



UNIVERSITI PUTRA MALAYSIA

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

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

By

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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

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Projek ini mengkaji kesan komersial serbuk *Ganoderma* (GF) dan micelia *Ganoderma* (GM) yang tumbuh pada ekstrak kacang soya ke atas barah dan tikus hiperklosterolemik 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 lipoprotein ketumpatan rendah (LDL-C) dan seterusnya menurunkan paras serum kolesterol lipoprotein 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 kumpulan 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 malondelhid (MDA), aktiviti katalase dan glutathion 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 nekrosis 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.

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TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xviii
LIST OF FIGURES	xix
LIST OF ABBREVIATIONS	xxiii
 CHAPTER	
1 GENERAL INTRODUCTION	1
 2 LITERATURE REVIEW	 6
2.1 Mycology of <i>G. lucidum</i>	6
2.2 Bioactive Substances of <i>G. lucidum</i>	8
2.2.1 <i>Ganoderma</i> Polysaccharides.....	8
2.2.2 Triterpenes.....	10
2.3 Biomedical Applications.....	11
2.3.1 Immunostimulation with <i>Ganoderma</i> Polysaccharides.....	12
2.3.2 <i>Ganoderma</i> as a Cardiotonic.....	13
2.3.3 Other Medicinal Efficacies of <i>Ganoderma</i> spp.....	15
2.4 Concept and Mechanisms of Bioactivity.....	17
2.5 Cholesterol Metabolism.....	19
2.6 Management of Plasma Cholesterol Levels.....	19
2.6.1 High Serum Cholesterol and Low Density Lipoprotein.....	20
2.6.2 Hypercholesterolaemia.....	22
2.6.3 Oxidized LDL and Atherosclerosis.....	22
2.6.4 Theories of Atherosclerotic Plaque Development.	25
2.6.5 Arteriosclerosis.....	26
2.7 Liver Function.....	27
2.7.1 Fatty Liver.....	27
2.8 Enzyme Tests in Liver Disease.....	28
2.8.1 Alanin Aminotransferase (ALT).....	28
2.8.2 Gamma Glutamyltransferase (GGT).....	29
2.9 Renal Function.....	31
2.9.1 Nonprotein Nitrogenous Metabolite Elimination...	32
2.10 Creatine Kinase (CK).....	33
2.11 Reduction of Hydrogen Peroxide.....	35
2.11.1 Antioxidants.....	36

2.11.2	Enzymes that Catalyze Antioxidant Reactions....	36
2.11.3	Antioxidant Chemicals.....	38
2.11.4	Malondialdehyde (MDA).....	39
2.11.5	Glutathione S-Transferase (EC 2.5.1.18).....	39
2.12	Skin Cancer.....	40
2.13	Benzo[a]pyrene (B[a]p).....	42
2.14	Lung Cancer.....	44
2.15	Antineoplastic Agents.....	45
2.15.1	Natural Product.....	46
2.16	Animal Cell Cultures.....	47
2.16.1	Apoptosis and Cellular Senescence.....	47
2.16.2	Necrosis.....	48
3	GENERAL MATERIALS AND METHODS.....	51
3.1	Materials.....	51
3.2	Methods.....	54
3.2.1	Procedure of Blood Sampling and Processing....	54
3.2.2	Cobas Intrega Multipurpose Auto-analyser Machine.....	55
3.2.3	Measurement of Serum Malondialdehyde (MDA) Concentration.....	56
3.2.4	Measurement of Erythrocytes Catalase activity...	57
3.2.5	Measurement of Whole Blood Glutathione Peroxidase (GSH-Px) Activity.....	58
3.2.6	Determination Vitamin C.....	59
4	THE ANTI-CHOLESTEROL EFFECTS OF COMMERCIAL GANODERMA FRUITING BODY POWDER (GF) AND SOY WASTE GROWN <i>G. LUCIDUM</i> MYCELIUM(GM) ON HYPERCHOLESTEROLAEMIC RATS	62
4.1	Introduction.....	62
4.2	Materials and Methods.....	66
4.2.1	Commercial <i>Ganoderma</i> Fruiting Body Powder (GF).....	66
4.2.2	<i>G. lucidum</i> Mycelium Grown in Soy Waste.....	68
4.2.3	Serum Lipid Profile, Enzyme Antioxidant, MDA and Vitamin C.....	73
4.2.4	Statistical Analysis.....	74
4.3	Results and Discussion.....	74
4.3.1	The Effect of Feeding 0.1% GF Powder and 10% GM on the Body Weight of Rats.....	75
4.3.2	The Effect of Feeding 0.1% GF Powder and 10% GM on the Serum Total Cholesterol (TC) in Rats	77
4.3.3	The Effect of Feeding 0.1% GF and 10% GM on the Serum Triglyceride (TG) in Rats.....	80

