UNIVERSITI PUTRA MALAYSIA

EFFECTS OF THYMOQUINONE AND THYMOQUINONE-LOADED NANOSTRUCTURED LIPID CARRIER ON HEPATOCELLULAR CARCINOMA CELL MODELS, HEP3B AND HEPG2

AMINAH SUHAILA BINTI HARON

FPSK(M) 2018 39

EFFECTS OF THYMOQUINONE AND THYMOQUINONE-LOADED NANOSTRUCTURED LIPID CARRIER ON HEPATOCELLULAR CARCINOMA CELL MODELS, HEP3B AND HEPG2

By

AMINAH SUHAILA BINTI HARON

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

December 2017

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

EFFECTS OF THYMOQUINONE AND THYMOQUINONE-LOADED NANOSTRUCTURED LIPID CARRIER ON HEPATOCELLULAR CARCINOMA CELL MODELS, HEP3B AND HEPG2

By

AMINAH SUHAILA BINTI HARON

December 2017

Chairman : Sharifah Sakinah Binti Syed Alwi, PhD
Faculty : Medicine and Health Sciences

Hepatocellular carcinoma (HCC) is the fourth most common solid tumor. Dysregulated cell proliferation and tumorigenesis of HCC is commonly associated with chronic hepatitis B infection. Thymoquinone (TQ), a bioactive compound found in *Nigella sativa* has been shown to exhibit anti-tumor properties. However, due to some limitations of discomfort, high cost and sterility, it was synthesized into Thymoquinone-loaded nanostructured lipid carrier (TQ-NLC) (PATENT NO: PI2012001818) to improve the bioavailability and cytotoxicity of TQ. As cellular models for *in vitro* liver cancer and toxicity studies, HepG2 and Hep3B are the two most frequently used liver cancer cells. This study aims to determine the differential effects of TQ and TQ-NLC on the different genomic sequence of liver cancer (Hep3B and HepG2 cell lines) as well as to identify the molecular mechanisms underlying its anticancer activities. TQ or TQ-NLC inhibited the growth of both models of human liver cancer cells. The IC_{50} obtained for Hep3B treated TQ or TQ-NLC at 24 hours was 16.7±2.86 μM and 13.5±3.58 μM respectively. While in HepG2 treated TQ or TQ-NLC at 24 hours, the IC_{50} obtained was 47.7±0.64 μM and 24.0±0.70 μM respectively. TQ-NLC was observed to be more toxic towards Hep3B cells with IC_{50} of 9.20 ± 2.25 μM compared to TQ with IC_{50} of 11.1 ± 0.41 μM at 72 hours of treatment. Meanwhile, IC_{50} of Hepg2 treated TQ-NLC was 25.5 ± 8.40 μM compared to TQ which was 41.8 ± 6.20 μM at 72 hours of treatment. Overall treatment showed both TQ and TQ-NLC were more toxic towards liver cancer cells compared to normal 3T3 with IC_{50} >30uM ($P<0.05$). The increased effectiveness of TQ-NLC maybe related to the encapsulation of TQ that increased its efficiency and toxicity. There was significantly higher percentage of apoptotic cells in Hep3B cells treated with TQ-NLC compared to HepG2 cells. Molecular analysis demonstrated response of Hep3B may be influenced by the level of GSH and Nrf2/Keap1 expression that governed by the ROS status. In HepG2 cells, ROS levels increased with the increased of GSH and Nrf2

upon treatment with TQ or TQ-NLC. By contrast, TQ-NLC reduced the level of ROS in Hep3B cells. TQ also increased GSH level as early as 12 hours in Hep3B cells. The expression of caspase-3 and caspase-7 suggested that both TQ and TQ-NLC may induce apoptosis via intrinsic pathway in both liver cancer cell models. Thus, this study demonstrated TQ and TQ-NLC has *in vitro* anti-cancer effects in human liver cancer cells and the differential effects appear to be linked to the differential sensitivity towards ROS production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN THYMOQUINONE DAN THYMOQUINONE-LOADED BERNANOSTRUKTUR LIPID CARRIER KE ATAS MODEL SEL HEPATOCELLULAR KARSINOMA, HEP3B DAN HEPG2

Oleh

AMINAH SUHAILA BINTI HARON

Disember 2017

Pengerusi : Sharifah Sakinah Binti Syed Alwi, PhD
Fakulti : Perubatan dan Sains Kesihatan

Hepatocellular karsinoma (HCC) adalah tumor pepejal yang keempat paling biasa. Percambahan dysregulated sel dan tumorigenesis HCC adalah biasanya dikaitkan dengan jangkitan kronik hepatitis B. Thymoquinone (TQ), satu sebatian bioaktif yang ditemui dalam *Nigella sativa* telah ditunjukkan mempamerkan sifat-sifat anti tumor. Walau bagaimanapun, disebabkan oleh beberapa kekangan iaitu ketidakselesaan, kos yang tinggi dan kemandulan, ia disintesis ke dalam Thymoquinone-loaded nanostructured lipid carrier (TQ-NLC) (paten NO: PI2012001818) untuk meningkatkan bioavailabiliti dan sitotoksisiti TQ. Sebagai model sel kanser hati *in vitro* dan kajian ketoksikan, HepG2 dan Hep3B adalah kedua-dua sel kanser hati yang paling kerap digunakan. Kajian ini bertujuan untuk menentukan kesan berbeza TQ dan TQ-NLC atas jujukan penghibridan yang berbeza daripada kanser hati (Hep3B dan HepG2 sel) serta untuk mengenal pasti mekanisma molekul yang mendasari aktiviti-aktiviti anti-kanser. TQ atau TQ-NLC menghalang pertumbuhan kedua-dua model sel kanser hati manusia. IC_{50} yang diperolehi untuk Hep3B dirawat TQ atau TQ-NLC pada 24 jam adalah masing-masing 16.7±2.86 μM dan 13.5±3.58 μM. Sementara HepG2 dirawat TQ atau TQ-NLC pada 24 jam, IC_{50} yang diperolehi adalah masing-masing 47.7±0.64 μM dan 24.0±0.70 μM. TQ-NLC adalah diperhatikan lebih toksik kepada sel Hep3B dengan IC_{50} 9.20 μM ± 2.25 berbanding TQ dengan IC_{50} 11.1 ± 0.41 μM pada 72 jam selepas rawatan. Sementara itu, IC_{50} Hepg2 dirawat TQ-NLC adalah ialah 25.5 μM ± 8.40 berbanding TQ yang 41.8 ± 6.20 μM pada 72 jam selepas rawatan. Keseluruhan rawatan menunjukkan kedua-dua TQ dan TQ-NLC adalah lebih toksik kepada sel-sel kanser hati berbanding 3T3 sel biasa dengan IC_{50} > 30uM ($P<0.05$). Peningkatan keberkesanan TQ-NLC mungkin berkaitan dengan encapsulation daripada TQ yang meningkatkan kecekapan dan ketoksikan. Terdapat peratusan yang lebih tinggi daripada sel apoptosis dalam sel Hep3B yang dirawat dengan TQ-NLC berbanding dengan sel HepG2. Analisis molekul menunjukkan

tindak balas yang berbeza daripada Hep3B dipengaruhi oleh tahap GSH dan pengekspresan Nrf2/Keap1 yang dipengaruhi status ROS. Dalam sel HepG2, tahap ROS meningkat dengan peningkatan GSH dan Nrf2 dengan rawatan TQ atau TQ-NLC. Sebaliknya, TQ-NLC mengurangkan tahap ROS dalam sel Hep3B. TQ juga didapati memberi kesan peningkatan terhadap aras GSH seawal 12 jam dalam sel Hep3B. Pengekspresan caspase-3 dan caspase-7 mencadangkan bahawa kedua-dua TQ dan TQ-NLC mungkin didorong melalui laluan intrinsik dalam kedua-dua model sel kanser hati. Oleh itu, kajian ini menunjukkan TQ dan TQ-NLC mempunyai kesan anti-kanser *in vitro* dalam sel-sel kanser hati manusia dan kesan sensitiviti yang berbeza terhadap pengeluaran ROS.

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious, the Most Merciful, Praise to Allah for the blessing, courage and guidance that have been given to me in completing my Master research project.

First of all, I wish to show my deepest gratitude and appreciation to my upmost beloved supervisor, Dr Sharifah Sakinah Syed Alwi for being such a dedicated mentor and is gracious enough to guide me along the whole process in completing my research. She is the best for being there whenever I have any doubts, offering time and valuable knowledge in her area of professional practices. She gave me a lot of knowledge about cell culture and some techniques to complete the works. She also gave me emotional support. Honestly, Dr Sharifah Sakinah is a very nice lecturer and supervisor that I ever met before. Also, not forget to my respectful co-supervisor, Associate Prof. Dr Latifah Saiful Yazan for her great guidance, encouragement and constructive advises that lead to the success of this research. I would like to thank the rest of my beloved lecturer, Dr. Huzwah Khaza'ai, Dr. Abdah Md Akim, Dr Suhaili, Dr. Hasiah Hamid and Dr. Siti Farah for the support and encouragements.

A genuine appreciation to Faculty of Medicine and Health Science, Universiti Putra Malaysia for giving me the opportunity and facilities to amass the essential practical skills and the keen in fulfilling the research. Not to forget, my appreciation to all staffs of the Laboratory of Biochemistry, Cell Signaling, Pharmacology, Physiology, Immunology, Hematology, Human Anatomy, Molecular Biology, Radioisotopes and Nutrition Laboratory at this faculty for their constructive assistance while grasping the handiness laboratory tasks.

My big thanks to my fellow lab mates: Amirah, Afifah, Henna, Najwa, Aisha, Dr Zainal, Asma, and Sakirah for stimulating discussions, the sleepless nights spent working together to meet deadlines and for all tears that for the last 2 years. Also, I thank my best friends: Farizatul, Siti Zawanah, Ana Masara, Amielia, Fatin Hannani, Baizura, Nur Arina, Fatin Najwa, and Anis Syafiqah for the motivations and courage. Special thanks go to my research partners and friends, Dr. Foo Jhi Biau, Ong Yong Sze, Aida, Sarah, Hidayat, Napsiah, Yim Sim, Faizul for their support, and for sharing the moment of joy and hardship with me.

I would also like to acknowledge the financial support from Ministry of Education Malaysia for the MyBrain15 (MyMaster) Scholarship and Universiti Putra Malaysia for Graduate Research Fellowship (GRF) Scholarship for my study.

Last but not least, my heartfelt gratitude goes to my beloved husband Mr. Mohamad Yuhanizal Yahaya for the endless supports and love. Also, thanks to my lovely mother Puan Suri binti Md Noor, parents in law and family members who shower me with their unconditional love and encouragement when I need them most.

Thank very much. I truly appreciate all of the kindness that had been given to me. Hopefully Allah will grant all your wishes.

