UNIVERSITI PUTRA MALAYSIA

ANTI-MALARIAL ACTIVITY OF GONIOthalamus ScorTechinII KING

NOOR AZIAN BT. MD YUSUF

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ANTI-MALARIAL ACTIVITY OF GONIOTHALAMUS SCORTECHINII KING

By

NOOR AZIAN BT. MD YUSUF

Thesis Submitted to the School of Graduates Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

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ANTI-MALARIAL ACTIVITY OF *GONIOTHALAMUS SCORTECHINII* KING

By

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February 2006

Chairman: Associates Professor Khozirah Shaari, PhD

Faculty: Institute of Bioscience

Malaria remains the most devastating infectious parasitic disease, inflicting both death and economic loses on at least half the world population. Numerous attempts have been made to control the disease by using vector control measures or/and chemoprophylaxis, but they have had limited success. Immunoprophylaxis hold promises but effective vaccines are still not available. Presently, the most effective way of dealing with malaria is the administration of chemotherapeutic agents. Although drugs treatments of malaria are currently the best means of disease management, there is an urgent need for the development of effective anti-malarial drugs.

Earlier assessment of *Goniothalamus scortechinii* King showed to possess significant anti-malarial properties, *in vitro*. A phytochemical study of *G. schortechinii* King was thus carried out and has led to the isolation and
characterization of two compounds, goniothalamin and pinocembrine, from the bioactive chloroform fraction. Both compounds were assayed for anti-malarial activity using the pLDH method. Both exhibited anti-malarial activity against *P. falciparum* in different degrees, goniothalamin gave an IC$_{50}$ value of 4.0824 µg/ml while pinocembrine gave 19.308 µg/ml.

**Goniothalamin** was evaluated for its anti-malaria activity *in-vivo* using 4-Day Suppressive Test against *Plasmodium berghei* ANKA strain in *Swiss Albino Mice*. The 4DT was carried out by inoculating the clean mice with *P. berghei* ANKA strain and the infected mice were then treated orally and subcutaneously with goniothalamin. The suppression of parasite parasitemia and the ED$_{90}$ value of goniothalamin were determined. Control drug used in this study was Chloroquine. Results showed that goniothalamin when given orally at a dose of 90 and 120 mg/kg mice body weight, exhibited suppressions of *P. berghei* infection of 98% and 99.7%, respectively. Meanwhile, goniothalamin given subcutaneously at a dose 120 mg/kg mice body weight gave 90.5% suppression of *P. berghei* infection.

**Ex vivo** assay was carried out to investigate the effect of goniothalamin towards *P. falciparum in vitro* using the mouse serum treated with goniothalamin. This was done to prove that goniothalamin reaction toward *P. falciparum* should same as reaction towards *P. berghei* in *in vivo*
reaction. *Ex vivo* test was carried out using pLDH assay with serum of mice given goniothalamin orally and subcutaneously. A graph to determine the 90% inhibition of drugs-serum towards *P. falciparum* was plotted for each treated mice serum. Results showed the IS$_{90}$ of mice serum given goniothalamin orally was ranging from 0.050 to 4.00 µg/ml, for subcutaneous route the IS$_{90}$ was ranging from 0.009-4.750 µg/ml. A graph for estimating the length of time goniothalamin can remain in the blood was plotted. This gave the estimated time of goniothalamin both given orally and subcutaneously can remained a minimum of 6 hours in the blood.

In conclusion, goniothalamin does strongly inhibit *P. falciparum*, although it is not as potent as the standard drugs in use. More investigations such as drug combination, cytotoxicity, mechanism of action and toxicology studies, need to be carried out in order to determine its full potential as an anti-malarial.

Penyelidikan fitoubatan secara in-vitro ke atas pokok Goniothalamus scortechinii King telah manunjuk bahawa ia mempunyai khasiat sebagai

Pengujian keatas aktiviti goniothalamin sebagai ubat anti-malaria secara in vivo telah dijalankan dengan kaedah “4 Day Suppressive Test” terhadap P. berghei strain ANKA di dalam mencit Swiss Albino. Khlorokuin telah digunakan sebagai kawalan dalam kajian ini. Keputusan telah menunjukkan bahawa pada dos 90 mg/kg dan 120 mg/kg yang diberikan secara oral, goniothalamin telah menindas peningkatan parasitemia parasit masing-masing sebanyak 98% dan 99.7%. goniothalamin apabila diberikan secara ‘subcutaneous’, telah menindas peningkatan parasitemia parasit sebanyak 90.5% apabila diberikan dos 120 mg/kg.