4.3.4	The Effect of Feeding 0.1% GF and 10% GM on the Serum High Density Lipoprotein (HDL-C) in Rats.....	82
4.3.5	The Effect of Feeding 0.1% GF and 10% GM on the Serum Low Density Lipoprotein (LDL-C) in Rats.....	86
4.3.6	The Effect of Feeding 0.1% GF and 10% GM on the Serum Alanine Aminotransferase (ALT) Level in Rats.....	90
4.3.7	The Effect of Feeding 0.1% GF and 10% GM on the Serum Gamma Glutamyltransferase (GGT) level in Rats.....	93
4.3.8	The Effect of Feeding 0.1% GF and 10% GM on the Serum Urea Level in Rats.....	96
4.3.9	The Effect of Feeding 0.1% GF and 10% GM on the Serum Creatinine Level in Rats.....	100
4.3.10	The Effect of Feeding 0.1% GF and 10% GM on the Serum Uric Acid Level in Rats.....	104
4.3.11	The Effect of Feeding 0.1% GF and 10% GM on the Serum Creatine Kinase (CK) Level in Rats...	106
4.3.12	The Effect of Feeding 0.1% GF and 10% GM on the Serum Malondialdehyde (MDA) Level in Rats.....	109
4.3.13	The Effect of Feeding 0.1% GF and 10% GM on the Erythrocyte Catalase Activity in Rats.....	111
4.3.14	The Effect of Feeding 0.1% GF and 10% GM on the Whole blood Glutathione Peroxidase (GSH-Px) Activity in Rats.....	113
4.3.15	The Effect of Feeding 0.1% GF and 10% GM on the Serum Vitamin C Content of Rats.....	116
4.3.16	Morphology of Heart and Liver	118
4.4	Conclusion.....	124

5	A COMPARATIVE STUDY OF CYTOTOXIC ACTIVITY WITH COMMERCIAL <i>GANODERMA</i> FRUITING BODY POWDER AND THREE <i>GANODERMA SPP.</i> MYCELIUM WATER SOLUBLE CRUDE EXTRACTS ON MOUSE MYELOMA, LEUKAEMIA AND HUMAN BREAST CANCER CELL-LINE.....	126
5.1	Introduction.....	126
5.2	Materials and Methods.....	128
5.2.1	Cell-Culture.....	128
5.2.2	<i>Ganoderma</i> Crude Extract.....	131
5.2.3	Microculture Tetrazolium Assay (MTT).....	134
5.2.4	Scanning Electron Microscopy (SEM).....	135
5.2.5	Transmission Electron Microscopy (TEM).....	136