I certify that a Thesis Examination Committee has met on 6 December 2017 to conduct the final examination of Aminah Suhaila binti Haron on her thesis entitled "Effects of Thymoquinone and Thymoquinone-Loaded Nanostructured Lipid Carrier on Hepatocellular Carcinoma Cell Models, Hep3B and HepG2" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Abdah binti Md Akim, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Suhaili binti Abu Bakar @ Jamaludin, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Nor Fadilah Rajab, PhD
Associate Professor
Universiti Kebangsaan Malaysia
Malaysia
(External Examiner)

RUSLI HAJI ABDULLAH, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 30 August 2018

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Sharifah Sakinah Syed Alwi, PhD
Senior Lecturer
Faculty Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Latifah Saiful Yazan, PhD
Associate Professor
Faculty Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: _____ Date: _____

Name and Matric No.: Aminah Suhaila Binti Haron, GS41004

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Dr. Sharifah Sakinah Syed Alwi

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Latifah Saiful Yazan

TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iii
ACKNOWLEDGEMENTS		v
APPROVAL		vii
DECLARATION		ix
LIST OF TABLES		xiv
LIST OF FIGURES		xv
LIST OF APPENDICES		xvii
LIST OF ABBREVIATIONS		xviii
CHAPTER		
1	**INTRODUCTION**	1
	1.1 Background of study	1
	1.2 Problem statement	3
	1.2.1 General objective	4
	1.2.2 Specific objectives	4
	1.3 Hypothesis	4
2	**LITERATURE REVIEW**	5
	2.1 Cancer	5
	2.2 Liver cancer	6
	2.2.1 Type of primary liver cancer	7
	2.2.1.1 Hepatoblastoma	7
	2.2.1.2 Cholangiocarcinoma	7
	2.2.1.3 Angiosarcoma	7
	2.3 Hepatocellular carcinoma	7
	2.4 Hepatitis B virus and liver cancer	8
	2.4.1 Pathological in liver cancer	9
	2.5 Signaling pathways in cancer	10
	2.5.1 Apoptosis	10
	2.5.1.1 The intrinsic apoptosis pathway	11
	2.5.1.2 The extrinsic apoptosis pathway	12
	2.5.2 Caspase	12
	2.5.2.1 Caspase 3	13
	2.5.2.2 Caspase 7	14
	2.5.3 Necrosis	14
	2.5.4 Nrf2/Keap1	16
	2.6 Glutathione	19
	2.7 Treatment of hepatocellular carcinoma	20
	2.7.1 Surgical resection	20
	2.7.2 Liver transplantation	21
	2.7.3 Radiofrequency ablation (RFA)	21
	2.7.4 Transarterial chemoembolization (TACE)	21

2.7.5 Radiotherapy 21
2.7.6 Chemotherapy 22
2.8 *Nigella sativa* (Habbatus sauda) 22
2.8.1 Molecular target of Thymoquinone in Cancer 26
2.9 Nanoparticles as Drug Carrier 29
2.9.1 Fullerenes 29
2.9.2 Solid lipid nanoparticles (SLNs) 29
2.9.3 Liposomes 30
2.9.4 Nanoshells 30
2.9.5 Quantum dots (QD) 30
2.9.6 Superparamagnetic nanoparticles 31
2.9.7 Dendrimers 31
2.9.8 Nanostructured lipid carriers (NLC) 31
2.10 Thymoquinone-loaded nanostructured lipid carrier (TQ-NLC) 32
2.11 Cell Lines 33
2.11.1 Human hepatocellular carcinoma cell integrated hepatitis B virus (Hep3B) 33
2.11.2 Human hepatocellular carcinoma cell (HepG2) 33
2.11.3 Normal mouse fibroblast cell (3T3) 34

3 MATERIALS AND METHODOLOGY 35
3.1 Chemical and reagents 35
3.2 Cell lines 35
3.3 Cell Culture 35
3.3.1 Media preparation 35
3.3.2 Cell recovery and thawing 36
3.3.3 Changing media 36
3.3.4 Subculturing of cells 36
3.3.5 Cells cryopreservation 37
3.3.6 Cell counting and seeding 37
3.4 Growth inhibition assay 37
3.5 Detection of mode of cell death 38
3.6 Glutathione measurement 39
3.7 Western Blotting 39
3.7.1 Preparation of protein lysates 39
3.7.2 Polyacrylamide gel preparation 39
3.7.3 Protein loading and gel electrophoresis 40
3.7.4 Transfer of protein from gel to membrane 40
3.7.5 Blocking and incubation with antibodies 40
3.7.6 Detection 41
3.8 Statistical data analysis 41

4 RESULT 42
4.1 Cytotoxicity effect of TQ and TQ-NLC on selected liver cancer cells 42
4.2 Effect of TQ and TQ-NLC on apoptosis 43
4.3 Effect of TQ and TQ-NLC on Caspase-3 48
4.4 Effect of TQ and TQ-NLC on Caspase-7 51

4.5 Effects of TQ and TQ-NLC on glutathione. 53
4.6 Effect of TQ and TQ-NLC on Nrf2 56
4.7 Effect of TQ and TQ-NLC on Keap1 59

5 DISCUSSION 62

6 SUMMARY, CONCLUSION, AND RECOMMENDATION FOR FUTURE RESEARCH 68
6.1 Summary and conclusion 68
6.2 Recommendation for Future Research 68

REFERENCES 70
APPENDICES 91
BIODATA OF STUDENT 97

LIST OF TABLES

Table		Page
2.1	Comparison between apoptosis and necrosis	16
2.2	Molecular targets of Thymoquinone in Cancer	26
3.1	Lists of primary and secondary antibody used in western blotting	41
4.1	Cytotoxicity effects of TQ and TQ-NLC on human liver cancer cell models and normal cell line reflected by the IC_{50} values using MTT assay	43
4.2	Percentage of viable, necrotic and apoptotic cells of HepG2 as determined by flow cytometer	45
4.3	Percentage of viable, necrotic and apoptotic cells of Hep3B as determined by flow cytometer	47

LIST OF FIGURES

Figure		Page
2.1	Gene structure and life cycle of HBV. (A) An illustration of the small HBV genome with its unique overlapping gene structure. (B) Life cycle of HBV	9
2.2	The apoptosis pathway	11
2.3	Schematic overview of the apoptotic pathways via caspase activation	13
2.4	Necrosis, cell death results from irreversible injury to the cell	15
2.5	Structure of Nrf2 and Keap1	17
2.6	Schematic model of the Nrf2–Keap1 signaling pathway18	
2.7	Structure of glutathione	20
2.8	*Nigella sativa* (A) Whole plant, (B) Flower (C) Seeds	23
2.9	Chemical structures of some major compounds isolated from *Nigella sativa*	24
2.10	Targets of thymoquinone in different sites in inflammatory diseases and cancer	25
2.11	Structure of NLC	32
2.12	Image of HepG2 cells	33
2.13	Image of 3T3 cells	34
4.1	Apoptosis analysis of HepG2 cells induced by TQ or TQ-NLC as determined by FITC-Annexin V staining	44
4.2	Quantitation of apoptosis in HepG2 cells treated with NLC, TQ and TQ-NLC for indicated time points (24, 48 and 72 hours)	45
4.3	Apoptosis analysis of Hep3B cells induced by TQ or TQ-NLC as determined by FITC-Annexin V staining	46
4.4	Quantitation of apoptosis in Hep3B cells treated with NLC, TQ and TQ-NLC for indicated time points (24, 48 and 72 hours)	47
4.5	Caspase-3 expression in HepG2 cells upon treatment with TQ or TQ-NLC	49

4.6 Caspase-3 expression in Hep3B cells upon treatment with TQ or TQ-NLC 50

4.7 Caspase-7 expression in HepG2cells upon treatment with TQ or TQ-NLC 52

4.8 Caspase-7 expression in Hep3B cells upon treatment with TQ or TQ-NLC 53

4.9 GSH levels in HepG2 and Hep3B cells 55

4.10 Nrf2 expression in HepG2 cells upon treatment with TQ or TQ-NLC 57

4.11 Nrf2 expression in Hep3B cells upon treatment with TQ or TQ-NLC 58

4.12 Keap1 expression in HepG2 cells upon treatment with TQ or TQ-NLC 60

4.13 Keap1 expression in Hep3B cells upon treatment with TQ or TQ-NLC 61

LIST OF APPENDICES

Appendix		Page
A1	Cell viability percentage in 3T3 cells against TQ	91
A2	Cell viability percentage in 3T3 cells against TQ-NLC	91
A3	Cell viability percentage in HepG2 cells against TQ	92
A4	Cell viability percentage in HepG2 cells against TQ-NLC	92
A5	Cell viability percentage in Hep3B cells against TQ	93
A6	Cell viability percentage in Hep3B cells against TQ-NLC	93
B1	Percentage of viable, apoptotic and necrotic cells of 3T3	94
B2	Percentage of viable, apoptotic and necrotic cells of HepG2	94
B3	Percentage of viable, apoptotic and necrotic cells of Hep3B	95
C1	GSH standard curve	95
D1	Western blot standard curve	96

LIST OF ABBREVIATIONS

%	Percentage
<	Less than
±	More or less
AFP	Alpha fetoprotein
Akt	Protein kinase B
Annexin-V−/PI−	Percentage of viable cells
Annexin-V+/PI−	Percentage of early apoptotic cells
Apaf-1	Apoptotic Protease Activating Factor 1
APS	Ammonium Persulfate
ARE	Antioxidant response element
ASR	Age-standardised rates
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
C3, C4	Complement
CAT	Catalase
cccDNA	Covalently closed circular DNA
CHEK1	Serine/threonine-protein kinase Chk1
CO_2	Carbon Dioxide
Cul3	Cullin3
CXCR1	C-X-C motif chemokine receptor 1
CXCR2	C-X-C motif chemokine receptor 2
Cyt. c	Cytochrome c
d-ATP	Deoxyadenosine triphosphate
DMEM	Dulbecco's Modified Eagle's Medium

DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DRs	Death receptors
EDTA	Ethylenediaminetetraacetic acid
EGFR	Epidermal Growth Factor Receptor
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinase
FACS	Fluorescence Activated Cell Sorted
FAK	Focal adhesion kinase
FasL	Fas ligand
FBS	Fetal Bovine Serum
FITC	Fluorescein isothiocyanate
GCL	Glutamate cysteine ligase
GCLM	Glutamate-cysteine ligase modifier
GSH	Glutathione
GSK 3β	Glycogen synthase kinase 3β
GSSG	Oxidized glutathione
GST	Glutathione S-transferase
H_2O_2	Hydrogen Peroxide
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HBx	Hepatitis B virus X
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HFSR	Hand foot skin reaction
HO-1	Heme oxygenase

HPLC	High-performance liquid chromatography
IARC	International Agency for Research on Cancer
IC_{50}	The concentration of an inhibitor where the response (or binding) is reduced by half
IgG	Immunoglobulin G
IL-13	Interleukin 13
IL-5	Interleukin 5
IMS	Mitochondrial intermembrane space
IVR	Intervening region
Jak2	Janus kinase
JNK	c-Jun N-terminal kinase
Keap1	Kelch-like ECH-associated protein 1
LPO	Lipid peroxidation
MAPK	Mitotic-activated protein kinase
Mcl-1	Induced myeloid leukemia cell differentiation protein
MEK	Methyl Ethyl Ketone
MMP-9	Matrix metalloproteinase-9
MOMP	Mitochondrial outer membrane permeabilization
MPO	Myeloperoxidase
mRNA	Messenger RNA
MRP1	Multi-drug resistance associated protein 1
Mtor	Mechanistic target of rapamycin
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
MUC4	Mucin 4
NaCl	Sodium chloride

NADP+	Nicotinamide adenine dinucleotide (reduced form)
NASH	Nonalcoholic steatohepatitis
NF-kB	Nuclear factor-kappa B
NK	Natural killer
NKT cells	Natural killer T cells
NLC	Nanostructured lipid carrier
NO	Nitric oxide
NP	Not performed
NQO	NAD(P)H:quinone oxidoreductase
NQO1	NAD(P) H:quinone oxidoreductase 1
Nrf2	Nuclear factor erythroid 2-related factor 2
ORR	Objective response rate
OS	Overall survival
p53	Tumor protein
PARP	Poly ADP ribose polymerase
PDK1	Phosphoinositide-dependent kinase-1
PGE2	Prostaglandin E2
PI+	Percentage of necrotic cells
PI3K	Phosphoinositide 3-kinase
Plk1	Serine/threonine-protein kinase
PMSF	Phenylmethylsulfonyl fluoride
PPARγ	Peroxisome proliferator-activated receptor gamma
PS	Phosphatidylserine
PTEN	Phosphatase and tensin homolog
PVDF	Polyvinylidene Difluoride

QD	Quantum dots
RECIST	Response evaluation criteria in solid tumors
RFA	Radiofrequency ablation
RIPA	Radioimmunoprecipitation assay buffer
ROS	Reactive oxygen species
SD	Standard deviation
SDS	Sodium dodecyl sulfatepolyacrylamide
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
–SH	Sulphydryl group
SLN	Solid lipid nanostructured
SOD	Superoxide Dismutase
TACE	Transarterial chemoembolization
TBS	Tris buffer saline
TBS-T	Tris buffer saline containing 0.1% of Tween-20
TEMED	Tetramethylethylenediamine
TNF	Tumour necrosis factor
TNFα	Tumor necrosis factor alpha
TQ	Thymoquinone
TQ-NLC	Thymoquinone loaded nanostructured lipid carrier
TRAIL	TNF-related apoptosis-inducing ligand
Tris	tris(hydroxymethyl)aminomethane
TUNNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
UV	Ultraviolet
VEGF	Vascular endothelial growth factor

WHO	World Health Organization
XIAP	X-linked inhibitor of apoptosis protein
γ -GCS	γ-glutamylcysteine synthetase
µg	Microgram
µL	Microlitre

CHAPTER 1

INTRODUCTION

1.1 Background of study

Cancer is categorized as the most fatal diseases by the uncontrolled growth of abnormal cells (Pérez-Herrero & Fernández-Medarde, 2015). By generating growth factor ligands themselves, cancer has the ability to preserve signal of cell proliferation. They also can react by cognate receptors expression, which result in autocrine proliferative stimulation. On the other hand, cancer cells supply various growth factors which may generate signals to stimulate normal cells within the supporting tumor-associated stroma (Cheng et al., 2008; Bhowmick et al., 2004). Alteration or cell structures dysregulation, can lead to the primary tumor to detach and increased potential for spreading and metastatic dissemination of cancer cells to secondary site throughout the body (Jiang et al., 2015). According to World Cancer Report in the year 2014, cancers figure among the top causes of mortality and morbidity worldwide, with 8.2 million cancers related deaths and approximately 14 million new cases. The most common causes of cancer death are cancer of lung, stomach, liver, colorectal, breast and oesophageal (WHO, 2015).

