UNIVERSITI PUTRA MALAYSIA

PRECLINICAL EVALUATION OF *ANDROGRAPHIS PANICULATA* (BURM. F.) WALL. EX NEES AS HERBAL MEDICINE FOR TREATMENT OF BREAST AND PROSTATE CANCER

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DOCTOR OF PHILOSOPHY
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IMPORTANT

The following manuscript "Preclinical Evaluation of *Andrographis paniculata* as an Herbal Medicine for the Treatment of Breast and Prostate Cancers" is submitted to the School of Graduate Studies, Universiti Puta Malaysia, in fulfilment of the requirements for the degree of Doctor of Philosophy by Audrey Yong Chee Hui. This manuscript can only be used for personal viewing and no part of this manuscript may be reprinted, linked to, or otherwise redistributed, in any form or by any means, without first obtaining the prior written consent of the author.

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By

AUDREY YONG CHEE HUI

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

August 2011

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Chairman : Associate Professor Johnson Stanslas, PhD

Faculty : Medicine and Health Sciences

Most Malaysians use some forms of herbal remedy to manage their diseases and ailments. Unfortunately, most of the time, the science behind the perceived benefits of this alternative therapy has not grown in proportion to its popular usage. The Malaysian National Pharmaceutical Control Bureau (NPCB) requires all herbal medicines to meet acceptable standards for quality, safety and efficacy for the purpose of product registration. Quality control in the phytopharmaceutical products is a multistep process that covers all stages from 'seed to pill'. It must be correctly authenticated, assessed for relative free from contamination and assayed for active

principles. In this study, *Andrographis paniculata* (Burm. F.) Nees (AP) or locally known as Hempedu bumi was evaluated preclinically for its quality, safety and efficacy towards development into a herbal medicine for the treatment of cancer. It is also a promising herb for the treatment of various diseases. There are three main diterpenoid lactones (DL) found in the aerial parts of AP, namely andrographolide (AGP, cytotoxic), 14-deoxy-11, 12-didehydroandrographolide (DDAG, non-cytotoxic) and neoandrographolide (NAG, non-cytotoxic). These three DL were used as marker compounds in assessing the quality of the plant materials and for subsequent *in vitro* and *in vivo* pharmacological studies. The stability and quality of dried AP material and extracts were evaluated in various storage temperatures (4 ± 2°C, 25 ± 2°C and 35 ± 2°C) based on of the content of the marker compounds by high performance liquid chromatography (HPLC). In a preliminary study to select the best extraction solvent (ethanol, ethanol: water (1:1), dichloromethane and water), these dried extracts were tested for their cytotoxicity against cancer cell lines using the MTT cell proliferation assay. Dichloromethane extract was found to be most active. Dichloromethane extract was then standardised to contain 15-20% w/w of AGP, 1-5%w/w of DDAG and 1-3% w/w of NAG of the extract weight. This extract was used throughout the *in vitro* and *in vivo* studies. It was found that the extract had improved cytotoxicity when the amount of AGP is equivalent to the pure AGP. This prompted an investigation of whether the DLs have combination cytotoxic effect in cancer cell lines. AGP-NAG and AGP-DDAG combinations produced profound synergistic cytotoxic effect in MCF-7 (breast) and DU 145 (prostate) cancer cells. The acute toxicity of this extract was determined in mice and rats, with escalating dosages from 100 mg/kg to 2 g/kg by oral administration, and no reduction of weight in the animals was observed. The plasma DL levels were determined in mice and rats