5.2.6	Acridine Orange (AO) and Propidium Iodide (PI) Staining.....	137
5.2.7	DNA Fragmentation Assay.....	137
5.3	Results and Discussion.....	138
5.3.1	MTT Assay.....	138
5.3.2	Morphological Assessment of Apoptosis.....	145
5.4	General Discussion and Conclusion.....	167
6	THE EFFECTS OF COMMERCIAL <i>GANODERMA</i> FRUITING BODY CRUDE EXTRACT ON BENZO[A]PYRENE IN HYPERCHOLESTEROLAEMIC RAT SKIN FOLLOWING TOPICAL APPLICATION.....	171
6.1	Introduction.....	171
6.2	Materials and Methods.....	173
6.2.1	Animal.....	173
6.2.2	Blood Samples Collecting.....	174
6.2.3	Serum Chemistry	174
6.2.4	Clinical Observation.....	175
6.2.5	Histological Staining Method.....	175
6.2.6	Hepatocytes Counting.....	177
6.3	Results and Discussion.....	177
6.3.1	Body Weight.....	177
6.3.2	Serum Lipid and Lipoprotein.....	178
6.3.3	Serum Gamma Glutamyltransferase (GGT) and Alanine Aminotransferase (ALT) level.....	181
6.3.4	Urea and Creatinine.....	183
6.3.5	Uric Acid.....	184
6.3.6	Malondialdehyde	186
6.3.7	Catalase.....	187
6.3.8	Glutathione peroxidase (GSH-Px).....	188
6.3.9	Vitamin C.....	190
6.3.10	Glutathione S-Transferase Activity (GST).....	191
6.3.11	Pathology.....	193
6.3.11	Histological Examination.....	195
6.4	Conclusion.....	197
7	EFFECT OF <i>G. LUCIDUM</i> MYCELIUM (GM) ON BENZO[A]PYRENE-INDUCED EARLY ALTERATIONS OF RESPIRATORY EPITHELIUM THE HYPERCHOLESTEROLAEMIC RATS.....	198
7.1	Introduction.....	198
7.2	Materials and Methods.....	200
7.2.1	Animals.....	200
7.2.2	Inoculum.....	201
7.2.3	Experimental Design.....	201
7.2.4	Intratracheal Administration.....	201

7.2.5	Excision of Lung and Histological Staining.....	202
7.2.6	Determination of Marker Enzyme Activities.....	202
7.2.7	Determination of Lipid Peroxidation and Antioxidant Enzyme.....	203
7.2.8	Glutathione S-Transferase (GST) Estimation in Lung Tissue.....	203
7.3	Results and Discussion.....	204
7.3.1	Body and Lung Weight.....	204
7.3.2	Serum Chemistry.....	205
7.3.3	Serum Marker Enzymes.....	207
7.3.4	Antioxidant and Peroxidation Status.....	208
7.3.5	Clinical Signs.....	217
7.3.6	Pathology.....	218
7.4	Conclusion.....	224
8	GENERAL DISCUSSION.....	225
	REFERENCES.....	228
	APPENDICES.....	262
	BIOGRAPHICAL SKETCH.....	267

LIST OF TABLES

Table		Page
2.1	Biomedical applications of <i>G. lucidum</i>	11
4.1	The contents (kg) of the experimental diets.....	67
4.2	The formulation for the cholesterol and <i>Ganoderma</i> mycelium diet	72
4.3	Ratio of serum TG/HDL of rats fed with (A) 0.1% GF powder and (B) 10% GM.....	85
4.4	Ratio of serum LDL/HDL of rats fed with (A) 0.1% GF powder and (B) 10% GM.....	90
4.5	Vitamin C content of different samples of feed.....	118
4.6	The effects of different treatments on the body weight and liver-to-body weight ratio. (A) 0.1% GF powder (B) 10% GM.....	120
4.7	Percentage of normal hepatocytes after 13 months of treatment in different groups.....	122
5.1	The 50% inhibition concentration (IC ₅₀) of various <i>Ganoderma</i> extracts against 4 types of cells determined by using MTT assay (µg/ml).....	139
5.2	Percentage of viable, apoptotic and necrotic cells of J558 and MDA-MB-435 at IC ₅₀ after treated with commercial GF powder hot water crude extract.....	152
6.1	Gross findings of liver and skin in rats and number of rats alive at the end.....	194
7.1	An outline of the anti-lung tumour (lung) experimental design.....	201
7.2	The effects of the different treatments on body weight and lung-to-body weight ratio.....	205
7.3	Serum lipid profiles, urea, creatinine and uric acid level.....	206
7.4	Serum ALT and GGT level in rats.....	207
7.5	The activity of GST in lung lavage and liver of rats at necropsy (U/mol)...	215