Hepatocellular carcinoma (HCC) is one of the cancers that normally affecting human worldwide which is the third ranking of cancer-related death (Siegel et al., 2013). The major histological subtype among all types of primary liver cancers is HCC, about 70%– 85% of the all types of liver cancer worldwide (Perz et al., 2006). The incidence of HCC is arising annually about 75,000 cases worldwide (Jemal et al., 2011). However, the epidemiology of HCC in Asia became worse. In 2012, the age-standardised rates (ASR) of HCC in males in Eastern Asia were 31.9 per 100,000 as compared to previously 35.5 per 100,000 in 2008 (Ferlay et al., 2013; Jemal et al., 2011). The annual mortality rate per 100,000 people from liver cancer in Malaysia has been increased by 42.8% since 1990. Hepatocellular carcinoma (HCC) is a global public concern as one of the most common and deadly cancers worldwide. HCC accounts for 85%-90% of primary liver cancer and it is the third most frequent cause of cancer-related mortality. HCC continue to be the most progressive diseases worldwide (Center & Jemal, 2011). Persistent infections by HBV is the main recognized risk factor for HCC in Malaysia which about more than 50% of other causes including HCV and alcohol intake. In Malaysia, liver cancer among males is the tenth most common cancer with the incidence of 378 per 100,000 (National Cancer Registry, 2004). The better results in cancer control are yield by down-staging of cancer. Though, the diagnosis of cancer commonly delayed due to factors such as frequent reliance on unorthodox medical remedies at initial presentation (Melor & Zabedah, 1997). Shortage of adequate cancer care continues to be a problem. In Malaysia, the ratio of megavoltage machines to the population is 1.2 per million (taking into account machines in both government and private sectors) (Lim, 2002).

HCC is caused by viral infection, cirrhosis, heavy alcoholism, non-alcoholic fatty liver disease, aflatoxin, infection of hepatitis B virus (HBV) and hepatitis C virus (HCV). Almost 80% of HCC is caused by HBV and HCV which lead to about 250,000 new cases of HCC every year (Arzumanyan et al., 2013). Epidemiologically, evidence has exposed the causative role of HBV in liver cancer with 54% of all HCC cases worldwide are related with chronic HBV infection (Szmuness, 1978). HBV is a hepadnavirus transmitted by exposure to infectious blood or body fluids, leading to hepatitis B infection which can be classified as either acute or chronic infection. Chronic HBV infections mostly are asymptomatic, but liver cancer and cirrhosis could develop over time (Di Bisceglie, 2000). However, still there is no approved systemic management for patients with advanced metastatic HCC or unresectable (Cheng et al., 2009; Llovet et al., 2008). Approximately 90% of patients treated with sorafenib will demonstrate cutaneous side effects such as hand-foot skin reaction, erythematous rash, scalp dysesthesia, subungual splinter hemorrhages, skin neoplasms and precancerous lesions (Ara & Pastushenko, 2014). Standard systemic chemotherapy (doxorubicin, cisplatin, tamoxifen, leuprorelin, and flutamide) are typically not effective for HCC and still poses major challenges to the available therapeutic regimes (Davis et al., 2008). Thus, alternative therapies are now drawing attention of researchers worldwide.

Natural products are a rich source of cancer chemotheraphy drugs which have clinical potential to prevent and treat diseases of cancer (Aziz et al., 2003). *Nigella sativa*, is an annual flowering plant can be found in Mediterranean countries, Pakistan and India, commonly called as black cumin (Gali-muhtasib et al., 2006). Traditionally, oil from the black seed had been used as herbal medicine in Arab country for treatment of arthritis, lung diseases and hypercholesterolemia (Khader et al., 2009). Thymoquinone (2-Isopropyl-5-methylbenzo-1,4-quinone; TQ) is an active compound of *Nigella sativa* also extensively consumed as a condiment in Ayurvedic medicine. Previously, TQ exerts its anti-neoplastic effects through modes of action including stimulation of apoptosis, inhibition of cell proliferation, cell cycle arrest, production of reactive oxygen species (ROS) and inhibition of angiogenesis and metastasis (Banerjee et al., 2010). Interestingly, despite having anti-proliferative effect, it may also exert its pro-apopototic effect through induction of p38 and ROS signaling pathway in human breast carcinoma (Woo et al., 2013). TQ also contain anti-inflammatory and antioxidative effects (Banerjee et al., 2010). However, intraperitoneal route of TQ is restricted by high discomfort and costly and sterility issues. TQ is limited by the solubility-related poor oral bioavailability (Pathan et al., 2011) and the pure TQ is relatively less soluble in water (Khader et al., 2009).

Thus, drug carrier systems will improve the therapeutic effectiveness and offer great promise safety level of cancer chemotherapy drug. The advantages offers by nanostructured lipid carrier (NLC) as one of the drug carrier systems including ability to increase bioavailability of poorly soluble drugs, provide safe protection and encapsulation for sensitive active compounds or drugs as well as assist the drugs controlled release (How et al., 2011). Recently, NLCs have been developed as alternative colloidal drug carrier systems in the process of reduction of problems of solid lipid nanostructured (SLN). NLC is composed of solid lipid and liquid lipid

mixture and has shown an increased drug loading compared with SLN (Müller et al., 2002). In spite of the presence of liquid lipid, NLC matrix will be solidify at room/body temperature (Chen et al., 2010). The drug expulsion during storage is expected to be reduced due to the imperfect crystal lattice, the drug-loading capacity will be improved and modulation of drug release profile by changing the arrangement of lipid matrix (Müller et al., 2002a; Rad- tke et al., 2005).

In previous study, Thymoquinone loaded nanostructured lipid carrier (TQ-NLC) (PATENT NO: PI201200181) has been successfully synthesized by the high pressure homogenization method which also showed high encapsulation efficiency and drug loading capacity (Ng et al., 2015). TQ-NLC improved bioavailability and exhibited strong cytotoxicity towards cell lines of cervical and breast cancer and significantly enhanced anti-cancer effect in MDA-MB-231 that induced specific cell cycle arrest and. TQ-NLC also inhibits growth in different cancer cell lines such as HeLa, SiHa, and MCF-7 (Ng et al., 2015). However, TQ-NLC was relatively non-cytotoxic and no effect towards normal cells (3T3-L1 and Vero) (Ng et al., 2015). Based on the *in vivo* toxicity study, TQ-NLC shows less toxic as compared to free TQ because the lipid carrier that encapsulates TQ has minimize toxic effect of the compound (Ong et al., 2016).

The evidences proved that TQ-NLC has great promise as the potential anti-cancer agent against several types of cancers. However, the role of TQ-NLC in HCC integrated with hepatitis B genome has yet to be investigated. Therefore, this study is designed to determine the cytotoxicity of TQ and TQ-NLC on hepatocellular carcinoma cell lines (Hep3B and HepG2) and the underlying mechanisms that involved in the mode of cell death, the level of total GSH production as well as Nrf2/Keap1 and caspase protein expression in both cell lines.

1.2 Problem statement

Liver cancer predominantly HCC is one of the most common solid tumors. The prevalence of liver cancer and mortality rate suffered from this disease is increasing every year. Despite several approaches for liver cancer treatment, HCC continue to be the most progressive diseases worldwide (Center & Jemal 2011). Persistent infection by HBV is the main recognized risk factor for HCC in Malaysia which accounts for more than 50% of other causes including HCV and alcohol intake. Current effective therapies for HCC included chemotherapy, radiotherapy and surgical treatment (Gong and Li 2011). However, these therapies may lead to several side effects such as damage in idney and hair as well as hearing loss (Liu et al. 2018).

Thus, a great deal research has been conducted to explore natural products from plants which have the potential to inhibit cancer progression. TQ is known with its ability to exert its anticancer effects in many types of cancers (Gullet et al., 2010; Attoub et al., 2013). It has also been reported able to inhibit cancer cell proliferation via several

signaling pathways (Cyclin dependent kinases (CDKs), cyclins and inhibiting telomerase activity) (Schneider-Stock et al., 2014). Moreover, almost all cancer development resulted from the uncontrolled growth of malignancy which surpasses cell death and has a deregulation of apoptosis. Hence, for *in vitro* study in this particular cancer type, HepG2 (non-Hepatitis B genome sequence) and Hep3B (with Hepatitis B genome sequence) cell lines were chosen to represent the cellular reference model as a platform for comparisons.

Research objectives

1.2.1 General objective

To investigate the cytotoxicity effects of TQ and TQ-NLC on liver cancer cell lines, Hep3B and HepG2 as well as eludicating the molecular mechanism of action underlying its anticancer activities.

1.2.2 Specific objectives

1. To determine the cytotoxicity effect of TQ and TQ-NLC towards normal mouse fibroblast cell (3T3), and human liver cancer cell lines (HepG2 and Hep3B) in time and concentration dependent manner.
2. To explore the mechanism of cell death induced by TQ and TQ-NLC on liver cancer cell lines using Annexin V staining.
3. To elucidate the involvement of the of GSH production in liver cancer cell lines upon treatment of TQ and TQ-NLC.
4. To assess the level of protein expression of caspase-3, caspase-7, Nrf2 and Keap1 upon TQ and TQ-NLC treatment on liver cancer cell lines.

1.3 Hypothesis

TQ and TQ-NLC exhibit cytotoxic effect towards human liver cancer cells with the involvement of apoptosis and antioxidant signaling pathway.

REFERENCES

Abdel-Rahman, O. (2013). Systemic therapy for hepatocellular carcinoma (HCC): from bench to bedside. *Journal of the Egyptian National Cancer Institute,* 25(December (4)):165–71.

Abdelwahab, S. I., Sheikh, B. Y., Taha, M. M. E., How, C. W., Abdullah, R., Yagoub, U Eid, E.E.M. (2013). Thymoquinone-loaded nanostructured lipid carriers: Preparation, gastroprotection, in vitro toxicity, and pharmacokinetic properties after extravascular administration. *International Journal of Nanomedicine,* 8, 2163–2172.

Abou-Alfa, G.K., Johnson, P., & Knox, J.J. (2010). Doxorubicin plus sorafenib vs doxorubicin alone in patients with advanced hepatocellular carcinoma. *The Journal of the American Medical Association,* 304(19): 2154–60.

