UNIVERSITI PUTRA MALAYSIA

POTENTIAL PHARMACOLOGICAL EFFECTS OF ETHANOLIC EXTRACT OF *Annona muricata* L. LEAVES IN ANIMAL MODELS

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

POTENTIAL PHARMACOLOGICAL EFFECTS OF ETHANOLIC EXTRACT OF *Annona muricata* L. LEAVES IN ANIMAL MODELS

By

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Annona muricata L, locally known as "durian belanda" has been used in folklore medicine to treat fever, cough, diarrhea, sedative, rash, ring worm and lactation for women afterbirth. The aims of this study were to investigate the anti-inflammatory, anti-nociceptive and anti-ulcerogenic activities and the mechanisms involve of the ethanolic leaves extract of *Annona muricata* L (EEAM) in various animal models. The anti-inflammatory effects of EEAM were tested against xylene-induced ear edema in mice and complete Freund's adjuvant (CFA)-induced arthritis in rats. Anti-nociceptive activity of EEAM was evaluated using acetic acid-induced abdominal writhing in mice, formalin test in rats and hot plate test in mice. Furthermore, the anti-ulcerogenic effect of EEAM was studied in ethanol-induced ulcer model in rats, ethanol-induced gastric lesions in N-Nitro-L-arginine methyl ester hydrochloride (L-NAME)-pre-treated rats as well as ethanol-induced gastric lesions in *N*-ethylmaleimide (NEM)-pre-treated rats test model to determine its mechanism. LD_{50} of EEAM was found to be 890 mg/kg when screened for its toxicity. At 100 and 300 mg/kg orally, EEAM produced significant dose-dependent inhibition in xylene-induced ear edema in mice by 63.10

and 72.41%, respectively. In CFA-induced arthritis model, administration of EEAM at the highest dose of 100 mg/kg significantly attenuated the development of swelling by 86.07%. Furthermore, at the similar dose i.e. 100 mg/kg EEAM also significantly suppressed the release of IL-1β and TNF-α in local arthritic tissue in ELISA test by inhibition of 43.06 and 46.37%, respectively. Effects produced by EEAM were significantly higher than those produced by indomethacin (10 mg/kg, p.o.) in inhibiting both cytokines in the CFA-induced arthritis model by 41.67 and 37.27%, respectively. For anti-nociceptive effect, the inhibition in the acetic acid-induced writhing test produced by both 100 (53.04%) and 300 mg/kg (95.30%) of EEAM were greater than that produced by 20 mg/kg of indomethacin (55.44%). Experimental evidence obtained in this study indicates that EEAM significantly reduced acetic acid-induced writhes in mice and the formalin-induced paw licking in rats dose-dependently. Administration of EEAM at 300 mg/kg (66.21%) exhibited significant and comparable anti-nociceptive activity with acetylsalicylic acid (ASA) (64.35%) in the formalin test. EEAM at 100 and 300 mg/kg also increased the latency time of mice exposed to the hot plate. Based on the results shown, we suggested that its anti-nociceptive effect may be mediated via both peripheral and central mechanisms. This has been supported by findings in a nociceptive study with pretreatment of naxolone. Results showed that there is also a possibility of the involvement of opioid mechanism in the effect of EEAM. EEAM decreased the ulcerative lesion produced by ethanol in rats in a dose-dependent manner. EEAM at 30 mg/kg exhibited an inhibitory effect by 51.77%, which is slightly greater than the effect of lansoprazole by 46.95%. The prior administration of L-NAME, a NO-synthase inhibitor, did not reduce the anti-

ulcerogenic effect of EEAM in ethanol-induced ulcer model, suggesting that there is no participation of nitric oxide (NO) in its pharmacological mechanism. Pre-treatment with NEM, a thiol blocker, including mucosal non-protein sulfhydryl groups, reduced the anti-ulcerogenic effect of EEAM in the same ulcer model. For histology study, inflammatory features which consist of edema, congestion, hemorrhage and necrosis were investigated. EEAM at all doses showed significant histological changes excluding the features in edema. As a conclusion, EEAM possesses anti-inflammatory, anti-nociceptive and anti-ulcerogenic effects.

