UNIVERSITI PUTRA MALAYSIA

ANTI-ACUTE INFLAMMATORY EFFECT OF HIBISCUS ROSA-SINENSIS L AND HIBISCUS ROSA-SINENSIS VAR ALBA FLOWER AND LEAF ETHANOL EXTRACTS AND ITS MECHANISM OF ACTION IN RATS

SITI ZALEHA RADUAN

FPSK(m) 2013 28

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SITI ZALEHA BINTI RADUAN

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2013

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By

SITI ZALEHA BINTI RADUAN

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

June 2013

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

ANTI-ACUTE INFLAMMATORY EFFECT OF *Hibiscus rosa-sinensis* L. AND *Hibiscus rosa-sinensis* var. *alba* FLOWER AND LEAF ETHANOL EXTRACTS AND ITS MECHANISM OF ACTION IN RATS

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June 2013

Chair: Prof Nazrul Hakim Abdullah, PhD

Faculty: Medicine and Health Sciences

Hibiscus rosa-sinesis L. is a plant having medicinal properties especially its flower and leaf but not much on its variant; *alba*. The study was carried out to determine and compare the anti-acute inflammatory activities and its mechanism of action of ethanol extract of flower and leaf of *Hibiscus rosa-sinensis* var *alba* (white) and *Hibiscus rosa-sinensis* L. (red) in rats.

Phytochemical screening was performed with 95% ethanolic crude extracts. Flavanoids, saponins and steroids presence in all extracts. Acute dose response was determined using Fixed Dose Procedure with fixed level of dose. Any signs of toxicity were observed within 14 days. Supplementation of 500 mg/kg of all extracts caused toxicity. The blood samples were collected and liver and kidney were isolated at day 15. No significant changes ($p>0.05$) in liver enzyme levels and histologically no presence of lesions at the organs as response up to 500 mg/kg.

For anti-inflammatory properties, edema and polymorphonuclear leukocyte (PNL) infiltration induced by carrageenan and licking time induced by formalin were studied. 0.1ml of carrageenan was injected subplantarly 30 min before administration of each extracts (5, 50 or 100 mg/kg). Supplementation of 50 and 100 mg/kg of flower and leaf of *Hibiscus rosa-sinensis* L. caused significant inhibition ($p<0.05$) of edema. Flower and leaf of *Hibiscus rosa-sinensis* var *alba* significantly inhibited ($p<0.05$) edema in all range of testing dose. It varies significantly ($p<0.05$) with variant, plant parts and doses. The animals were killed after 6 hrs and PNLs in paw tissues were counted. Supplementation of all extracts at various concentration caused significant reduction ($p<0.05$) on PNL infiltration. It varies significantly ($p<0.05$) with dose but no significant ($p>0.05$) with variant and plant parts. 50µl of formalin was injected subplantarly 30 min before administration of 100 mg/kg of each extracts. Supplementation of all extracts showed significant reduction ($p<0.05$) on the duration of licking response. It varies significantly ($p<0.05$) with variant but no significant ($p>0.05$) with plant parts and phase.

For anti-acute inflammatory mechanism, edema and PNL infiltration induced by bradykinin (BK) and histamine were studied. 0.1ml of BK was injected subplantarly 15 min before administration of 100 mg/kg of each extracts. Supplementation of all extracts showed significant inhibition ($p<0.05$) of edema. It varies significantly ($p<0.05$) with variant but no significant ($p>0.05$) with plant parts. The animals were killed after 6 hrs and PNLs in paw tissues were counted. Supplementation of all extracts showed significant reduction ($p<0.05$) on PNL infiltration. It varies significantly ($p<0.05$) with variant and plant parts. 0.1ml of histamine was injected subplantarly immediately before administration of 100 mg/kg of each extracts.

Supplementation of all extracts showed significant inhibition ($p<0.05$) of edema. It varies significantly ($p<0.05$) with variant but no significant ($p>0.05$) with plant parts. The animals were killed after 2 hrs and PNLs in paw tissue were counted. Supplementation of all extracts showed significant reduction ($p<0.05$) on PNL infiltration. It varies significantly ($p<0.05$) with variant but no significant ($p<0.05$) with plant parts.