Kajian ex vivo pula dijalankan bagi melihat keberkesanan goniothalamin terhadap parasit P. falciparum secara in-vitro dengan menggunakan serum mencit yang telah diberikan goniothalamin. Ujian ini dijalankan bagi
membuktikan bahawa tindakbalas goniothalamin terhadap *P. falciparum* secara *in vitro* ini adalah sama kesannya apabila dijalankan secara *in vivo*. Goniothalamin telah diberikan secara oral dan ‘subcutaneous’, dan assai pLDH digunakan untuk menentukan 90% penyekatan serum-dadah terhadap peningkatan parasitemia *P. falciparum*. Graf untuk menentukur 90% penyekatan diplotkan bagi melihat tindakkan serum dadah ini terhadap *P. falciparum*. Keputusan menunjukan IS90 goniothalamin apabila diberikan goniothalamin secara oral telah menyekat peningkatan parasitemia pada kepekatan yang berbeza bermula daripada julat kepekatan 0.050 hingga 4.00 µg/ml, manakala untuk ‘subcutaneous’ IS90 berjulat daripada 0.009- 4.750 µg/ml. Graf untuk melihat berapa lama serum-dadah boleh bertahan didalam dadah turut plotkan. Minimum masa untuk serum-dadah bertahan didalam darah dianggarkan selama 6 jam.

Kesimpulannya, goniothalamin telah menunjukan keupayaannya untuk menyekat *P. falciparum* walaupun keupayaannya tidak sekuat standard dadah yang digunakan. Kajian lanjut perlu dilakukan seperti kombinasi dengan dadah lain, sitotoksisiti, mekanisma tindakan, kajian keracunan (toxicology) demi menentukan keupayaan sebenarnya sebagai agen anti-malarial.
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Noor Azian Bt Md Yusuf

Sik, Kedah

February 2006
I certify that an Examination Committee met on 24 February 2006 to conduct the final examination of Noor Azian Bt. Md Yusuf on her Master of Science thesis entitled “Anti-malarial Activity of *Goniothalamus Scortechinii* King” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citation 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.

NOOR AZIAN BT MD YUSUF

Date: 10 May 2006
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<th>Description</th>
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<tr>
<td>APAD</td>
<td>Analog 3 acetyl pyridine dinucleotide</td>
</tr>
<tr>
<td>BC</td>
<td>Before Century</td>
</tr>
<tr>
<td>BH</td>
<td>Beta-haematin</td>
</tr>
<tr>
<td>CQ</td>
<td>Chloroquine</td>
</tr>
<tr>
<td>CM</td>
<td>Culture Medium</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>COSY</td>
<td>Correlated Spectroscopy</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxynucleic Acid</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl-sulphate</td>
</tr>
<tr>
<td>dHFR</td>
<td>Dehydrofolate Reductase</td>
</tr>
<tr>
<td>dHPS</td>
<td>Dehydropteroate Synthase</td>
</tr>
<tr>
<td>ED₉₀</td>
<td>Effective Dose at 90%</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>EIR</td>
<td>Erythrocyte Infection Rate</td>
</tr>
<tr>
<td>EIMS</td>
<td>Electrospray Ionization Mass Spectrometry</td>
</tr>
<tr>
<td>FPIX</td>
<td>Free ferrisoporphyrin IX hydroxide</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>HMRC</td>
<td>Herbal Medicine Research Centre</td>
</tr>
<tr>
<td>HMQC</td>
<td>Heteronuclear Multiple Quantum Correlation</td>
</tr>
<tr>
<td>HSQC</td>
<td>Heteronuclear Single Quantum Correlation</td>
</tr>
<tr>
<td>HMBC</td>
<td>Heteronuclear Multiple Bond Correlation</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>----------------------------------</td>
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<tr>
<td>HS</td>
<td>Human Serum</td>
</tr>
<tr>
<td>IRBC</td>
<td>Infected Red Blood Cell</td>
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<tr>
<td>IMR</td>
<td>Institute for Medical Research</td>
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<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
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<td>IV</td>
<td>Intra-veneous</td>
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<tr>
<td>Kb</td>
<td>Kilo-base</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate Dehydrogenase</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectroscopy</td>
</tr>
<tr>
<td>MQ</td>
<td>Milipore Quality water</td>
</tr>
<tr>
<td>ml</td>
<td>Mili-liter</td>
</tr>
<tr>
<td>mg/kg</td>
<td>Mili-gram per kilo-gram</td>
</tr>
<tr>
<td>NBT</td>
<td>Nitroblue Tetrozolium</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>NaOH</td>
<td>Natrium Hydroxide</td>
</tr>
<tr>
<td>NaCl</td>
<td>Natrium Chloride</td>
</tr>
<tr>
<td>N&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>O</td>
<td>Oral Route</td>
</tr>
<tr>
<td>O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Oxygen</td>
</tr>
<tr>
<td>PRBC</td>
<td>Peripheral Red Blood Cell</td>
</tr>
<tr>
<td>PABA</td>
<td>p-amino Benzoic Acid</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
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</table>
PES  Phenazine ethhiosulphate
Pre-4DT  Preliminary Four Day Test
pLDH  Parasite Lactate Dehydrogenase
RBCs  Red Blood Cells
SC  Subcutaneous Route
SP  Sulfadoxine/ Phyrimethamin
TLC  Thin Layer Chromatografi
WHO  World Health Organisation
\(0^\circ\)C  Degrees Celcius
µl  Micro-liter
4DT  Four Day Test
\(\lambda_{max}\)  In UV spectroscopy, the wavelength at which maximum absorption occurs
Malaria