Abou-Alfa, G.K., Schwartz, L., Ricci, S., Amadori, D., Santoro, A., Figer, A., De Greve, J., Douillard, J.Y., Lathia, C., Schwartz, B., Taylor, I., Moscovici, M., & Saltz, L.B. (2006). Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. *Journal of Clinical Oncology,* 24(26):4293–4300.

Acharya, B.R., Chatterjee, A., Ganguli, A., Bhattacharya, S., & Chakrabarti, G. (2014). Thymoquinone inhibits microtubule polymerization by tubulin binding and causes mitotic arrest following apoptosis in A549 cells. *Biochimie.* 97:78–91.

Ahmad, A., Husain, A., Mujeeb, M., Khan, S.A., Najmi, A.K., Siddique, N.A., Damanhouri, Z.A., & Anwar, F. (2013). A review on therapeutic potential of Nigella sativa: a miracle herb. *Asian Pacific Journal of Tropical Biomedicine,* 3:337–352.

Ahrens, W. Timmer, A. Vyberg, M. Fletcher, T. Guenel, P. Merler, E. (2007). Risk factors for extrahepatic biliary tract carcinoma in men: medical conditions and lifestyle: results from a European multicentre case–control study. *European Journal of Gastroenterology & Hepatology,* 19:19623–19630.

Al-Bukhari, M.I., & Al-Bukhari, S. (1976). The collection of authentic sayings of Prophet Mohammad (peace be upon him), division 71 on medicine. Hilal Yayinlari, Ankara, Turkey.

Aleksunes, L. M., & Manautou, J. E. (2007). Emerging role of Nrf2 in protecting against hepatic and gastrointestinal disease. *Toxicologic Pathology,* 35(4): 459-473.

Alhosin, M., Abusnina, A., Achour, M., Sharif, T., Muller, C., Peluso, J. (2010). Induction of apoptosis by thymoquinone in lymphoblastic leukemia Jurkat cells is mediated by a p73-dependent pathway which targets the epigenetic integrator UHRF1. *Biochemical Pharmacology,* 79:1251–60.

Alvarez, A., Lacalle, J., & Alvarez, F. J. (2010). Cell death. A comprehensive approximation. *Necrosis,* 1017–1024.

An, M.J., Cheon, J.H., Kim, S.W., Kim, E.S., Kim, T.I., & Kim, W.H. (2009). Guggulsterone induces apoptosis in colon cancer cells and inhibits tumor growth in murine colorectal cancer xenografts. *Cancer Letters,* 279(1):93-100.

Antonsson, B., & Martinou, J.C. (2000). The Bcl-2 protein family. *Experimental Cell Research,* 256:50–7.

Acharya, B. R., Chatterjee, A., Ganguli, A., Bhattacharya, S., & Chakrabarti, G. (2014). Thymoquinone inhibits microtubule polymerization by tubulin binding and causes mitotic arrest following apoptosis in A549 cells. *Biochimie,* 97:78–91.

Arzumanyan, A., Reis, H.M.G.P.V., & Feitelson, M.A. (2013). Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nature Reviews Cancer,* 13(2):123–135.

Ashkenazi, A., & Herbst, R.S. (2008). To kill a tumor cell: the potential of proapoptotic receptor agonists. *Journal of Clinical Investigation*, 118:1979–1990.

Ashour, A. E., Abd-Allah, A. R., Korashy, H. M., Attia, S. M., Alzahrani, A. Z., Saquib, Q., & Rishi, A. K. (2014). Thymoquinone suppression of the human hepatocellular carcinoma cell growth involves inhibition of IL-8 expression, elevated levels of TRAIL receptors, oxidative stress and apoptosis. *Molecular and Cellular Biochemistry*, *389*(1-2):85–98.

ATCC (https://www.atcc.org/products/all/HB-8065.aspx). Retrieved 2th December, 2016.

Attoub, S., Sperandio, O., Raza, H., Arafat, K., Al-salam, S., Ahmed, M., & Adem, A. (2013). Thymoquinone as an anticancer agent : evidence from inhibition of cancer cells. *Fundamental & Clinical Pharmacology,* 27:557–569.

Aziz, M.H., Kumar, R., Ahmad, N. (2003). Cancer chemoprevention by resveratrol: in vitro and in vivo studies and the underlying mechanisms (review). *International Journal of Oncology,* 23:17–28.

Azmi, A.S., & Mohammad, R.M. (2009). Non-peptidic small molecule inhibitors against Bcl-2 for cancer therapy. *Journal of Cellular Physiology,* 218:13–21.

Azmi, A.S., Masood, A., & Mohammad, R.M. (2011). Small molecule inhibitors of bcl-2 family proteins for pancreatic cancer therapy. *Cancers (Basel)*, 3:1527–49.

Badr, G., Mohany, M., & Abu-Tarboush, F. (2011). Thymoquinone decreases F-actin polymerization and the proliferation of humanmultiple myeloma cells by suppressing STAT3 phosphorylation and Bcl2/Bcl-XL expression. *Lipids in Health and Disease,* 10:236.

Baig, S., Seevasant, I., Mohamad, J., Mukheem, A., Huri, H. Z., & Kamarul, T. (2016). Potential of apoptotic pathway-targeted cancer therapeutic research : Where do we stand ? *Nature Publishing Group,* 1–11.

Baird, L., & Dinkova-Kostova, A.T. (2011). The cytoprotective role of the Keap1-Nrf2 pathway, Arch. *Toxicology,* 85:241–272.

Banerjee, S., Kaseb, A.O., Wang, Z., Kong, D., & Mohammad, M. (2009). Antitumor activity of gemcitabine and oxaliplatin is augmented by thymoquinone in pancreatic cancer. *Cancer Research,* 69:5575–5583.

Banerjee, S., Padhye, S., Azmi, A., Wang, Z., Philip, P.A., & Kucuk, O. (2010). Review on molecular and therapeutic potential of thymoquinone in cancer. *Nutrition and Cancer,* 62(9):38-46.

Bertoletti, A., & Ferrari, C. (2011). Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection. *Gut,* gutjnl-2011.

Bhoori, S., Toffanin, S., & Sposito, C. (2010). Personalized molecular targeted therapy in advanced, recurrent hepatocellular carcinoma after liver transplantation: a proof of principle. *Journal of Hepatology,* 52:771.

Bhowmick, N.A., Neilson, E.G., & Moses, H.L. (2004). Stromal fibroblasts in cancer initiation and progression. *Nature,* 432:332–337.

Boatright, K. M., & Salvesen, G. S. (2003). Mechanisms of caspase activation. *Current Opinion in Cell Biology,* 15(6):725–731.

Boffetta, P., & Hashibe, M. (2006). Alcohol and cancer, *Lancet Oncol.* 7:149–156.

Bradford, M.T. (1976). Protein measurement with the follin phenol reagent. *Biochemistry,* 72:248-254.

Brechot, C. (2004). Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: old and new paradigms. *Gastroenterology,* 127:S56-S61.

Brentnall, M., Rodriguez-Menocal, L., De Guevara, R. L., Cepero, E., & Boise, L. H. (2013). Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. *BMC cell biology,* 14(1):32.

Bruix, J., & Sherman, M. (2011). Management of hepatocellular carcinoma: an update. *Hepatology,* 53:1020-2.

Bruix, J., Sala, M., & Llovet, J.M. (2004). Chemoembolization for hepatocellular carcinoma. *Gastroenterology,* 127(5 Suppl 1):S179–S188.

Bulterys, M., Goodman, M.T., Smith, M.A., Buckley, JD. (1999). Cancer Incidence and Survival among Children and Adolescents (NIH Publication No. 99-4649), National Cancer Institute, Bethesda.

Burits, M., & Bucar, F. (2000). Antioxidant activity of Nigella sativa essential oil. *Phytotherapy Research,* 14:323–8.

Buzea, C., Pacheco, I., & Robbie, K. (2007). Nanomaterials and nanoparticles: sources and toxicity. *Biointerphases,* 2:MR17– MR71.

Carr, B.I., & Nagalla, S. (2010). In Hepatocellular Carcinoma: *Diagnosis and Treatment, Brain Carr (Ed). Humana Press, New York, USA,* p. 527.

Castell, J.V., Jover, R., Martnez-Jimnez, C.P., & Gmez-Lechn, M. J. (2006). Hepatocyte cell lines: their use, scope and limitations in drug metabolism studies. *Expert opinion on drug metabolism & toxicology,* 2(2):183-212.

Cepero, E., King, A.M., Coffey, L.M., Perez, R.G., & Boise, L.H. (2005). Caspase-9 and effector caspases have sequential and distinct effects on mitochondria. *Oncogene,* 24(42):6354–6366.

Cereghetti, G.M., & Scorrano, L. (2006). The many shapes of mitochondrial death. *Oncogene,* 25:4717–24.

Chan, S.L., & Yeo, W. (2012). Targeted therapy of hepatocellular carcinoma: present and future. *Journal of gastroenterology and hepatology,* 27(5):862-872.

Chautan M., Chazal, G., Cecconi, F., Gruss, P., & Golstein, P. (1999). Interdigital cell death can occur through a necrotic and caspase- independent pathway. *Current Biology,* 9:967-970.

Chemin, I., & Zoulim, F. (2009). Hepatitis B virus induced hepatocellular carcinoma. *Cancer Letters*, 286:52-59.

Chen, C. C., Tsai, T. H., Huang, Z. R., & Fang, J. Y. (2010). Effects of lipophilic emulsifiers on the oral administration of lovastatin from nanostructured lipid carriers: physicochemical characterization and pharmacokinetics. *European Journal of Pharmaceutics and Biopharmaceutics,* 74(3):474-482.

Cheng, A.L., Kang, Y.K., Chen, Z., Tsao, C.J., Qin, S., & Kim, J.S. (2009). Efficacy and safety of sorafenib in patients in the Asia–Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncology*, 10(1):25–34.

Cheng, N., Chytil, A., Shyr, Y., Joly, A., & Moses, H.L. (2008). Transforming growth factor-beta signaling-deficient fibroblasts enhance hepatocyte growth factor signaling in mammary carcinoma cells to promote scattering and invasion. *Molecular Cancer Research,* 6:1521–1533.

Chiou, H.E., Wang, T.E., Wang, Y.Y., & Liu, H.W. (2006). Efficacy and safety of thalidomide in patients with hepatocellular carcinoma. *World Journal of Gastroenterology,* 12(43):6955–6960.

Chiuh, C., Prem, A., Sethi, G., Huat, K., & Tan, B. (2012). Thymoquinone : Potential cure for inflammatory disorders and cancer. *Biochemical Pharmacology,* 83(4):443–451.

Cragg, G. M. & Newman, D. J. (2005). Plants as a source of anti-cancer agents. *Journal of Ethnopharmacology,* 100:72-79.

Curado, M.P., Edwards, B., Shin, H.R. (2007) Cancer incidence in five continents, vol. IX. *IARC Scientific Publications* No. 160. Lyon: IARC.

Dal-Cim, T., Molz, S., Egea, J., Parada, E., Romero, A., Budni, J., Martin de Saavedra, M. D., del Barrio, L., Tasca, C. I., & Lopez, M. G. (2012). Guanosine protects human neuroblastoma SH-SY5Y cells against mitochondrial oxidative stress by inducing heme oxigenase-1 via PI3K/ Akt/GSK-3 beta pathway. *Neurochemistry International,* 61:397−404.

Das, A., & Maini, M.K. (2010). Innate and adaptive immune responses in hepatitis B virus infection. *Digestive Diseases,* 28:126-132.

Das, S., Dey, K.K., Dey, G., Pal, I., Majumder, A., MaitiChoudhury, S., Kundu, S.C., & Mandal, M. (2012). Antineoplastic and apoptotic potential of traditional medicines thymoquinone and diosgenin in squamous cell carcinoma. *PLoS ONE,* 7, e46641.

Davis, G.L., Dempster, J., Meler, J.D., Orr, D.W., Walberg, M.W., Brown, B., & Goldstein, R.M. (2008). Hepatocellular carcinoma: management of an increasingly common problem. *Proceedings (Baylor University. Medical Center),* 21(3):266–80.