LIST OF FIGURES

Figure		Page
2.1	Morphology of <i>G. lucidum</i>	7
2.2	Schematic of a hypothetical sequence in which lipoprotein oxidation causes atherosclerosis.....	23
2.3	The detoxification and metabolic activation of benz[a]pyrene.....	40
2.4	Schematic diagram of morphological characteristics of necrosis and apoptosis.....	50
3.1	Schematic diagram depicting blood sample processing and analyses.....	55
3.2	Machine Cobas Interga multipurpose auto-analyser from Hospital Universiti Kebangsaan Malaysia.....	56
4.1	<i>G. lucidum</i> mycelium grown in cooked wheat grain after 15 days at 25°C.....	69
4.2	<i>G. lucidum</i> mycelium grown in soy waste after 2½ months at 25°C.....	71
4.3	Body weight of rats fed with (A) 0.1% GF powder and (B) 10% GM.....	76
4.4	Serum Total Cholesterol (TC) of rats fed with (A) 0.1% GF powder and (B) 10% GM.....	79
4.5	Serum triglycerides (TG) of rats fed with (A) 0.1% GF powder and (B) 10% GM.....	81
4.6	Serum high-density lipoprotein cholesterol (HDL-C) of rat fed with (A) 0.1% GF powder and (B) 10% GM.....	83
4.7	Serum low density lipoprotein cholesterol (LDL-C) of rats fed with (A) 0.1% GF powder and (B) 10% GM.....	87
4.8	Serum alanin aminotransferase (ALT) level of rats fed with (A) 0.1% GF powder and (B) 10% GM.....	92
4.9	Serum gamma glutamyltransferase (GGT) level of rats fed with (A) 0.1% GF powder and (B) 10% GM.....	94
4.10	Serum urea level of rat fed with (A) 0.1% GF powder and (B) 10% GM.....	97

4.11	Serum creatinine level of rats fed with (A) 0.1% GF powder and (B) 10% GM.....	101
4.12	Photomicrographs showing the glomerular and tubules in kidney of rats fed with GF powder.....	104
4.13	Serum uric acid level of rats fed with (A) 0.1% GF powder and (B) 10% GM.....	105
4.14	Serum creatine kinase (CK) level of rats fed with commercial GP.....	107
4.15	Photomicrographs showing different degree of thickening blood vessel in heart from different groups.....	108
4.16	Serum Malondialdehyde (MDA) level of rats fed (A) 0.1% GF powder and (B) 10% GM.....	110
4.17	Erythrocyte catalase activity of rats fed (A) 0.1% GF powder and (B) 10% GM.....	112
4.18	Whole blood glutathione peroxidase (GSH-Px) of rats fed with (A) 0.1% GF powder and (B) 10% GM.....	114
4.19	Serum vitamin C (ascorbic acid) of rats fed with (A) 0.1% GF powder and (B) 10% GM.....	116
4.20	Photographic showing the heart with lung of rat fed with GM in different groups.....	119
4.21	Photomicrograph showing liver of rat from different groups.....	123
5.1	<i>G. lucidum</i> mycelium mat grown in soy waste milk for 20 days.....	132
5.2	The cytotoxic effect of various <i>Ganoderma</i> extracts against J558.....	141
5.3	The cytotoxic effect of various <i>Ganoderma</i> extracts against MDA-MB-435.....	142
5.4	The cytotoxic effect of various <i>Ganoderma</i> extracts against PN6.....	143
5.5	Photomicrographs showing phase-contrast microscopy examination of J558 with commercial <i>Ganoderma</i> powder hot water crude extract after 72 hours.....	147

