



***EFFECT OF ZERUMBONE - LOADED NANOSTRUCTURED LIPID
CARRIER ON CANINE MAMMARY GLAND TUMOUR CELL LINE***

FOONG JIA NING

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EFFECT OF ZERUMBONE-LOADED NANOSTRUCTURED LIPID CARRIER
ON CANINE MAMMARY GLAND TUMOUR CELL LINE.

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CERTIFICATION

It is hereby certified that we have read this project paper entitled “Effect Of Zerumbone-Loaded Nanostructured Lipid Carrier On Canine Mammary Gland Tumour Cell Line”, by Foong Jia Ning and in our opinion is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999 – Project.

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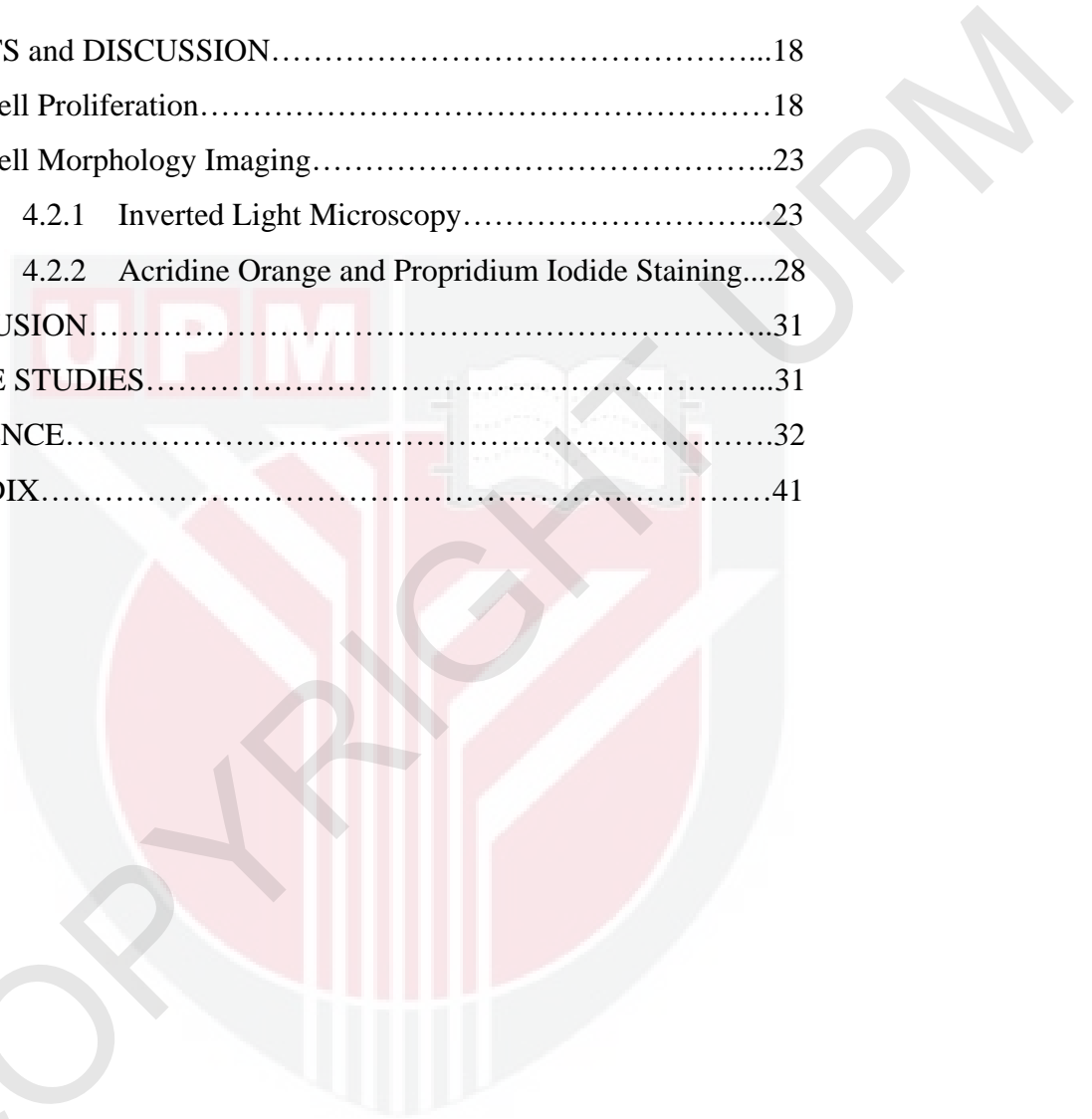
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LIST OF ABBREVIATIONS

