

# **UNIVERSITI PUTRA MALAYSIA**

ANTI-LEUKEMIC EFFECTS OF ZERUMBONE NANOPARTICLE ON HUMAN JURKAT T LYMPHOBLASTOID CELL LINES IN VITRO AND MURINE LEUKEMIC WEHI-3B MODEL IN VIVO

**HESHU SULAIMAN RAHMAN MUHAMMAD** 

FPV 2014 10



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By

HESHU SULAIMAN RAHMAN MUHAMMAD

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

February 2014

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## **DEDICATION**

This Thesis is Dedicated to

My Beloved Husband Hemn Hassan Othman

My Lovely Triple Sons Mabast, Paiwast and Bahast

My Lovely Parents and Siblings

All My Kind Hearted Teachers, Lecturers and Friends

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

## ANTI-LEUKEMIC EFFECTS OF ZERUMBONE NANOPARTICLE ON HUMAN JURKAT T LYMPHOBLASTOID CELL LINES *IN VITRO* AND MURINE LEUKEMIC WEHI-3B MODEL *IN VIVO*

By

#### HESHU SULAIMAN RAHMAN MUHAMMAD

#### February 2014

#### Chairman: Professor Rasedee Bin Abdullah, PhD

#### **Faculty: Veterinary Medicine**

Zerumbone (ZER) is a crystalline, monocyclic, sesquiterpene, phytochemical, natural dietary substance was isolated firstly from essential volatile oil of rhizomes of the edible wild ginger, Zingiber zerumbet (L.) Smith. Recent studies showed that ZER has antiproliferative properties on several cancers. ZER has poor aqueous solubility that has inhibited cancers development as a therapeutic compound. In this study, it is postulated that ZER incorporation into nanostructured lipid carriers (NLC) will improve solubility and delivery of the compound while not comprise its therapeutic effects. Thus, the objective of the current study is to improve the therapeutic potential of ZER by incorporation into NLC and to determine the effect of ZERloaded NLC (ZER-NLC) on a human T-lymphoblastic leukemia (Jurkat) cell line and on WEHI-3B (myelomonocytic leukaemia) cell-induced murine leukemia. The ZER-NLC produced using the high pressure homogenization (HPH) technique contained 5% lipid. The ZER-NLC was characterised by zetasizer, reverse phase high performance liquid chromatography (RP-HPLC), transmission electron microscopy (TEM), wide angle X-ray diffraction (WAXR), differential scanning colorimeter (DSC) and Franz Diffusion Cell (FDC) system analyses and shown to be physically stable, particle size (PS) of  $52.68 \pm 0.1$  nm, zeta potential (ZP) of - 25.03  $\pm$  1.24 mV and polydipersity index (PDI) of 0.29  $\pm$  0.0041 µm. These are all characteristics of an excellent drug-carrier and delivery system.

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Jurkat cells were used to determine the anticancer properties of ZER-NLC. MTT assay, fluorescent microscopy, scanning and transmission electron microscopy, flow cytometric analysis after annexinV-FITC staining, cell cycle and TUNEL assay, and caspase -3, -8 and -9 assays were also employed in the study. The study showed that ZER-NLC significantly (P<0.05) suppress proliferation of Jurkat cells *in vitro* in a time-dependent manner with an IC<sub>50</sub> of 12.5 ± 0.1, 9.09 ± 0.14 and 5.64 ± 0.38 µg/mL at 24, 48 and 72 h, respectively. The antiproliferative effect of ZER-NLC on Jurkat cells was attributed to induction of apoptosis via the mitochondrial (intrinsic) pathway. BALB/c mice were induced to develop leukemia with a single intraperitoneal injection of WEHI-3B cells (1 × 10<sup>6</sup> cells/animal). The *in vivo* study showed that oral ZER-NLC at doses of 60 mg/kg inhibited the proliferation of

leukemic cells in leukemic BALB/c mice as evidenced by the decrease in leukemic cell population in the spleen. Based on histological, electron microscopic, immunochemical evaluations and TUNEL assay, the effect of ZER-NLC in the inhibition of leukemia was via apoptosis. Using Western blot and qRT-PCR, the spleen cells of ZER-NLC-treated leukemic mice also showed increased expression of Bax, Cyt-c, and PARP proteins while the expression of Bcl-2 protein decreased. At the same time, PARP protein cleaved from 116 kDa to 85 kDa. These findings also suggested that the *in vivo* effect of ZER-NLC on murine leukemia is apoptosis via the mitochondrial pathway.

