UNIVERSITI PUTRA MALAYSIA

IN VITRO AND IN VIVO EFFECTS OF SELECTED MALAYSIAN MEDICINAL PLANT EXTRACTS ON Toxoplasma gondii

KUMARESWARAN A/L DEVANTHRAN

FPSK(M) 2016 78
COPYRIGHT

All materials contained within this thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

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

**IN VITRO AND IN VIVO EFFECTS OF SELECTED MALAYSIAN MEDICINAL PLANT EXTRACTS ON Toxoplasma gondii**

By

KUMARESWARAN A/L DEVANTHRAN

April 2016

Chairman: Professor Hj Wan Omar Abdullah, PhD

Faculty : Medicine and Health Sciences

The sporozoan parasite *Toxoplasma gondii* is tissue parasite commonly causing infection to humans and animals, particularly in the immunocompromised hosts. Latest report stated that approximately one third of the global population has been infected with *T. gondii*. The World Health Organisation considers toxoplasmosis as one of the major parasitic diseases infecting human in the developing countries. General therapeutic drug for toxoplasmosis is pyrimethamine. Since it presents several adverse side effects, the need to develop new drugs for this condition is critical.

The aim of this study is to investigate anti-toxoplasma effect of some selected medicinal plants. Assays were developed to determine the anti-toxoplasma effects *in vitro* and *in vivo*. Four medicinal plants have been used, which are *Tinospora crispa*, *Piper sarmentosum*, *Andrographis paniculata* and *Curcuma longa*. The plants are reputed in traditional medicine for many treatments of diseases.

These medicinal plants reported in literature as antimalarial agent, were evaluated for their *in vitro* cytotoxicity activity against mammalian cell lines, Vero cell line, which was used as host cell in this anti-toxoplasma activity study. Methyl thiazolyl diphenyl tetrazolium (MTT) assay was used to measure *in vitro* cytotoxicity activity. The cell culture-based assays were performed in this study to evaluate the plant extract and determine their effectiveness in inhibiting the growth of *T. gondii* *in vitro*.

In *in vivo* experiment, survival analysis was conducted to estimate the survival time of *T. gondii* infected mice treated with plant extract. *T. gondii* brain cysts were inoculated orally in mice. In each study, three groups of mice were assigned to treatment with plant extract prior to *T. gondii* infection (prophylactic), after infection (therapeutic), or left untreated (infected untreated control). The plant extract effect on toxoplasmosis was evaluated by the assessment of survival rate and brain cyst burden.
All four plant extracts were found non toxic towards Vero cells. The IC\textsubscript{50} value for all plant ethanolic extracts were above 100µg/ml. The least toxicity was the extract of \textit{C. longa} followed by \textit{T. crispa}, \textit{P. sarmentosum} and \textit{A. paniculata}. \textit{T. gondii} tachyzoites was inhibited by plant extracts in Vero cells by concentration-dependent manner. Even at low concentration as at 25µg/ml, \textit{T. crispa} and \textit{P. sarmentosum} extract dramatically inhibited \textit{T. gondii} tachyzoites in Vero cells. \textit{T. crispa} extract showed the greatest inhibition on \textit{T. gondii} tachyzoites growth in Vero cells followed by \textit{P. sarmentosum}, \textit{A. paniculata} and \textit{C. longa}. \textit{T. crispa} and \textit{P. sarmentosum} extracts were comparable with positive control, clindamycin.

\textit{T. crispa} extract was found as a potent anti-toxoplasma agent followed by \textit{P. sarmentosum}, \textit{A. paniculata} and \textit{C. longa} for both \textit{in vitro} and \textit{in vivo} studies. In infection induced by inoculation of cysts of \textit{T. gondii}, plant extract in prophylactic or therapeutic regimens significantly enhanced protection of infected mice against death. Delayed deaths of treated mice compare to untreated mice were observed throughout the 60 days observation period. The brains of infected mice treated with plant extract prophylactic or therapeutic groups showed low brain cyst burden compared to the infected untreated control. \textit{T. crispa} and \textit{P. sarmentosum} extracts reduced the brain cyst count almost to 25% compared to untreated infected mice.

