



UNIVERSITI PUTRA MALAYSIA

**CYTOTOXICITY OF GONIOTHALAMIN ON THE HUMAN
HEPATOCELLULAR CARCINOMA HEPG2 CELL LINE**

**MOTHANNA SADIQ OBAID AL-QUBAISI
FBSB 2009 31**



**CYTOTOXICITY OF GONIOTHALAMIN ON THE HUMAN
HEPATOCELLULAR CARCINOMA HEPG2 CELL LINE**

By

MOTHANNA SADIQ OBAID AL-QUBAISI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Masters of Science**

October 2009



DEDICATION

I wish to dedicate this thesis to my mother and father for their love and giving me the genes for research. They have always believed in me and have always encouraged me not only during this master period but throughout life.



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

**CYTOTOXICITY OF GONIOTHALAMIN ON THE HUMAN
HEPATOCELLULAR CARCINOMA HEPG2 CELL LINE**

By

MOTHANNA SADIQ OBAID AL-QUBAISI

October 2009

Chairman: Noorjahan Banu Mohamedd Alitheen, Ph.D

Faculty: Biotechnology and Biomolecular Sciences

Goniothalamine is a biologically active styrylpyrone derivative isolated from various *Goniothalamus* sp., belonging to the Annonaceae family. This plant extract has been reported to be cytotoxic towards several tumor cell lines such as pancreas carcinoma (PANC-1), gastric carcinoma (HGC-27) and breast carcinoma (MCF-7). The purpose of this study was to examine and characterize the *in vitro* cytotoxicity effect of goniothalamine on the human hepatocellular carcinoma HepG2 cells and normal liver Chang cells and also to study the morphological and biochemical changes of goniothalamine-treated HepG2 and Chang cells. Goniothalamine (2.3 -150 μM ; 24, 48 and 72 hours) treatment to HepG2 and Chang cells resulted in a dose and time dependent inhibition of cell growth as assessed by MTT and LDH assays. The data suggest that goniothalamine selectively inhibits HepG2 cells (IC_{50} of MTT= 4.6(\pm 0.23) μM ; IC_{50} of LDH= 5.20(\pm 0.01) μM for 72 hours) with less inhibition of growth in Chang cells (IC_{50} of MTT= 35.0(\pm 0.09) μM ; IC_{50} of LDH= 32.5(\pm 0.04) μM for 72 hours). The cytotoxic activity of goniothalamine on HepG2 cells was confirmed by Trypan blue dye exclusion



assay. Goniotalamin reduced the number of viable cells (non-stained) associated with an increase on the number of non-viable cells (stained) and the Viability Indexes were $52 \pm 1.73\%$ for HepG2 cells and $62 \pm 4.36\%$ for Chang cells at IC_{50} after 72 hours. Cells were exposed to goniotalamin at lowest concentration ($2.3 \mu\text{M}$), IC_{50} (of MTT results), and highest concentration ($150 \mu\text{M}$) for 24, 48, or 72 hours and then examined for effects on cell cycle (using the flow cytometry) or proliferation (using the BrdU ELISA assay). The cytotoxic activity of goniotalamin was related to the inhibition of DNA synthesis, as revealed by the reduction of BrdU incorporation. At 72 hours with the lowest goniotalamin concentration of $2.3 \mu\text{M}$, the normal liver Chang cells retained 97.6% of control proliferation while the liver cancer HepG2 cells were reduced to 19.8% of control proliferation. Goniotalamin caused the accumulation of hypodiploid apoptotic cells in cell cycle analysis by flow cytometry. Goniotalamin arrested HepG2 and Chang cells in the G2/M phase with different degrees. Light microscopy examination of HepG2 and Chang cells exposed to different concentrations of goniotalamin up to 72 h demonstrated changes in cellular morphology; i.e. cell rounding followed by a loss of adherence with subsequent cell shrinkage and blebbing. In addition, the apoptotic cells were more abundant in goniotalamin-treated HepG2 cells ($84 \pm 4.58\%$) for 72 hours than in untreated cell ($4 \pm 2.65\%$) upon measurement by TUNEL staining. In view of the toxicity of goniotalamin, the kind of cell death, namely apoptosis or necrosis, was assessed. Therefore, staining with fluorescence labeled annexin V in combination with propidium iodide was performed on HepG2 and Chang cells exposed to goniotalamin. The laser scanning cytometry of propidium iodide and annexin V-stained cells indicated that the growth inhibiting effect of goniotalamin was consistent with a strong induction of apoptosis at late stage. This is because the cellular