ANOVA	Analysis Of Variance
AOPI	Acridine Orange and Propodium Iodide
CMT	Canine mammary gland tumour
DOX	Doxorubicin
DMSO	Dimethylsulphoxide
FBS	Foetal Bovine Serum
FPV	Faculty of Veterinary Medicine
GI50	Growth Inhibition Dose 50
hr	Hour
IBS	Institute Bioscience
IU	International Unit
LD50	Lethal Dose 50
μ L	Micro-litre
mL	Millilitre
M	Molar
NLC	Nanostructure Lipid Carrier
PBS	Phosphate Buffer Solution
RPMI	Roswell Park Memorial Institute
SLN	Solid Lipid Nanoparticles
SPSS	Statistical Package for the Social Sciences
UPM	University Putra Malaysia
UVH	University Veterinary Hospital
ZER	Zerumbone
ZER-NLC	Zerumbone-loaded Nanostructure Lipid Carrier

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan VPD 4999-Projek.

**KESAN PEMBAWA LIPID NANOSTRUKTUR TERISI ZERUMBON
TERHADAP TITISAN SEL TUMOR KELENJAR MAMA KANIN**

Oleh

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Penyelia: Prof. Dr Rasedee Abdullah

Zerumbon (ZER) daripada rizom halia liar, *Zingiber zerumbet* (L.) Smith adalah suatu sebatian diet semula jadi lipofilik yang diketahui mempunyai sifat antitumor, antikeradangan, antioksidan, antimikrob, antinosiseptif, pelindung hati dan imunopemodulatan. Bagaimanapun, penggunaan terapi ZER diganggu oleh kelarutan air buruk yang mengakibatkan kepada penyerapan and biokeperolehan and penghantaran kepada tisu sasar rendah. Untuk mengatasi kelemahan ini, ZER telah diisikan ke dalam pembawa lipid nanostuktur (NLC) (ZER-NLC). Dalam kajian ini kesan antikanser ZER-NLC ditentukan terhadap titisan sel (CMT-stylo) kelenjar mama kanin. Adalah dipostulat bahawa pengisian ZER ke dalam NLC tidak menjejaskan sifat

antikanser ZER. Menguna assai MTT (3-(4,5-dimetiltiazol-2-yl)-2,5-difeniltetrazolium bromida), daya hidup titisan sel CMT-stylo selepas perlakuan dengan 1.7 mM of ZER adalah 64.30 ± 9.87 , 42.06 ± 9.00 , $37.81 \pm 10.04\%$ dan dengan 1.8 mM ZER-NLC adalah 102.77 ± 12.68 , 38.42 ± 9.16 , $41.13 \pm 11.72\%$, masing-masing pada 24, 48 and 72 jam perlakuan. Dos maut separuh maksimum (LD50) untuk ZER dan ZER-NLC selepas 72 perlakuan, masing-masing ialah 100 dan 90 μM . Dos perencatan pertumbuhan separuh maksimum (GI50) untuk ZER dan ZER-NLC selepas 72 jam perlakuan, masing-masing adalah 20 dan 25 μm . Kesan antikanser ZER dan ZER-NLC juga digambarkan menguna kaedah pewarnaan dedua akridina jingga/propidium iodida. Zerumbon dan ZER-NLC mengaruh apoptosis terhadap sel CMT-stylo yang ternyata sebagai pemleban membran, penjidaran nukleus dan pengkondensasian kromatin. Kajian ini kali pertamanya menunjukkan yang ZER-NLC sebagai sistem penghantar drug ada potensi berkesan dalam rawatan tumor kelenjar mama kanin. ZER-NLC adalah inovatif, novel, selamat untuk terapi kanser.

Kata kunci: zerumbon, pembawa lipid nanostruktur, pembawa lipid nanostruktur terisi zerumbon, tumor kelenjar mama kanin, antikanser, apoptosis.

ABSTRACT

An abstract of the project paper presented to Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999-Project.

