



UNIVERSITI PUTRA MALAYSIA

***THE EFFECT OF GENDARUSSA VULGARIS METHANOLIC
EXTRACT ON THE REPRODUCTIVE ORGANS OF FEMALE MICE***

WAN NURHAKIMAH BINTI WAN ZAKARIA

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FACULTY OF VETERINARY MEDICINE
UNIVERSITI PUTRA MALAYSIA
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2015

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ON THE REPRODUCTIVE ORGANS OF FEMALE MICE**

WAN NURHAKIMAH BINTI WAN ZAKARIA

A project submitted to the Faculty of Veterinary Medicine, Universiti Putra Malaysia

in partial fulfilment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

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It is hereby certified that we have read this project paper entitled “The Effect of *Gendarussa vulgaris* Methanolic Extract on the Reproductive Organs of Female Mice”, by Wan Nurhakimah binti Wan Zakaria and in our opinion it is satisfactory in terms of scope, quality and presentation as partial requirement for the course VPD 4999- Final Year Project.

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DEDICATION

I would like to dedicate this project to the loved ones who contributed directly and indirectly to my project

MY FAMILY

Abah, Ma, Kak long, Angah, Kak, Akim, Nain

MY SUPERVISOR

Dr Wan Mastura Shaik Mohamed Mossadeq

MY CO-SUPERVISOR

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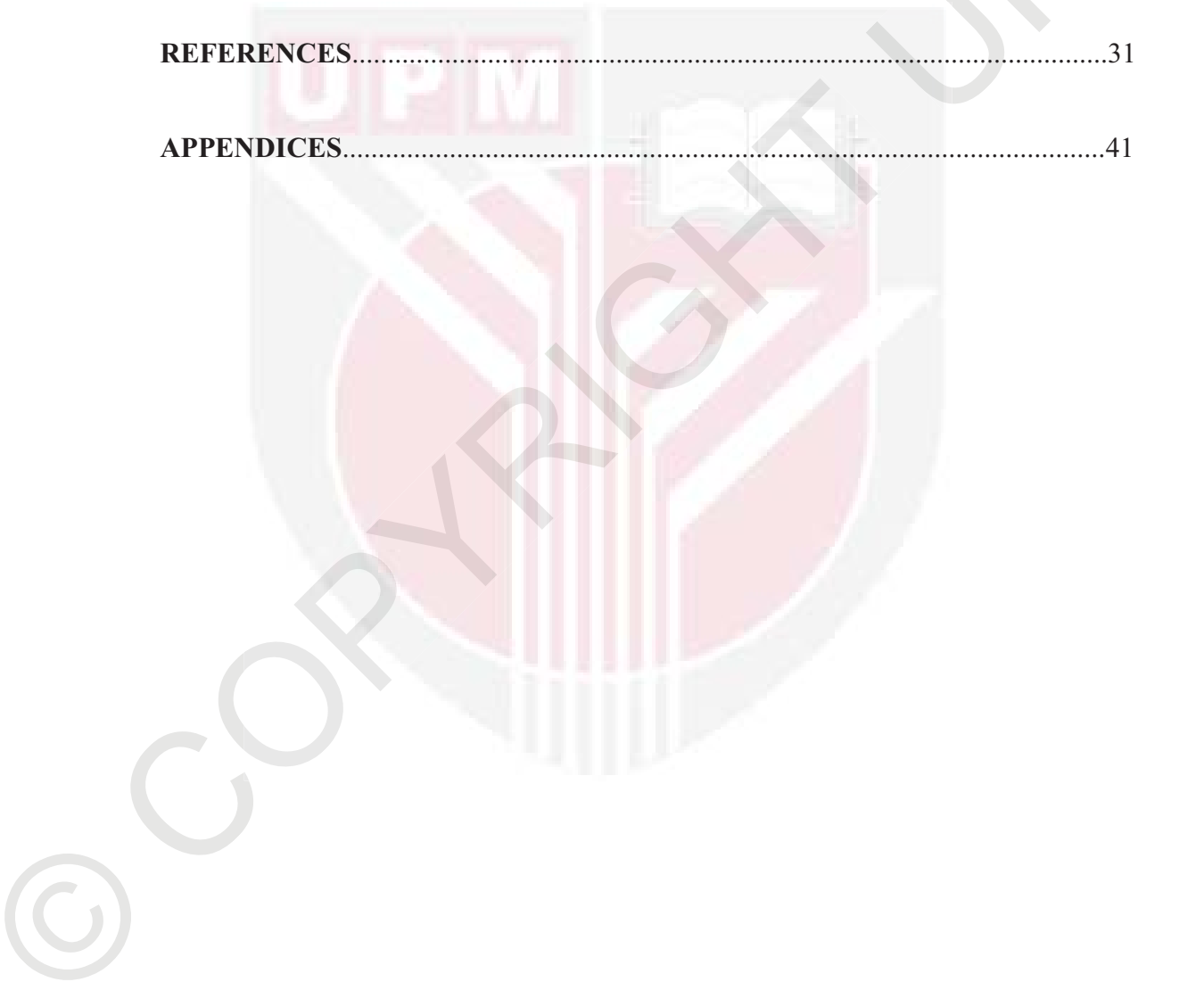
My deepest gratitude goes to my parents; Mr. Wan Zakaria Wan Salleh, Mrs. Zauyah Abdullah and my siblings for their endless love, prayers, encouragement and always there cheering me up and stood by me through out my life.