membrane integrity was lost, so the cells exhibited annexin V- and propidium iodide-double positive up to 85.87 ± 0.78 and 57.69 ± 1.12 in HepG2 and Chang cells after 24 hours, respectively. In order to confirm apoptotic mechanism in the goniotalamin-treated cells, caspase 3 activity upon the same treatment conditions was carried out. The results indicate that caspase 3 activity was significantly elevated early in IC_{50} treated Chang cells (574% of control) after 24 hours and late in IC_{50} treated cells after 72 hours in HepG2 cells (879% of control). Our findings suggest a potential mechanism for the strong growth inhibitory effect of goniotalamin on this HepG2 liver cancer cells. However, less sensitivity to normal liver Chang cell line was observed by this compound. An important feature of the cytotoxicity by goniotalamin is that it is mediated through apoptosis.

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

**SITOTOKSISITI GONIOHALAMIN TERHADAP SEL ASAS KARSINOMA
HEPAR HEPG2 PADA MANUSIA**

Oleh

MOTHANNA SADIQ OBAID AL-QUBAISI

Oktober 2009

Pengerusi: Noorjahan Banu Mohamed Alitheen, Ph.D

Fakulti: Bioteknologi dan Sains Biomolekul

Goniothalamine adalah molekul aktif terbitan styrylpyrone secara biologi yang telah diasingpisahkan daripada spesies *Goniothalamus* dari Famili Annonaceae. Ekstrak tumbuhan ini dilaporkan memberi kesan sitotoksik terhadap beberapa sel tumor asas seperti sel karsinoma pankreas (PANC-1), sel karsinoma gastrik (HGC-27) dan sel karsinoma payudara (MCF-7). Tujuan kajian ini adalah untuk memeriksa dan mencirikan kesan sitotoksiti goniothalamine pada sel karsinoma hepar manusia (HepG2) dan sel Chang secara *in vitro* dan juga mengkaji morfologi dan perubahan biokimia pada sel HepG2 dan sel Chang yang dirawat dengan goniothalamine. Rawatan goniothalamine (2.3-150 μM ; 24, 48 dan 72 jam) pada sel HepG2 dan sel Chang dengan menggunakan pengujian MTT dan LDH, menghasilkan keputusan perencatan pertumbuhan sel yang berkadaran dengan dos dan masa. Data mencadangkan goniothalamine merencatkan sel HepG2 (IC_{50} MTT=4.6 (± 0.23) μM ; IC_{50} LDH=5.20(μM) untuk 72 jam) dengan sedikit perencatan pertumbuhan pada sel Chang (IC_{50} MTT=35.0 (± 0.09) μM ; IC_{50} LDH=32.5(± 0.04) μM untuk 72 jam). Aktiviti sitotoksiti goniothalamine pada sel HepG2 telah



juga dipastikan menggunakan pengujian pewarna biru Trypan. Goniothalamine telah mengurangkan bilangan sel hidup (tidak berwarna) yang berhubung dengan pertambahan bilangan sel mati (berwarna) dan indeks viabiliti pada pengukuran IC_{50} adalah $52 \pm 1.73\%$ bagi sel HepG2 dan $62 \pm 4.36\%$ untuk sel Chang selepas 72 jam. Sel-sel yang didedahkan pada goniothalamine pada kepekatan terendah ($2.3 \mu\text{M}$), IC_{50} (keputusan MTT), dan kepekatan tertinggi ($150 \mu\text{M}$) pada 24, 48 atau 72 jam dan kemudian diperiksa kesan pada kitaran sel (menggunakan aliran sitometrik) atau pertumbuhan sel (menggunakan pengujian BrdU ELISA). Aktiviti sitotoksik goniothalamine adalah berkait dengan perencatan sintesis DNA, seperti yang ditunjukkan oleh pengurangan penggabungan BrdU. Pada 72 jam terakhir untuk goniothalamine berkepekatan $2.3 \mu\text{M}$, peningkatan sel normal hati Chang kekal pada 97.6% berbanding pertumbuhan sel kawalan, sementara sel kanser hati HepG2 telah menurun kepada 19.8% berbanding pertumbuhan sel kawalan. Goniothalamine menyebabkan pengumpulan sel apoptotik hipodiploid pada kitar sel yang dianalisis menggunakan aliran sitometri. Goniothalamine menghentikan sel HepG2 dan sel Chang pada fasa G2/M pada darjah yang berbeza. Pemeriksaan melalui mikroskop cahaya pada sel HepG2 dan sel Chang yang terdedah terhadap goniothalamine pada kepekatan yang berbeza hingga 72 jam menunjukkan perubahan pada morfologi sel; i.e. sel membulat dan diikuti dengan kehilangan sifat pelekatan antara sel seterusnya menghasilkan sel yang kecut dan mengerut. Tambahan pula, sel apoptotik adalah lebih banyak dalam sel HepG2 yang dirawat dengan goniothalamine ($84 \pm 4.58\%$) untuk 72 jam berbanding sel-sel yang tidak dirawat ($4 \pm 2.65\%$) yang diukur dengan teknik warna TUNEL. Melalui kajian toksisiti goniothalamine, jenis kematian sel iaitu apoptosis atau nekrosis perlu dinilai. Oleh itu, pewarnaan dengan fluoresen yang dilabelkan dengan annexin V dengan