To determine potential toxicity of ZER-NLC, human peripheral blood mononuclear cells (PBMC) were treated *in vitro* with serial concentrations ZER-NLC up to 100 mg/mL and normal BALB/c mice treated orally with ZER-NLC at doses up to 200 mg/kg. The treatment did not produce any sign of toxicity in either normal human peripheral mononuclear cells or mice at any of the doses used, indicating that ZER-NLC is safe for parenteral use.

In conclusion, the study shows that loading of ZER into NLC did not reduce the therapeutic potential of compound and the *in vitro* effects of ZER-NLC on leukemic cells and *in vivo* effect on induced murine leukemia is apoptosis via the mitochondrial pathway. The ZER-NLC thus has excellent potential to be developed into a drug-carrier and delivery system for the treatment of cancers.

Abstrak tesis yang dikemukakan kepda Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

## KESAN ANTILEUKEMIA NANOZARAH ZERUMBONE TERHADAP TITISAN SEL LIMFOBLASTOID T JURKAT MANUSIA *IN VITRO* DAN MODEL LEUKEMIA WEHI-3B MENCIT *IN VIVO*

Oleh

#### HESHU SULAIMAN RAHMAN MUHAMMAD

#### Februari 2014

#### Pengerusi: Professor Rasedee Bin Abdullah, PhD

#### Fakulti : Perubatan Veterinar

Zerumbon (ZER), suatu bahan hablur, monosiklik, seskuiterpen, fitokimia dan makanan semula jadi, dipencil daripada minyak pati mudah meruap daripada rizom halia liar boleh makan, Zingiber zerumbet (L.) Smith. Kajian terkini menunjukkan ZER mempunyai sifat antiproliferatif terhadap beberapa kanser. Kelarutan ZER adalah buruk dan ini telah menjejas perkembangannya sebagai sebatian terapeutik. Dalam kajian ini apa yang telah dipostulat ialah, pemuatan ZER ke dalam pembawa lipid nanostruktur (NLC) akan meningkat kelarutan dan penghantaran sebatian ini sambil tidak menjejaskan kesan terapeutiknya. Justeru itu, objektif kajian ini ialah untuk memperbaiki potensi terapeutik ZER dengan memuatkannya ke dalam NLC dan untuk menentukan kesan NLC termuat ZER (ZER-NLC) terhadap titisan sel leukemia T-limfoblas manusia (Jurkat) dan terhadap leukemia murin teraruh titisan sel WEHI-3B (leukemia mielomonosit). ZER-NLC telah yang dihasilkan melalui teknik kehomogenan tekanan tinggi (HPH) diciri mengandungi 5% lipid. ZER-NLC telah dicirikan melalui analisis zetasizer, kromatografi cecair prestasi tinggi fasa terbalik (RP-HPLC), mikroskopi elektron pancaran (TEM) dan imbasan (SEM), belauan X-sinaran sudut lebar (WAXR), kolorimeter imbasan pembezaan (DSC) dan sistem sel resapan Franz (FDC) dan hasil menunjukkan ianya stabil fizikal, saiz zarah (PS)  $52.58 \pm 0.10$  nm, potensi zeta (ZP) -  $25.03 \pm 1.24$  mV dan indeks polidispersiti (PI)  $0.290 \pm 0.004$  µm. Ini semua adalah ciri suatu sistem pembawa dan penghantar drug yang unggul.

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Sel Jurkat diguna untuk menentukan sifat antikanser ZER-NLC. Assai MTT, mikroskopi pendarfluor, elektron imbasan dan pancaran, analisis sitometri aliran selepas pewarnaan annexin V-FITC, assai kitaran sel dan TUNEL, assai kaspase -3, -8, -9 telah diguna. Kajian ini menunjukkan ZER-NLC secara tererti menindas (P<0.05) bersandarkan masa pemproliferatan sel Jurkat *in vitro* dengan IC<sub>50</sub> 12.5 ± 0.1, 9.09 ± 0,14 dan 5.64 ± 0.38 µg/mL, masing-masing pada jam 24, 48 dan 72. Kesan antipemproliferatan ZER-NLC terhadap sel Jurkat disabitkan dengan pengaruhan apoptosis melalui arah laluan mitokondrion (intrinsik). Mencit BALB/c diaruh untuk mendapat leukemia dengan satu suntikan WEHI-3B cell (1 × 10<sup>6</sup>)

