EVALUATION OF THE REPRODUCTIVE AND DEVELOPMENTAL TOXICITIES OF THE AQUEOUS EXTRACT OF *Labisia pumila var. alata* IN FEMALE RATS

by

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DECLARATION

I declare that the contents presented in this thesis are my own work, which was done at School of Medical Sciences, Universiti Sains Malaysia unless stated otherwise. The thesis has not been previously submitted for any other degree.

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LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA	Analysis of variance
CFSAN	Center for Food Safety and Applied Nutrition
CMBW	Corrected maternal body weight
°C	Degree of Celcius
D	Diestrus
DCA	Drug Control Authority
Ddel	Day upon delivery
Dkill	Day upon sacrifice
DW	Distilled water
E	Estrus
EU	European Union
FBW	Female body weight
FRIM	Forest Research Institute of Malaysia
FSH	Follicle stimulating hormone
F. tes.	Free testosterone
g	gram
GMP	Good Manufacturing Practice
GnRH	Gonadotropin-releasing hormone
GUW	Gravid uterine weight
H & E	Haematoxylin and eosin
HKL	Kuala Lumpur Hospital
HUKM	Hospital Universiti Kebangsaan Malaysia
HUSM	Hospital Universiti Sains Malaysia
ICH	International Conference on Harmonization
IMR	Institute for Medical Research
IQR	Interquartile range
KF	Kacip Fatimah
kg	Kilogram
LH	Luteinizing hormone
LPA	*Labisia pumila var. alata*
LD_{50}	Median lethal dose

LPL	*Labisia pumila var. lanceolata*
LPP	*Labisia pumila var. pumila*
M	Metestrus
MARDI	Malaysian Agricultural Research and Development Institute
MBW	Maternal body weight
mg	milligram
ml	millilitre
MOH	Ministry of Health
n	Sample size of animal
NCTR	National Center for Toxicological Research
NOAEL	No observable adverse effect level
NPCB	National Pharmaceutical Control Bureau
NRDHM	National Committee for Research and Development in Herbal Medicines
OECD	Organization for Economic and Cooperation and Development
P	Proestrus
pc	Post-coitus
Prog.	Progesterone
SEM	Standard error of mean
SD	Sprague Dawley
T/CM	Traditional and complimentary medicine
TLC	Thin layer chromatography
UH	Universiti Hospital, Universiti Malaya
UK	United kingdom
UKM	Universiti Kebangsaan Malaysia
UM	Universiti Malaya
UMS	Universiti Malaysia Sabah
UTM	Universiti Teknologi Malaysia
US	United States
USA	United State of America
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration

USM	Universiti Sains Malaysia
WHO	World Health Organization
11 β-HSD1	11 Beta Hydroxysteroid Dehydrogenase type 1
17-β oestradiol	17-Beta oestradiol

KAJIAN KETOKSIKAN REPRODUKTIF DAN PERTUMBUHAN EKSTRAK AIR *Labisia pumila var. alata* PADA TIKUS BETINA

ABSTRAK

Labisia pumila var. alata (LPA) atau Kacip Fatimah disenaraikan sebagai salah satu spesis utama oleh kerajaan Malaysia untuk dijalankan kajian saintifik pelbagai disiplin ke arah pembangunan ubatan herba tempatan berasaskan bukti saintifik. Kajian yang dihuraikan di dalam tesis ini adalah sebahagian daripada kajian pra klinikal ke atas LPA untuk menilai profil keselamatannya. Tujuan kajian ini adalah untuk menentukan potensi kesan sediaan komersial ekstrak air LPA ke atas ketoksikan reproduktif dan pertumbuhan pada tikus betina Sprague Dawley. Kajian permulaan dijalankan dengan menggunakan ekstrak air tidak terpiawai LPA pada dos 2, 20, 200 dan 400 mg/kg/hari. Sediaan komersial ekstrak air terpiawai LPA, didaftarkan sebagai Biolabisia® pada dos 2, 20, 200, 400, 1000 mg/kg/hari atau air suling (kawalan) diberikan kepada tikus secara gavaging dalam kajian utama. Kajian Teratogenik dijalankan dengan memberikan rawatan Biolabisia® kepada tikus hamil semasa peringkat organogenesis. Rawatan ke atas tikus diberi sejak sebelum tempoh mengawan sehingga hari ke-7 waktu laktasi dalam kajian Ketoksikan reproduktif dan sebelum mengawan sehingga hari ke-15 kehamilan bagi kajian Kesuburan. Tikus dara diberikan rawatan dengan Biolabisia® selama tiga minggu dalam kajian ketoksikan am. Beberapa parameter reproduktif dan pertumbuhan dinilai di sepanjang tempoh kajian. Semua data numerikal dianalisa menggunakan SPSS versi 12.0.1. Keputusan diperolehi menunjukkan