gabungan propidium iodida telah dilakukan pada sel HepG2 dan sel Chang yang terdedah pada goniothalamine. Imbasan pancaran laser sitometri propidium iodida dan annexin V pada sel yang diwarnakan telah menunjukkan bahawa perencatan pertumbuhan akibat goniothalamine adalah konsisten dengan aruhan kuat proses apoptosis pada peringkat akhir disebabkan oleh kehilangan integriti membran, maka sel-sel tersebut telah mempamerkan peningkatan bacaan dwi-positif untuk annexin V dan propidium iodida sehingga 85.87 ± 0.78 dan 57.69 ± 1.12 untuk sel HepG2 dan sel Chang masing-masing selepas 24 jam. Dalam menastikan mekanisma apoptotik bagi sel yang dirawat dengan goniothalamine, pengukuran aktiviti caspase 3 telah dijalankan dengan keadaan ujikaji yang sama. Keputusan ujikaji menunjukkan aktiviti caspase 3 adalah meningkat awal dengan signifikan dalam sel Chang yang dirawat IC_{50} (574% berbanding kawalan) iaitu selepas 24 jam dan akhir pada sel HepG2 yang dirawat IC_{50} iaitu selepas 72 jam (879 % berbanding kawalan). Hasil kajian ini mencadangkan suatu mekanisma yang mungkin untuk perencatan kuat pertumbuhan akibat goniothalamine pada sel cancer hati (HepG2) dengan sensitiviti yang rendah pada sel asas hati normal Chang terhadap bahan ini. Suatu ciri penting sitotoksiti goniothalamine adalah pengantaraannya adalah melalui proses apoptosis.

ACKNOWLEDGEMENTS

Praise be to Allah the Almighty, and peace be upon our prophet Mohammed,

At the beginning, I must thank ALLAH swt for His numerous blessings among which the completion of this thesis.

I would like to express my sincerest gratitude to my mentor and advisor, Dr. Noorjahan Banu Mohamed Alitheen. Her support, guidance, and especially her patience throughout this project were invaluable. I would also like to thank Prof.Dr. Abdul Manaf Ali and Dr. Abdul Rahman Omar, who have provided valuable guidance and support for both this project and my education.

My deepest thanks to Dr. Rozita Rosli for providing the equipment, facilities, for supporting me and my work through these years, for helpful discussions, suggestions and ideas of how to improve my work. I would never have been able to finish my dissertation without her guidance.

Special thanks to Ms. Rohaya bt Ibrahim, Mr. Yeap Swee Keong, Mrs. Norhaszalina Md Isa and Ms. Nurfarhana bt Ferdaos for all of their technical help, friendship, and valuable comments during this project. I would also like to thank my fellow students, especially Azwan B Aziz, for their support during my time at Universiti Putra Malaysia.

Thank you.



I certify that a Thesis Examination Committee has met on 12 October 2009 to conduct the final examination of Mothanna Sadiq Obaid Al-Qubaisi on his thesis entitled "Cytotoxicity of Goniotalamin on the Human Hepatocellular Carcinoma HepG2 Cell Line" 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:

Tan Soon Guan, PhD

Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Chairman)

Shuhaimi Mustafa, PhD

Associate Professor

Institute of Halal Products Research

Universiti Putra Malaysia

(Internal Examiner)

Muhajir Hamid, PhD

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Internal Examiner)

Kamarudin Mohd Yusoff, PhD

Professor

Faculty of Medicine

Universiti Malaya

Malaysia

(External Examiner)



BUJANG BIN KIM HUAT, PhD

Professor and Deputy Dean

School of Graduate Studies

Universiti Putra Malaysia

Date: 15 January 2010



This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Sciences.