De Nicola, G.M., Karreth, F.A., Humpton, T.J., Gopinathan, A., Wei, C., Frese, K., Mangal, D., Yu, K.H., Yeo, C.J., Calhoun, E.S., Scrimieri, F., Winter, J.M., Hruban, R.H., Iacobuzio-Donahue, C., Kern, S.E., Blair, I.A., & Tuveson, D. A. (2011). Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature*, 475:106−128.

Debatin, K.M., & Krammer, P.H. (2004). Death receptors in chemotherapy and cancer. *Oncogene,* 23:2950–66.

Degenhardt, K., Mathew, R., Beaudoin, B., Bray, K., Anderson, D., Chen, G., Mukherjee, C., Shi, Y., Gélinas, C., Fan, Y., Nelson, D.A., Jin, S., & White, E. (2006). Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell,* 10:51-64.

Dehghani, H., Hashemi, M., Entezari, M., & Mohsenifar, A. (2015). The Comparison of Anticancer Activity of Thymoquinone and Nanothymoquinone on Human Breast Adenocarcinoma, *14(October 2013),* 539–546.

Desoize, B., & Madoulet, C. (2002). Particular aspects of platinum compounds used at present in cancer treatment. *Critical Reviews in Oncology/Hematology,* 42:317-325.

Di Bisceglie, A.M. (2000). Natural history of hepatitis C: its impact on clinical management. *Hepatology (Baltimore, Md.),* 31:1014–1018.

Du, T.A. (2013). Cell death: balance through a bivalent regulator. *Nature Reviews Molecular Cell Biology,* 14:546.

Duprez, L., Wirawan, E., Vanden Berghe, T., & Vandenabeele, P. (2009). Major cell death pathways at a glance. *Microbes and Infection,* 11:1050-1062.

Duthie, J.F. (1960). Flora of the Upper Gangetic Plain and of the Adjacent Siwalik and Sub-Himalayan Tracts, Vol. I, Botanical Survey of India, Calcutta, pp.19-20.

Ekshyyan, O., & Aw, T.Y. (2004). Apoptosis: a key in neurodegenerative disorders. *Current Neurovascular Research,* 1:355–71.

El Gazzar, M.A., El Mezayen, R., Nicolls, M.R., & Dreskin, S.C. (2007). Thymoquinone attenuates proinflammatory responses in lipopolysaccharide-activated mast cells by modulating NF-kappaB nuclear transactivation. *Biochimica et Biophysica Acta,* 1770: 556-564.

El-Beltagi, H.S., & Mohamed, H.I. (2013). Reactive oxygen species, lipid peroxidation and antioxidative defense mechanism. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca,* 41(1):44–57.

El-Mahdy, M.A., Zhu, Q., Wang, Q.E., Wani, G., & Wani, A.A. (2005). Thymoquinone induces apoptosis through activation of caspase-8 and mitochondrial events in p53-null myeloblastic leukemia HL-60 cells. *International Journal of Cancer,* 117:409–417.

El-Najjar, N., Chatila, M., Moukadem, H., Vuorela, H., Ocker, M., Gandesiri, M., Schneider-Stock, R., & Gali-Muhtasib, H. (2010). Reactive oxygen species mediate thymoquinone-induced apoptosis and activate ERK and JNK signaling. *Apoptosis* 15(2):183–195.

Elsherbiny, N.M., & El-Sherbiny, M. (2014). Thymoquinone attenuates Doxorubicin-induced nephrotoxicity in rats: Role of Nrf2 and NOX4. *Chemico-Biological Interactions,* 223, 102–108.

Eskes, R., Desagher, S., Antonsson, B., & Martinou, J.C. (2000). Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane. *Molecular and Cellular Biology,* 20(3):929–935.

Falk, H., Herbert, J., Crowley, S., Ishak, K.G., Thomas, L.B., & Popper, H. (1981). Epidemiology of hepatic angiosarcoma in the United States 1964–1974. *Environmental Health Perspectives,* 41:107–113.

Fallot, G., Neuveut, C., & Buendia, M.A. (2012). Diverse roles of hepatitis B virus in liver cancer. *Current Opinion in Virology,* 2(4):467–473.

Fang, C.L., Al-Suwayeh, S.A, & Fang, J.Y. (2013). Nanostructured lipid carriers (NLCs) for drug delivery and targeting. *Recent Patents on Nanotechnology,* 7:41–55.

Fayez, A. M., Awad, A. S., El-Naa, M. M., Kenawy, S. A., & El-Sayed, M. E. (2014). Beneficial effects of thymoquinone and omega-3 on intestinal ischemia/reperfusion-induced renal dysfunction in rats. *Bulletin of Faculty of Pharmacy, Cairo University,* 52(2):171–177.

Ferlay, J., Bray, F., Pisani, P., & Parkin, D. M. (2004). Cancer incidence, mortality and prevalence worldwide. *IARC Cancer Base* No. 5, version 2.0. IARC Press, Lyon.

Ferlay, J., Parkin, D.M., & Steliarova-Foucher, E. (2010). Estimates of cancer incidence and mortality in Europe in 2008. *European Journal of Cancer,* 46(4):765–81.

Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., & Bray, F. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International journal of cancer,* 136(5):E359-E386.

Ferlay, J., Steliarova-Foucher, E., Lortet-Tieulent, J., Rosso, S., Coebergh, J.W.W., Comber, H., & Bray, F. (2013). Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. *European Journal of Cancer,* 49(6):1374–1403.

Fernald, K., & Kurokawa, M. (2013). Evading apoptosis in cancer. *Trends in Cell Biology,* 23(12):620–633.

Festjens, N., Vanden Berghe, T., Cornelis, S., & Vandenabeele, P. (2007). RIP1, a kinase on the crossroads of a cell's decision to live or die. *Cell Death & Differentiation,* 14:400-410.

Flohe´, L., & Günzler, W.A. (1984). Assays of glutathione peroxidase. *Methods Enzymol,* 105:114–21.

Fong, Y., Sun, R.L., Jarnagin, W., & Blumgart, L.H. (1999). An analysis of 412 cases of hepatocellular carcinoma at a Western center. *Annals of Surgery,* 229(6):790– 799.

Forman, L.M., Lewis, J.D., Berlin, J.A., Feldman, H.I., & Lucey, M.R. (2002).The association between hepatitis C infection and survival after orthotopic liver transplantation. *Gastroenterology,* 122(4):889–896.

Fulda, S. (2010). Evasion of apoptosis as a cellular stresss response in cancer. *International Journal of Cell Biology,* 2010(370835).

Fuss, M., Salter, B.J., Herman, T.S., & Thomas, C.R. (2004). External beam radiation therapy for hepatocellular carcinoma: potential of intensity-modulated and image-guided radiation therapy. *Gastroenterology,* 127(5 Suppl 1):S206–S217.

Gali-Muhtasib, H., El-Najjar, N., & Schneider-Stock, R. (2006). The medicinal potential of black seed (Nigella sativa) and its components. *Advances in Phytomedicine,* 2:133-153.

Gali-Muhtasib, H., Ocker, M., Kuester, D., Krueger. S., El-Hajj, Z, Diestel, A. (2008). Thymoquinone reduces mouse colon tumor cell invasion and inhibits tumor growth in murine colon cancer models. *Journal of Cellular and Molecular Medicine*, 12:330–42.

Gali-Muhtasib, H., Roessner, A., & Schneider-Stock, R. (2006). Thymoquinone: a promising anti-cancer drug from natural sources. *The international journal of biochemistry & cell biology,* 38(8):1249-1253.

Gali-Muhtasib, H.U., Abou Kheir, W.G., Kheir, L., Darwiche, N., & Crooks, P. (2004). Molecular pathway for thymoquinone-induced cell-cycle arrest and apoptosis in neoplastic keratinocytes. *Anticancer Drugs*, 15:389–399.

Gomez-Martin, C., Bustamante, J., Castroagudin, J.F. (2012). Efficacy and safety of sorafenib in combination with mammalian target of rapamycin inhibitors for recurrent hepatocellular carcinoma after liver transplantation. *Liver Transplantation*, 18:45–52.

Gonzalvez, F., Ashkenazi, A. (2010). New insights into apoptosis signaling by Apo2L/TRAIL. *Oncogene*, 29:4752–4765.

Goreja, W. G. (2003). Black seed. Nature's Miracle, Remedy. *Amazing Herbs Press, New York,* 1-64.

Gullet, N.P., Ruhul Amin, A.R., Bayraktar, S., Pezzuto, J.M., Shin, D.M., Khuri, F.R., Aggarwal, B.B., Surh, Y.J., Kucuk, O. (2010). Cancer prevention with natural compounds. *Seminars in Oncology,* 37:258-281.

Gurung, R.L., Lim, S.N., Khaw, A.K., Soon, J.F., Shenoy, K., Mohamed Ali, S., Jayapal, M., Sethu, S., Baskar, R., Hande, M.P. (2010). Thymoquinone induces telomere shortening, DNA damage and apoptosis in human glioblastoma cells. *PLoS One,* 5(8).

Hajhashemi, V., Ghannadi, A., Jafarabadi, H. (2004). Black cumin seed essential oil, as a potent analgesic and anti-inflammatory drug. *Phytotherapy Research,* 18:195–199.

Han, S.I., Kim, Y.S., & Kim, T.H. (2008). Role of apoptotic and necrotic cell death under physiologic conditions. *BMB Reports,* 41(1):1-10.

Hanahan, D, Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. *Cell,* 144(5):646–674.

Hanahan, D., & Weinberg, R.A. (2000). The hallmarks of cancer. *Cell,* 100:57–70.

Hollebecque, A., Malka, D., Ferté, C., Ducreux, M., & Boige, V. (2015). Systemic treatment of advanced hepatocellular carcinoma: From disillusions to new horizons. *European Journal of Cancer,* 51, 327–339.

Holmgren, A. (1989). Thioredoxin and glutaredoxin systems. *Journal of Biological Chemistry,* 264:13963–13966.

How, C. W., Abdullah, R., & Abbasalipourkabir, R. (2011). Physicochemical properties of nanostructured lipid carriers as colloidal carrier system stabilized with polysorbate 20 and polysorbate 80. *African Journal of Biotechnology,* 10(9):1684-1689.

Hsu, C. H., Shen, Y. C., Lin, Z. Z., Chen, P. J., Shao, Y. Y., Ding, Y. H., & Cheng, A. L. (2010). Phase II study of combining sorafenib with metronomic tegafur/uracil for advanced hepatocellular carcinoma. *Journal of hepatology,* 53(1):126-131.

Huang, C. H., Mandelker, D., Gabelli, S. B., & Amzel, L.M. (2008). Insights into the oncogenic effects of /PIK3CA/ mutations from the structure of p110 alpha/p85 alpha. *Cell Cycle*, 7: 1151−1156.

Huang, Q., Li, F., Liu, X., Li, W., Shi, W., Liu, F.F. & Li, C.Y. (2011). Caspase 3–mediated stimulation of tumor cell repopulation during cancer radiotherapy. *Nature Medicine,* 17(7), 860–866.

Huitzil-Melendez, F.D., Capanu, M., & O'Reilly, E.M. (2010). Advanced hepatocellular carcinoma: which staging systems best predict prognosis? *Journal of Clinical Oncology,* 28:2889.

Hussain, A. R., Ahmed, M., Ahmed, S., Manogaran, P., Platanias, L. C., Alvi, S. N., Uddin, S. (2011). Thymoquinone suppresses growth and induces apoptosis via generation of reactive oxygen species in primary effusion lymphoma. *Free Radical Biology and Medicine,* 50(8):978–987.

Hwang, Y.P., & Jeong, H.G. (2010). Ginsenoside Rb1 protects against 6-hydroxydopamine-induced oxidative stress by increasing heme oxy- genase-1 expression through an estrogen receptor-related PI3K/Akt/ Nrf2-dependent pathway in human dopaminergic cells. *Toxicology and Applied Pharmacology*, 242:18−28.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, & International Agency for Research on Cancer. (1994). Hepatitis viruses (Vol. 59). World Health Organization.

Irving, B. (2007). Nanoparticle drug delivery systems. *Innovation in Pharmaceutical Biotechnology,* 24:58–62.

