UNIVERSITI TEKNOLOGI MARA

GENE EXPRESSION ANALYSIS OF CHRONICALLY STRESSED DANIO RERIO TREATED WITH AQUEOUS ETHANOLIC MORINGA OLEIFERA LEAVES EXTRACT

MUHAMMAD FAIQ NAJMI BIN TAJUDIN

Thesis submitted in fulfillment of the requirements for the degree of **Master of Science** (Molecular Biology)

Faculty of Applied Sciences

December 2023

ABSTRACT

Anxiety disorder is a mental health condition that affects millions of people worldwide. Common treatments for anxiety include psychotherapy and medications, such as benzodiazepines and selective serotonin reuptake inhibitors (SSRIs). However, these treatments have adverse effects and may not be suitable for everyone. As a result, there is a growing interest in exploring natural remedies for anxiety, including those derived from plants. Moringa oleifera, a plant known for its medicinal properties, has recently gained attention for its potential anxiolytic effects. This plant has been traditionally used to treat various ailments, including anxiety. Nevertheless, there is still a lack of understanding on the mechanism of action of M. oleifera in anxiolytic effects. The aim of this study was to investigate the anxiolvtic effects and mechanism of action of ethanolic extracts of *M. oleifera* (MOLE) leaves, by observing behavioural changes in Danio rerio (zebrafish) and analyzing differential gene expression. For 14-day chronic behavior study, zebrafish were grouped into different treatment groups, including a non-stressed untreated group, stressed untreated group, a positive control group treated with fluoxetine and MOLE treatments group. MOLE was administered at three different concentrations (500 mg/L, 1000 mg/L, and 2000 mg/L) in the stressed treatment groups. The anxiolytic effects were evaluated using the novel tank test (NTT) and light-dark test (LDT). After the behaviour study, RNA was extracted from the fishes' brains for gene expression analysis using a custom RT² Profiler PCR array. 33 compounds were profiled after LCMS-QTOF analysis. LC50 values of MOLE, 1231 mg/L, were deemed save to be used for this research. Meanwhile, behavior study results showed that 1000 mg/L MOLE significantly reduced anxiety levels in the stress-induced zebrafish group compared to the other treatment groups. The fish also exhibited significant changes in the expression of crhb, faah2a, mao, and pah genes; which potentially affected by MOLE compounds such as quercetin and kaempferol. This study suggests that MOLE may serve as a potential new supplement for anxiety treatment, given its anxiolytic effect on the zebrafish model. However, the result of this study would need to be replicated in other animal models and human before it is marketed as treatment for anxiolytic effects.

ACKNOWLEDGEMENT

Firstly, I wish to thank Allah for allowing me to embark on my postgraduate study and for completing this long and challenging journey successfully. My gratitude and thanks to, Prof. Dato' Dr Mohd Zaki Bin Salleh, who is my supervisor and the Director of Integrative Pharmacogenomics Institute (iPROMISE), and Prof Dr. Farida Zuraina, my co-supervisor for their patience, motivation, enthusiasm and immense knowledge. Thank you for the support, patience and ideas in assisting me with this project. I would also like to express my gratitude to Prof. Dr. Teh Lay Kek and the Supervisory Committee iPROMISE. Their guidance helped me in my research and writing of this thesis. I would also like to thank the administrative and technical staff members of iPROMISE who have been kind to advise and help me in their respective roles.

Finally, this thesis is dedicated to my father, Tajudin bin Karim and to my mother, for supporting me financially and emotionally throughout the journey. This piece of victory is dedicated to both of you. Alhamdulilah.

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	X
LIST OF FIGURES	xi
LIST OF SYMBOLS	xiii
LIST OF ABBREVIATIONS	xiv
CHAPTER ONE INTRODUCTION	1
1.1 Background of Study	1
1.2 Problem statement	3
1.3 Objectives	3
1.4 Significance of Study	3
CHAPTER TWO LITERATURE REVIEW	5
2.1 Mental Health	5
2.1.1 Anxiety disorders	6
2.1.2 Potential cause of anxiety disorders	7
2.1.3 Treatment options for anxiety disorders	8
2.2 Natural remedies for anxiety disorders treatment	10

CHAPTER ONE INTRODUCTION

1.1 Background of Study

Anxiety disorder is a widespread mental health concern affecting people globally. In 2017, it was estimated that 260 million people were living with anxiety, and the number was expected to increase every year (World Health Organization, 2017). Malaysia is not an exception, as a survey conducted by the Institute for Public Health Malaysia in 2017 found that approximately 40% of Malaysians suffered from depression and anxiety disorder. Anxiety is the body's natural response to stress, and while it is a normal process for everyone, intense feelings of fear or nervousness can lead to an anxiety disorder. Anxiety disorders can include generalized anxiety disorder (GAD), phobia disorder, obsessive-compulsive disorder (OCD), separation anxiety disorder (SAD), post-traumatic stress disorder (PTSD), and panic disorder (Baldwin et al., 2014). Left untreated, anxiety disorder may lead to suicidal thoughts, highlighting the importance of timely diagnosis and treatment.

Thankfully, there are some treatment methods available for anxiety disorder, including psychopharmacological drugs and psychological therapy. Psychopharmacological drugs include antidepressants, benzodiazepines, and beta-blockers, which can help regulate mood and reduce anxiety symptoms (Bandelow et al., 2008). Psychological therapy, such as cognitive-behavioural therapy (CBT) and exposure therapy, can also help individuals manage and overcome their anxiety disorder by changing their behaviour and thought patterns (Baldwin et al., 2014; National Institute for Health and Clinical Excellence (NICE), 2011). It is worth noting that treatment plans may vary depending on the severity and types of anxiety disorder, and it is best to consult with a mental health professional for personalized treatment.

Many patients with anxiety disorder prefer anxiolytic drugs over psychological therapy due to their perceived efficiency. However, some commonly used drugs like buspirone, benzodiazepines, and pregabalin have been associated with adverse side effects such as headache, stomachache, and nausea (Davis, 2017). In addition, some of