administered with AP extract at the doses of 250 mg/kg. The 250 mg/kg and 500 mg/kg doses were applied in the therapeutic studies using nude mice xenografted with breast and prostate tumours. The results from the stability study showed that the suitable storage temperature for dry AP material was at 35ºC up to 12 months to maintain content of AGP ≥ 70% of the initial content, and for the crude ethanol extract was at 35ºC for up to 6 months. For pharmacokinetic study in rodents (BALB/c, NU/Nu mice and rats), when 250 mg/kg dose of AP extract was administered orally, the maximum plasma concentrations (C_{max}) of DLs were found to be in the following ranges: AGP (2.1 – 5.3 µM), NAG (2.3 – 6 µM) and DDAG (3.1 – 4.7 µM). Preclinical therapeutic study revealed antitumor activity of AP extract against DU 145 prostate but not against MDA-MB-231 breast tumour xenografts. It is highly suggestive that the synergistic cytotoxic effect of DLs may have contributed to the *in vivo* antitumour activity of AP extract. This study also established suitable storage conditions for AP dry materials to preserve the quality in order to obtain reliable and consistent amounts of the DLs in extracts for *in vitro* and *in vivo* studies.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijazah Doktor Falsafah

PENILAIAN PRAKLINIKAL *ANDROGRAPHIS PANICULATA* (BURM. F.) WALL. EX NEES SEBAGAI UBATAN HERBA UNTUK RAWATAN KANSER PAYUDARA DAN PROSTAT

Oleh

AUDREY YONG CHEE HUI

Ogos 2011

Pengerusi : Profesor Madya Johnson Stanslas, PhD

Fakulti : Perubatan dan Sains Kesihatan

Kebanyakan rakyat Malaysia menggunakan pelbagai ubatan herba untuk menguruskan penyakit dan kesihatan mereka. Malangnya, sebahagian besar ilmu di sebalik manfaat yang dirasakan daripada terapi alternatif sumber tumbuhan in belum berkembang setanding dengan penggunaan popularnya. Biro Pengawalan Farmaseutikal Kebangsaan Malaysia (BPFK) menghendaki semua ubatan herba untuk mencapai tahap kepiawaian kualiti, keselamatan dan efikasi (keberkesanan) untuk tujuan pendaftaran produk. Kawalan kualiti dalam penghasilan produk fitofarmaseutikal adalah proses yang merangkumi semua peringkat pengembangan dari 'benih sampai pil'. Ubatan herba harus disahkan tulen dan benar, bebas daripada kecemaran dan diuji mengandungi prinsip-prinsip yang aktif. Dalam kajian ini, *Andrographis paniculata* (Burm. F.) Nees (AP) atau lebih dikenali sebagai Hempedu bumi dinilai secara praklinikal dari aspek kualiti, keselamatan dan efikasi (keberkesanan) terhadap pengembangan menjadi ubatan herba untuk rawatan kanser.

Tumbuhan herba ini adalah ramuan yang menjanjikan rawatan pelbagai penyakit. Terdapat tiga lakton diterpenoid (DL) utama yang diasingkan dari bahagian hijau, di atas tanah (batang and daun) AP, iaitu andrografolid (AGP, sitotoksik), 14-deoksi-11, 12-didehydroandrographolide (DDAG, tidak sitotoksik) dan neoandrographolide (NAG, tidak sitotoksik). Ketiga-tiga DL digunakan sebagai sebatian penunjuk dalam penilaian kualiti bahan mentah tumbuhan AP kering dan untuk selanjutnya, dalam kajian farmakologi pra-klinikal *in vitro* dan *in vivo*. Kestabilan dan kualiti bahan mentah tumbuhan kontang dan ekstrak AP dinilai dalam pelbagai suhu simpanan (4 ± 2°C, 25 ± 2°C dan 35 ± 2°C) dengan penentuan kandungan sebatian penanda dengan kromatografi cecair prestasi tinggi (HPLC). Dalam satu kajian pendahuluan untuk memilih pelarut ekstraksi terbaik (etanol, etanol: air (1:1), diklorometana dan air), ekstrak kontang diuji kesitotoksikan terhadap sel-sel kanser dengan menggunakan asai proliferasi sel MTT. Ekstrak diklorometana didapati paling aktif. Dalam kajian ini, ekstrak piawai diklorometana AP mengandungi AGP dalam julat 15-20%, DDAG dalam julat 1-5% dan 1-3% adalah NAG. Ekstrak ini digunakan dalam kajian farmakokinetik dan terapeutik *in vivo*. Didapati bahawa kesitotoksikan ekstrak AP lebih tinggi dibandingkan dengan jumlah setara AGP tulin. Perkara ini mendorong kita untuk menyiasat aakah ketiga-tiga DL ini akan memberi kesan kombinasi sitotoksik terhadap se-sel kanser. Kombinasi AGP-NAG dan AGP-DDAG didapati menghasilkan kesan sinergistik ke atas sel-sel kanser MCF-7 (payudara) dan DU 145 (prostat). Untuk menentukan ketoksikan akut ekstrak ini dalam mencit dan tikus, dos meningkat dari 100 mg/kg sehingga 2 g/kg diberikan secara oral dan tidak berlaku pengurangan berat badan haiwan tersebut diamati. Paras plasma DL ditentukan dalam plasma mencit dan tikus yang diberikan ekstrak AP pada dos 250 mg/kg. Dos yang sama dan 500 mg/kg diterapkan dalam kajian kesan terapeutik