pemberian semua tahap dos Biolabisia® tidak menyebabkan kematian atau tanda-tanda ketoksikan yang ketara pada keadaan fizikal dan perlakuan keseluruhan tikus yang dikaji. Berat badan tikus betina menunjukkan corak perubahan yang sama tetapi tiada keputusan yang signifikan di dalam semua eksperimen kecuali bagi penurunan signifikan berat badan dan berat badan sebenar ibu di dalam kajian Kesuburan. Rawatan dengan Biolabisia® tidak menyebabkan kesan teratogenik ke atas tikus di mana tiada fetus yang mempamerkan kecacatan kongenital luaran. Jumlah bilangan korpora lutea, bilangan tapak implantasi, peratus kehilangan embrio sebelum implantasi, peratus kematian embrio selepas implantasi, bilangan resorpsi fetus, bilangan fetus hidup, berat badan fetus dan nisbah jantina fetus adalah setanding di kalangan semua kumpulan rawatan. Tempoh kitaran estrus dan berat organ reproduktif tidak terjejas oleh Biolabisia® secara statistik. Walaubagaimanapun, peningkatan bilangan tikus dengan kitaran estrus tidak teratur, kemunculan sis folikel ovari dan corak peningkatan aras hormon progesteron and testosterone bebas bagi kumpulan yang menerima dos Biolabisia® 1000 mg/kg/hari mencetuskan kebimbangan. Dengan mengambil kira semua data terkumpul, penemuan kajian ini mencadangkan bahawa rawatan oral Biolabisia® sehingga ke dos 1000 mg/kg/hari ke atas tikus betina tidak menunjukkan kesan ketoksikan yang signifikan. Walaubagaimanapun, berat badan dan kitaran estrus tikus berkemungkinan terjejas jika rawatan dengan Biolabisia® pada dos tinggi diberikan untuk jangkamasa panjang. Penyelidikan berterusan menggunakan spesis haiwan bukan roden perlu dijalankan untuk melengkapkan profil keselamatan herba ini.

EVALUATION OF THE REPRODUCTIVE AND DEVELOPMENTAL TOXICITIES OF THE AQUEOUS EXTRACT OF *Labisia pumila var. alata* IN FEMALE RATS

ABSTRACT

Labisia pumila var. alata (LPA) or Kacip Fatimah has been listed by the government of Malaysia as one of the priority species for a multi-disciplinary scientific study towards the development of scientific, evidence-based herbal medicine. The present study described in this thesis is part of the preclinical assessment of LPA to evaluate its safety profile. The aims of this study were to evaluate the potential effects of aqueous extract of LPA on reproductive and developmental toxicities in Sprague Dawley rats. A preliminary study was conducted using unstandardised extract of LPA at 2, 20, 200 and 400 mg/kg/day. Commercially prepared standard aqueous extract of LPA, registered as Biolabisia® at the doses of 2, 20, 200, 400, 1000 mg/kg/day or distilled water (control) were administered by gavaging to animals during the main studies. The Teratogenic study was conducted by treating pregnant animals with Biolabisia® during the period of organogenesis. Treatments of animals began from the time prior to mating until lactational periods of seven days in the Reproductive toxicity study and from before mating up to day 15 of pregnancy in the Fertility study. Virgin rats were treated with Biolabisia® for duration of three weeks in the Female toxicity study. A substantial number of reproductive and developmental parameters were assessed throughout this study. All numerical data were analysed using SPSS version 12.0.1. Results obtained indicated that

the administration of Biolabisia® at all dose levels did not cause mortality nor show noticeably any treatment-related signs of toxicity on the physical appearance and behaviour of all the rats studied. There was a consistent trend with body weight changes of females in all the experiments performed but they showed no significant differences except for the significant reduction in the actual and corrected maternal body weights of Biolabisia®-treated animals in the Fertility study. Treatment with Biolabisia® did not result in teratogenicity in rats since no foetus exhibited external congenital malformations. The total number of corpora lutea, number of implantation sites, percentages of pre-implantation loss and post-implantation death, number of resorptions, number of live foetuses, foetal body weight and foetal sex ratio were comparable among all experimental groups. The length of oestrous cycles and reproductive organs weights of rats were statistically unaffected by Biolabisia®. However, high number of animals with irregular cycles, presence of ovarian follicular cysts and a trend of increased serum progesterone and free testosterone levels that were observed in the Biolabisia® 1000 mg/kg/day dose group do raised some concern. Taking all the cumulative data together, the current findings suggest that oral treatment of Biolabisia® of up to 1000 mg/kg/day in female rats is not associated with significant deleterious effects. However, the body weight and oestrous cycle of the females may be affected if higher doses of Biolabisia® are administered for a longer duration. Studies involving non-rodent animal species need to be performed to complete the safety profile of this herb.

