# UNIVERSITI TEKNOLOGI MARA

# THE CYTOPROTECTIVE EFFECT OF PURE PHENOLIC COMPOUNDS ON HepG2 CELL LINES AGAINST tert-BUTYLHYDROPEROXIDEINDUCED OXIDATIVE STRESS

# **NURUL HIDAYAH BT ADNAN**

Dissertation submitted in partial fulfillment of the requirements for the Bachelor of Pharmacy (Hons.)

Faculty of Pharmacy

November 2008

## ACKNOWLEDGEMENT

In the name of Allah, the most gracious the most merciful.

First of all, I would like to begin my thanking to my supervisor, Dr. Mizaton Hazizul Hasan for being such a wonderful guidance. Without your supervision, I might not able to complete my thesis paper.

My gratitude also goes to all the members of Pharmaco-Toxicology Research lab, UiTM Malaysia in guiding and monitoring me doing the lab works. You guys had being such wonderful people and I wish you all the best in your ongoing research and future.

For all my classmates and friends, thank you so much for your cooperation and it was lucky for me for being one of the team members. I wish all of you good luck and all the best in your future undertaking.

Special thanks for my family members for their immense moral support and advice and this thesis is especially dedicated to all of you. Once again I would like to thank all the people that had involved directly or indirectly in this thesis.

Last but not least, I would like to express my gratitude for being a student of Faculty of Pharmacy of Universiti Teknologi MARA. Thank you.

# TABLE OF CONTENTS

			Page
TITI	E OF P	AGE	
	ROVAL		
ACKNOWLEDGEMENT			ii
TALBE OF CONTENTS			iii
LIST OF FIGURES			V
LIST OF ABBREVIATIONS			vi
ABS	TRACT		vii
СНА	PTER C	ONE (INTRODUCTION)	1
1.1	Backg	round of the study	1
1.2	Object	tive of the study	4
1.3	Hypot	hesis of the study	4
СНА	PTER T	TWO (LITERATURE REVIEW)	5
2.1	Oxidative stress		5
	2.1.1	<i>tert</i> -butylhydroperoxide (t-BOOH) as an oxidative stress -induced agent	6
2.2	Antiox	sidant enzymes (catalase and superoxide dismutase)	8
2.3	Phenolic compounds		9
		Catechin	12
	2.3.2	Ouercetin	13
	2.3.3	Resveratrol	14
СНА	PTER T	THREE (MATERIALS AND METHODS)	17
3.1	Materials		17
3.2	Instrur	nents	17
3.3	Methods		18
	3.3.1	Plating cells	18
	3.3.2	Cell treatment	21
		3.3.2(a) Treatment with pure phenolic compounds	23
		3.3.2(b) Treatment with t-BOOH	24
	3.3.3	and superconduction	25
		3.3.3(a) Determination of catalase activity	25
		3.3.3(b) Determination of superoxide dismutase activity	26
	3.3.4	Statistical analysis	27

# **ABSTRACT**

Reactive oxygen species (ROS) such as tert-butylhydroperoxide (t-BOOH) is a product of normal cellular metabolism and are recognized to be harmful or beneficial to living systems. Antioxidants such as superoxide dismutase (SOD) and catalase efficiently remove ROS surplus to maintain the normal cell homeostasis. When the exposure to oxidative stress becomes extended, antioxidant enzymes naturally occurring in the cells might not be able to protect the cells anymore. There are many studies regarding phenolic compounds nowadays. This is may be due to low cost in discovery and developing stage or to find an alternative to agents used in medication therapy yet producing many side effects. Cytoprotective effects of three different phenolic compounds which are resveratrol, catechin and quercetin had been studied and the outcome obtained showed that each of them affected catalase activities in the cells. HepG2 cell lines were plated and treated by different concentrations (1, 10, 100 and 1000 μM) of pure phenolic compounds to provide the protection before the cells were exposed to 200 µM of t-BOOH. This study showed that 1000 µM of resveratrol, catechin and quercetin significantly increased catalase activities as compared to treat with 200 µM t-BOOH, without phenolic compounds treatment. Resveratrol, catechin and quercetin at different concentration (1-1000 µM) showed some tendency to increase SOD activity but the differences as compared to 0 µM was not significant. In conclusion, resveratrol, catechin and quercetin showed protection against t-BOOH (200 μM) in HepG2 cell lines.

# **CHAPTER 1**

### INTRODUCTION

# 1.1 Background of study

Free radical reactions, especially with participation of oxidative radicals, have been shown to be involved in many biological processes that cause damage to lipids, proteins, membranes and nucleic acids, thus giving rise to a variety of diseases. Reactive oxygen species (ROS) have been recognized as playing an important role in the initiation and/or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular disease. Damage mediated by free radicals results in the disruption of membrane fluidity, protein denaturation, lipid peroxidation, oxidative DNA and alteration of platelet functions. The organic hydroperoxide, *tert*-butylhydroperoxide (t-BOOH), causes oxidative damage in a number of cell types. This compound of interest in this study is widely used in industrial field and research-based institutes. Apprehensive exposure to this kind of oxidative stress-induced agent may give rise to undesired effects. Antioxidant enzymes which are naturally present in cells help in protecting cells from harm induced by free radical reaction. These enzymes may no longer be able to provide cytoprotectivity to the cells when the exposure to oxidative stress is too high and persistent.