Jafri, S.H., Glass, J., Shi, R., Zhang, S., Prince, M., & Kleiner-Hancock, H. (2010). Thymoquinone and cisplatin as a therapeutic combination in lung cancer: in vitro and in vivo.J. Exp.Clin. *Cancer Research,* 29:87.

Jain, A.K., & Jaiswal, A.K. (2006). Phosphorylation of tyrosine 568 controls nuclear export of Nrf2. *Journal of Biological Chemistry,* 281:12132− 12142.

Jaramillo, M., & Zhang, D. (2013). The emerging role of the Nrf2–Keap1 signaling pathway in cancer. *Genes & Development,* 2179–2191.

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA: Cancer Journal for Clinicians,* 61(2):69–90.

Jeong, W.S., Jun, M., & Kong, A.N. (2006). Nrf2: A potential molecular target for cancer chemoprevention by natural compounds. *Antioxidants & Redox Signaling,* 8:99–106.

Jiang, W.G., Sanders, A.J., Katoh, M., Ungefroren, H., Gieseler, F., Prince, M., & Sliva, D. (2015). Tissue invasion and metastasis: molecular, biological and clinical perspectives. *In Seminars in cancer biology,* 35:S244-S275. Academic Press.

Johnson, B.W., Cepero, E., & Boise, L.H. (2000). Bcl-xL inhibits cytochrome c release but not mitochondrial depolarization during the activation of multiple death pathways by tumor necrosis factor-alpha. *Journal of Biological Chemistry,* 275(40):31546–31553.

Jonathan, M., Schwartz, Robert, L., & Carithers. (2001). Epidemiology and etiologic associations of hepatocellular carcinoma; last updated: January 4, 2011.

Joshi, M.D., & Müller, R.H. (2009). Lipid nanoparticles for parenteral delivery of actives. *European Journal of Pharmaceutics and Biopharmaceutics,* 71:161-172.

Kalayarasan, S., Prabhu, P.N., Sriram, N., Manikandan, R., Arumugam, M., & Sudhandiran, G. (2009). Diallyl sulfide enhances antioxidants and inhibits inflammation through the activation of Nrf2 against gentamicin-induced nephrotoxicity in Wistar rats. *European Journal of Pharmacology,* 606:162–171.

Kanduc, D., Mittelman, A., Serpico, R., Sinigaglia, E., Sinha, A.A., Natale, C., Santacroce, R., Di Corcia, M.G., Lucchese, A., Dini, L., Pani, P., Santacroce, S., Simone, S., Bucci, R. & Farber, E. (2002). Cell death: apoptosis versus necrosis. *International Journal of Oncology,* 21(1):165-170.

Kanter, M., Coskun, O., Korkmaz, A., & Oter, S. (2004). Effects of Nigella sativa on oxidative stress and β-cell damage in streptozotocin-induced diabetic rats. *The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology,* 279(1):685-691.

Kaplowitz, N., Aw, T.Y., & Ookhtens, M. (1985). The regulation of hepatic glutathione. *Annual Review of Pharmacology and Toxicology,* 25(1):715-744.

Kaseb, A.O., Chinnakannu, K., Chen, D., Sivanandam, A., Tejwani, S., Menon, M., Dou, Q.P., & Reddy, G.P. (2007). Androgen receptor-and E2F-1-targeted thymoquinone therapy for hormone refractory prostate cancer. *Cancer Research,* 67(16):7782–7788.

Ke, A.W., Shi, G.M., Zhou, J., Huang, X.Y., Shi, Y.H., Ding, Z. B., Wang, X. Y., Devbhandari, R.P., & Fan, J. (2011). CD151 Amplifies signaling by integrin alpha 6 beta 1 to PI3K and induces the epithelial- mesenchymal transition in HCC Cells. *Gastroenterology,* 140:1629−U389.

Kelvin, J.F. & Thyson, L.B. (2010). Cancer symptoms and cancer treatment side effects. *Cancer and cancer treatment* (9pp. 1-30). 2nd edition: Jones and Bartlett Publishers.

Kensler, T.W., Wakabayashi, N., & Biswal, S. (2007). Cell survival responses to environmental stresses via the Keap1–Nrf2– ARE pathway. *Annual Review of Pharmacology and Toxicology,* 47: 89–116.

Khader, M., Bresgen, N., & Eckl, P. M. (2009). In vitro toxicological properties of thymoquinone. *Food and Chemical Toxicology,* 47(1):129-133.

Khan, S.A., Thomas, H.C., Davidson, B.R., & Taylor-Robinson, S.D. (2005). Cholangiocarcinoma. *Lancet*, 366:1303–1314.

Khosravi-Far, R., & Esposti, M.D. (2004). Death receptor signals to mitochondria. *Cancer Biology and Therapy,* 3(11): 1051-1057.

Knasmüller, S., Parzefall, W., Sanyal, R., Ecker, S., Schwab, C., Uhl, M., & Darroudi, F. (1998). Use of metabolically competent human hepatoma cells for the detection of mutagens and antimutagens. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* 402(1):85-202.

Knowles, B.B., Howe, C.C., & Aden, D.P. (1980). Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. *Science,* 209:497–499.

Kobayashi, A., Ohta, T., & Yamamoto, M. (2004). Unique function of the Nrf2-Keap1 pathway in the inducible expression of antioxidant and detoxifying enzymes. *Methods Enzymol,* 378:273–286.

Kohle, C., Badary, O.A., Nill, K., Bock-Henning, B.S., & Bock, K.W. (2005). Serotonin glucuronidation by Ah receptor- and oxidative stress inducible human UDP-glucuronosyltransferase (UGT) 1A6 in Caco-2 cells. Biochemical Pharmacology, 69(9):1397-1402.

Koike, K., Takaki, A., Tatsukawa, M., Suzuki, M., Shiraha, H., Iwasaki, Y., Sakaguchi, K., Shiratori, Y. (2006). Combination of 5-FU and IFN alpha enhances IFN signaling pathway and caspase-8 activity, resulting in marked apoptosis in hepatoma cell lines. *Int J Oncol,* 29:1253–1261.

Kolli-Bouhafs, K. (2012). Thymoquinone reduces migration and invasion of human glioblastoma cells associated with FAK, MMP-2 and MMP-9 down-regulation. *Investigational New Drugs,* 30:2121–2131.

Kundu, J., Chun, K.S., Aruoma, O.I., & Kundu, J.K. (2014). Mechanistic perspectives on cancer chemoprevention/chemotherapeutic effects of thymoquinone. *Mutation Research/Fundamental and Molecular,* 768:22–34.

Kundu, J., Kim, D.H., Kundu, J.K., & Chun, K.S. (2013). Thymoquinone induces heme oxygenase-1 expression in HaCaT cells via Nrf2/ARE activation: Akt and AMP- Kalpha as upstream targets. *Food and Chemical Toxicology,* 65C:18–26.

Kundu, J.K., Liu, L., Shin, J.W., & Surh, Y.J. (2013). Thymoquinone inhibits phorbol ester- induced activation of NF-kappaB and expression of COX-2, and induces expression of cytoprotective enzymes in mouse skin in vivo. *Biochemical and Biophysical Research Communications,* 438:721–727.

Lakhani, S.A., Masud, A., Kuida, K., Porter, G.A., Booth, C.J., Mehal, W.Z., Inayat, I., & Flavell, R.A. (2006). Caspases 3 and 7: key mediators of mitochondrial events of apoptosis. *Science,* 311(5762):847–851.

Lamkanfi, M., & Kanneganti, T.D. (2010). Caspase-7: a protease involved in apoptosis and inflammation. *International Journal of Biochemistry,* 42(1):21-24.

Latifah, S.Y., Ng, W.K., Al-naqeeb, G., & Ismail, M. (2009). Cytotoxicity of thymoquinone (TQ) from Nigella sativa towards human cervical carcinoma cells (HeLa). Journal of Pharmacy Research, 2(4):585–589.

Lau, A., Villeneuve, N.F., Sun, Z., Wong, P.K., & Zhang, D.D. (2008). Dual roles of Nrf2 in cancer. *Pharmacological Research,* 58:262–270.

Lavanchy, D. (2008). Chronic viral hepatitis as a public health issue in the world. *Best Practice & Research Clinical Gastroenterology,* 22:991–1008.

Lee, J.M., Li, J., Johnson, D.A., Stein, T.D., Kraft, A.D., Calkins, M.J., Jakel, R.J., & Johnson, J.A. (2005). Nrf2, a multi-organ protector? *The FASEB Journal*, 19:1061-1066.

Li, F., Rajendran, P., & Sethi, G. (2010). Thymoquinone inhibits proliferation, induces apoptosis and chemosensitizes human multiple myeloma cells through suppression of signal transducer and activator of transcription 3 activation pathway. *British Journal of Pharmacology,* 161:541–554.

Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., & Wang, S.I. (1997). PTEN, a putative protein tyrosine phosphatase genemutated in human brain, breast, and prostate cancer. Science, 275(5308):1943–1947.

Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S.M., Ahmad, M., Alnemri, E.S., & Wang, X. (1997). Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell,* 91(4):479–489.

Lim, G. C. C. (2002). Overview of Cancer in Malaysia. *Japanese Journal of Clinical Oncology,* 32(suppl 1), S37–S42.

Liu, L., Cao, Y., Chen, C., Zhang, X., McNabola, A., Wilkie, D., Wilhelm, S., Lynch, M., & Carter, C. (2006). Sorafenib blocks the RAF/MEK/ERK pathway, inhibits tumor angiogenesis, and induces tumor cell apoptosis in hepatocellular carcinoma model PLC/PRF/5. *Cancer Research,* 66(24):11851– 11858.

Liu, Z. N., & Roberts, T.M. (2006). Human tumor mutants in the p110 alpha subunit of PI3K. *Cell Cycle*, 5:675−677.

Llovet, J., Ricci, S., Mazzaferro, V., Hilgard, P., Raoul, J., Zeuzem, S., Pou-lin-Costello, M., Moscovici, M., Voliotis, D., & Bruix, J. (2007). Sorafenib improves survival in advanced hepatocellular carcinoma (HCC): results of a phase III randomized placebo-controlled trial (SHARP trial). *Journal of Clinical Oncology,* 25(18S):LBA1.

Llovet, J.M., & Bruix, J. (2003). Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. *Hepatology,* 37(2):429-442.

Llovet, J.M., & Bruix, J. (2003). Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. *Hepatology,* 37(2):429-442.

Llovet, J.M., Ricci, S., Mazzaferro, V., Hilgard, P., Gane, E., Blanc, J.F., de Oliveira, A.C., Santoro, A., Raoul, J.L., & Forner, A. (2008). Sorafenib in advanced hepatocellular carcinoma. *The New England Journal of Medicine,* 359:378-390.

London, W.T., & McGlynn, K.A. (2006). Liver cancer, in: D. Schottenfeld, J.F. Fraumeni (Eds*.), Cancer Epidemiology and Prevention*, 3rd Ed., Oxford University Press, Inc., New York, 763–786.

Mahoney, J.A., & Rosen, A. (2005). Apoptosis and autoimmunity. *Current Opinion in Immunology,* 17:583–8.

Malich, G., Markovic, B., & Winder, C. (1997). The sensitivity and specificity of the MTS tetrazolium assay for detecting the in vitro cytotoxicity of 20 chemicals using human cell lines. *Toxicology,* 124:179–192.

Manju, V., Balasubramaniyan, V., & Nalini, N. (2005). Rat colonic lipid peroxidation and antioxidant status: the effects of dietary luteolin on 1,2-dimethylhydrazine challenge. *Cell Mol Biol Lett,* 10:535–551.

Martin-Cordero, C., Leon-Gonzalez, A.J., Calderon-Montano, J.M., Burgos-Moron, E., & Lopez-Lazaro, M. (2012). Pro-oxidant natural products as anticancer agents. *Current Drug Targets,* 13:1006-1028.

Mayer, M.P., & Bukau, B. (2005). Hsp70 chaperones: cellular functions and molecular mechanism. *Cellular and Molecular Life Sciences,* 62:670–684.

