UNIVERSITI PUTRA MALAYSIA

IN VIVO AND IN VITRO STUDIES OF ANTI-CHOLESTEROL AND ANTI-CARCINOGENIC EFFECTS OF GANODERMA CRUD EXTRACT

CHOONG YEW KEONG

FSAS 2003 47

IN VIVO AND IN VITRO STUDIES OF ANTI-CHOLESTEROL AND ANTI-CARCINOGENIC EFFECTS OF *GANODERMA* CRUDE EXTRACT

By

CHOONG YEW KEONG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

April 2003

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

IN VIVO AND IN VITRO STUDIES OF ANTI-CHOLESTEROL AND ANTI-CARCINOGENIC EFFECTS OF *GANODERMA* CRUDE EXTRACT

By

CHOONG YEW KEONG

April 2003

Chairman: Associate Professor Dr. Tong Chow Chin

Faculty: Science and Environmental Studies

The *in vivo* and *in vitro* effect of a local, commercially available *Ganoderma* fruiting body powder (GF) and *G. lucidum* mycelium (GM) grown in soy waste on tumour and hypercholesterolaemic rats were studied.

Administration of 1% cholesterol diet in the cholesterol (Chol) group caused a significant ($p<0.05$) increase in the serum total cholesterol (TC), triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) levels while reducing serum high density lipoprotein cholesterol (HDL-C) level. In the case of the Chol+GF and Chol+GM groups, the initial serum TC, TG and LDL-C levels showed a much higher levels ($p<0.05$) compared to the Chol group. However, the levels gradually decreased towards the end of the experiment. There was no significant difference in the lipid profiles amongst the Control, GF and GM groups. Serum alanine transferase (ALT), gamma glutamyltransferase (GGT) and creatine kinase (CK) in the Chol+GF group as well as the ALT, GGT level in the

Chol+GM group were found to be significantly ($p<0.05$) lower than those in the Chol group for both the experiments using GF and GM. Despite the fact that all groups showed levels of serum urea and creatinine within the normal range, mild degenerative changes in the glomeruli were seen in the Chol group. In addition, higher level of serum uric acid was observed in the Chol group compared to the Chol+GF and Chol+GM groups. In term of the lipid peroxidation and antioxidant enzymes analyses, the Chol+GF and Chol+GM groups showed a significantly ($p<0.05$) lower level of malondialdehyde (MDA), catalase and glutathione peroxidase (GSH-Px) activities but higher vitamin C level compared to the Chol group.

G. lucidum crude extract exhibited the highest cytotoxic activity (lower IC_{50}) towards J558 Balb/C mouse myeloma, MDA-MB-435 human breast ductal carcinoma, PN6 leukemia T-cell but not against 3T3 mouse fibroblast normal cell-line as compared to crude extract from *G. tsugae* and *G. tropicum*. Among these cancer cell-lines, J558 cells was the most sensitive to the cytotoxic effects of all the three *Ganoderma* spp. Examination by acridine-orange/propidium iodine staining, electron microscopy and transmission electron microscopy showed that the *Ganoderma* crude extract caused both apoptosis and necrosis in the cancer cell-line.

The anti-carcinogenesis test indicated that hypercholesterolaemic rats (the Chol group) demonstrated a significantly ($p<0.05$) higher serum MDA level as compared to the GF and Chol+GF groups. Furthermore, *Ganoderma* supplemented groups had significantly ($p<0.05$) lower levels of serum MDA, catalase, GSH-Px and GGT activities but higher in

the ascorbic acid level as compared to the Chol group. Histologically, the Chol+GF group showed a much reduced thickened coronary vessel wall as well as normal hair growth in the anti-carcinogenesis test. The Chol+GM group registered 61.9% normal hepatocytes as compared to only 5.6% in the Chol group. The presence of epithelisation of lung epithelium in the Chol group which were not severe in the Chol+GM group indicated the anti-tumour effect of 10% GM.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KESAN KAJIAN ANTI-KOLESTEROL DAN ANTI-KARCINOGENIK BAGI EKSTRAK MENTAH *GANODERMA* SECARA IN VIVO DAN IN VITRO

Oleh

CHOONG YEW KEONG

April 2003

Pengerusi: Profesor Madya Dr. Tong Chow Chin

Fakulti : Sains dan Pengajian Alam Sekitar

Projek ini mengkaji kesan komersial serbuk *Ganoderma* (GF) dan micelia *Ganoderma* (GM) yang tumbuh pada ekstrak kacang soya ke atas barah dan tikus hiperkloresterolemik secara *in vivo* dan *in vitro*.