These results suggest that all four plant extracts have potent anti-toxoplasma effect against \textit{T. gondii} especially \textit{T. crispa} and \textit{P. sarmentosum} extracts. These plant extracts can be exploited for development of alternative medicine to treat \textit{T. gondii} infections.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai
memenuhi keperluan untuk ijazah Master Sains

KESAN IN VITRO DAN IN VIVO EKSTRAK TUMBUHAN HERBA
MALAYSIA TERHADAP PARASIT Toxoplasma gondii

Oleh

KUMARESWARAN A/L DEVANTHRAN

April 2016

Pengerusi: Profesor Hj Wan Omar Abdullah, PhD
Fakulti: Perubatan dan Sains Kesihatan

Parasit Toxoplasma gondii adalah parasit tisu yang menyebabkan jangkitan kepada
manusia dan haiwan, terutamanya dalam perumah yang mempunyai sistem imun yang
lemah. Satu pertiga daripada jumlah penduduk dunia kini telah dijangkiti parasit ini.
Pertubuhan Kesihatan Dunia mempertimbangkan toksoplasmosis sebagai salah satu
penyakit parasit utama menjangkiti manusia di kalangan negara membangun. Ubat
umum terapeutik untuk merawat toksoplasmosis adalah ‘pyrimethamine’. Memandangkan
ubat tersebut menyebabkan beberapa kesan sampingan yang buruk, keperluan untuk
menghasilkan dan menilai ubat-ubatan baru adalah penting.

Tujuan kajian ini adalah untuk mengkaji kesan anti-toksoplasma daripada herba.
Kajian telah direka untuk menentukan kesan anti-toksoplasmal in vitro dan in vivo.
Empat herba telah digunakan dalam kajian ini. Herba tersebut ialah Tinospora crispa,
Piper sarmentosum, Andrographis paniculata dan Curcuma longa. Herba tersebut
biasa digunakan dalam perubatan tradisional untuk merawat pelbagai penyakit.

Herba tersebut telah dilaporkan mempunyai kesan terhadap parasit malaria. Oleh yang
demikian, herba tersebut telah dimilai aktiviti ketoksikan secara in vitro terhadap sel
mamalia; sel Vero yang digunakan sebagai sel perumah untuk kajian aktiviti anti-
toksoplasma. Aktiviti ketoksikan in vitro dikenalpasti melalui kajian ‘methyl thiazolyl
diphenyl tetrazolium’. Kajian berasaskan kultur sel telah dijalankan untuk menilai
ekstrak herba yang berkesan untuk merencat aktiviti T. gondii dalam sel Vero melalui
kaedah in vitro.

Dalam eksperimen in vivo, analisis jangka hayat hidup telah dijalankan untuk
menganggarkan jangka hayat tikus yang dijangkiti T. gondii yang dirawat dengan
ekstrak herba. Sista otak yang mengandungi T. gondii disuntik melalui mulut pada
tikus. Dalam setiap kajian, tiga kumpulan rawatan tikus telah direka. Kumpulan
rawatan tersebut adalah rawatan ekstrak herba sebelum jangkitan (pencegahan),
Selepas jangkitan (terapeutik), dan kumpulan tanpa rawatan herba. Kesan ekstrak herba terhadap toksoplasmosis telah dinilai melalui pemerhatian jangka hayat hidup tikus dan beban sista otak tikus.


Ekstrak *T. crispa* menghasilkan kesan anti-toksoplasma terbaik diikuti oleh *P. sarmentosum*, *A. paniculata* dan *C. longa* dalam kedua-dua kajian *in vitro* dan *in vivo*. Dalam jangkitan yang disebabkan oleh sista otak *T. gondii*, ekstrak herba dalam kumpulan pencegahan dan terapeutik telah meningkatkan perlindungan tikus yang dijangkiti daripada kematian. Pengurangan jangka hayat hidup tikus tanpa rawatan berbanding tikus yang dirawat telah diperhatikan sepanjang tempoh pemerhatian 60 hari. Tikus yang dijangkiti dan dirawat dengan ekstrak herba dalam kumpulan pencegahan dan terapeutik menunjukkan beban sista otak yang rendah berbanding dengan kumpulan tikus yang dijangkiti tanpa rawatan. Ekstrak *T. crispa* dan *P. sarmentosum* telah mengurangkan beban sista otak hampir 25% berbanding dengan tikus yang dijangkiti tanpa rawatan.