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ABSTRAK

Abstrak daripada kertas kerja yang dikemukakan kepada Fakulti Perubatan Veterinar, UPM untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Tahun Akhir.

**KESAN EKSTRAK METANOL *GENDARUSSA VULGARIS* TERHADAP
ORGAN PEMBIAKAN MENCIT BETINA**

Oleh

Wan Nurhakimah binti Wan Zakaria

2015

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Penyelia bersama : Dr Md Sabri Mohd Yusoff

Gendarussa vulgaris (syn. *Justicia gendarussa*) dikenali sebagai gandarusa atau daun rusa oleh masyarakat Melayu. Tumbuhan ini berasal dari keluarga Acanthaceae and kebiasaannya dijumpai di kawasan tropika Asia termasuk Malaysia. Pokok ini digunakan secara tradisional untuk merawat demam, patah tulang, reumatisma, artritis, sakit otot dan banyak lagi. Pokok ini telah terbukti mempunyai ciri-ciri antibakteria, antiparasitik, antinosiseptif, antioksidan, antiangiogenik, anti-keradangan, antiviral dan antitumor. Tambahan lagi, daun pokok dikomersialkan dalam bentuk pil dan kebiasaannya digunakan di Indonesia sebagai agen kontraseptif untuk lelaki. Gendarusin A dan B yang terdapat di dalam pokok ini dapat merangsang kemandulan dengan menghalang penembusan sperma ke dalam ovum

secara *in vitro*. Walau bagaimanapun, kesan daun ini terhadap organ pembiakan wanita masih tidak diketahui. Kesan pokok in terhadap organ pembiakan mencit betina dikaji. Ekstrak metanol *Gendarussa vulgaris* (GVME) telah disediakan daripada daun matang yang dikutip di Kulim, Kedah. Sebanyak 24 ekor mencit (n=8) telah dibahagikan kepada 3 kumpulan. Kumpulan kawalan positif telah diberikan 0.35 mg/kg chlomiphene sitrat, kumpulan kawalan negatif diberikan 0.9% NaCl (10ml/kg) manakala kumpulan rawatan diberikan dos GVME 300mg/kg (p.o.) sekali sehari untuk tempoh 10 hari. Kesan GVME terhadap fungsi organ pembiakan mencit betina diperhatikan berdasarkan tanda-tanda yang ditunjukkan sepanjang tempoh rawatan seperti tabiat makan dan minum, ketidakselesaan, hilang/ kurang pergerakan, postur rehat yang tidak normal, keadaan yang tidak terurus, menjilat, menggigit, menggaru atau menggeletar, kematian, sakit ketika dipegang dan perubahan berat badan. Organ-organ pembiakan iaitu ovari dan uterus serta hati dan buah pinggang juga diambil selepas rawatan. Keputusan menunjukkan tiada perubahan tidak normal atau kematian diperhatikan sepanjang tempoh rawatan. Tiada perubahan yang signifikan $p < 0.05$ pada berat badan untuk ketiga-tiga kumpulan. Keputusan skor lesi menunjukkan tiada perbezaan signifikan untuk lesi kongesi dan keabnormalan sel granulosa ovari sebelah kanan dan kiri; dan kongesi pada uterus di antara kumpulan kawalan dan kumpulan rawatan pada $p < 0.05$. Selain itu, tiada perbezaan signifikan pada kongesi hati dan buah pinggang dan nekrosis buah pinggang pada $p < 0.05$. Walau bagaimanapun, skor lesi untuk keradangan uterus di antara kumpulan menunjukkan beza yang signifikan pada $p < 0.05$. Fungsi sistem pembiakan haiwan betina boleh dikaji dengan menilai kadar kebuntingan dan peratusan kelahiran yang tidak normal. Selain itu, rawatan menggunakan ekstrak

