



**UNIVERSITI PUTRA MALAYSIA**

***THE EFFECTS OF ENROFLOXACIN IN COMBINATION OF  
DOXORUBICIN ON CELL VIABILITY IN CANINE MAMMARY GLAND  
TUMOR CELLS IN VITRO***

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**FPV 2017 65**

**The Effects of Enrofloxacin in Combination of Doxorubicin on Cell Viability in  
Canine Mammary Gland Tumor Cells *in Vitro***

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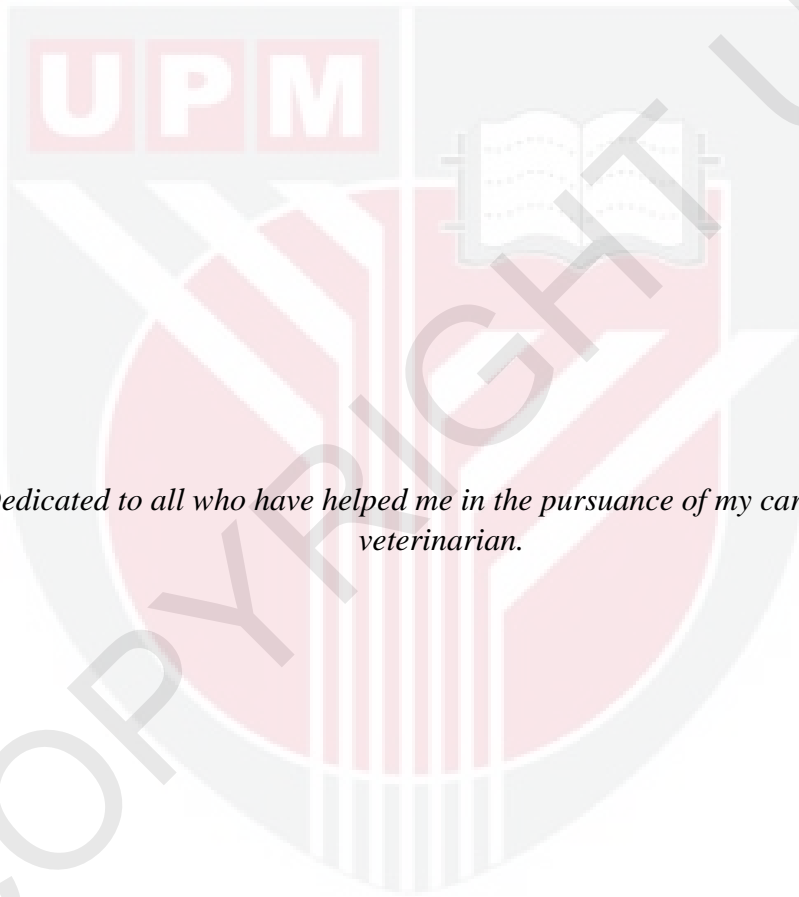
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*Dedicated to all who have helped me in the pursuance of my career as a  
veterinarian.*

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**LIST OF ABBREVIATIONS**

ABC-t	ATP- dependent binding cassette transporter
AO	Acridine Orange
BCRP	breast cancer resistance gene
CMT-Stylo	Canine Mammary Tumor – Stylo
DMSO	dimethyl sulfoxide
DMEM	Dulbecco's Modified Eagle Medium
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
PARP	Poly [ADP-ribose] polymerase
PGP	P- Glycoprotein
PBS	phosphate buffer saline
PI	Propidium Iodide



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## ABSTRAK

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

### **Kesan Rawatan Enrofloxacin Dalam Kombinasi Doxorubicin Pada Daya Maju Sel di Dalam Sel Ketumbuhan Kelenjar Mamari Anjing *in Vitro***

Oleh

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2017

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Ketumbuhan kelenjar mamari adalah biasa di kalangan anjing betina dewasa. Ketumbuhan utama boleh dibuang melalui pembedahan bagaimanapun, metastasis ke kelenjar limfa dan paru-paru boleh wujud dalam peringkat lanjutan yang merupakan titik akhir yang biasa pada akhirnya membawa kepada kematian atau keputusan untuk euthanasia. Kemoterapi menggunakan ubat sitotoksik seperti doxorubicin intravena kepada anjing diperingkat akhir atau dengan ketumbuhan adenokarsinoma gred tinggi. Peranan doxorubicin adalah untuk mencegah metastasis jauh dan berulang tempatan, walaupun cardiotoxicity adalah dos biasa yang menghadkan kesan sampingan ubat ini. Baru-baru ini, enrofloxacin agen antibiotik bakteria didapati menunjukkan aktiviti anti-proliferatif dalam kanser kolon manusia dan sel-sel osteosarcoma anjing *in vitro*. Dengan