Kajian ini menunjukkan bahawa kesemua ekstrak herba mempunyai kesan anti-toksoplasma terhadap *T. gondii* terutamanya ekstrak *T. crispa* dan *P. sarmentosum*. Ekstrak herba ini boleh dieksploitasi untuk pembangunan perubatan alternatif bagi merawat jangkitan *T. gondii*. 
ACKNOWLEDGEMENTS

Firstly, I would like to express my appreciation to my respected supervisor Professor, Dr. Hj. Wan Omar Abdullah and my utmost gratitude to him for his supervision throughout my study. Professor Wan was my research mentor in providing me with guidance, expertise and encouragement without which I could have never completed this study. His enthusiasm and support were the great factors contributing to all of my physical, intellectual, moral and spiritual upliftments. I can never thank him enough for all the time he has invested in me, and he will be an unforgettable figure in my life.

I am delighted to express my gratitude to my co supervisor Dr Ngah Zasmy Unyah for his patience, progressive advices and guidance throughout my project, without him I won’t be able to complete this research. Special appreciation for all the people in my lab; Medical Parasitology lab especially Cik Norhanim and Pn. Siti Farah for helping me with my research. Special thanks also to my dear lab mates who were part of my research life in laboratory as well as outside campus life

I extend my sincere thanks to Animal Experimental Unit, Virology Lab, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Institute of Medical Research, Universiti Malaya and Forest Research Institute Malaysia for providing great assistance for my research. I would like to acknowledge UPM for providing Graduate Research Fellowship (UPM/SPS/GS37441), and Research University Grant Scheme, UPM (RUGS 04-02-12-1763RU) as well as Ministry of Higher Education (MOHE) Malaysia for providing MyMaster [KPM(B) 880730055563] scholarship. Without the funding and support this research would not become a reality.

My special thanks to my family for patiently putting up with me emotionally and financially throughout my studies. Also not forgetting their love and continuous support and encouragement throughout my study. Last but never the least, is God Almighty for allowing me to finish this thesis, overcoming many obstacles and preserve my sanity through my research period.
I certify that a Thesis Examination Committee has met on (date of viva voce) to conduct the final examination of Kumareswaran A/L Devanthran on his thesis entitled “In vitro and in vivo effects of extracts of selected Malaysian plants on *Toxoplasma gondii*” 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:

**Prof. Dr. Zamberi Sekawi, MPath**  
Professor  
Department of Medical Microbiology and Parasitology  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Rusliza Basir, PhD**  
Associate Professor  
Department of Anatomy  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Nasuruddin Hj. Abdullah, MMed**  
Professor  
Department of Pathology and Laboratory Medicine  
Kulliyah of Medicine  
International Islamic University Malaysia  
(External Examiner)

(Prof. Dr. Zulkarnain Zainal)  
Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia  
Date:
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the supervisory committee are as follows:

**Hj. Wan Omar Abdullah, PhD**  
Professor  
Faculty of Medicine and Health Sciences  
University Putra Malaysia  
(Chairman)

**Ngah Zasmy A/L Unyah, PhD**  
Senior Lecturer  
Faculty of Medicine and Health Sciences  
University Putra Malaysia  
(Member)

**BUJANG KIM HUAT, 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 other 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 the 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 software.

Signature: ------------------------- Date: ----------------------------

Name and Matric No.: Kumareswaran A/L Devanthran, GS37441
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) are adhered to:

Signature: _____________________________

Name of Chairman of Supervisory Committee: Prof. Dr. Hj. Wan Omar Abdullah

Signature: _______________________________

Name of Member of Supervisory Committee: Dr. Ngah Zasmy Unyah
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xvi</td>
</tr>
</tbody>
</table>

CHAPTER

1 INTRODUCTION
1.1 Objectives 1
1.2 Specific Objectives 2

2 LITERATURE REVIEW
2.1 Introduction 3
2.2 Apicomplexan Parasites 3
2.3 Toxoplasmosis 4
2.4 Mode of transmission 4
2.5 Life cycle 5
2.6 T. gondii host cell invasion 7
2.7 Signs and symptoms 8
2.8 Epidemiology 9
2.9 Associated risk factors 10
2.10 Morphology of T. gondii 11
2.11 Diagnosis of toxoplasmosis 13
2.12 Histological findings 14
2.13 Prevention of Toxoplasma gondii infection 14
2.14 Treatment for toxoplasmosis 14
2.15 Clinical manifestation 15
  2.15.1 Healthy individuals 15
  2.15.2 Pregnant women 15
  2.15.3 Infants 15
  2.15.4 Immune deficiency individuals 16
2.16 Plant selection 16
  2.16.1 Tinospora crispa 16
  2.16.2 Piper sarmentosum 17
  2.16.3 Andrographis paniculata 18
  2.16.4 Curcuma longa 19

