

THE TOXICITY STUDY OF *AQUILARIA MALACCENSIS*  
(AGARWOOD) LEAVES AQUEOUS EXTRACT ON  
MALE REPRODUCTIVE SYSTEM IN SPRAGUE  
DAWLEY RATS

BY

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## ABSTRACT

*Aquilaria malaccensis* (AM) or locally known as ‘gaharu’ (agarwood) is a species of *Aquilaria* genus and belongs to the Thymelaeaceae family. It is widely distributed in Malaysia, Indonesia, and the Borneo Islands. Traditionally, its leaves were used to relieve bruises and studies have shown that they function as an antioxidant, aphrodisiac, and tranquilizer. Despite its proven beneficial medicinal properties, information regarding its toxicity is limited. Therefore, this study was conducted to investigate the male reproductive toxicity of AM. Prior to the toxicity study, the quality and antioxidant property of standardized *A. malaccensis* leaves aqueous extract (AMLAE) was determined by a set of phytochemical screening, High-Performance Liquid Chromatography (HPLC) analysis, heavy metal, microbial contamination and *in vitro* antioxidant tests. The general toxicity of AMLAE was evaluated based on acute and sub-acute oral administration in Sprague Dawley (SD) rats according to the Organization for Economic Cooperation and Development (OECD) Guideline 420 and 407 respectively. The OECD Guideline 421 was selected to evaluate the male reproductive toxicity analysis which comprised of control group and three AMLAE-treated groups (100, 300 and 500mg/kg) respectively. In total of 63 days of oral administration was carried out prior to the one-to-one mating activity with female. Male necropsy was conducted upon sperm-positive vaginal smear to evaluate the male reproductive parameters. Pregnant female rats were necropsied on day 21 to evaluate the reproductive outcomes via caesarean hysterectomy. The extraction protocol successfully yielded 17.64% powder extract. Phytochemical analyses revealed the presence of saponins, phenolics, tannins, flavonoids and aromatic compounds. No microbial and heavy metal contamination was detected. HPLC analysis of the AMLAE revealed that it contained mangiferin (31.08mg/g) as one of its major constituents. AMLAE indicated strong cupric ion reducing power and potent scavenging activity with 740.83mmol Trolox equivalent/g and  $1.24 \pm 0.27\mu\text{g/ml}$  respectively. The assessment of acute toxicity revealed that AMLAE did not influence mortality, clinical behaviours, body weight gain, or necropsy findings at a dose of 2000mg/kg body weight. In the sub-acute toxicity, both male and female rats had shown abnormalities in the liver and kidney histology at the dose of 2000mg/kg. No significant findings were recorded in male reproductive parameters and reproductive outcomes on pregnant rats except significant elevations in the *in vivo* antioxidant activity, hormonal concentration, testicular histology, protamination level and protamine 1 gene expression. Data from present results revealed that AMLAE did not exhibit toxicity on male reproductive system and the no observed adverse effect level for male reproductive toxicity was >500mg/kg via oral route.

## خلاصة البحث

عود (خشب) أو المعروف محلياً باسم agarwood هو نوع من جنس العود *Aquilaria* وينتمي إلى عائلة ويتم توزيعه على نطاق واسع في ماليزيا وإندونيسيا وجزر بورنيو. وتقليدياً، تم استخدام أوراقها لتخفيف الكدمات وأظهرت الدراسات أنها تعمل كمضاد للأكسدة، كمنشط جنسي، ومهدئ. وعلى الرغم من الخصائص الطبية المفيدة التي أثبتت جدواها، إلا أن المعلومات المتعلقة بسميتها محدودة. لذلك، أجريت هذه الدراسة للتحقيق في سمية الذكور الإنجابية من أقويلاريا ملاسينسيس. قبل دراسة السمية، تم تحديد جودة ومقاومة مضادات الأكسدة الخاصة بمعيار أقويلاريا ملاسينسيس المستخلص المائي من خلال مجموعة من الفحوصات الكيميائية النباتية وتحليل كروماتوجرافيا السائل عالي الأداء والمعادن الثقيلة والتلوث الميكروبي واختبارات مضادات الأكسدة المختبرية. وتم تقييم السمية العامة لهذا التحليل استناداً إلى الإعطاء الفموي الحاد وشبه الفرعي في فئران سبراغ داوولي وفقاً لمبدأ منظمة التعاون الاقتصادي والتنمية التوجيهي على التوالي. تم اختيار المبدأ التوجيهي 421 لمنظمة التعاون الاقتصادي والتنمية لتقييم تحليل السمية التناسلية للذكور والذي يتكون من مجموعة المراقبة وثلاث مجموعات المعالجة (100 ، 300 و 500 ملغ / كلغ) على التوالي. وتم تنفيذ ما مجموعه 63 يوماً من تناوله عن طريق الفم قبل نشاط التزاوج الفردي مع الإناث. وأجريت عملية التشريح الذكري عند التشويه المهيلي الإيجابي للحيوانات المنوية لتقييم العوامل التناسلية للذكور. وتم استحضار الفئران الأنثى الحامل في اليوم 21 لتقييم النتائج الإنجابية عن طريق استئصال الرحم القيصري. وحقق بروتوكول بنجاح 17.6% من استخراج المسحوق. وكشفت التحليلات الكيميائية النباتية وجود الصابونين والفينول والعفص والفلافونويد والمركبات العطرية. ولم يتم الكشف عن تلوث المعادن الميكروبية والثقيلة. وكشف تحليل ايج.بي.ايل.سي ل أم.لا.بي أنه يحتوي على مانجيفيرين (31.08mg/g) كأحد مكوناته الرئيسية. وأشارت أم.لا.بي إلى أن أيونات الكبريت القوية تقلل من القدرة ونشاط التنظيف القوي مع مكافئ 740.83 مكافئ ترولوكس/غرام و  $0.27 \pm 1.24$  ميكروغرام/مل على التوالي. وكشف تقييم السمية الحادة أن أم.لا.بي لم يؤثر على الوفيات، أو السلوكيات السريرية، أو زيادة وزن الجسم، أو نتائج التشريح بجرعة 2000 مغ/كغ من وزن الجسم. في السمية دون الحادة، أظهر كل من ذكور وإناث الفئران تشوهات في الأنسجة الكبدية والكلية بجرعة 2000 ملغرام/غرام. ولم تسجل أي نتائج ذات دلالة إحصائية في البارامترات التناسلية للذكور والنتائج الإنجابية على الفئران الحوامل باستثناء ارتفاعات كبيرة في نشاط مضادات الأكسدة في الجسم الحي، والتركيز الهرموني والأنسجة الخصية ومستوى البروتامين والتعبير الجيني. وكشفت المعطيات من النتائج الحالية أن أم.لا.بي م تظهر سمية على الجهاز التناسلي الذكري ولم يكن مستوى التأثير السلبي الملحوظ للسمية التناسلية للذكور > 500/غرام ملغرام عن طريق الفم.

## **APPROVAL PAGE**

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## DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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