5.6	Photomicrographs showing phase-contrast microscopy examination of MDA-MB-435 with commercial <i>Ganoderma</i> powder hot water crude extract after 72 hours.....	147
5.7	Photomicrographs showing phase-contrast microscopy examination of PN6 with commercial <i>Ganoderma</i> powder hot water crude extract after 72 hours.....	148
5.8	Photomicrographs showing phase-contrast microscopy examination of 3T3 with commercial <i>Ganoderma</i> powder hot water crude extract after 72 hours.....	148
5.9	Photomicrographs showing fluorescence microscopy examination of J558 cells.....	150
5.10	Photomicrographs showing fluorescence microscopy examination of MDA-MB-435 cells.....	151
5.11	Agarose-gel-electrophoretic patterns of DNA isolated from J558 cells after incubation at 37°C for 72 hours with or without commercial <i>Ganoderma</i> powder hot water crude extract.....	154
5.12	Agarose-gel-electrophoretic patterns of DNA isolated from MDA-MB-435 cells after incubation at 37°C for 72 hours with or without commercial <i>Ganoderma</i> powder hot water crude extract.....	155
5.13	Electronmicrographs (SEM) of J558 cells treated with commercial <i>Ganoderma</i> powder hot water crude extract at IC ₅₀ for 72 hours.....	157
5.14	Electronmicrographs (SEM) of PN6 cells treated with commercial <i>Ganoderma</i> powder hot water crude extract at IC ₅₀ for 72 hours.....	158
5.15	Electronmicrographs (SEM) of adherent MDA-MB-435 cells treated with commercial <i>Ganoderma</i> powder hot water crude extract at IC ₅₀ for 72 hours.....	159
5.16	Electronmicrographs (SEM) of MDA-MB-435 cells treated with commercial <i>Ganoderma</i> powder hot water crude extract at IC ₅₀ for 72 hours.....	160
5.17	Electronmicrographs (TEM) of J558 cells treated with commercial <i>Ganoderma</i> powder hot water crude extract at IC ₅₀ for 72 hours.....	163
5.18	Electronmicrographs (TEM) of MDA-MB-435 cells treated with commercial <i>Ganoderma</i> powder hot water crude extract at IC ₅₀ for 72 hours.....	165

6.1	Rat in preparation for topical application of B[a]p.....	174
6.2	Body weight of rats fed with 0.1% commercial GF.....	178
6.3	Serum lipid profile in rat.....	179
6.4	Serum (A) gamma glutamyltransferase (GGT) and (B) alanine aminotransferase (ALT) level in rats.....	182
6.5	Serum (A) urea and (B) creatinine level in rats.....	184
6.6	Serum uric acid level in rats.....	185
6.7	Serum malondialdehyde (MDA) concentration in rats.....	186
6.8	Erythrocyte catalase activity in rats.....	187
6.9	Whole blood glutathione peroxidase (GSH- Px) activity in rats.....	189
6.10	Serum vitamin C content in rats.....	191
6.11	Glutathione S-Transferase activity of rats from (A) Liver (B) Lung.....	192
6.12	Photomicrographs showing cross section of skin from different groups after six months.....	196
7.1	Serum malondialdehyde concentration in rats.....	208
7.2	Erythrocytes catalase activity in rats.....	210
7.3	Whole blood glutathione peroxidase (GSH-Px) activity in rats.....	211
7.4	Serum vitamin C content in rats.....	214
7.5	Photograph showing the lung and heart of different groups of rats that killed 90 days post instillation.....	219
7.6	Photomicrograph of lung tissue from different groups of rats killed 90 days of post instillation.....	221

LIST OF ABBREVIATIONS

AAT	Alanine aminotransferase
anti-SRBC	Anti-Sheep Red Blood Cell
AST	Aspartate aminotransferase
ATCC	American Type Culture Collection
Ca	Calcium
CCl₄	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	<i>Ganoderma</i> fruiting body

GGT	Gamma glutamyl transferase
GM	<i>Ganoderma mycelium</i>
GP	<i>Ganoderma polisaccharide</i>
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
H₂O₂	Hydrogen peroxide
IC₅₀	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