UNIVERSITI PUTRA MALAYSIA

TOXICITY AND ANTIPYRETIC EFFECT OF Hibiscus rosa-sinensis L. AND Hibiscus rosa-sinensis var. Alba FLOWER AND LEAF ETHANOL EXTRACTS ON RATS

MUHAMMAD WAHIZUL HASWAN BIN ABDUL AZIZ

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By

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

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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

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January 2013

Chair: Prof. Muhammad Nazrul Hakim Abdullah, PhD

Faculty: Medicine and Health Sciences

Hibiscus rosa-sinensis has been traditionally used by local communities to treat fever. However, there are only limited data have been published to support the antipyretic effects. The objective of this study is to investigate the antipyretic properties and possible mechanism of the ethanol extracts of *Hibiscus rosa-sinensis* L. (red) and *Hibiscus rosa-sinensis* var. *Alba* (white) flower and leaf. Phytochemical analysis, heavy metals screening and acute toxicity test were done to evaluate the safety of extracts. The first model ran induced fever in rats by injecting Brewer's Yeast subcutaneously and then treated with 4 extracts at dosage 5 & 50 mg/kg. The dosages used for the study were obtained by the acute toxicity test. Ibuprofen was used as a reference drug, with dose 100 mg/kg. The results of the study showed that white flower extract 5 mg/kg and 50 mg/kg significantly ($p<0.05$) reduced the total temperature when compared to positive control group. For the second model, 50 mg/kg dosage was chosen based on the first model. The rats were induced to fever by injecting 100 µg/kg lipopolysaccharide (LPS) intraperitoneally. LPS model is divided into pre-treatment and post-treatment studies. Pre-treatment was done with ethanol extract treated prior to fever induced by LPS whereas post-treatment will be

induced by LPS, and then treated with the extracts. Temperatures of rats were measured using a digital thermometer. The results were expressed as mean ± S.E.M. and analyzed using the SAS system. In pre-treatment, the ethanol extract of H. rosa-sinensis (red) flower indicate a significant impediment of temperature rise ($p<0.05$) in rectal temperature when compared to control at all times. In post-treatment, statistical analysis revealed that only *H. rosa-sinensis* (red) flower ethanol extract of have significant ($p<0.05$) antipyretic effect in abolishing the LPS-induced fever in rats and the values were comparable to Ibuprofen. Similarly, the *H. rosa-sinensis* (red) flower extract showed more potency than ibuprofen at the first and second hour, but less potent at third hour until the fifth hour. The data showed that both extracts of *H. rosa-sinensis* var. *Alba* (white) flowers and *H. rosa-sinensis* (red) flowers have antipyretic property on pyrexia models. To determine the correlation of Prostaglandin E_2 (PGE_2) in the physiology of fever, peripheral blood of rats was taken and the serum was measured for PGE_2 metabolite content. The results of the pre-treatment study showed extracts of *H. rosa-sinensis* var. *Alba* (white) flower 50 mg/kg have significantly lower PGE_2 (1.43 ± 0.64 pg/ml) than control (4.03 ± 0.07 pg/ml). For post-treatment, Ibuprofen, *H. rosa-sinensis* (red) flower and *H. rosa-sinensis* var. Alba (white) flower 50 mg/kg extracts showed significant ($p>0.05$) decrease of PGE_2 when compared to control. Therefore, this research suggest the probability for its therapeutic effectiveness as plant-based antipyretic agent as claimed by traditional medicine practitioners of our local community.

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

TOKSISITI DAN KESAN ANTIPIRETIK EKSTRAK ETANOL BUNGA DAN DAUN *Hibiscus rosa-sinensis* L. DAN *Hibiscus rosa-sinensis* var. *Alba* KEATAS TIKUS

Oleh

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Pengerusi: Prof. Muhammad Nazrul Hakim Abdullah, PhD