terhadap xenograft tikus telanjang yang membawa tumor payudara dan prostat manusia. Hasil dari kajian kestabilan menunjukkan bahawa suhu simpanan yang sesuai untuk bahan AP kering adalah pada 35ºC hingga 3 bulan untuk mempertahankan kandungan AGP 70% ≥ dari kandungan awal dan suhu simpanan yang sesuai untuk etanol ekstrak kasar di 35º C hingga 6 bulan. Dos untuk kajian farmakokinetik pada haiwan roden (mencit-mencit BALB/c dan NU/NU serta tikus), apabila 250 mg/kg ekstrak AP yang diberikan secara oral kepada mencit dan tikus menunjukkan kepekatan maksimum plasma-DL (C_{max}) ditemui dalam julat berikut: AGP (2.1-5.3 µM), NAG (2.3-6 µM) dan DDAG (3.1-4.7 µM). Dalam kajian terapeutik *in vivo*, ekstrak AP telah menunjukkan kesan merencatkan pertumbuhan tumor terhadap DU 145 tumor prostat tetapi tidak terhadap MDA-MB-231 tumor xenografts mencit telanjang. ini mencadangkan bahawa kesan sitotoksik sinergistik DL mungkin telah menyumbang dalam aktiviti antitumor *in vivo* AP ekstrak. Penyelidikan ini turut juga mengenalpasti keadaan simpanan yang sesuai untuk bahan AP kontang untuk menjamin kualiti dan seterusnya memberi jumlah DL yang kukuh dan konsisten dalam ekstrak untuk menjalankan kajian *in vitro* dan *in vivo*.

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I certify that an Examination Committee has met on 15th August 2011 to conduct the final examination of Audrey Yong Chee Hui on her PhD thesis entitled "Preclinical Evaluation of *Andrographis paniculata* as an Herbal Medicine for the Treatment of Breast and Prostate Cancer" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertaninan Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the Doctor of Philosophy degree. Members of the Thesis Examination Committee were as follows:

Fauziah Othman, PhD
Professor
Department of Human Anatomy,
Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia
(Chairman)

Dr. Muhammad Nazrul Hakim Abdullah, PhD
Professor
Department of Biomedical Sciences
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Latifah Saiful Yazan, PhD
Lecturer
Department of Biomedical Sciences
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Mohd Zaini Asmawi, PhD
Professor
School of Pharmaceutical Sciences
Universiti Sains Malaysia
(External Examiner)

SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This was thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy
The members of the Supervisory Committee were as follows:

Johnson Stanslas, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Md Nordin Lajis, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Member)

Khozirah Shaari, PhD
Associate Professor
Institute of Bioscience
Universiti Putra Malaysia
(Member)

Mohd Said Saad, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Nashiru Billa, PhD
Associate Professor
School of Pharmacy
Faculty of Health and Biological Sciences
The University of Nottingham Malaysia Campus
(Member)

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

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institution.

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AUDREY YONG CHEE HUI

Date: 15 August 2011

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