cells/mencit) secara intraperitoneum. Kajian *in vivo* ini menunjukkan dos oral ZER-NLC pada 60 mg/kg telah merencat pemproliferatan sel leukemia dalam mencit BALB/c yang ternyata dengan penurunan populasi sel leukemia dalam limpa. Berasaskan penilaian histologi, mikroskopi elektron, imunokimia dan assai TUNEL, kesan ZER-NLC dalam perencatan leukemia ialah melalui apoptosis. Melalui sap Western blot dan qRT-PCR, sel limpa pada mencit leukemia yang diperlakukan ZER-NLC juga menunjukkan peningkatan penyataan protein Bax, Cyt-c, dan PARP, sambil penyataan protein Bcl-2 menurun. Pada masa sama, protein PARP dibelah daripada 116 kDa kepada 85 kDa. Penemuan ini menyarankan kesan *in vivo* ZER-NLC terhadap leukemia murin adalah juga/apoptosis melalui arah laluan mitokondrion.

Untuk menentukkan ketoksikan potensi ZER-NLC, sel mononukleus darah periferi manusia (PBMC) telah diperlakukan *in vitro* dengan kepekatan bersiri ZER-NLC sehingga 100 mg/mL dan mencit BALB/c diperlaku secara oral dengan ZER-NLC pada dos setinggi hingga 200 mg/kg. Perlakuan ini tidak menghasilkan sebarang petanda ketoksikan sama ada terhadap PBMC manusia atau mencit normal pada mana-mana dos yang diguna, menunjukkan ZER-NLC adalah selamat untuk diguna secara parenteral.

Sebagai kesimpulan, kajian ini menunjukkan pemuatan ZER ke dalam NLC tidak menjejas potensi terapeutik ZER dan kesan *in vitro* ZER-NLC terhadap sel leukemia manusia dan *in vivo* terhadap leukemia murin ialah apoptosis melalui arah laluan mitokondrion. Kajian ini menunjukkan ZER-NLC mempunyai potensi yang unggul untuk dikembangkan sebagai sistem pembawa dan penghantar drug dalam rawatan kanser.

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 $\bigcirc$ 

I certify that a Thesis Examination Committee has met on 21 February 2014 to conduct the final examination of Heshu Sulaiman Rahman on his thesis entitled "Anti-Leukemic Effects Of Zerumbone Nanoparticle On Human Jurkat T Lymphoblastoid Cell Lines *In Vitro* And Murine Leukemic WEHI-3B Model *In Vivo*" 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 Doctor of Philosophy.

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> **BUJANG BIN KIM HUAT, PhD** Professor and Dean School of Graduate Studies Universiti Putra Malaysia

Date:

# DECLARATION

## **Declaration by the student**

I hereby confirm that:

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# 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 slated in Rule 41 in Rules 2003 (Revision 2012-2013) were adhered to.

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- revealed significant (P < 0.05) increases in apoptotic cells after 6, 12, 24 and 48 h in ZER-NLC treated groups compared to that of untreated control group. Whereas significant (P < 0.05) (\*\*) reduction of viable cells in 6, 12, 24 and 48 h in ZER-NLCtreated groups was observed. (Comparisons were made with untreated control groups). ZER-NLC = zerumbone-loaded nanostructured lipid carrier.
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# LIST OF ABBREVIATIONS

	°C	degree Celsius
	®	trade mark
	μg	microgram
	μl	microlitre
	μm	micro meter
	2X	two fold
	Å	Angstrom
	AA	arachidonic acid
	AB	apoptotic body
	ACUC	animal care and use committee
	ADP	adenosine di phosphate
	Alb	albumin
	ALL	acute lymphocytic leukemia
	ALP	alkaline phosphatase
	ALT	alanine aminotransferase
	AML	acute myelogenous leukemia
	AO	acridine orange
	AST	aspartate aminotransferase
	ATCC	American type culture collection
	Bax	Bcl <sub>2</sub> associated x protein
	B-cell	B lymphocyte
	Bcl-2	B cell lymphoma 2
	Bcl-xL	B cell lymphoma extra large
	BDMA	benzyl dimethyl amine
	BHT	butylated hydroxytoulene
	BL	blebbing of cell membrane
	BSA	bovine serum albumen
	CB	conjugated bilirubin
	CC	chromatin condensation
	CD	cluster of differentiation