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Hibiscus rosa-sinensis telah digunakan secara tradisional oleh masyarakat tempatan untuk merawat demam. Namun, data untuk menyokong kesan antipiretik untuk tumbuhan tersebut adalah terhad. Objektif kajian ini adalah untuk menyiasat kesan ekstrak etanol bunga dan daun *H. rosa-sinensis* (merah) serta bunga dan daun *H. rosa-sinensis* var. *Alba* (putih) dalam menurunkan suhu badan. Analisa fitokimia, logam berat dan toksisiti akut telah dijalankan untuk memgetahui tahap keselamatan ekstrak-ekstrak tersebut. Model pertama dijalankan dengan menginduksikan demam terhadap tikus dengan menyuntik 'Brewer's Yeast' secara subkutan dan setelah itu menerima dos rawatan sebanyak 5 & 50 mg/kg empat jenis ekstrak *H. rosa-sinensis* yang berlainan. Dos yang dipilih untuk ekstrak diperolehi selepas kajian toksisiti akut dijalankan. Ibuprofen diguna sebagai ubat rujukan, dengan dos 100 mg/kg. Hasil kajian mendapati ekstrak bunga *H. rosa-sinensis* var. *Alba* (putih) 5 mg/kg dan 50 mg/kg berjaya menurunkan suhu demam dengan siknifikan ($p<0.05$) berbanding dengan kumpulan kawalan positif. Kemudiannya dos 50 mg/kg dipilih untuk setiap ekstrak dalam model kedua kajian pireksia. Tikus diinduksi kepada demam dengan menyuntik 100 μg/kg lipopolisakarida (LPS) secara intraperitoneal. Setiap kumpulan

tikus menerima dos rawatan sebanyak 50 mg/kg secara intraperitoneal dengan empat jenis ekstrak yang berlainan. Model LPS ini terbahagi kepada kajian pra-rawatan dan pasca-rawatan. Pra-rawatan dirawat dengan ekstrak etanol terlebih dahulu, kemudian diikuti dengan induksi kepada demam dengan LPS. Pasca-rawatan diinduksi kepada demam dengan LPS, dan dirawat dengan ekstrak kemudian. Suhu tikus diukur dengan menggunakan termometer digital. Keputusan dinyatakan sebagai min ± S.E.M. dan dianalisis dengan menggunakan system SAS. Dalam kumpulan pra-rawatan, ekstrak etanol bunga *H. rosa-sinensis* (merah) menunjukkan halangan kenaikan suhu yang signifikan ($p<0.05$) dalam suhu rektal berbanding dengan kumpulan kawalan pada kesemua masa kajian. Dalam kumpulan pasca-rawatan, ekstrak etanol bunga *H. rosa-sinensis* (merah) menunjukkan penurunan yang signifikan dalam suhu rektal dan mempunyai kepotenan yang agak sama dengan Ibuprofen. Demikian juga dalam pasca-rawatan, ekstrak bunga merah lebih poten daripada Ibuprofen pada jam pertama dan kedua, tetapi kurang berkesan pada jam ketiga dan seterusnya. Sebagai kesimpulan, data dari pra-rawatan dan pasca-rawatan menunjukkan bahawa ekstrak bunga putih dan bunga merah *H. rosa-sinensis* mempunyai kesan antipiretik terhadap model-model pireksia yang telah dijalankan. Untuk mengkaji kaitan Prostaglandin E_2 (PGE_2) dalam fisiologi demam, darah periferi tikus diambil dan diukur kandungan metabolit PGE_2 dalam serum. Hasil kajian menunjukkan ekstrak bunga *H. rosa-sinensis* var. *Alba* (putih) pra-rawatan dengan dos 50 mg/kg yang telah berjaya menghalang kenaikan suhu dengan siknifikan ($p<0.05$), mengandungi paling sedikit PGE_2 iaitu 1.43 ± 0.64 pg/ml berbanding dengan kawalan 4.03 ± 0.07 pg/ml. Untuk pasca-rawatan pula, Ibuprofen, bunga *H. rosa-sinensis* dan *H. rosa-sinensis* var. *Alba* dos 50 mg/kg menurunkan kandungan PGE_2 dengan siknifikan ($p<0.05$) berbanding dengan kawalan.

Kesimpulannya, kajian ini menunjukkan bahawa ekstrak memberi keberkesanan terapeutik sebagai agen antipiretik sebagaimana yang dinyatakan oleh pengamal perubatan tradisional.

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

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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.

MUHAMMAD WAHIZUL HASWAN BIN ABDUL AZIZ

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