3 MATERIAL AND METHODS 20
3.1 Materials 20
  3.1.1 Plant material 20
  3.1.2 Host cell 20
  3.1.3 Reagents and chemicals 20
3.1.4 Parasite
3.1.5 Animal
3.2 Methods
3.2.1 Plant preparations
3.2.2 Extraction
3.2.3 Culturing Vero cell
3.2.4 Parasites
3.2.4.1 Maintenance of RH strain of *T. gondii* in mice
3.2.4.2 Maintenance of ME-49 strain of *T. gondii* in mice
3.2.5 Culturing of *T. gondii* in Vero cell
3.2.6 Trypsin/EDTA treatment of host cells
3.2.7 Freezing and defrosting of *T. gondii* parasite
3.2.8 Determination of amount of parasites
3.2.9 Preparation of extract dilutions for *in vitro* experiment
3.2.10 Evaluating drug and plant extracts toxicity
3.2.10.1 MTT assay
3.2.10.2 Evaluating anti toxo-plasma activity
3.2.11 Microscopic observation
3.2.11.1 Observation of Vero cell
3.2.11.2 Observation of the tachyzoites
3.2.12 Inoculum preparations
3.2.13 Experiment Grouping
3.2.14 Survival analysis on mice
3.2.15 Brain cyst burden
3.2.16 Statistical analysis

4 RESULTS
4.1 Determination of cytotoxicity
4.1.1 Toxicity of clindamycin on Vero cell
4.1.2 Toxicity of plant extract on Vero cell
4.2 Observing the effect of treatment on Vero cell
4.3 Anti-Toxoplasma activity
4.4 Observing the effect of anti-*T. gondii* under microscope
4.5 Clinical behaviour
4.6 Survival analysis
4.7 Brain cyst burden

5 DISCUSSION
5.1 Determination of cytotoxicity
5.2 Anti-Toxoplasma activity of plant extracts
5.3 Other antiparasitic effect of plant extracts
5.4 Microscopic observation of cell line and parasite
5.5 *In vivo* experiment
5.6 Drug resistance in *T. gondii* treatment

6 CONCLUSION, LIMITATION AND FUTURE RECOMMENDATIONS
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>REFERENCES</td>
<td>58</td>
</tr>
<tr>
<td>APPENDICES</td>
<td>73</td>
</tr>
<tr>
<td>BIODATA OF STUDENT</td>
<td>85</td>
</tr>
<tr>
<td>LIST OF PUBLICATIONS</td>
<td>86</td>
</tr>
</tbody>
</table>