CHAPTER ONE

INTRODUCTION

1.1 Preface

During the 21st century, there has been an elevated level of hazardous risk due to xenobiotics and toxic substances towards the reproductive function of the human (Osamu, 2005). These groups of substances which include synthetics, naturally occurring chemicals, natural products, food additives, drugs, heavy metals, environmental pollutants, natural toxin and so forth have been included in the list of possible hazards (Sharara *et al.*, 1998). Some of the substances are recognised (Hutz *et al.*, 2006; Llanos and Ronco, 2009) and some are suspected (Sharara *et al.*, 1998) as reproductive toxicants. Yet, there are still marketed products that use materials or chemicals, which have not been analyzed for its reproductive toxicity.

In many instances, women are believed to be more vulnerable and at a higher risk than men in experiencing negative responses while being assessed with the reproductive effects of some of the aforementioned agents (Hoyer, 2001; Butter, 2006; Vahter *et al.*, 2007). Episodic exposure to high levels of some of these agents is responsible for the acute and chronic female reproductive and developmental health problems. Disorders of reproduction reported in females include infertility, menstrual disorders, reduction in libido, pregnancy complications, adverse outcomes of pregnancy, spontaneous abortion, low birth weight, heritable defects, premature reproductive

senescence, lactation disorders, various genetic diseases, and cancers (Daston *et al.*, 1997; Drozdowsky and Whittaker, 1999; Hutz *et al.*, 2006).

The prevailing risks from the above-mentioned inputs towards female reproductive health have caused much public concern and regarded as a relatively recent medical challenges raising considerable attention from scientific communities and legislators (Daston *et al.*, 1997; Kimmel and Makris, 2001). Stemming from conventional toxicology, this area of interest has evolved and ramified into a field known as female reproductive and developmental toxicology (Neubert *et al.*, 1999a; Christian, 2001c; Collins, 2006).

In Malaysia, the field of reproductive toxicology is comparatively new as an area of research. To date, Malaysia is not a member in any of the international toxicological organization. Nevertheless, Malaysia through its Ministry of Health (MOH) has adapted various existing international guidance and has come out with its own regulation for conducting toxicological testing of materials (Ministry of Health Malaysia, 2003a). Researchers in this country are now ushering the community to take steps towards exploring this field upon realizing its enormous importance. These actions are genuinely taken in an attempt to improve the general health of the nation. Reproductive toxicology is currently experiencing a surge significance as there is a thrust in this area by the Malaysian government to encourage the production of high quality herbal products and nutraceuticals derived from local bioresources. The toxicological testings required in the development of these products will help to reduce public anxiety over the possible deleterious effects associated with herbal use.

1.2 Reproductive and developmental toxicology

Toxicology is the study of poisons, their adverse effects on biological systems and methods of treatment for conditions they produce (Friis, 2006). The challenging science of toxicology has been in existence for many centuries. It has evolved from an expression of thoughts concerning poisons, victims and plants or chemicals as sources of ills to the current scientific discipline that it is. This field continues to establish and expand its principles into a range of sub-specializations, for instance, reproductive and development toxicology, food and chemical toxicology, occupational medicine and industrial toxicology, forensic toxicology and so forth. All share one main goal that is to provide valuable information in the pursuit of ideals to protect people from harmful effects of hazardous agents (Borzelleca, 2001). Among the wide range of potential health risks, a prominent concern is that the agents may interfere with the functions of the reproductive system vis-à-vis reproductive and developmental toxicology.

Reproductive and developmental toxicology investigate the effects of drug and other xenobiotics, which collectively show potential to impair human reproductive health. The consequences of even minor impairment during the reproductive process and / or during the development of the growing foetus can result in devastating outcomes to the organism (Miller *et al.*, 2004). The complexity of component within the reproductive unit comprising the dam, sire and the progenies has driven the field of reproductive toxicology to be one of the vital scientific specialization recognised globally (Hoyer, 2001). According to Hayes (2001a), reproductive toxicology is concerned with the causes, mechanism and sequelae of adverse effects of substances on the reproductive

process. The adverse effects may include alterations to the sexual organs and their functions, related endocrine regulation or pregnancy outcomes. They can be manifested as aberrations or modifications on sexual maturation, gamete production and transport, oestrous cycle, sexual behaviour, fertility, gestation, parturition, lactation, pregnancy outcomes and reproductive senescence (Christian, 2001c). The field is separated into areas related to male and female fertility, and developmental toxicology (sometimes referred to as teratogenicity).