yang berbeza seperti etanol dan akuas perlu dijalankan untuk membandingkan kesannya terhadap sistem pembiakan yang mana ekstrak yang berbeza memiliki kompaun bioaktif yang berbeza. Penilaian fungsi pembiakan juga boleh dilakukan dengan mengukur tahap hormon pembiakan yang dihasilkan oleh mencit sebelum dan selepas rawatan. Akhir sekali, status kesihatan haiwan yang berbeza perlu diketahui untuk memastikan mencit yang diguna dalam kajian adalah sihat untuk mengelakkan pemerhatian berat sebelah yang tidak disengajakan.

Kata kunci: *Gendarussa vulgaris*, ekstrak metanolik, fungsi pembiakan, mencit

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine, UPM in partial requirement of the course VPD 4999 -Final Year Project.

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ON THE REPRODUCTIVE ORGANS OF FEMALE MICE**

By

Wan Nurhakimah binti Wan Zakaria

2015

Supervisor : Dr Wan Mastura Shaik Mohamed Mossadeq

Co-supervisor : Dr Md Sabri Mohd Yusoff

Gendarussa vulgaris (GV) (syn. *Justicia gendarussa*) is locally known as gandarusa or daun rusa in Malay. This plant belongs to Acanthaceae family and is commonly found in tropical Asian regions including Malaysia. This plant is traditionally being used to treat fever, bone fracture, rheumatism, arthritis and muscle pain, and amongst others. The plant has been proven to have antibacterial, antiparasitic, antinociceptive, antioxidant, antiangiogenic, anti-inflammatory, antiviral and antitumor properties. Moreover, the leaves of the plant in the form of pills are commercially being used in Indonesia as contraceptive agent in men. Gendarusin A and B that are present in the plant have been shown to induce sterility *in vitro* by preventing penetration of sperm into the ovum. However, the effect of the plant on the female reproductive organs is still unknown. This study

embarks on the effect of plant on the reproductive organs of female mice. *Gendarussa vulgaris* methanolic extract (GVME) was prepared from the mature leaves collected from its natural habitat in Kulim, Kedah. A total of 24 mice (n=8) were divided into 3 groups. The positive control group was treated with 0.35mg/kg clomiphene citrate; the negative control group was treated with 0.9% NaCl at 10ml/kg bodyweight while the third group was treated with 300mg/kg GVME (p.o.) once daily for 10 days. Behaviour of mice in each group such as feeding and drinking, defecating and urinating, restlessness, loss/reduce mobility, abnormal resting posture, unresponsiveness, failure to groom/unkempt appearance, licking, biting, scratching or shaking, mortality, pain upon restraining and body weight changes were observed during the treatment period. At the end of the treatment period, mice were euthanised and their reproductive organs were harvested. No abnormal behavioural changes or mortality were observed during the treatment period. The results were not significantly different $p < 0.05$ for the body weight changes for all the 3 groups. Results for the lesion scoring showed no significant difference for congestion and granulosa cell abnormality of the right ovary, left ovary and congestion in uterus between control groups and treatment group at $p < 0.05$. The liver and kidney congestion and kidney necrosis showed no significant difference at $p < 0.05$. However, the lesion score of uterine inflammation between groups and within groups showed significant difference at $p < 0.05$. The lesion score for the liver necrosis is significantly different between control groups and treatment group at $p < 0.05$. The function of the female reproductive system may be assessed by monitoring the pregnancy rate and the percentage of abnormality among the newborns. Furthermore, treatment using different extracts such as ethanol and

aqueous should be conducted to compare the effects of these 2 extracts on the reproductive system, as different extract possess different bioactive compounds. The assessment of the reproductive function may also be done by measuring the levels of the reproductive hormones produced at pre- and post-treatment periods. Finally, the health status of the mice need to be determined to ensure that the mice used for the study are healthy to avoid unintentional observational bias.