xii
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Voucher specimen of plants</td>
</tr>
<tr>
<td>4.1</td>
<td>Cytotoxicity against Vero cell</td>
</tr>
<tr>
<td>4.2</td>
<td>Cytotoxicity against <em>T. gondii</em> tachyzoites</td>
</tr>
<tr>
<td>4.3</td>
<td>Brain cyst count in mice infected with <em>T. gondii</em></td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>2.1</td>
<td>The life cycle of <em>Toxoplasma gondii</em></td>
</tr>
<tr>
<td>2.2</td>
<td>The life cycle of <em>Toxoplasma gondii</em> inside organism</td>
</tr>
<tr>
<td>2.3</td>
<td>Worldwide seroprevalence of the parasite <em>Toxoplasma gondii</em></td>
</tr>
<tr>
<td>2.4</td>
<td>Tachyzoite stage of <em>T. gondii</em></td>
</tr>
<tr>
<td>2.5</td>
<td>Cyst stage of <em>T. gondii</em></td>
</tr>
<tr>
<td>3.1</td>
<td>Experimental design of herbal against <em>T. gondii in mice</em></td>
</tr>
<tr>
<td>4.1</td>
<td>Cytotoxicity assay against Vero cell</td>
</tr>
<tr>
<td>4.2</td>
<td>Morphology of Vero cells after cytotoxicity assay</td>
</tr>
<tr>
<td>4.3</td>
<td>Anti-<em>Toxoplasma</em> assay</td>
</tr>
<tr>
<td>4.4</td>
<td>Anti-<em>T. gondii</em> activity of RPMI medium</td>
</tr>
<tr>
<td>4.5</td>
<td>Anti-<em>T. gondii</em> activity of clindamycin</td>
</tr>
<tr>
<td>4.6</td>
<td>Anti-<em>T. gondii</em> activity of <em>T. crispa</em></td>
</tr>
<tr>
<td>4.7</td>
<td>Anti-<em>T. gondii</em> activity of <em>P. sarmentosum</em></td>
</tr>
<tr>
<td>4.8</td>
<td>Anti-<em>T. gondii</em> activity of <em>A. paniculata</em></td>
</tr>
<tr>
<td>4.9</td>
<td>Anti-<em>T. gondii</em> activity of <em>C. longa</em></td>
</tr>
<tr>
<td>4.10</td>
<td>Survival analysis of mice treated with clindamycin</td>
</tr>
<tr>
<td>4.11</td>
<td>Survival analysis of mice treated with <em>T. crispa</em></td>
</tr>
<tr>
<td>4.12</td>
<td>Survival analysis of mice treated with <em>P. sarmentosum</em></td>
</tr>
<tr>
<td>4.13</td>
<td>Survival analysis of mice treated with <em>A. paniculata</em></td>
</tr>
<tr>
<td>4.14</td>
<td>Survival analysis of mice treated with <em>C. longa</em></td>
</tr>
<tr>
<td>4.15</td>
<td>Survival analysis of prophylactic treatment mice</td>
</tr>
<tr>
<td>4.16</td>
<td>Survival analysis of therapeutic treatment mice</td>
</tr>
<tr>
<td>4.17</td>
<td>Brain cyst burden in mice</td>
</tr>
</tbody>
</table>
# LIST OF APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Animal Use and Care Committee Approval</td>
</tr>
<tr>
<td>II</td>
<td><em>Piper sarmentosum</em> washing and drying</td>
</tr>
<tr>
<td>III</td>
<td><em>Andrographis paniculata</em> collection and drying</td>
</tr>
<tr>
<td>IV</td>
<td><em>Tinospora crispa</em> collection and drying</td>
</tr>
<tr>
<td>V</td>
<td><em>Curcuma longa</em></td>
</tr>
<tr>
<td>VI</td>
<td>Plant extraction and filtration</td>
</tr>
<tr>
<td>VII</td>
<td>Evaporating plant extract</td>
</tr>
<tr>
<td>VIII</td>
<td>Cell culture</td>
</tr>
<tr>
<td>IX</td>
<td>Cytotoxicity assay</td>
</tr>
<tr>
<td>X</td>
<td>Healthy and sick mice</td>
</tr>
<tr>
<td>XI</td>
<td>Intraoesophageal and intraperitoneal injection</td>
</tr>
<tr>
<td>XII</td>
<td>Statistical analysis</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACUC</td>
<td>Animal Care and Use Committee</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired Immune Deficiency Syndrome</td>
</tr>
<tr>
<td>CD4</td>
<td>Cluster of differentiation 4</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton</td>
</tr>
<tr>
<td>DHFR</td>
<td>Dihydrofolate Reductase</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immuno assay</td>
</tr>
<tr>
<td>FAS</td>
<td>Fatty Acid Synthesis</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal Bovine Serum</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hanks’ balanced salt solution</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>IC50</td>
<td>Half maximal inhibitory concentration</td>
</tr>
<tr>
<td>IFA</td>
<td>Indirect fluorescent antibody</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>LC50</td>
<td>Half maximal lethal concentration</td>
</tr>
<tr>
<td>LD</td>
<td>Lethal Dose</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>mg/ml</td>
<td>milligram/ mililitre</td>
</tr>
<tr>
<td>ml</td>
<td>millilitre</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin-resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>MTT</td>
<td>(3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide)</td>
</tr>
<tr>
<td>PABA</td>
<td>Para-Aminobenzoic Acid</td>
</tr>
<tr>
<td>PAS</td>
<td>Periodic Acid Schiff</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffer Solution</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PI</td>
<td>post-infection</td>
</tr>
<tr>
<td>ppm</td>
<td>part per million</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>RPMI</td>
<td>Roswell Park Memorial Institute</td>
</tr>
<tr>
<td><em>T. gondii</em></td>
<td><em>Toxoplasma gondii</em></td>
</tr>
<tr>
<td>µg/ml</td>
<td>microgram/ mililitre</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

Toxoplasmosis caused by the ubiquitous obligatory intracellular coccidian protozoan, *Toxoplasma gondii* (Negash et al., 2008) that has many forms. There are three infectious stages of *Toxoplasma gondii*. They are tachyzoites (rapidly multiplying form), bradyzoites (tissue cyst form), and sporozoites (in oocysts). Tachyzoites are found in the acute phase of the disease and are responsible for clinical manifestations. They are susceptible to the immunological response of the host and to drug action. Cysts are the resistant form of the parasite, persisting for the host's entire life. Cyst walls are resistant to both drugs and the immune system (Hill et al., 2002).