The members of the Supervisory Committee were as follows:

Noorjahan Banu Mohamed Alitheen, PhD

LECTURER

Faculty of Biotechnology and Biomolecular Sciences

University Putra Malaysia

(Chairperson)

Abdul Manaf Ali, PhD

Professor

Faculty of Biotechnology and Biomolecular Sciences

University Putra Malaysia

(Member)

Abdul Rahman Omar, PhD

Associate Professor

Faculty of Veterinary Medicine

University Putra Malaysia

(Member)

Rozita Rosli, PhD

Associate Professor

Faculty of Medicine and Health Sciences

University Putra Malaysia

(Member)

HASANAH MOHD. GHAZALI, PhD

Professor and Dean

School of Graduate Studies

Universiti Putra Malaysia

Date: 11 February 2010



DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

MOTHANNA AL-QUBAISI

Date: 3 March 2010



TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xvii
LIST OF APPENDICES	xviii
LIST OF ABBREVIATIONS	xix
CHAPTERS	
I INTRODUCTION	1
II LITERATURE REVIEW	4
2.1 Cancer	4
2.2 Liver Cancer	5
2.2.1 Intrahepatic cholangiocarcinoma	5
2.2.2 Extrahepatic bile duct and gallbladder cancer	6
2.2.3 Hepatoblastoma	7
2.2.4 Angiosarcoma	7
2.3 Hepatocellular Carcinoma	8
2.3.1 Background	8
2.3.2 Epidemiology and frequency	8
2.3.3 Malaysian Experiments	10
2.3.4 Molecular basis of Hepatocellular Carcinoma	11
2.3.5 HepG2 and Chang cell lines	12
2.3.6 Drugs	13
2.4 Drug- resistance	14
2.5 The turning toward plant extracts	14
2.6 <i>Goniothalamus macrophyllus</i>	16
2.7 Goniothalamine	17
2.7.1 Activities	18
2.7.2 Anti-cancer role of goniothalamine	18
2.8 Cell Death Pathways	19
2.9 <i>In Vitro</i> Methods for Detecting Cytotoxicity	19
2.9.1 Cytotoxicity tests	21
2.9.2 Proliferation tests	23
2.9.3 Cellular metabolic Tests	25
2.9.4 Quantification of Caspase-3 activity	27

III	MATERIAL AND METHODS	28
3.1	Materials and Reagents	28
3.2	Cell Lines	28
3.3	Medium Preparation	29
3.4	Inactivation of fetal bovine serum FBS	29
3.5	Buffer Preparation	29
3.6	Trypsin/EDTA solution	30
3.7	Cryopreservation	30
3.8	Maintenance of Cell Culture	31
3.9	Recovery and Thawing	31
3.10	MTT Cytotoxicity Assay	31
3.11	Lactate Dehydrogenase (LDH) Assay	32
3.12	Cell Cycle Analysis	33
3.13	Bromodeoxyuridine (BrdU) Cell Proliferation Assay	34
3.14	Viable Cell Counts Using Trypan Blue	34
3.15	Microscopic examination of nuclei and cell morphology	35
3.16	Annexin V–FITC Assay	36
3.17	Caspase-3 Assay	37
3.18	Statistical analysis	37
IV	RESULTS	38
4.1	Cellular sensitivity of HepG2 and Chang cells to goniothalamine	38
4.2	Cell cycle analysis	47
4.3	Goniothalamine and Cell Proliferation (BrdU ELISA)	53
4.4	Trypan blue dye exclusion	56
4.5	Measurements of apoptosis by morphology and TUNEL assay	59
4.6	Characterization of cell death	66
4.7	Detection of caspase-3 activity	70
V	DISCUSSION	72
5.1	Cellular sensitivity of HepG2 and Chang cells to goniothalamine	72
5.2	Cell cycle analysis	76
5.3	Goniothalamine and Cell Proliferation (BrdU ELISA)	80
5.4	Trypan blue dye exclusion	84
5.5	Measurements of apoptosis by morphology and TUNEL assay	86
5.6	Characterization of cell death	91
5.7	Detection of caspase-3 activity	93
5.8	General discussion	96
VI	CONCLUSION	100
	REFERENCES	102