Keywords: *Gendarussa vulgaris*, methanolic extract, reproductive function, mice



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1.0 INTRODUCTION

In Veterinary Medicine, induction of sterility is done to control the population of pest (Kennelly and Converse, 1993) such as screw-worm fly (Bushland and Hopkins, 1951) and Norway rat (Ericsson, 1970); wildlife such as feral pig (Elder, 1964), red foxes in Alaska (Bailey, 1992) and Canada geese (Akesson and Ravelling, 1982); and stray animals such as cats (Baran et al., 2010) and dogs (Jana and Samanta, 2007). Sterility is also induced to enhance the production efficiency of aquaculture (Gall, 1997) either by surgical intervention or non-surgical intervention using antifertility agent (Kennelly and Converse, 1993), immunocontraceptives (Hedge, 2013) and targeted gene silencing (Dissent et al., 2012) However, the cost of surgical procedures such as castration (Neville, 1983), vasectomy (Mech and Fritts, 1993), ovariectomy, tubal ligation, ovariectomy, salpingectomy or subtotal hysterectomy (Bloomberg, 1996) is high compared to non-surgical procedures. Moreover, surgical intervention gives a permanent effect which means that animals will become sterile forever while non-surgical intervention gives temporary effect which means that the animal still has a chance to reproduce.

The antifertility agent is further divided into natural herbs or chemicals. Example of natural herbs that possess antifertility effect are: *Hibiscus rosasinensis* (bunga raya in Malay), *Momordica charantia* (bitter melon or peria in Malay), *Azadirachta indica* (neem or semambu in Malay), *Aristolochia indica* (Indian birthwort or hempedu beruang in Malay) (Farnsworth and Walker, 1982) and *Gendarussa vulgaris* (black adusa or gandarusa in Malay) (Prajogo, 1988). Separately, examples of chemicals used to induce fertility include, intratesticular

injection of calcium chloride (Baran et al., 2010), zinc gluconate compound buffered with arginine (Zeuterin™) and megestrol acetate (Bloomberg, 1996) in addition to immunocontraceptives such as GnRH-targeted vaccine (GonaCon™) (Miller et al., 2013) and GnRH agonist (Suprelorin™) (Herbert and Trigg, 2005). Although all the methods described before may prevent births, they may also induce unintended changes to behaviour and physiology of the animal (Nettles, 1997; Gray and Cameron, 2010). This is because the body's homeostasis is not functioning as normal as previously and adaptation to the new condition may alter the physical and biological processes in the animal.

Currently, a vast body of research is being catered towards using natural herbs as an alternative medicine as the source of materials are easily available and the cost of production is reasonable. In future, the use of alternative medicine may benefit the owner of the animal especially companion animal owners who are planning to prevent their animals from unwanted breeding using cheaper alternatives. The use of natural herbs to induce sterility in aquaculture for example can save cost and time as the herbs can be mixed together in the feed.

The native people of Indonesia used *Gendarussa vulgaris* as a traditional medicine for various ailments and especially as a natural source of contraceptive agent for men. In addition, the male contraceptive pills are commercially available and has long been used by the local people for family planning. To date, extensive research have been conducted to assess the effect of this plant on male reproduction especially in Indonesia but studies on the effect of this plant on the female reproductive organs are currently lacking. We investigated the effect of *G. vulgaris* on the reproductive organs of female mice.

1.1 Objective

To determine the effect of *Gendarussa vulgaris* methanolic extract on the reproductive organs of female mice.

1.2 Hypothesis

Gendarussa vulgaris methanolic extract exert adverse effects on the reproductive organs of female mice.



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