Penggunaan makanan kolesterol 1% bagi kumpulan kolesterol (Chol) menyebabkan signifikan peningkatan paras serum kolesterol keseluruhan (TC), trigliserid (TG), kolesterol lipoprotin ketumpatan rendah (LDL-C) dan seterusnya menurunkan paras serum kolesterol lipoprotin ketumpatan tinggi (HDL-C). Dalam hal kumpulan Chol+GF dan Chol+GM, paras serum TC, TG dan LDL-C lebih tinggi daripada kumpulan Chol pada mulanya, tetapi parasnya beransur-ansur menurun pada akhir eksperimen. Tidak terdapat perbezaan yang signifikan bagi paras profil lipid antara kumpulan kawalan, GF dan GM. Paras serum alanin transferase (ALT), gama glutamil transferase (GGT) dan

kinase (CK) dalam kumpulan Chol+GF dan paras ALT, GGT dalam kumpulan Chol+GM didapati signifikan ($p<0.05$) rendah daripada kumpulan Chol dalam eksperimen yang menggunakan GF dan GM. Walaupun semua kumpulan menunjukkan paras urea dan creatinine dalam lingkungan yang normal, terdapat juga sedikit degenerasi pada glomeruli dalam kumpulan Chol setelah diperhatikan melalui kajian histologi. Paras serum asid urik yang tinggi dalam kumpluan Chol berbanding dengan kumpulan Chol+GF dan Chol+GM. Dalam analysis peroksidasi lipid dan enzim antioksidan, kumpulan Chol+GF dan Chol+GM menunjukkan signifikan ($p<0.05$) paras malondeldehid (MDA), aktiviti katalase dan glutation peroksidase (GSH-Px) yang rendah, tetapi paras vitamin C yang lebih tinggi jika dibandingkan dengan kumpulan Chol.

Ekstrak mentah *G. lucidum* menunjukkan aktiviti sitotoksik yang paling berkesan (IC_{50} yang terendah) terhadap J558 "myeloma" tikus Balb/C, MDA-MB-435 duktul kanser buah dada manusia, PN6 sel-T leukimia tetapi tidak berkesan terhadap 3T3 sel normal fibroblast berbanding dengan ekstrak mentah *G. tsugae* dan *G. tropicum*. Bagi ketiga-tiga sel kultur kanser, sel J558 pula yang paling sensitif terhadap kesan sitotoksik. Kajian dengan menggunakan perwarna flurasen AOPI dan pemeriksaan dibawah mikroskop elektron "scanning" dan mikroskop elektron "transmission" menunjukkan bahawa ekstrak mentah *G. lucidum* dapat mengakibatkan apoptosis dan neukrosis pada sel kultur kanser.

Kajian anti-kasinogenik yang dijalankan menunjukkan bahawa tikus hiperkolesterolemik

(kumpulan Chol) menghadapi secara signifikan ($p<0.05$) paras serum MDA yang tinggi dibandingkan dengan kumpulan GF, GM, Chol+GF dan Chol+GM. Seterusnya, kumpulan yang dibekalkan *Ganoderma* mengandungi paras serum MDA, katalase, GSH-Px dan aktiviti GGT yang rendah secara signifikan dan kandungan serum vitamin C yang tinggi dibandingkan dengan kumpulan Chol. Dalam kajian histologi, kumpulan Chol+GF menunjukkan dinding arteri koronari yang kurang tebal dan pertumbuhan bulu yang normal dalam kajian anti-karsinogenik. Kumpulan Chol+GM mendedahkan 61.9% sel hepar yang normal berbanding cuma 5.6% dalam kumpulan Chol. Kejadian epitelisasi dalam epitelium peparu pada kumpulan Chol adalah tidak seteruknya dalam kumpulan Chol+GM membuktikan kesan anti-barah 10% GM.