APPENDICES

Appendix A	MTT assay	115
Appendix B	LDH assay	119
Appendix C	Cell Cycle analysis	123
Appendix D	BrdU assay	129
Appendix E	Trypan blue dye exclusion	131
Appendix F	Cellular membrane PS externalization	133
Appendix G	Caspase-3 activity	135
BIODATA OF STUDENT		137



LIST OF TABLES

Table		Page
2.1	Risk factors (carcinogens and cocarcinogens) regarding hepatocellular carcinoma	10
2.2	Features of Apoptosis and Necrosis	20
4.1	The IC ₅₀ values after different intervals of drug treatment in HepG2 cells	41
4.2	The IC ₅₀ values after different intervals of goniotalamin and doxorubicin treatment in Chang cells	41
4.3	The selective index (SI) of goniotalamin and doxorubicin treatment	44
4.4	Comparison of IC ₅₀ values for HepG2 and Chang cells	46



LIST OF FIGURES

Figure		Page
2.1	Pictures a and b show the plant and leaves of <i>Goniothalamus macrophyllus</i> .	17
2.2	The structure of goniothalamine	17
4.1	Effects of goniothalamine treatment in the human hepatocellular carcinoma cell line, HepG2	39
4.2	Effects of doxorubicin treatment in the human hepatocellular carcinoma cell line, HepG2	40
4.3	Effects of goniothalamine treatment in the normal human Chang liver cell line	42
4.4	Effects of doxorubicin treatment in the normal human Chang liver cell line	43
4.5	LDH leakage in HepG2 cells treated with goniothalamine	45
4.6	LDH leakage in Chang cells treated with goniothalamine	46
4.7	Effect of goniothalamine on cell-cycle distribution in HepG2	48
4.8	Effect of doxorubicin on cell-cycle distribution in HepG2 cells	49
4.9	Effect of goniothalamine on cell-cycle distribution in Chang cells	51
4.10	Effect of doxorubicin on cell-cycle distribution in Chang cells	52
4.11	Effect of goniothalamine and doxorubicin on the proliferation of HepG2 cells <i>in vitro</i>	54
4.12	Effect of goniothalamine and doxorubicin on the proliferation of Chang cells <i>in vitro</i>	55
4.13	Cell viability (HepG2 cells)	56
4.14	Cell viability (Chang cells)	58
4.15	Morphological changes of HepG2 cells after the exposure to goniothalamine	62
4.16	Typical fluorescence images of apoptotic cell in goniothalamine-treated HepG2 cells	63
4.17	Percentages of HepG2 cell death via apoptosis after goniothalamine treatment	64
4.18	Morphological changes of HepG2 cells after the exposure to goniothalamine	65
4.19	Flow cytometry analysis of apoptosis in HepG2 cells treated with goniothalamine	68
4.20	Flow cytometry analysis of apoptosis in Chang cells treated with goniothalamine	69
4.21	Treatment of HepG2 and Chang cells with goniothalamine results in activation of caspase 3	71



LIST OF APPENDICES

Appendix		Page
A.	MTT assay	115
B.	LDH assay	119
C.	Cell Cycle analysis	123
D.	BrdU assay	129
E.	Trypan blue dye exclusion	131
F.	Cellular membrane PS externalization	133
G.	Caspase-3 activity	135



LIST OF ABBREVIATIONS

Abrivation	Full name
AFB1	Aflatoxin B1
ATCC	The American Type Culture Collection
BrdU	Bromodeoxyuridine
Chang cells	Normal liver cell line
CO ₂	Carbon Dioxide
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
dUTP	2'-deoxyuridine 5'-triphosphate
EDTA	Ethylendiaminetetraacetic acid
ELISA	Enzyme-linked Immunosorbent Assay
EtOH	Ethanol
FACS	Fluorescence-Activated Cell Sorting
FCS	Fetal Calf Serum
FITC	Fluorescein isothiocyanate
G ₀	Resting phase
G ₁	Gap between mitosis and DNA synthesis
G ₂	Gap between DNA synthesis and mitosis
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HCl	Hydrochloric acid
HepG2	Human hepatocellular liver carcinoma cell line
HRP	Horseradish Peroxidase