In conclusion, the study showed flower and leaf of *Hibiscus rosa-sinensis* var *alba* and *Hibiscus rosa sinensis* L. produced anti-acute inflammatory activity. It may involve the inhibition of cyclooxygenase, bradykinin, histamine, and reduce polymorphonuclear infiltration.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN ANTI-INFLAMASI AKUT EKSTRAK ETANOL BUNGA DAN DAUN *Hibiscus rosa-sinensis* L. DAN *Hibiscus rosa-sinensis* var. *alba* DAN MEKANISME TINDAKANNYA PADA TIKUS.

Oleh

SITI ZALEHA BINTI RADUAN

Jun 2013

Pengerusi: Prof Nazrul Hakim Abdullah, PhD

Fakulti: Perubatan dan Sains Kesihatan

Hibiscus rosa-sinesis L. adalah tumbuhan yang mempunyai ciri-ciri perubatan terutama dalam bunga dan daun tetapi tidak kepada varian; *alba*. Kajian ini dijalankan untuk menentu dan membandingkan aktiviti anti-inflamasi akut dan mekanisme tindakan oleh ekstrak etanol bunga dan daun *Hibiscus rosa-sinensis* var *alba* (putih) dan *Hibiscus rosa-sinensis* L. (merah) pada tikus.

Pemeriksaan fitokimia telah dijalankan dengan menggunakan 95% ekstrak mentah etanol. Terdapat kehadiran flavanoids, saponins dan steroids dalam kesemua ekstrak. Tindak balas akut terhadap dos telah ditentukan dengan menggunakan 'Fixed Dose Procedure' dengan tahap dos yang tetap. Tanda toksik diperhatikan dalam tempoh 14 hari. Rawatan pada 500 mg/kg oleh kesemua ekstrak menyebabkan toksik. Sampel darah telah dikumpul manakala hati serta buah pinggang telah diasingkan pada hari ke-15. Tiada perubahan yang signifikan ($p>0.05$) pada takat enzim hati dan tiada tanda kerosakan secara histologi pada organ sebagai tindak balas sehingga 500 mg/kg.

Untuk ciri anti-inflamasi, edema dan penyusupan polimorphonukleus leukosit (PNL) disebabkan oleh carrageenan dan masa penjilatan disebabkan oleh formalin telah dikaji. 0.1ml carrageenan telah disuntik pada subplantar 30 minit sebelum penggunaan setiap extrak (5, 50 atau 100 mg/kg). Rawatan oleh 50 dan 100 mg/kg bunga dan daun *Hibiscus rosa-sinensis* L. menyebabkan perencatan edema yang signifikan ($p<0.05$). Bunga dan daun *Hibiscus rosa-sinensis* var *alba* merencatkan edema secara signifikan ($p<0.05$) dalam pelbagai ujian dos. Ia berbeza secara signifikan ($p<0.05$) dengan varian, bahagian tumbuhan dan dos. Haiwan dibunuh selepas 6 jam dan PNL dalam tisu tapak kaki telah dikira. Rawatan oleh kesemua ekstrak pada pelbagai kepekatan menyebabkan pengurangan penyusupan PNL yang signifikan ($p<0.05$). Ia berbeza secara signifikan ($p<0.05$) dengan dos tetapi tidak signifikan ($p>0.05$) dengan varian dan bahagian tumbuhan. 50µl formalin telah disuntik pada subplantar 30 minit sebelum penggunaan 100 mg/kg setiap ekstrak. Rawatan oleh kesemua ekstrak menunjukkan pengurangan tempoh penjilatan yang signifikan ($p<0.05$). Ia berbeza secara signifikan ($p<0.05$) dengan varian tetapi tidak signifikan ($p>0.05$) dengan bahagian tumbuhan dan fasa.

Mekanisme anti-inflamasi akut, edema dan penyusupan PNL disebabkan oleh bradikinin (BK) dan histamin telah dikaji. 0.1ml BK telah disuntik pada subplantar 15 minit sebelum penggunaan 100 mg/kg setiap ekstrak. Rawatan oleh kesemua ekstrak menunjukkan perencatan edema yang signifikan ($p <0.05$). Ia berbeza secara signifikan ($p<0.05$) dengan varian tetapi tidak signifikan ($p>0.05$) dengan bahagian tumbuhan. Haiwan dibunuh selepas 6 jam dan PNL dalam tisu tapak kaki telah dikira. Rawatan oleh kesemua ekstrak menunjukkan pengurangan pada penyusupan