Meddah, B., Ducroc, R., El Abbes Faouzi, M., Eto, B., Mahraoui, L., Benhaddou-Andaloussi, A., Martineau, L.C., Cherrah, Y., & Haddad, P.S. (2009). Nigella sativa inhibits intestinal glucose absorption and improves glucose tolerance in rats. *Journal of Ethnopharmacology,* 121:419–424.

Mellor, M.M & Zabedah, O. (1997). Alternative medicine – friend or foe? *Presented at the 14th Asia Pacific Cancer Conference, Hong Kong.*

Mitsuishi, Y., Taguchi, K., Kawatani, Y., Shibata, T., Nukiwa, T., Aburatani, H., Yamamoto, M., & Motohashi, H. (2012). Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. *Cancer Cell,* 22:66–79.

Mohammad, R.M., Muqbil, I., Lowe, L., Yedjou, C., Hsu, H.Y., Lin, L.T., & Azmi, A.S. (2015). Broad targeting of resistance to apoptosis in cancer. *Seminars in Cancer Biology,* 1–26.

Mohanraj, V.J., & Chen, Y. (2006). Nanoparticles: a review. *Tropical Journal of Pharmaceutical Research,* 5(1):561–573.

Motaghed, M., Hassan, F.M., & Hamid, S.S. (2014). Thymoquinone regulates gene expression levels in the estrogen metabolic and interferon pathways in MCF7 breast cancer cells. *International Journal of Molecular Medicine,* 33:8–16.

Motohashi, H., & Yamamoto, M. (2004). Nrf2-Keap1 defines a physiologically important stress response mechanism. *Trends in Molecular Medicine,* 10:549–57.

Mudshinge, S.R., Deore, A.B., Patil, S., & Bhalgat, C.M. (2011). Nanoparticles: Emerging carriers for drug delivery. *Saudi Pharmaceutical Journal,* 19(3):129–141.

Muller, R.H., Radtke, M., & Wissing, S.A. (2002). Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Advanced Drug Delivery Reviews,* 54:131-155.

Müller, R.H., Radtke, M., & Wissing, S.A. (2002a). Nanostructured lipid matrices for improved microencapsulation of drugs. *International Journal of Pharmaceutics,* 242:121–128.

Murphy, K.M., Ranganathan, V., Farnsworth, M.L., Kavallaris, M., & Lock, R.B. (2000). Bcl-2 inhibits Bax translocation from cytosol to mitochondria during drug induced apoptosis of human tumor cells. *Cell Death & Differentiation,* 7:102–111.

Nagata, S. (2000). Apoptotic DNA fragmentation. *Experimental Cell Research*, 256:12–18.

National Cancer Registry: Second Report of the National Cancer Registry on Cancer Incidence in Malaysia (2003). *Malaysia, Ministry of Health,* 2004.

Neuveut, C., Wei, Y., & Buendia, M.A. (2010). Mechanisms of HBV-related hepatocarcinogenesis. *J Hepatol*, 52:594–604.

Newell, P., Toffanin, S., & Villanueva, A. (2009). Ras pathway activation in hepatocellular carcinoma and anti-tumoral effect of combined sorafenib and rapamycin in vivo. *Journal of Hepatology,* 51:725

Ng, W.K., Yazan, L.S., & Ismail, M., (2011). Thymoquinone from Nigella sativa was more potent than cisplatin in eliminating of SiHa cells via apoptosis with down- regulation of Bcl-2 protein. *Toxicology in Vitro,* 25:1392–1398.

Ng, W.K., Yazan, L.S., Yap, L.H., Abd, W., Wan, G., Hafiza, N., & Abdullah, R. (2015). Thymoquinone-Loaded Nanostructured Lipid Carrier Exhibited Cytotoxicity towards Breast Cancer Cell Lines (MDA-MB-231 and MCF-7) and Cervical Cancer Cell Lines (HeLa and SiHa). *BioMed Research International,* 2015:10.

Normura, M., Shimizu, S., Ito, T., Narita, M., Matsuda, H., & Tsujimoto, Y. (1999). Apoptotic cytosol facilitates Bax translocation to mitochon- dria that involves cytosolic factor regulated by Bcl-2. *Cancer Research,* 59:5542–5548.

Ong, Y.S, Yazan, L.S, Ng. W.K, Noordin, M.M, Sapuan, S., Foo, J.B, & Tor, Y. (2016). Acute and subacute toxicity profiles of thymoquinone-loaded nanostructured lipid carrier in BALB / c mice. *International Journal of Nanomedicine,* 5905–

5915.

Paarakh, P. M. (2010). Nigella sativa Linn. A comprehensive review. *Indian Journal of Natural Products and Resources,* 1(4):409–429.

Palmer, D.H., Hussain, S.A., & Johnson, P.J. (2004). Systemic therapies for hepatocellular carcinoma. *Expert Opinion on Investigational Drugs,* 13(12):1555–1568.

Pan, J.S., Hong, M.Z., & Ren, J.L. (2009). Reactive oxygen species: A double-edged sword in oncogenesis. *World Journal of Gastroenterology,* 15:1702-1707.

Paramasivam, A., Sambantham, S., Shabnam, J., Raghunandhakumar, S., Anandan, B., Rajiv, R., Vijayashree Priyadharsini, J., & Jayaraman, G. (2012). Anti-cancer effects of thymoquinone in mouse neuroblastoma (Neuro-2a) cells through caspase-3 activation with down-regulation of XIAP. *Toxicology Letters,* 213:151–159.

Pardeike, J., Hommoss, A., & Muller, R.H. (2009). Lipid nanoparticles (SLN: NLC) in cosmetic and pharmaceutical dermal products. *International Journal of Pharmaceutics,* 366:170–184.

Parkin, D.M., Bray, F., Ferlay, J., & Pisani, P. (2005).Global cancer statistics, 2002. *CA: A Cancer Journal for Clinicians,* 55:74-108.

Pathan, S.A., Jain, G.K., & Zaidi, S.M.A. (2011). Stability- indicating ultra-performance liquid chromatography method for the estimation of thymoquinone and its application in biopharmaceutical studies. *Biomedical Chromatography,* 25(5):613–620.

Peng, L., Liu, A., Shen, Y., Xu, H.Z., Yang, S.Z., Ying, X.Z., Liao, W., Liu, H.X., Lin, Z.Q., Chen, Q.Y., Cheng, S.W., & Shen, W.D. (2013). Antitumor and anti-angiogenesis effects of thymoquinone on osteosarcoma through the NF-kappaB pathway. *Oncology Reports,* 29:571–578.

Pérez-Herrero, E., & Fernández-Medarde, A. (2015). Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy. *European Journal of Pharmaceutics and Biopharmaceutics,* 93(March):52–79.

Perz, J.F., Armstrong, G.L., Farrington, L.A., Hutin, Y.J., & Bell, B.P. (2006). The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *Journal of Hepatology,* 45:529–538.

Petrini, I., Lencioni, M., & Ricasoli, M. (2012). Phase II trial of sorafenib in combination with 5-fluorouracil infusion in advanced hepatocellular carcinoma. *Cancer Chemotherapy and Pharmacology,* 69:773–80.

Plati, J., Bucur, O., & Khosravi-Far, R. (2008). Dysregulation of apoptotic signaling in cancer: molecular mechanisms and therapeutic opportunities. *Journal of Cellular Biochemistry,* 104(4):1124–1149.

Qiu, G.H, Xie, X., Xu, F., Shi, X., Wang, Y., & Deng, L. (2015). Distinctive pharmacological differences between liver cancer cell lines HepG2 and

Hep3B. *Cytotechnology,* 67(1):1-12.

Rademaker, J., Widjaja, A., & Galanski, M. (2000). Hepatic hemangiosarcoma: imaging findings and differential diagnosis. *European Radiology,* 10:129–133.

Radtke, M., Souto, E.B., & Müller, R.H. (2005). Nanostructured lipid carriers: a novel generation of solid lipid drug carriers. *Pharmaceutical Technology Europe,* 17:45–50.

Raffeles, J., Griffin, M., & Dickinson, J.M. (2004). Activation of ERK1/2, JNK and PKB by Hydrogen peroxide in human SH-SY5Y neuroblast cells, Role of ERK 1/2 IN Hydrogen peroxide induced cell death. *European Journal of Pharmacology,* 483:163–173.

Rahman, I. (2000). Regulation of nuclear factor-kappa B, activator protein-1, and glutathione levels by tumor necrosis factor-alpha and dexamethasone in alveolar epithelial cells. *Biochemical Pharmacology,* 60:1041–1049.

Rajput, S., Kumar, B. N. P., & Sarkar. S. (2013). Targeted apoptotic effects of thymoquinone and tamoxifen on XIAPmediated Akt regulation in breast cancer. *PLoS ONE,* 8(4):61342.

Reed, J.C. (1997). Double identity for protein of BCL 2 family. *Nature,* 387:773–776.

Reindl, W., Yuan, J., Kramer, A., Strebhardt, K., & Berg, T. (2008). Inhibition of polo-like kinase 1 by blocking polo-box domain-dependent protein–protein interactions. *Chemistry & Biology*, 15:459–466.

Rodriguez, H., Holmquist, G.P., D'Agostino, R., Keller, J., & Akman, S.A. (1997). Metal ion-dependent hydrogen peroxide-induced DNA damage is more sequence specific than metal specific. *Cancer Research,* 57:2394–2403.

Roepke, M., Diestel, A., Bajbouj, K., Walluscheck, D., Schonfeld, P., Roessner, A., Schneider-Stock, R., & Gali-Muhtasib, H. (2007). Lack of p53 augments thymoquinone-induced apoptosis and caspase activation in human osteosarcoma cells. *Cancer Biology and Therapy,* 6(2):160–169.

Rojo, A.I., Medina-Campos, O. N., Rada, P., Zuniga-Toala, A., Lopez-Gazcon, A., Espada, S., Pedraza-Chaverri, J., & Cuadrado, A. (2012). Signaling pathways activated by the phytochemical nordihydroguaiaretic acid contribute to a Keap1-independent regulation of Nrf2 stability: Role of glycogen synthase kinase-3. *Free Radical Biology and Medicine,* 52:473−487.

Rooney, S., & Ryan, M. F. (2005). Modes of action of alpha-hederin and thymoquinone, active constituents of *Nigella sativa*, against HEp-2 cancer cells. *Anticancer Research,* 25(6B): 4255–4259.

Rotblat, B. Southwell, A.L. Ehrnhoefer, D.E. Skotte, N.H. Metzler Franciosi, M. Leprivier, S. (2014). HACE1 reduces oxidative stress and mutant Huntingtin toxicity by promoting the NRF2 response. *Proceedings of the National Academy of Sciences of the United States,* 111:3032–3037.

Rusyn, I., & Lemon, S.M. (2014). Mechanisms of HCV-induced liver cancer: What did we learn from in vitro and animal studies? *Cancer Letters,* 345(2):210–215.

Sakalar, C., Yuruk, M., Kaya, T., Aytekin, M., Kuk, S., & Canatan, H. (2013). Pronounced transcriptional regulation of apoptotic and TNF-NF-kappa-B signaling genes during the course of thymoquinone mediated apoptosis in HeLa cells. *Molecular and Cellular Biochemistry,* 383:243–251.

Sala, M., Llovet, J.M., Vilana, R., Bianchi, L., Solé, M., Ayuso, C., Brú, C., Bruix, J. (2004). Barcelona Clinic Liver Cancer Group. Initial response to percutaneous ablation predicts survival in patients with hepatocellular carcinoma. *Hepatology,* 40(6):1352–1360.

Sala, M., Llovet, J.M., Vilana, R., Bianchi, L., Solé, M., Ayuso, C., Brú, C., Bruix, J. Salem, M.L. (2005). Immunomodulatory and therapeutic properties of the Nigella sativa L. seed. *International Immunopharmacology,* 5(13-14):1749-1770.