**EFFECT OF ZERUMBONE-LOADED NANOSTRUCTURED LIPID CARRIER
ON CANINE MAMMARY GLAND TUMOUR CELL LINE.****By****Foong Jia Ning****2015****Supervisor: Prof. Dr. Rasedee Abdullah**

Zerumbone (ZER) from the rhizomes of the wild ginger, *Zingiber zerumbet* (L.) Smith, is a natural dietary lipophilic compound with antitumour, antiinflammatory, antioxidant, antimicrobial, antinociceptive, hepatoprotective and immunomodulatory properties. However, therapeutic application of zerumbone is plagued by poor water-solubility and subsequent poor absorption, bioavailability and delivery to target tissues. To overcome this limitation, ZER was loaded into nanostructured lipid carrier (NLC) (ZER-NLC). In this study the anticancer effect of ZER-NLC was determined on a canine mammary gland tumour (CMT-stylo) cell line. It is postulated that loading of ZER into NLC does not compromise the anticancer properties ZER. Using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, the proliferation of CMT-stylo cells after treatment with 1.7 mM of ZER was at 64.30 ± 9.87 , 42.06 ± 9.00 ,

37.81±10.04% while with 1.8 mM ZER-NLC at 102.77±12.68, 38.42±9.16, 41.13±11.72% after 24, 48 and 72 hr treatment, respectively. The half maximal lethal dose (LD50) for ZER and ZER-NLC at 72 hr was 100 and 90 µM, respectively. The half maximal growth inhibition dose (GI50) for ZER and ZER-NLC after 72 hr treatment was 20 and 25 µM, respectively. The anticancer effect of ZER and ZER-NLC was also visualised using the acridine orange/propidium iodide double staining method. Zerumbone and ZER-NLC induced apoptosis of CMT-stylo cells as shown by the membrane blebbing, nucleus margination and chromatin condensation. This study, for the first time, shows that ZER-NLC is a potentially effective drug delivery system for the treatment of canine mammary gland tumours. The ZER-NLC is innovative, novel, and safe, for cancer therapy.

Keywords: zerumbone, nanostructured lipid carrier, zerumbone-loaded nanostructured lipid carrier, canine mammary gland tumour, anticancer, apoptosis.

1.0 INTRODUCTION

Zerumbone (ZER), is a major compound from the essential volatile oil of edible wild ginger rhizomes, *Zingiber zerumbet* (L.) Smith (Kitayama *et al.*, 2003; Rahman *et al.*, 2013). It has been shown to possess antitumor, antiinflammatory, antioxidant, antimicrobial, antinociceptive, hepatoprotective and immunomodulatory activity (Abdelwahab *et al.*, 2009; 2011; Rahman *et al.*, 2013). However, therapeutic application of zerumbone has been plagued by poor water solubility and subsequent poor absorption, bioavailability and delivery to target tissues and organs eventhough it has been credited with numerous pharmacological potentials (Rahman *et al.*, 2013; Rahman *et al.*, 2014). Therefore, alternative method of drug administration; for example, a drug carrier system is needed to overcome this problem. Beginning in 1990s, advances in nanotechnology has resulted in the production of a lipid carrier, nanostructured lipid carriers (NLC) that is able to load lipophilic drug to enhance drug delivery, loading stability, controlled-release and tolerability of drugs (Abbasalipourkabar *et al.*, 2011b).

In order to overcome ZER poor water-solubility and subsequently increase its therapeutic application, ZER can be loaded into NLC, which is an effective drug carrier for the compound (Abdelwahab *et al.*, 2011; Abbasalipourkabar *et al.*, 2011b; Souto, 2004; Müller *et al.*, 2000). A study

of apoptogenic effect of ZER-loaded NLC (ZER-NLC) on an acute human lymphoblastic leukemia (Jurkat) cell line *in vitro* showed that ZER-NLC is a potentially effective drug delivery system for the treatment of leukemia (Rahman *et al.*, 2014). To date, there is no study done on the anticancer effect of ZER-NLC on canine mammary gland tumour cell line.

Therefore, the objective of this project is to determine the anticancer effect of ZER-NLC on a canine mammary gland tumour cell line (CMT-stylo). We hypothesised that ZER-NLC has cytotoxic effect on the CMT-stylo cell line via the induction of apoptosis and the incorporation of ZER into NLC does not alter the anticancer effect of ZER.

The objectives of this study are to:

- 1) determine the anticancer effect of ZER-NLC on the mammary gland tumour (CMT-stylo) cell line.
- 2) compare the cytotoxic effects of ZER and ZER-NLC.
- 3) determine the mechanism of cytotoxicity of ZER-NLC on CMT-stylo cell line.

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