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CLL	chronic lymphocytic leukemia
Cm	centimeter
cm <sup>2</sup>	square centimetre
CML	chronic myelogenous leukemia
$CO_2$	carbon dioxide
COX-2	cyclooxygenase -2
CPD	critical point drier
Creat	creatinine
Cu Ka	copper anode
CXCR4	chemokine receptor type 4
Cyt-c	cytochrome c
DAB	3,3'-diaminobenzidine
DD	death domain
DDSA	dodecenyl succinic anhydride
DMSO	dimethyl sulphoxide
DNA	de <mark>oxyribo nucle</mark> ic acid
dNTP	deoxyribonucleotide
DPX	mounting media and section adhesive
DSC	differential scanning calorimetry
DTBN	5, 5 dithiohis-2-nitrobenzoic acid
DTT	dithiothreitol
Ε	intracellular redox potential
EA	early apoptosis
EBV	Epstein-Bar virus
ECL	enhanced chemiluminescence
EDTA	ethyl diamine tetra acetic acid
EE	entrapment efficiency
ELISA	enzyme linked immunosorbant assay
EtBr	ethidium bromide
FAS	TNF superfamily receptor 6
FCS	fetal calf serum

	FDC	franz diffusion cell
	FITC	fluorescein isothiocyanate
	FADD	Fas associated protein with death domain
	FLICE	FADD-like Interleukin-1β-converting enzyme
	FLIP	FLICE-inhibitory protein
	G	gage
	g	gram
	G0/G1	quiescent/ gap 1
	G2/M	gap 2/mitosis
	GGT	γ-Glutamyl transferase
	GSH	glutathione
	H&E	haematoxylin and eosin
	h	hour (s)
	Hcl	hydrochloric acid
	Hg	Mercury
	HPLC	high performance liquid chromatography
	HPO	hydrogenated palm oil
	HRP	horse radish peroxidase
	IAP	inhibitor of apoptosis protein
	IBS	Institute of Bioscience
	IC <sub>50</sub>	half-maximal inhibitory concentration
	ICAM-1	intercellular Adhesion Molecule 1
	IDT	Integrated DNA Technologies
	IDTE	Integrated DNA Technologies EDTA
	IgG	immunoglobulin
	IHC	immunohistochemistry
	IKK	inhibitor of nuclear factor kappa-B kinase
	Inc	Incorporation
	ΙκΒα	nuclear factor of kappa light polypeptide gene enhancer in B-cells
		inhibitor, alpha
	Kcps	kilo counts per second
	kDa	kilo Dalton
	Kg	Kilogram

	KH <sub>2</sub> PO <sub>4</sub>	potassium dihydrogen phosphate
	Kv	kilo volt
	L	litre
	LA	late apoptosis
	LD	loading capacity
	$LD_{50}$	lethal dose
	LIVES	Laboratory of Immunotherapeutic and Vaccines
	mA	milliamp
	MAKNA	National Cancer Council Malaysia
	MDA	malondialdehyde
	MeOH	methanol
	mg	milligram
	min	minute
	mL	millilitre
	Mm	micromolar
	mm	millimetre
	MMP-9	matrix metallopeptidase 9
	MNA	methyl nadic anhydride
	MN	mariginated nucleus
	MOSTI	Ministry of Science, Technology and Innovation
	MRI	magnetic resonance imaging
	MTT	3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide
	n	number
	NaCN	sodium cyanide
	NADPH	nicotinamide adenine dinucleotide phosphate
	NaOH	sodium hydroxide
	NBT	nitro blue tetrazolium
	NBT	nitro blue tetrazolium
	NF-ĸB	nuclear factor kappa-light-chain-enhancer of activated B cells
	NH <sub>4</sub> Cl	ammonium chloride
	NIK	NF-κB inducing kinase

	NK	natural killer
	NLC	nanostructured lipid carrier
	nm	nanometer
	nmol	nanomole
	NMR	nuclear magnetic resonance
	NTC	no template control
	OD	optical density
	P < 0.05	Probability values of less than alpha 0.05
	PARP	peroxisome proliferator activated receptor
	PBS	phosphate buffer saline
	PBST	phosphate buffer solution with triton X-100
	PCS	photon correlation spectroscopy
	PDI	Polydispersity Index
	pН	measurement for hydrogen ion concentration
	PhD	doctor of philosophy
	PI	propidium iodide
	PMSF	phenylmethanesulfonylfluoride or phenylmethylsulfonyl fluoride
	PS	Particle size
	PTA	phosphotungstic acid
	RIPA	radio immune precipitation assay
	RNAase	ribonuclease enzyme
	RP	reverse phase
	rpm	round per minute
	RPMI	Roswell park memorial institute medium
	RT	Reverse transcriptase
	S	synthesis phase
	SD	standard deviation
	SDS-	sodium dodecyl sulphate-polyacrylamide gel electrophoresis.
	PAGE	
	Sec	second (s)
	SEM	scanning electron microscope
	SN	secondary necrosis
	SOD	superoxide dismutase

