## **UNIVERSITI TEKNOLOGI MARA**

# FORENSIC TOXICOLOGICAL STUDY OF CITALOPRAM LETHAL POISONING USING BIOLOGICAL AND ENTOMOLOGICAL SAMPLES

SITI AISYAH BINTI SHAMSUDDIN

Thesis submitted in fulfilment of the requirements for the degree of Master of Science

**Faculty of Applied Sciences** 

December 2017

#### ABSTRACT

Entomological evidence plays a significant role in most cases of decomposed body which provide beneficial information related to forensic investigation, especially in estimation of the minimal post-mortem interval as well as the cause of death. This study was designed to investigate the effect of citalopram on the decomposition rates of rabbit carcasses and faunal succession as well as the development rates of blowfly Chrysomya megacephala and Chrysomya rufifacies. The goal of the study was also to develop and optimized QuEChERS extraction method and applied the validated method to determine the concentration of target analytes in biological and blowflies specimens. Correlation between both of the samples were also analysed to relate the suitability of the entomological specimens used in toxicological analysis. For indoor decomposition, six New Zealand White rabbits weighing between 2.5 kg to 3.0 kg, were equally divided into control and test groups. The control group was sacrificed by isoflurane while test group was force fed with two lethal doses of citalopram, T1 (800 mg/kg) and T2 (1600 mg/kg) before euthanized with isoflurane. Toxicological analysis by high performance liquid chromatography coupled with diode array detector was performed using C<sub>8</sub> column under isocratic elution of 1 mL/min running through the mobile phase consisting of 60% (v/v) 10 mM ammonium acetate buffer (pH 7) and 40% (v/v) acetonitrile at 40°C. Study revealed that the decomposition rates of the treated-citalopram rabbit carcasses were accelerated from control carcasses. Entomofauna attracted to the carcasses were also found abundant on the citalopram treated group compared to control. The study found that the development rates of the treated larvae were faster than the control. The length and weight of the treated larvae were observed bigger and attained maximum size earlier than control. This clearly proved that citalopram could disturb the PMI estimation based on the decomposition rates and blowflies development rates. Modification of QuEChERS method has successfully optimized and validated to obtain highest linearity of regression coefficient ( $R^2 > 0.999$ ) estimated from the calibration curve range from 0.05 µg/mL to 50.0  $\mu$ g/mL, with the high precision (RSD <5%) and accuracy (75% – 110%). The method was revealed high sensitivity with limit of detection (LOD) and limit of quantification (LOQ) range between 0.02  $\mu$ g/mL to 0.15  $\mu$ g/mL and 0.08  $\mu$ g/mL to 0.34 µg/mL, respectively. The target analytes of citalopram and its metabolites desmethylcitalopram and didesmethylcitalopram were successfully achieved using QuEChERS method analysed by high performance liquid chromatography diode array detector. Study indicated that the entomological sample could be valuable as alternative specimens used in toxicology for qualitative analysis, but unreliable in quantification.

### ACKNOWLEDGEMENT

Upon completion of this project, I would like to express my gratitude to many parties. First of all, I am grateful to The Almighty and Merciful Allah SWT and his messenger Prophet Muhammad SAW that by His will has conferred me to observe one of the universe mysteries and gave me strength to finish up my project excellently.

Firstly, I would like to offer my sincerest gratitude is my main supervisor, Associate Professor Dr Farida Zuraina Mohd Yusof. I would like to thank with full of my heart for her supervision and support throughout my project, laboratory works and during thesis writing. I would also like to show my gratitude to my co-supervisor, Pn. Rumiza Abd Rashid for her guidance and assistance during the completion of my research. This study would not have been successful without her support and guidance.

I would also like to express my gratitude to staff of the Institute for Medical Research, especially Dr. Lee Han Lim, Dr. Nazni Wasi Ahmad and Mr. Chew Wai Kian for providing the facilities, knowledge and assistance on species identification.

Not forgotten to thanks Mr. Dzahir Dzaidanee Nasarudin and Mrs. Norhaida as Forensic Lab assistant for their guidance in using the instrument during the laboratory work. Thousands of thanks expressed to the staff at Institute of Forensic Science, Jalan Klang Lama for their support.

Finally, I would like to show my endless gratitude to my family members, colleagues and friends for their support throughout my project. They had encouraged me to keep on focusing when I was struggling to finish up this research. Without their encouragement, maybe I wouldn't have finished this research.

### **TABLE OF CONTENTS**

	Page
CONFIRMATION BY PANEL OF EXAMINERS	ii
AUTHOR'S DECLARATION	iii
ABSTRACT	iv
ACKNOWLEDGEMENT	v
TABLE OF CONTENTS	vi
LIST OF TABLES	xi
LIST OF FIGURES	xiv
LIST OF PLATES	xvi
LIST OF SYMBOLS	xvii
LIST OF ABBREVIATIONS	xviii
LIST OF NOMENCLATURES	xix

CH	APTER ONE: INTRODUCTION	1
1.1	Background of Study	1
1.2	Problem Statement	3
1.3	Research Question	4
1.4	Significance of Study	4
1.5	Hypothesis	5
1.6	Objectives of the Study	5
1.7	Scope and Limitation	6
СН	APTER TWO: LITERATURE REVIEW	7
2.1	Drug Poisoning	7
2.2	Antidepressant As a Fatal Toxic Agent	8
	2.2.1 Citalopram	8
	2.2.2 Pharmacology of Citalopram	10
2.3	Forensic Entomology	12
	2.3.1 Application of Forensic Entomology	13
2.4	Decomposition Process and Insect Succession	15

	2.4.1 Stage in Decomposition	15
	2.4.2 Faunal Succession along Decomposition Stages	17
	2.4.3 Factor Affecting the Decomposition and Faunal Succession	18
2.5	Important Carrion Insects for Forensic Purposed in Malaysia	20
2.6	Analysis of Drugs in Entomological Samples	21
2.7	Sample Preparation Techniques	22
	2.7.1 Solid Phase Extraction (SPE)	22
	2.7.2 Dispersive Liquid-Liquid Micro-Extraction (DLLME)	25
h	2.7.3 Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS)	
	Extraction Method	27
	2.7.3.1 Application in Extraction of Pesticides Residue	28
	2.7.3.2 Application in Extraction of Polyacromatic Hydrocarbon	
	(PAH) and Mycotoxins	28
	2.7.3.3 Application in Extraction of Drugs and Toxins Residue	29
СН	APTER THREE: MATERIALS AND METHODS	33
3.1	Chemicals and Reagents	33
3.2	Materials and Apparatus	33
3.3	Equipments	34
3.4	Animal Model	34
	3.4.1 New Zealand White Rabbits (Oryctolagus cuniculus)	
	As Animal Model	34
	3.4.2 Animal Handling	34
	3.4.3 Acute Exposure of Citalopram Overdose into Rabbits	35
	3.4.4 Simulation Site for Decomposition Study	35
	3.4.5 Observation of Decomposition Process and Faunal Succession	36
	3.4.6 Samples Collection and Preservation	36
	3.4.7 Measurement of the Larvae and Pupa Length and Width	37
	3.4.8 Morphological Observation of Instar Larvae	38
	3.4.9 Sampling Procedure for Toxicological Analysis	38
	3.4.9.1 Sampling Procedure for Biological Samples	38
	3.4.9.2 Sampling Procedure for Entomological Samples	39
3.5	Chemical Analysis of Citalopram and Metabolites	39