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

POTENSI KESAN FARMAKOLOGI OLEH ESTRAK ETANOL DAUN *Annona muricata* L. DALAM MODEL HAIWAN

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Annona muricata L, atau nama tempatannya, durian belanda adalah tumbuhan yang digunakan di dalam perubatan tradisional dalam merawat demam, batuk, cirit- birit, sebagai sedatif, ruam, jangkitan cacing gelang dan meningkatkan laktasi wanita selepas bersalin. Kajian ini bertujuan untuk mengkaji aktiviti anti-radang, anti-nosiseptif dan anti-ulser ke atas ekstrak etanol dari *Annona muricata* L (EEAM) dan menentukan mekanisma yang terlibat di dalam aktiviti tersebut di dalam pelbagai model haiwan ekperimen. Kesan anti-radang EEAM diuji ke atas edema telinga mencit yang diaruh menggunakan xilena dan juga ke atas kesan artritis diaruh menggunakan adjuvan pelengkap Freund pada tikus. Aktiviti antinosiseptif ke atas EEAM dilakukan dengan menggunakan kesan pencerutan pada abdomen mencit diaruh menggunakan asid asetik, ujian formalin pada tikus dan ujian plet panas pada mencit. Manakala kesan anti-ulser ke atas EEAM dikaji dengan menggunakan model ulser secara aruhan oleh etanol pada tikus, ujian lesi gastrik diaruh oleh etanol dalam tikus yang dirawat terdahulu dengan N-Nitro-L-arginin metil ester hidroklorik (L-NAME) dan ujian lesi gastrik diaruh oleh etanol dalam tikus yang dirawat terdahulu dengan *N*-etilmaleimide (NEM) untuk menentukan mekanisma yang terlibat di dalam

aktiviti tersebut. Daripada penyaringan ketoksikan ke atas EEAM, LD_{50} yang diperolehi ialah 890 mg/kg. Pada dos 100 dan 300 mg/kg, yang diberikan secara oral, EEAM menghasilkan peratus perencatan yang signifikan masing-masing sebanyak 63.10% dan 72.14% terhadap edema telinga mencit diaruh dengan xilena. Dalam model artritis diaruh dengan adjuvan pelengkap Freund, rawatan EEAM pada dos tertinggi iaitu 100 mg/kg berjaya mengurangkan pembentukan pembengkakan sebanyak 86.05% pada tapak kaki tikus secara signifikan. Selanjutnya, pada dos yang sama iaitu 100 mg/kg, EEAM juga merencat penghasilan IL-1β dan TNF-α secara signifikan, masing masing sebanyak 43.06% dan 46,37% di dalam tisu artritik menggunakan ujian ELISA. Kesan yang dihasilkan oleh EEAM, secara signifikannya jauh lebih tinggi berbanding dengan kesan indomethacin pada dos 10 mg/kg, yang diberikan secara oral dalam merencat kedua-dua sitokin pada model artritis diaruh dengan adjuvan pelengkap Freunds iaitu masing-masing sebanyak 41.67 dan 37.27%. Untuk kesan antinosiseptif, peratus perencatan ke atas ujian pencerutan abdomen mencit diaruh dengan asid asetik oleh EEAM pada dos 100 dan 300 mg/kg masing-masing sebanyak 53.04% dan 95.30%. Kesan EEAM pada dos 300 mg/kg adalah jauh lebih tinggi daripada yang dihasilkan oleh indometasin pada dos 20 mg/kg iaitu sebanyak 55.44%. Bukti eksperimental yang diperoleh dalam kajian ini menunjukkan bahawa EEAM mampu mengurangkan pencerutan abdomen mencit diaruh dengan asid asetik dan juga mampu mengurangkan jilatan tapak kaki pada tikus diaruh dengan formalin. Pengambilan EEAM pada dos 300 mg/kg yang memberikan peratus perencatan sebanyak 66.21% menunjukkan aktiviti anti-nosiseptif yang signifikan dan setara dengan kesan ditunjukkan oleh asid asetilsalisilik (ASA) sebanyak 64.35% di

dalam ujian formalin. EEAM juga meningkatkan masa pendam terhadap mencit yang didedahkan pada plet panas. Berdasarkan keputusan yang diperoleh, adalah disarankan bahawa kesan anti-nosiseptif EEAM berkemungkinan diperantarai melalui kedua-dua mekanisma periferi dan pusat. Ini telah disokong oleh penemuan dalam kajian dengan prarawatan dengan naxolone. Keputusan kajian juga menunjukkan bahawa terdapat kemungkinan penglibatan mekanisme opioid ke atas kesan EEAM. EEAM mengurangkan lesi berulser yang dihasilkan oleh etanol pada tikus secara bergantung kepada dos. Pada dos 30 mg/kg, EEAM menunjukkan kesan perencatan sebanyak 51.77% yang lebih tinggi sedikit daripada kesan yang ditunjukkan oleh lansoprazole dengan perencatan sebanyak 46.95%. Pra-rawatan dengan L-NAME, sejenis perencat nitrik oksida sintase, tidak mengurangkan kesan anti-ulser ke atas EEAM dalam model ulser diaruh dengan etanol, mencadangkan bahawa tiada penglibatan nitrik oksida (NO) dalam mekanisme farmakologi EEAM. Sebaliknya, pra-rawatan dengan *N*-etilmaleimide, sejenis penghalang thiol, termasuk kumpulan sulfhidril nonprotein bermukosa, berjaya mengurangkan kesan anti-ulser ke atas EEAM dalam model ulser yang sama. Ciri-ciri inflamasi seperti edema, kongesi, pendarahan dan nekrosis dikaji di dalam kajian histologi. Pada kesemua dos yang diuji ke atas EEAM menunjukkan perubahan histologi secara signifikan dalam semua ciri kecuali edema. Sebagai kesimpulannya, EEAM mempunyai kesan anti-radang, anti-nosiseptif dan anti-ulser.

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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.

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