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation and unqualified gratitude to the chairman of my supervisory committee Associate Professor Dr. Tong Chow Chin of the Department of Biochemistry and Microbiology, Universiti Putra Malaysia for his understanding, patience, invaluable guidance, suggestions and constant encouragement throughout the planning and execution of the project.

My grateful thanks also to Associate Professor Dr. Noordin Mohamed Mustapha of Faculty of Veterinary Medicine, Universiti Putra Malaysia for conscientiously serving as member of my supervisory committee. The support, suggestions and insightful comments given by other members of my supervisory committee Professor Dr. Suhaila Mohamed of the Department of Food Science, Faculty of Food Science and Biotechnology is gatefully acknowledged. My heartfelt appreciation also goes to Dr. Nor Aini Umar of Department of Chemistry Pathology, Hospital Universiti Kebangsaan Malaysia, UKM for her generous advice on the clinical chemistry studies.

My thanks and appreciation are also due to the Head of Department Professor Datin Dr. Kathijah Mohd Yusoff, Department of Biochemistry and Microbiology and Professor Dr. Abdul Manaf bin Ali, Department of Biotechnology, Faculty of Food Science and Biotechnology for their kindness in allowing me to pursue pertinent works in their laboratories.

My sincere gratitude is also extended to Institute Medicinal Research Center, Unit

Pemakanan for providing the materials and methods in vitamin C analysis. I would like to thank Miss Yang, Miss Khor for their generous advice on this experiment.

I also wish to thank all the staffs of the Department of Biochemistry and Microbiology, Faculty of Science and Environmental for their kind assistance and co-operation, special thanks are also extended to laboratory assistant Kak Rohaida and Encik Karim for their support, advice and delightful friendship.

Many thanks are also due to the following people. Mr Zhang, Miss Irine for their assistance in animal experimental work and antioxidant analysis; Antony, Lim Yen Moi and Boon Keat for their technical assistance in the cell-culture work and Mr. Siew Kok Phang for his unfailing support and commitment in this thesis necessitated. The assistance and support rendered by the staffs of the Hospital Universiti Kebangsaan Malaysia are duly acknowledged and appreciated.

Last but not least, I am greatly indebted to my beloved wife, son, daughter and my mother for their loves, patience, supports and sacrifices they have given.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAT	Alanine aminotransferase
anti-SRBC	Anti-Sheep Red Blood Cell
AST	Aspartate aminotransferase
ATCC	American Type Culture Collection
Ca	Calsium
CCl_4	Carbon tetrachloride
Chol	Cholesterol
CLL	Chronic leukemia
CTL	Cytotoxic T lymphocytes
cm	Centrimetre
dL	densilitre
DMEM	Dulbecco's modification of Eagle's medium
DMSO	Dimethyl sulfoxide
EDTA	Ethylene diamine tetraacetic acid
ELISA	Enzyme linked immunosorbent assay
EMEM	Eagle's minimum essential medium
FCS	Fetal Calf Serum
HBSS	Hank's Balanced Salt Solution
g	gram
grp	group
GF	*Ganoderma* fruiting body

GGT	Gamma glutamyl transferase
GM	*Ganoderma* mycelium
GP	*Ganoderma* polisaccharide
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase
GSSG	Oxidative glutathione
h	hour
H&E	Haematoxylin and eosin staining
hGSTP1-1	Human GST of class p subunit type 1
H_2O_2	Hydrogen peroxide
IC_{50}	Inhibition concentration of 50%
IEL	Internal Elastic Lamina
KCl	Potassium chloride
Kda	kilodalton
kg	kilograms
L	litre
LD	Lactate dehydrogenase
M	Molar
MDA	Malondialdehyde
ml	mililitre
mg	Milligrams
min	minute
mmol	Millimolar