Malaria continues to exact a substantial toll of human life and sufferings, particularly in the tropic and sub-tropic regions of the world. Human malaria has been recognized since the earliest period of man’s recorded history, and the discovery of mosquitoes trapped in amber suggests its prevalence in pre-historic times. A variety of names have been used to describe the disease such as the shakes, March, Roman, jungle, intermittent fever and ague chills. It was earlier thought that there was an etiological relationship between swamps and this fever. The name malaria is a misnomer and has originated from the Italian words *mala* (bad) and *aryia* (air) since in earlier days it was believed to be caused by breathing bad air (Ichpujani and Bathia, 1998 and Smyth, 1976).

Malaria is caused by single celled protozoa of the genus *Plasmodium*. *Plasmodium* does not only infect man but also apes, monkeys, birds and other vertebrate hosts. Four species of *Plasmodium* pathogenic to man are *P. falciparum* (malignant tertian or falciparum malaria), *P. vivax*
(benign tertian or vivax malaria, 48 hours cycles), P. malariae (quartant malaria, 72 hours cycles) and P. ovale (mild tertian or mild malaria).
Species parasitic to birds are P. gallinaceum (chicken), P. elongatum, P. reticulum and P. cathemerium. Simian malaria includes P. knowlesi, P. cynomolgi, P. inui, P. simium and P. lophure, while species parasitic to murine rodents are P. bergei, P. vinckei, P. chabaudi and P. yoelii (Ichpujani and Bathia, 1998; LaPage, 1963; Rosenthal, 2001).

**Epidemiology**

In 1955, WHO launched a program to eradicate malaria. This effort produced some important successes, but, for the most part, it has been a major disappointment. Indeed, over recent decades, morbidity and mortality caused by malaria have increased in many parts of the world with a large proportion of the world’s population remaining at risk of contracting this disease (Fig. 1.1). Hundred of millions of clinical episodes of malaria occur each year and it was estimated that 1.5-2.7 million deaths resulted from these infections. Numerous factors contribute to the persistence of the malaria problem and annually these include, among others:-
• efforts to control mosquito vectors, which were quite successful in some areas many years ago, have been limited by financial constraints and insecticide resistance

• programs to treat and control malaria, especially in highly vulnerable young children and pregnant women, are limited by poverty in most endemic regions

• despite many efforts, an effective malaria vaccine is not yet available and is unlikely to be available to those most at need in the near future

• malarial parasites have consistently demonstrated the ability to develop resistance to available drugs

• although great progress have been made in our understanding of malaria in recent years, our ability to develop new strategies to control the disease remain significantly limited by an incomplete understanding of the biology of the parasite and of the host’s response to parasite infection (Rosenthal, 2001)

Malaysia is no exception from the risk of malaria. Up to the days of the Malacca Sultanate, settlements had to be largely restricted to river mouths to avoid risks of malarial infections, thus curtailing population growth. In 1829, forty years after Penang Island was first occupied, one third of the deaths were caused by malaria (Lim, 2001).
Table 1.1 shows the number of malarial cases reported in 2001 and 2002 according to the infecting species in Malaysia. In 2001, *P. falciparum* and *P. vivax* account for just below 50% of malarial cases but in 2002, the cases increased to more than 50% for *P. falciparum* and 50% for *P. vivax*. In Sabah, malarial cases for 2001 were 54.87% and this increased to 64.2% in 2002. Malarial cases in Sarawak in 2001 and 2002 remained under 20% (MOH Annual Report, 2002).

![Figure 1.1: Regions of the world at risk of malarial infections.](image)

(The shrinking range of malaria is depicted by overlaying WHO maps for malaria risk for the year 1946 (yellow), 1966 (brown) and 1994 (red).)