PNL yang signifikan ($p<0.05$). Ia berbeza secara signifikan ($p<0.05$) dengan varian dan bahagian tumbuhan. 0.1ml histamin telah disuntik pada subplantar sebelum administrasi 100 mg/kg setiap ekstrak. Rawatan oleh kesemua ekstrak menunjukkan perencatan edema yang signifikan ($p<0.05$). Ia berbeza secara signifikan ($p<0.05$) dengan varian tetapi tidak signifikan ($p>0.05$) dengan bahagian tumbuhan. Haiwan dibunuh selepas 2 jam dan PNL dalam tisu tapak kaki telah dikira. Rawatan oleh semua ekstrak menunjukkan pengurangan pada penyusupan PNL yang signifikan ($p <0.05$). Ia berbeza secara signifikan ($p<0.05$) dengan varian tetapi tidak signifikan ($p<0.05$) dengan bahagian tumbuhan.

Kesimpulannya, kajian menunjukkan bunga dan daun *Hibiscus rosa-sinensis* var *alba* dan *Hibiscus rosa sinensis* L. menghasilkan aktiviti anti-inflamasi akut. Ia mungkin melibatkan perencatan cyclooxygenase, bradykinin, histamine dan mengurangkan penyusupan polimorphonukleus leukosit.

ACKNOWLEDGEMENTS

It was really a laborious task to accomplish a master research. Many people gave me support and help in the process of completing this research. I would like to express my deep sense of gratitude to my research supervisor, Professor Dr. Muhammad Nazrul Hakim bin Abdullah, for his insightful guidance and earnest help all through the information searching, paper-writing stages and invaluable comments. He was being the integral part of this thesis throughout all its stages, both intellectual and the practical level. I really appreciate his generosity in sharing his knowledge and time, and for being attentive to my every question.

Also, I would like to acknowledge my sincere thanks to my co-supervisor, Dr. Roslida Abd Hamid @ Abdul Razak and Asscociate Prof. Dr Zainul Amirudin Zakaria, for their generous assistance in my research. I am very thankful to Dr Mohamad Aris Mohd Moklas in his guidance throughout histopathology analysis and Dr Mohd Khairi Hussain for his helping mostly in methodology. Million of thanks to Associate Prof Dr Aziz bin Dollah and Associate Prof Dr Saidi bin Moin for helping in the statistical analysis for my research.

My special thanks also goes to my friend, Mr. Muhammad Wahizul Haswan bin Abdul Aziz for lending a hand helping my research. Furthermore, my deep appreciation is sincerely goes to all the staff of Faculty of Medicine and Health Sciences, especially Mr. Ramli (Animal House), Miss Nurhayati Zainal Abidin and Mr. Zain Zailan (Pharmacology and Toxicology Lab), Madam Juita Chupri (Histopathology Lab) and Madam Noridah Mat Top (Research Laboratory of

Anatomy) for providing me with the experimental animals, equipments and reagents needed. Special thanks for their warmhearted helps and cooperation in assisting my research project gone smoothly. I apologized to any unintended inconvenience caused.

Last but not least, I would like to acknowledge my heartfelt appreciation to all my friends. Thanks for sharing their time to facilitate me in various task of experiment. Thanks for being sharing their information and ideas with me in completing this thesis. Finally, I would like to dedicate my thesis to my beloved family. Whenever I was on the verge of giving up, they always stood by me and never hesitated to give me a hand.

I certify that a Thesis Examination Committee has met on 20 June 2013 to conduct the final examination of Siti Zaleha binti Raduan on her thesis entitled "Anti-Acute Inflammatory Effect of *Hibiscus rosa-sinensis* L. and *Hibiscus rosa-sinensis* var. *alba* Flower and Leaf Ethanol Extracts and Its Mechanism of Action in Rats" 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:

Dr. Cheah Yoke Kqueen, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Dr. Mohammad Aziz bin Dollah, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Dr. Latifah binti Saiful Yazan, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Dr. Vasudevan Mani, PhD
Associate Professor
Universiti Teknologi Mara
Malaysia
(External Examiner)

NORITAH OMAR, PhD
Assoc. Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 2 August 2013

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the Master of Science. The members of the Supervisory Committee were as follows:

Dr. Muhammad Nazrul Hakim, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Dr. Roslida Abd Hamid @ Abdul Razak, PhD
Senior Lecturer,
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Dr. Zainul Amirudin Zakaria, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

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

Date: 15 August 2013

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, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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SITI ZALEHA BINTI RADUAN

Date: 20 June 2013

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