Cairo University

Faculty of Veterinary Medicine

Neurobehavioral Toxicity of Propiconazole Fungicide and a Trial for Protection in Rats

A thesis submitted by

Peter Azmy Noshy

(BVSc, Cairo University, 2009; MVSc, Cairo University, 2015)

For the degree of the Ph.D.

(Toxicology and Forensic Medicine)

Under Supervision of

Prof. Dr. Mohamed A. Elhady

Prof. of Toxicology and Forensic Medicine, Vice Dean for Community Service and Environment Development, Faculty of Veterinary Medicine, Cairo University

Prof. Dr. Abdel-Azeim A. Khalaf

Prof. of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Cairo University

Prof. Dr. Mervat M. Kamel

Prof. of Animal, Poultry Behavior and Management, Faculty of Veterinary Medicine, Cairo University

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Approval Sheet

This thesis entitled **"Neurobehavioral Toxicity of Propiconazole Fungicide and a Trial for Protection in Rats"** prepared and submitted by **Peter Azmy Noshy** in partial fulfillment of the requirements for the degree of **Ph.D.** in **Toxicology and Forensic Medicine** has been examined and hereby recommended for approval and acceptance.

Approval Committee

Prof. Dr. Hesham S. Elsabbagh

Professor and Chairman of Forensic Medicine and Toxicology

Faculty of Veterinary Medicine, University of Sadat City

Prof. Dr. Fatma S. Salem

Professor of Toxicology and Forensic Medicine

Faculty of Veterinary Medicine, Cairo University

Prof. Dr. Mohamed A. Elhady

Professor of Toxicology and Forensic Medicine,

Vice Dean for Community Service and Environment Development,

Faculty of Veterinary Medicine, Cairo University

Prof. Dr. Abdel-Azeim A. Khalaf

Professor of Toxicology and Forensic Medicine

Faculty of Veterinary Medicine, Cairo University

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Prof. of Animal, Poultry Behavior and Management, Faculty of Veterinary Medicine, Cairo University

Name of Candidate: Peter Azmy Noshy **Degree: Ph.D.**

Title of Thesis: Neurobehavioral Toxicity of Propiconazole Fungicide and a Trial for Protection in Rats

Supervisors: Prof. Dr. Mohamed Ali Elhady – Prof. Dr. Abdel-Azeim Ali Khalaf – Prof. Dr. Mervat Mahmoud Kamel

Department: Toxicology and Forensic Medicine **Approval: 7/4/2019**

Abstract

Propiconazole (PCZ) is a triazole fungicide extensively used in agriculture. Carvacrol (CAR) is a naturally occurring phenolic monoterpene which has various biological and pharmacological effects. The present study was designed to investigate the neurobehavioral toxic effects of PCZ in albino rats and to evaluate the ameliorative role of CAR against such toxic effects. Sixty adult male rats were used in this investigation; they were randomly and equally divided into 4 groups: control group, PCZ group, CAR group and PCZ + CAR group. PCZ (75 mg/kg) and/or CAR (50 mg/kg) were administered daily by oral gavage for 8 weeks. Behavioral investigation clearly demonstrated the negative impact of PCZ on psychological, motor and cognitive brain functions. Exposure to PCZ also adversely affected the measured oxidative stress and lipid peroxidation parameters in brain tissue. A significant decrease in activity of AChE enzyme in neural tissue was also observed in PCZ-exposed rats. The comet assay revealed a high percent of DNA damage in the brain of rats exposed to PCZ. Histopathological examination of the cerebrum, cerebellum, and hippocampus showed various histopathological lesions after exposure to PCZ which were confirmed by immunohistochemical examination. On the other hand, concurrent administration of CAR ameliorated most of the undesirable effects of PCZ.

Keywords: Triazole fungicides – Oregano – Brain – Oxidative stress – Cholinesterase – Comet assay.

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List of Abbreviations

ACh	Acetylcholine
AChE	Acetylcholine esterase
AChEI	Acetylcholine esterase inhibitor
AD	Alzheimer's disease
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
Aβ	Amyloid β
CAR	Carvacrol
CAT	Catalase
CK	Creatine kinase
C_{max}	Maximum concentration
cLogP	Partition coefficient
COX	Cyclooxygenase
CYP	Cytochrome P450
DA	Dopamine
DEN	Nitrosodiethylamine
D-GalN	D-galactosamine
EPA	Environmental protection agency
GABA	Gamma amino butyric acid
GFAP	Glial fibrillary acidic protein
GLU	Glutamate
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione

GST	Glutathione S transferase
IL	Interleukin
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
LPO	Lipid peroxidation
MAO	Monoamine oxidase
MDA	Malondialdehyde
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NF	Nuclear factor
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NOS	Nitric oxide synthase
OHDA	Hydroxy dopamine
PC	Protein carbonyls
PCZ	Propiconazole
PND	Post-natal day
ROS	Reactive oxygen species
SDS	Sodium dodecyl sulfate
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TNF	Tumor necrosis factor
TP	Total protein

Chapter (1)

Introduction

Chapter (1)