ini hipotesis kajian ini ialah enrofloxacin akan mengurangkan daya maju sel dengan cara yang bergantung kepada dos dan ia juga akan meningkatkan kesan sitotoksik doxorubicin pada sel-sel ketumbuhan kelenjar mamari anjing. Objektif kajian ini adalah untuk menentukan kesan anti-proliferatif enrofloxacin dalam garis sel tumor mamari anjing, semata-mata dan dalam kombinasi dengan doxorubicin. MTT dan AO / asai PI dijalankan ke atas sel-sel CMT-Stylo untuk menentukan percambahan sel dan daya maju sel. Kepekatan doxorubicin (50nm, 100nm, 200nm, 400nm) dan enrofloxacin (1, 3,125, 6.25, 12.5, 25, 50, 100  $\mu\text{g} / \text{mL}$ ) telah dipilih berdasarkan kajian sebelum dan IC50 bagi *cell line*. Purata tiga eksperimen replika di 96 yang plat untuk 24, 48 dan 72 jam telah ditentukan dan analisis statistik telah dilakukan. Didapati kekurangan percambahan sel dalam sel-sel yang dirawat dengan enrofloxacin sahaja atau dalam kombinasi dengan doxorubicin dalam satu masa yang sama bergantung kepada dos. Eksperimen menyimpulkan bahawa gabungan ubat-ubatan ini mempunyai kesan sinergi dalam sel Kelenjar mamari anjing CMT-Stylo.

Kata Kunci: enrofloxacin, doxorubicin, tumor mamari, MTT, *in vitro*

**ABSTRACT**

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

**The Effects of Enrofloxacin Treatment in combination of Doxorubicin on Cell Viability in Canine Mammary Gland Tumor Cells *in Vitro*****By****Carolyn Kai-Ling Yang****2017****Supervisor: Dr. Gayathri Thevi Selvarajah**

Mammary gland tumors are common among intact adult female dogs. Primary tumors can be surgically removed however, metastasis to lymph nodes and lungs can be present in advanced stages which is a common end point ultimately leading to death or decision for euthanasia. Chemotherapy using cytotoxic drug such as intravenous doxorubicin is delivered to dogs with advanced stages or with high grade adenocarcinoma tumors. The role of doxorubicin is to prevent distant metastasis and local recurrence, although cardiotoxicity is a common dose limiting side effect of this drug. Recently, enrofloxacin a bactericidal antibiotic agent was found to demonstrate anti-proliferative activity in human colon cancer and canine osteosarcoma cells *in vitro*. With this the hypothesis of this study is that enrofloxacin will reduce cell viability in a dose dependent manner and it will also enhance the cytotoxic effects of doxorubicin on canine mammary

tumor cells. The objectives of this study are to determine the anti-proliferative effects of enrofloxacin in canine mammary tumor cell line, alone and in combination with doxorubicin. MTT and AO/PI assays were conducted on CMT-Stylo cells to determine cell proliferation and cell viability. Concentrations of doxorubicin (50nM, 100nM, 200nM, 400nM) and enrofloxacin (1, 3.125, 6.25, 12.5, 25, 50, 100  $\mu\text{g}/\text{mL}$ ) were chosen based on previous studies and IC50 for the cell line. Average of three replicate experiments in 96 well-plates for 24, 48 and 72 hours was determined and statistical analysis was done. There was reduced cell proliferation in cells treated with enroflocaxin alone or in combination with doxorubicin in a time and dose-dependent manner. The experiment concluded that the combination of these drugs has synergistic effects on canine mammary CMT-Stylo cells.

**Keywords:** enrofloxacin, doxorubicin, mammary tumor, MTT, *in vitro*

## 1.0 INTRODUCTION

Cancer has been a problem in today's society. Humans and animals alike have been plagued of developing cancer due to various reasons. So far, there is still no standard cure for cancer. In humans and dogs alike, breast cancer remains the number one cancer diagnosed (Caceres *et al.*, 2016). Mammary tumors are more common in intact and aged female dogs. Up to a total of 50% of swelling observed on the mammary glands in intact bitches can be diagnosed as hyperplasia and remaining neoplasia which includes chances of it being benign and malignant (Novosad, 2003). Further diagnostic procedures must be done to see if the tumor is extensive or not. This includes diagnostic imaging, cytology, as well as histopathology (Kabiru *et al.*, 2012). Based on the findings, the treatment will be made in order to battle the cancer cells.

Enrofloxacin has been in the market for many years. Coming from the Nalidixic family, enrofloxacin is the first fluoroquinolone to be developed and patented for animal use. It comes from the quinolone subfamily and the primary use of this drug is for its antibacterial properties. With the adding of the fluor atom, this improves the antibacterial spectrum and its effectiveness (Trouchon & Lefebvre, 2016). Through the extensive research of enrofloxacin, it has been found that enrofloxacin might have a way of being used for cancer as other studies suggested. Main theory of enrofloxacin is that it might have a way of potentiating the effect of the cytotoxic drug if added. This means that it helps further the process of killing the cancerous cells (Boudreaux *et al.*, 2012).

Therefore, the objective of this experiment is to explore the potential of the enrofloxacin for its anticancer effects. This will be done on canine mammary tumor cell line (CMT-Stylo). It was hypothesized that enrofloxacin will reduce cell viability in a dose-dependent manner in a canine mammary tumor cell line and that enrofloxacin would enhance the cytotoxic effects of chemotherapy on canine mammary gland tumors cells.

The objectives of this study are:

1. To determine the anti-cancer effects of Enrofloxacin in a canine mammary tumor cell line *in vitro*.
2. To evaluate the anti-cancer effects of enrofloxacin in combination with doxorubicin in a canine mammary tumor cell line.



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