IC ₅₀	Inhibition concentration at 50 percent
ICAM-1	Inter-Cellular Adhesion Molecule 1
KCl	Potassium Chloride
KH ₂ PO ₄	Potassium dihydrogen phosphate
LDH	Lactate Dehydrogenase
LDL	Low-density lipoproteins
M	Mitosis
mL	Mililiter
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaCl	Sodium Chloride
NADH	Nicotinamide adenine dinucleotide
NaHPO ₄	Disodium hydrogen phosphate anhydrous
NaOH	Sodium Hydroxide
Nm	Nanometer
PBS	Phosphate buffer saline
pH	Minus the decimal logarithm of the hydrogen ion activity in an aqueous solution
PI	Propidium iodide
PS	Phosphatidyl serine
RB 1	Retinoblastoma protein 1
S	DNA synthesis
SI	Selective Index
STAT	Signal Transducers and Activators of Transcription
TDT	Deoxynucleotidyl Transferase
TP53	Tumor protein p53
TUNEL	TdT-mediated dUTP Nick End Labeling

VCAM-1	Vascular cell adhesion molecule-1
VLDL	Very low-density lipoproteins
WNT	Proteins have roles in embryogenesis, cancer and in normal physiological processes
μg	Microgram



CHAPTER I

INTRODUCTION

Goniothalamus macrophyllus (locally named "Gajah beranak") is used traditionally as health tonic during pregnancy and to treat cold as well as fever (Burkill, 1953). The screening of this plant for bioactive compounds has resulted in the isolation of a large number of cytotoxic compounds, notably styryl-lactone derivatives, acetogenins, aporphine alkaloids and related alkaloids (Blasquez *et al.*, 1999). These compounds have also been found to possess strong antimicrobial (Khan *et al.*, 1999), larvicidal (Ee, 1998), antimalarial (Likhitwitayawuid *et al.*, 1997) and embryotoxic activities (Sam *et al.*, 1987).

Goniothalamine is a styryl-lactone compound isolated from the root and stem of *Goniothalamus macrophyllus* (Sam *et al.*, 1987). Cytotoxicity of goniothalamine was reported in a number of carcinoma cell types isolated from a variety of tissues such as colon cancer cell line (Ângelo *et al.*, 2005), breast cancer cell lines (Chen *et al.*, 2005) and lung carcinoma (Chatchai *et al.*, 2005). Skin fibroblast, human fibroblast and bovine kidney are normal cell lines that showed resistant to this compound (Chatchai *et al.*, 2005).

More than 80% of Hepatocellular carcinoma HCC cases occur in the Far East and Southeast Asia. Although immunization has been successful against hepatitis B virus (HBV), a changing disease burden of HCC has been observed in many parts of the



world because of the increasing prevalence and duration of hepatitis C virus (HCV) infection in these countries (Kao and Chen, 2005).

Hepatocellular carcinoma (HCC) is refractory to chemotherapy because of tumor heterogeneity and the development of multidrug resistance phenotypes (Huang *et al.*, 1992; Legoix *et al.*, 1999). The Hepatocellular Carcinoma HCC cells are presenting mutations of p53 (transcription factor works as a tumor suppressor that is involved in preventing cancer), which lead to more aggressive resistance to chemotherapy (Heinze *et al.*, 1999)

Doxorubicin is the best systemic chemotherapy with a variety of agents, including, epirubicin, mitoxantrone, cisplatin, and etoposide, either alone or in combination (Shah *et al.*, 1998). It is often used in patients with HCC disseminated beyond the liver, although the response rates are generally of the order of only 15 %. In addition to that, doxorubicin is expensive and has serious side effects such as nausea, vomiting, mucositis, ulceration, necrosis of the colon and acute myeloid leukemia with a preleukemic phase and may cause heart failure (British Medical Association and Royal Pharmaceutical Society of Great Britain RPSGB, 2006).

Plant bioactive compounds have fewer side effects with low-cost when used in chemotherapy. Thus, the gearing of compounds, extracted from plants, for medicinal purposes becomes a workable thing. Based on this, the objectives of the study are:

1. To assess toxicity and selectivity of goniotalamin against Hepatocellular Carcinoma HepG2 cell line in comparison with normal liver (Chang) cell line.
2. To determine the mechanism of cytotoxicity, the treated cells with goniotalamin, have behaved.