SPSS	statistical package for the social sciences
survivin	baculoviral inhibitor of apoptosis repeat-containing 5
TB	total bilirubin
ТВ	tuberculin
TBA	thiobarbituric acid
TCA	tri-chloro-acetic acid
T-cell	T lymphocyte
TEM	transmission electron microscopy
TEP	tetra-ethoxy propane
TNF	tissue necrotizing factor
ТР	total protein
TPA	12-O-tetradecanoylphorbol-13-acetate
TPA	tetradecanoylphorbol-13-acetate
TRADD	tumor necrosis factor receptor-associated associated death domain
TRAF	tumor necrosis factor receptor-associated factor
TUNEL	Tdt-mediated dUTP Nick-end labelling
UPM	Universiti Putra Malaysia
USA	United States of America
UV	ultra violet
v/v	volume to volume
VC	viable cell
w/v	weight to volume
WHO	world Health Organization
WXRD	wide-angle x-ray diffraction
XIAP	X-linked inhibitor of apoptosis protein
XO	xanthine oxidase
Z	Zingiber
ZER	zerumbone
ZER-NLC	zerumbone loaded nanostructure lipid carrier
ZP	zeta potential
β-actin	beta actin
	SPSS survivin TB TB TBA TCA TCA TCA TCA TCA TCA TCA TA TA TA TA TA TA TA TA TA TA TA TA TA

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#### **CHAPTER ONE**

#### **INTRODUCTION**

Cancer is the major killer and dreaded disease caused by abnormal proliferation of body cells. These abnormal cells can block circulations, and damage organ functions which may lead to death. The annual incidence of cancer worldwide is estimated at 30,000. While the most common killer among malignancies is lung cancer, breast cancer poses the biggest threat (Lim, 2002).

With the increasing prevalence of cancer, patient are now turning to complementary and alternative medicine (CAM) to treat the disease (Chrystal et al., 2003). The CAM includes medical herbs and plant foods such as fruits, vegetables, and spices containing many biologically active phytochemicals with various health promoting effects (Lampe, 1999; Surh, 2003). It has long been established that a diet rich in fresh fruits, vegetables, seeds, grains and legumes, antioxidants and other beneficial compounds may help in prevention of cancer. Although diet is not a cure for cancer, it may help prevent some cancers from developing, while avoiding the some of the adverse effects from conventional treatments (Montbriand, 2000). However, using natural compounds in diets and supplements is still not a substitute for regular medical care. In cancers surgery, radiotherapy and chemotherapy are still the treatments of choice. However natural compounds may be considered as complementary medicine in the treatment these diseases (Golbeck et al., 2011). Natural compounds are not only sources of drugs or drug templates but in many instances they had been a source of discovery of novel biology that provided better understanding of target and pathway involved in the diseases processes as well as in the majority of cancer drugs used today (Da Rocha et al., 2001; Bhattacharya et al., 2011a). Between 60 to 75% of anticancer drugs are derived from natural compounds. It has been claimed that drugs derived from natural compounds are more efficacious for cancer patients than those manufactured synthetically (Golbeck et al., 2011). Currently, it is known that approximately 10,000 out of 500,000 plant species are likely to have medicinal substances of which most located in the rain forests, grasslands and fields. However, only a fraction of these plants have been analysed and investigated for their therapeutic potential. It is unfortunate that as a result of deforestation, many valuable medicinal herbs and plants are becoming rare and these precious inheritances are now lost (Srujana et al., 2012).



