De Sousa RAL. Reactive gliosis in Alzheimer's disease: a crucial role for cognitive impairment and memory loss. Metab Brain Dis. 2022;37(4):851-7.
20.Ahmed MS, Massoud AH, Derbalah AS, Ismail AA. Pathological and Biochemical Assesment of the Fungicide (Metalaxyl) on Rats. Egypt J Comp Path &Clinic Path. 2011;24(1):136-54.
21.Ahmed MS, Massoud AH, Derbalah AS, Al-Brakati A, Al-Abdawani MA, Eltahir HA, et al. Biochemical and Histopathological Alterations in Different Tissues of Rats Due to Repeated Oral Dose Toxicity of Cymoxanil. Animals (Basel). 2020;10(12):2205.
22.Lafon PA, Wang Y, Arango-Lievano M, Torrent J, Salvador-Prince L, Mansuy M, et al. Fungicide residues exposure and β-amyloid aggregation in a mouse model of Alzheimer's disease. Environ Health Perspect. 2020;128(1):17011.
23.Regueiro J, Olguín N, Simal-Gándara J, Suñol C. Toxicity evaluation of new agricultural fungicides in primary cultured cortical neurons. Environ Res. 2015;140:37-44.
24.Abd El-Moneim Ibrahim K, Mohamed Abdelrahman S, Elhakim HK, Ali Ragab E. Single or combined exposure to chlorpyrifos and cypermethrin provoke oxidative stress and downregulation in monoamine oxidase and acetylcholinesterase gene expression of the rat's brain. Environ Sci Pollut Res Int. 2020;27(11):12692-703.
25.Faro LR. Neurotoxic Effects of Triazole Fungicides on Nigrostriatal Dopaminergic Neurotransmission. In: Carisse O, editors. Fungicide. IntechOpen; 2010. p. 405-20.
26.Fattahi E, Mousavi Moghadam M, Khanbabaei R. The Effect of Tricyclazole on Testosterone Changes and Testicular Structure in Mice. J Babol Univ Med Sci. 2015;17(2):43-9. [In Persian]
27.Morgan AM, Hassanen EI, Ogaly HA, Al Dulmani SA, Al-Zahrani FA, Galal MK, et al. The ameliorative effect of N-acetylcysteine against penconazole induced neurodegenerative and neuroinflammatory disorders in rats. J Biochem Mol Toxicol. 2021;35(10):e22884.
28.Hamdi H, Graiet I, Abid-Essefi S, Eyer J. Epoxiconazole profoundly alters rat brain and properties of neural stem cells. Chemosphere. 2022;288(Pt 3):132640.
29.Kumar V, Abbas A, Aster J. Robbins basic pathology, 10th ed. Elsevier; 2017. p. 32-5.
30.Ebedy YA, Hassanen EI, Hussien AM, Ibrahim MA, Elshazly MO. Neurobehavioral Toxicity Induced by Carbendazim in Rats and the Role of iNOS, Cox-2, and NF-κB Signalling Pathway. Neurochem Res. 2022;47(7):1956-71.