*Toxoplasma gondii* has a wide range of hosts which includes humans, mammals and marine mammals. About one third of the world population has been exposed to this parasite. Humans or animals can acquire *Toxoplasma gondii* infection post-natal by ingestion of undercooked or raw meat from infected animals, or ingestion of food or water contaminated with oocysts excreted by infected cats (Dubey et al., 2008). The population structure of *Toxoplasma gondii* consists of three main clonal lineages; Type I (including RH, a highly virulent strain), Type II (including avirulent strains like Me49), and Type III (including avirulent strains like NED) correlated with virulence expression in mice (Howe et al., 1995).

The treatment of toxoplasmosis is essential as *Toxoplasma gondii* causes serious mortality and morbidity in pregnant women and in immunocompromised patients who are suffering from the Acquired Immune Deficiency Syndrome (AIDS) or those undergoing chemotherapy. It is therefore clear that anti-Toxoplasmic therapy need to be potent against all strains of *Toxoplasma gondii*, be capable of killing tachyzoites and have a high ocular and cerebral penetration. However, their side effects, lack of efficacy against the tissue cyst of parasite and the potential appearance of resistant strains are particular drawback of the available treatments. (Mui et al., 2008)

Several treatment failures of toxoplastic encephalitis, chorioretinitis, and congenital toxoplasmosis have been reported (Baatz et al., 2006; Doliwa et al., 2013; Petersen 2007; Torres et al., 1997). In addition, long term use of these drugs may lead to hematologic and renal toxicity (Crespo et al., 2000) and the condition which lead to clinical failure by selecting drug resistant parasite variants. The current use of pyrimethamine for treatment of *T. gondii* infection is associated with suppression of bone marrow and may lead to neutropenia condition. Moreover, the combination of pyrimethamine with sulfadiazine can give rise to further concern due to allergy, kidney stones, or hepatic or renal complications (Mui et al., 2005).

Nowadays, there is an increase of awareness on therapeutic potential of natural products and medicinal plants. Natural products are usually considered to be less toxic
and free from side effects than synthetic drugs in treating various diseases. The importance of these plants as sources of natural product bioactive compounds to medicine lies not only in their pharmacological or chemotherapeutic effect, but also in their role as template compounds for the production of new drug compounds (Phillipson et al., 1994).

Previous studies showed that, lack of vaccine availability, and with the rise of parasite resistance towards therapeutic drugs, natural products can be efficient alternative against intracellular parasites such as Plasmodium falciparum and Trypanosoma cruzi. (Cui et al., 2007, Kolodziej et al., 2005) Natural products play an important role in the process of developing new drugs in the field of cancer research and infectious diseases (Newman et al., 2003). The medicinal value of these plants lies in some chemical compounds that induce a definite physiological activity on the human body (Balunas et al., 2005). In recent years, people have tended to use traditional medicine for the treatment of diseases. Medicinal plants are assumed to possess numerous secondary metabolites as flavonoids, alkaloids, terpenoids, tannins and others that help to protect the body from a variety of diseases.

Problem Statement

Standard therapies for toxoplasmosis involving combinations of pyrimethamine with sulfadiazine, clindamycin, azithromycin, or atavaquone. Drug treatment is often associated with severe side effects such as bone marrow suppression, cutaneous rash, leukopenia and thrombocytopenia. Alternative drugs with lesser side effects are needed to combat the disease by utilising medicinal plants.

1.1 Objectives

The general objective of this study is to evaluate the effects of selected Malaysian medicinal plants on Toxoplasma gondii infection.

1.2 Specific objectives

1. To determine in vitro activity of selected Malaysian medicinal plants against Toxoplasma gondii infection on Vero cell and the inhibitory concentration values for each treatment condition.
2. To demonstrate in vivo anti-toxoplasma activity in animal experiments for effective control of infection using infusions of selected Malaysian medicinal plants.
3. To determine cytotoxicity levels of selected Malaysian medicinal plants on mammalian cell.
REFERENCES


65