Introduction

Propiconazole (PCZ) (cis-trans-1-[2-(2,4-dichlorophenyl)4-propyl-1,3-dioxolan-2-ylmethyl]-1H-1,2,4-triazole) is a triazole antifungal pesticide belongs to the class of ergosterol biosynthesis inhibiting fungicides (EBIFs). It has moderate acute oral toxicity in rats and mice (LD_{50}, 1517 and 1490 mg/kg, respectively) **(JMPR, 2004).** PCZ is used as a fungicide on bananas, grapes, pineapples, peanuts, sugarcane, wheat, barley and other crops and is effective against different fungal species, for instance, *Erysiphe graminis*, *Leptosphaerian odorum*, *Puccinia spp.* and *Septoria spp.* **(Sun *et al.*, 2005).**

Several studies had investigated the neurobehavioral toxic effects of triadimefon, another triazole fungicide, in rats and mice and reported various behavioral effects and neurochemical alterations **(Reeves *et al.*, 2003; Xi *et al.*, 2012).** Moreover, another study had reported biochemical changes and pathological lesions in the cerebrum and cerebellum of rats exposed to penconazole – another triazole fungicide **(Chaâbane *et al.*, 2017).** Few studies had investigated the neurobehavioral toxicity of PCZ in fish **(Li *et al.*, 2010_a; 2011_a; Srivastava and Singh, 2014; Tabassum *et al.*, 2016_b)** and demonstrated that exposure to PCZ had resulted in behavioral changes as well as biochemical alterations and pathological lesions in the fish brain. However, to date, no researcher had explored the neurobehavioral toxicity of PCZ in rats and mice as animal models.

Carvacrol (2-methyl-5-[1-methylethyl]phenol) is a monoterpenoid phenol present mainly in essential oils produced by various aromatic plants such as oregano, thyme, pepperwort and wild bergamot **(Tang *et al.*, 2011; Jamali *et al.*, 2012; Kim *et al.*, 2013).** Many *in vitro* and *in*

vivo studies demonstrated various biological and therapeutic activities of CAR, including: antiseptic, antifungal, antibacterial, antiviral, anti-inflammatory, antioxidant, growth promoter, antispasmodic, antitussive, expectorant, immunomodulatory and chemo-preventive actions **(Soltanab *et al.*, 2011; Hashemipour *et al.*, 2013; Bravo *et al.*, 2014).**

Aside from the above mentioned pharmacological effects, several studies had investigated the neuroprotective effect of CAR **(Azizi *et al.*, 2012; Yu *et al.*, 2012; Deng *et al.*, 2013; Baluchnejadmojarad *et al.*, 2014; Wang *et al.*, 2017).** It was found that CAR has many beneficial effects on the nervous system, including anti-apoptotic, antioxidant, anti-inflammatory and cognitive enhancing activities. Cognitive function improvement had been attributed to acetylcholinesterase inhibitory effect of CAR **(Jukic *et al.*, 2007).** Additionally, CAR had been reported to have anxiolytic and anti-depressant effects mediated through modulation of GABAergic transmission and dopaminergic system **(Melo *et al.*, 2010; 2011).** Moreover, due to its small molecular size and lipophilicity, CAR can readily cross the blood-brain barrier and exert its therapeutic actions effectively **(Savelev *et al.*, 2004).**

In spite of the extensive use of PCZ as a fungicide, and the potential for harm due to exposure of human and animal populations, few studies have investigated its potential detrimental actions on the nervous system. Because of this, the current study was designed to explore the possible neurobehavioral harmful effects of PCZ in rats and to investigate its potential mechanisms of action. Also, several studies had revealed the antioxidant and anti-inflammatory effects of CAR in different organs including the nervous system. So, it was selected for evaluation of its neuroprotective effect against PCZ-induced toxic impacts.

Chapter (2)

Review of Literature

Chapter (2)

Review of Literature

A. Propiconazole

1. Introduction:

Fungicides are pesticides that particularly kill or destroy fungi responsible for several diseases associated to humans and other living organisms. Assessment of toxic effects and mechanisms of fungicide action is important because humans and domesticated animals get exposed to these pesticides through a wide variety of applications. Several fungicides are being used at a large scale for crop protection from the fungal invasion (**Tabassum *et al.*, 2016$_a$).**

Conazoles are azole antifungal agents used in agricultural and pharmaceutical products. They are applied as fungicides in fruit, vegetable, and cereal crop protection programs, and also in lawn care and wood preservation. Medically, they are widely used to treat local and systemic fungal and yeast infections; some with anti-estrogenic activity are also being used clinically for treatment of prostate and breast cancer **(Zarn *et al.*, 2003).**

The fungicidal properties of conazoles are due to their abilities to inhibit ergosterol biosynthesis. Since ergosterol is an essential component of fungal membranes, its inhibition leads to cell death. These chemicals are representative of a larger class of ergosterol biosynthesis inhibiting fungicides (EBIFs), which act primarily by inhibiting lanosterol 14α-demethylase (encoded by the CYP51 gene) enzyme activity, a metabolic pathway in ergosterol production.