In Malaysia, studies on plants and herbs as potential sources of cancer therapeutics are on the rise. Among the plants being investigated for their therapeutic potential include *Typhonium flagelliforme* for treatment of leukemias (Mohan *et al.*, 2010a; Mohan *et al.*, 2010b), chalcone from *Boesenbergia rotunda* for lung cancers (Isa *et al.*, 2012), and *Elephantopus scaber* for human breast cancers (Ho *et al.*, 2011). At the same time, several active principles have been identified and investigated to include girinimbine from roots of *Murraya koenigii* for liver cancers (Syam *et al.*, 2011), dentatin from wild shrub *Clausena excavata* Burm for prostate cancers (Arbab *et al.*, 2012), kenaf seed oil from *Hibiscus cannabinus* (Foo *et al.*, 2011) and phenylbutenoids (Anassamy *et al.*, 2013) from *Zingiber cassumunar* Roxb for leukemias. The Zingiberaceae family of plants is found in tropical and subtropical areas, and approximately 161 species from 18 genera of this family are found in Peninsular Malaysia. The plant species belonging to the Zingiberaceae family have been reported to possess biological activities responsible for medicinal value. The ginger rhizome is generally recognized as safe, and it is used traditionally in local folklore medicine for various ailments (Ruslay *et al.*, 2007). *Zingiber zerumbet* (L.) Smith belonging to this family is an edible ginger, originating from South-East Asia and been cultivated for thousands of years (Vimala *et al.*, 1999). Generally, the rhizomes and the leaves are used for spice, tea, beverages and medical purposes, while the milky, mucilaginous substances of the pinecones are as shampoo and natural hair conditioner, especially in Asia and Hawaii (Sabu, 2003) contained several types of phytochemicals and the rhizome, in particular, has been used in traditional Oriental medicine for various human ailments in different parts of the globe, especially for the treatment of digestive conditions (Prakash *et al.*, 2011a).

Zerumbone (ZER) is a crystalline, monocyclic, sesquiterpene, phytochemical substance that was first isolated in 1956 from the essential volatile oil of rhizomes of *Zingiber zerumbet* (L.) Smith (Kitayama *et al.*, 2003). Several pharmacological potentials of ZER identified through several test models include potent and strong anticancer activity. ZER is poorly soluble in water and consequently poor oral bioavailability and delivery (Shegokar and Müller, 2010). Thus, there is need to improve delivery of this compound before its therapeutic potential can be realized. One of the approaches to increase solubility of ZER is by incorporation into nanocarriers and nanoparticles (Jens and Rainer, 2008). For this purpose, there are several nanocarriers that can be used to include solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), as well as lipid drug conjugates (Shaji and Jain, 2010a; Mistry *et al.*, 2011).

Leukemia is a cancer of the blood-forming cells and the most common cancer among children (Campana and Pu, 2008) caused by the production of immature and abnormal blood cells that are unable to perform normal functions (Annino *et al.*, 2002). In 2010 alone, leukaemia was diagnosed 10 times more often in adults than in children and it is more common in males than females (American Cancer Society, 2012). While in Malaysia, the incidence of leukemia ranked fourth among all cancers in males and fifth among females (Yeoh *et al.*, 2010). Currently, the most widely used anti-leukemia therapies are chemotherapy, radiotherapy, hormonal therapy, immune therapy and bone marrow transplantation. Generally, most of these treatments will damage healthy cells and tissues with short- to long-term side-effects. To avoid these side-effects extensive research are being conduct to discover innocuous therapeutic compounds as candidates for next generation anti-leukemic drugs. Although pharmaceutical companies prefer synthetic compounds to natural materials, the search for new and effective natural therapeutic agents which offer better survival rates and fewer side-effects, still persists among researchers worldwide (Butler, 2008).

This study attempts to incorporate ZER in NLC (ZER-NLC) and determine its effect on Jurkat T-lymphoblastic leukemia cells and WEHI-3B cell-induced myelomonocytic leukemia in mice. This study aimed to investigate the antiproliferative and cytotoxic



effects of ZER-NLC on human lymphocytic leukemia (Jurkat) cell line and WEHI-3B cell-induced myelomonocytic leukemia in mice.

# Hypothesis of the Study

- 1. ZER-NLC is cytotoxic to human lymphoblastic leukemia cell line.
- 2. ZER-NLC induces apoptosis of leukemia cells in mice induced to develop leukemia.

# **Objectives of the Study**

# **Main Objective**

To prepare, characterise ZER-NLC and evaluate its in vitro and in vivo anti-leukemic activities.

# Specific Objectives of the Research

- 1. To isolate ZER crystals from *Zingiber zerumbet* (L.) Smith, incorporate into NLC and characterise the ZER-NLC.
- 2. To determine the cytotoxicity of ZER-NLC on a human T-lymphoblastic leukemia (Jurkat) cell line through the assessment of cell morphology and biochemical parameters.
- 3. To determine the apoptotic effect of ZER-NLC on WEHI-3B cell-induced myelomonocytic leukemia in mice through tissue morphological and chemical assessment.
- 4. To optimize the concentration of Bax, Bcl-2, Cyt-c, and PARP in leukemic cells from mice with WEHI-3B cell-induced myelomonocytic leukemia.

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