Sayed-Ahmed M.M., Aleisa, A.M., & Al-Rejaie, S.S. (2010). Thymoquinone attenuates diethylnitrosamine induction of hepatic carcinogenesis through antioxidant signaling. *Oxidative Medicine and Cellular Longevity,* 3(4):254-261.

Schalm, S. W. (2008). Clinical use of interferon in hepatitis B and C. *Verhandelingen-Koninklijke Academie voor Geneeskunde van Belgie,* 71(1-2):87-99.

Schütte, K., Bornschein, J., & Malfertheiner, P. (2009). Hepatocellular carcinoma-epidemiological trends and risk factors. *Digestive Diseases,* 27:80–92.

Schwartz, L.M., & Osborne, B.A. (1995). Cell Death. *Academic Press,* San Diego, 77–97.

Seeger, C., & Mason, W. S. (2015). Molecular biology of hepatitis B virus infection. *Virology,* 479-480:672–686.

Seitz, S., Urban, S., Antoni, C., & Bottcher, B. (2007). Cryo-electron microscopy of hepatitis B virions reveals variability in envelope capsid interactions. *EMBO Journal*, 26:4160-4167.

Sen, S., Sharma, H., & Singh, N., (2005). Curcumin enhances Vinorelbine mediated apoptosis in NSCLC cells by mitochondrial pathway. *Biochemical and Biophysical Research Communications* 295:24–30.

Sethi, G., Ahn, K.S. & Aggarwal, B.B. (2008). Targeting nuclear factor-kappa B activation pathway by thymoquinone: role in suppression of antiapoptotic gene products and enhancement of apoptosis. *Molecular Cancer Research,* 6:1059-1070.

Sharma, H., Sen, S., & Singh, N. (2005). Molecular pathways in the chemosensitization of Cisplatin by quercetin in human head and neck cancer.

Cancer Biology & Therapy, 4:945–949.

Sharma, N.K., Ahirwar, D., Jhade, D., & Gupta, S. (2009). Medicinal and Phamacological Potential of Nigella sativa : A Review. *Ethnobotanical Review,* 13:1–8.

Shlomai, A., de Jong, Y.P., & Rice, C.M. (2014). Virus associated malignancies: The role of viral hepatitis in hepatocellular carcinoma. *Seminars in Cancer Biology,* 26:78–88.

Shoieb, A.M., Elgayyar, M., Dudrick, P.S., Bell, J.L., Tithof, P.K. (2003). In vitro inhibition of growth and induction of apoptosis in cancer cell lines by thymoquinone. *International Journal of Oncology,* 22:10-13.

Siegel, R., Naishadham, D., Jemal, A. (2013). Cancer statistics 2013. *CA: A Cancer Journal for Clinicians*63:11–30.

Silverstein, A., Silverstein, V., &Nunn, L.S. (2006). Cells gone wild. *In Cancer* (pp. 7-12). United State of America: Lerner Publishing Group.

Slany, A., Haudek, V.J., Zwickl, H., Gundacker, N.C., Grusch, M., Weiss, T.S., Seir, K., Rodgarkia-Dara, C., Hellerbrand, C., & Gerner, C. (2010). Cell characterization by proteome profiling applied to primary hepatocytes and hepatocyte cell lines HepG2 and Hep3B. *J Proteome Res,* 9:6–21

Song, T.J., Ip, E.W., & Fong, Y. (2004). Hepatocellular carcinoma: current surgical management. *Gastroenterology,* 127(5 Suppl 1):S248–S260.

Sporn, M.B., & Liby, K.T. (2012). NRF2 and cancer: the good, the bad and the importance of context. *Nature Reviews Cancer,* 12:564–571.

Srinivasula, S.M., Ahmad, M., Fernandes-Alnemri, T., & Alnemri, E.S. (1998). Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. *Molecular Cell,* 1(7):949–957.

Sutherland, L.M., Williams, J.A., Padbury, R.T., Gotley, D.C., Stokes, B., & Maddern, G.J. (2006). Radiofrequency ablation of liver tumors: a systematic review. *Archives of Surgery,* 141(2):181–190.

Syed Alwi, S.S., Cavell, B.E., Donlevy, A., & Packham, G. (2012). Differential induction of apoptosis in human breast cancer cell lines by phenethyl isothiocyanate, a glutathione depleting agent. *Cell Stress Chaperones,* 17(5):529-538.

Szmuness, W. (1978). Hepatocellular carcinoma and the hepatitis B virus: evidence for a causal association. *Progress in Medical Virology,* 24:40-69.

Taguchi, K., Motohashi, H., & Yamamoto, M. (2011). Molecular mechanisms of the Keap1-Nrf2 pathway in stress response and cancer evolution. *Genes Cells,* 16:123–40

Tait, S.W.G., & Green, D.R. (2010). Mitochondria and cell death: outer membrane permeabilization and beyond. Nature Reviews. *Molecular Cell Biology,* 11(9):621–32.

Talalay, P., Dinkova-Kostova, A.T., & Holtzclaw, W.D. (2003). Importance of phase 2 gene regulation in protection against electrophile and reactive oxygen toxicity and carcinogenesis. *Advances in Enzyme Regulation,* 43:121–134.

Tanaka S, & Arii S. (2012). Molecular targeted therapies in hepatocellular carcinoma. *Seminars in Oncology,* 39:486–92.

Theret, N., Musso, O., Turlin, B., Lotrian, D., Bioulac-Sage, P., Campion, J. P., Boudjema, K., & Clement, B. (2001). Increased extracellular matrix remodeling is associated with tumor progression in human hepatocellular carcinomas. *Hepatology,* 34:82–88.

Tirunagari, S., & Shaik, D. (2013). Hepato Cellular Carcinoma. *Webmed Central CLINICAL TRIALS,* 4:2–10.

Torres, M.P., Ponnusamy, M.P., Chakraborty, S., Smith L.M., Das, S., Arafat, H.A., & Batra, S.K. (2010). Effects of thymoquinone in the expression of mucin 4 in pancreatic cancer cells: implications for the development of novel cancer therapies. *Molecular Cancer Therapeutics,* 9:1419–1431.

Tschopp, J., Martinon, F., & Burns, K. (2003). NALPs: a novel protein family involved in inflammation. *Nature Reviews Molecular Cell Biology,* 4:95-104.

Varshosaz, J., Minayian, M., & Moazen, E. (2010). Enhancement of oral bioavailability of pentoxifylline by solid lipid nanoparticles. *Journal of Liposome Research,* 20:115–123.

Vermes, I., Haanen, C., Steffens-Nakken, H., & Reutelingsperger, C. (1995). A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. *Journal of Immunological Methods,* 184(1):39–51.

Vermeulen, K., Van Bockstaele, D.R., & Berneman, Z.N. (2005). Apoptosis: mechanisms and relevance in cancer. *Annals of Hematology,* 84:627–39.

Walsh, J.G., Cullen, S.P., Sheridan, C., Luthi, A.U., Gerner, C., & Martin, S.J. (2008). Executioner caspase-3 and caspase-7 are functionally distinct proteases. *Proceedings of the National Academy of Sciences of the United States,* 35:12815–12819.

Wang, X.J., Sun, Z., Villeneuve, N.F., Zhang, S., Zhao, F., Li, Y., Chen, W., Yi, X., Zheng, W., & Wondrak, G.T. (2008b). Nrf2 enhances resistance of cancer cells to chemotherapeutic drugs, the dark side of Nrf2. *Carcinogenesis,* 29:1235–1243.

Wang, Z., Zhou, J., & Fan, J. (2008). Effect of rapamycin alone and in combination with sorafenib in an orthotopic model of human hepatocellular carcinoma. *Clinical Cancer Research,* 14:5124.

Warrier, P.K., Nambiar, V.P.K., Ramankutty, C., & Vasudevan N.R. (2004). Indian Medicinal Plants: A Compendium of 500 Species. Telangana: *Orient*

Longman, 139-142.

Wei, M.C., Lindsten, T., Mootha, V.K., Weiler, S., Gross, A., Ashiya, M., Thompson, C.B., & Korsmeyer, S.J. (2000). tBID, a membrane-targeted death ligand, oligomerizes BAK to release cytochrome c. *Genes & Development*, 14(16):2060–2071.

Wei, M.C., Zong, W.X., Cheng, E.H., Lindsten, T., Panoutsakopoulou, V., Ross, A.J., Roth, K.A., MacGregor, G.R., Thompson, C.B., & Korsmeyer, S.J. (2001). Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. *Science,* 292(5517):727–730.

WHO (2014, November 2014). Cancer. http://www.who.int/mediacentre/factsheets/fs297/en/. Retrieved 8th January, 2015

Wild, A.C., Moinova, H.R., & Mulcahy, R.T. (1999). Regulation of γ-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. *Journal of Biological Chemistry,* 274:33627–33636.

Williams, C.J., & Whitehouse, J.M. (1979). Cis-platinum: a new anticancer agent. *British Medical Journal,* 23:1689-1691.

Womack, K., Anderson, M., Tucci, M., Hamadain, E., & Benghuzzi, H. (2006). Evaluation of bioflavonoids as potential chemotherapeutic agents. *Biomedical Sciences Instrumentation*, 42:464-469.

Wong, G.L., Wong, V.W., & Chan, H.L. (2014). Combination therapy of interferon and nucleotide/nucleoside analogues for chronic hepatitis B. *Journal of Viral Hepatitis,* 21:825–834.

Woo, C.C., Hsu, A, Kumar, A.P., Sethi, G., & Tan, K.H. (2013). Thymoquinone inhibits tumor growth and induces apoptosis in a breast cancer xenograft mouse model: the role of p38 MAPK and ROS. *PLoS ONE,* 8(10):75356.

Woo, C.C., Loo, S.Y., Gee, V., Yap, C.W., Sethi, G., Kumar, A.L., Huat, K., & Tan, B. (2011). Anticancer activity of thymoquinone in breast cancer cells: possible involvement of PPAR-g pathway. *Biochemical Pharmacology,* 82:464–475.

Worthen, D.R., Ghosheh, O.A., & Crooks, P.A. (1998). The in vitro anti-tumor activity of some crude and purified components of black seed Nigella sativa L. *Anticancer Research,* 18:1527–32.

Wu, B., Chu, X., Feng, C., Hou, J., Fan, H., Liu, N., & Meng, S. (2015). Heat shock protein gp96 decreases p53 stability by regulating Mdm2 E3 ligase activity in liver cancer. *Cancer Letters,* 359:325–334.

Yi, T., Cho, S.G., Yi, Z., Pang, X., & Rodriguez, M. (2008). Thymoquinone inhibits tumor angiogenesis and tumor growth through suppressing AKT and extracellular signal-regulated kinase signaling pathways. *Molecular Cancer Therapeutics,* 7:1789–1796.

Yim, H.J., & Lok, A.S. (2006). Natural history of chronic hepatitis B virus infection:

what we knew in 1981 and what we know in 2005. *Hepatology,* 43:S173–S181.

Yuan, S., & Akey, C.W. (2013). Apoptosome structure, assembly, and procaspase activation. *Structure,* 21:501–15.

Zhang, D.D. (2006). Mechanistic studies of the Nrf2–Keap1 signaling pathway. *Drug Metabolism Reviews,* 38:769–789.

Zhang, Z., Cui, W., Li, G., Yuan, S., Xu, D., Hoi, M.P.M., Lin, Z., Dou, J., Han, Y., & Lee, S.M.Y. (2012). Baicalein protects against 6-OHDA- induced neurotoxicity through activation of Keap1/Nrf2/ HO-1 and involving PKC alpha and PI3K/AKT signaling pathways. *Journal of Agricultural and Food Chemistry,* 60:8171−8182.

Zhou, S. L., Dai, Z., Zhou, Z.J., Wang, X.Y., Yang, G.H., Wang, Z., Huang, X.W., Fan, J., & Zhou, J. (2012). Overexpression of CXCL5 mediates neutrophil infiltration and indicates poor prognosis for hepatocellular carcinoma. *Hepatology*, 56:2242−2254.

Zong, W.X., & Thompson, C.B. (2006). Necrotic death as a cell fate. *Genes & Development,* 20:1-15.