Developmental toxicology is the study of the causes, mechanisms and sequelae of perturbed developmental events in species of animals that undergo ontogenesis. The affected endpoints include death, delayed / retarded development, dysmorphology and functional impairment (Hayes, 2001b). Based on these definitions, developmental toxicology is considered synonymous with "teratology". Special emphasis is put on the developing conceptus, which include the development and function of that conceptus throughout its entire lifespan (Christian, 2001c). This field can be further narrowed down into prenatal and postnatal toxicology (Hayes, 2001c).

Presently toxicological evaluation and regulations of chemicals referred earlier were developed and reviewed as a consequence of three human tragedies that resulted from *in utero* exposure to drugs; (i) thalidomide – congenital malformations, (ii) Minimata disease – behavioural / functional alterations and (iii) diethylstilbestrol (DES) – cancer (Christian, 2001c). Before the "Thalidomide disaster" (1959-1962), no routine toxicological evaluation of

chemicals and drugs on reproduction and development was carried out (Neubert *et al.*, 1999a).

Only in mid-1960s, the systematic preclinical studies of the possible adverse effects on reproduction and development necessitated the need for the registration of new pharmaceutical products and certain types of environmentally occurring chemicals, such as pesticides (Neubert *et al.*, 1999a). However, the regulatory research and testing guidelines needed to be reviewed and improved especially after the first and second outbreak of Minimata disease in 1956 and 1965. This occurrence has increased concerns regarding adverse effects of highly toxic chemicals that can cause severe health problems and death to people and animals. Furthermore, cancer that resulted from exposure to DES in early 1970s has once again raised additional attention concerning deleterious effects that were not noticeable until after puberty. Thus, a broad range of endpoints in guidelines was required to evaluate various aspects of potential reproductive toxicity (Christian, 2001c).

1.3 Herbal medicine

Herbal medicine (phytomedicine / botanical medicine) constitutes one of the leading complementary therapies in this century. It is a common component in Ayurveda, pharmacotherapy, homeopathy, naturopathy and so forth. It refers to the use of finished, labelled medicinal products that contain active ingredients of aerial or underground parts of plants, or other plant material, or combinations, whether in the crude form or as plant preparations for the purposes of maintaining and the healing of various health conditions. Herbal medicines may

contain excipients in addition to the active ingredients. Medicines containing plant material combined with chemically defined substances are however, not considered to be herbal medicines (World Health Organization, 1996). The utilisation of herbs as medicine started since time immemorial. Its contributions in maintaining human health is very impressive and well recognised globally. The World Health Organisation (WHO) estimated that 80% of the world population rely on it for their health care needs (Shrivastava *et al.*, 2007).

In recent years, we are witnessing enormous public interests in the use of herbal remedies despite the advancement of modern medicine. The popularity of herbs is experiencing a renewed resurgence with new approaches and use. According to WHO, the herbal usage throughout the world exceeds that of the conventional drugs by two to three times (Pal and Shukla, 2003). The numerous varieties of herbal products in the market are testimony of the current upward trend in its use. They have been widely consumed as alternative medicines in conjunction with conventional synthetic drugs to treat a variety of medical conditions and reverse the progression of health disorders (Kamboj, 2000; Erah, 2002) including premature aging, obesity, body weakness, sexual dysfunction, menopause, women's problem, chronic diseases et cetera.

For various reasons, people often turn to herbal medicines when their long-lasting illness could not be cured by conventional medicine, also when these products can satisfy their needs and show no untoward side effects (Pal and Shukla, 2003). Herbal remedies are not only used in the prevention and treatment of diseases but are also utilized as alternative approaches to enhance

overall well being while they are still in a good health. The healing power of herbs is thought to sooth the soul and help people feel better and live longer due to the naturally derived substances present in them (Kamboj, 2000; Abas, 2001; Seeff, 2009). Moreover, the lower costs and the ease of self-medication with herbal products when compared to synthetic medicine give people a perceived ability to personally control their health (Pal and Shukla, 2003). Apart from the above, the holistic practices that is associated with herbal medicine like prayers (doa), incantation (jampi), abstinence (pantang) and other practices make the process of rehabilitation and health promotion alive and real (Zakaria and Mohd, 1994; Haliza, 2000). Additionally, the believe that herbal medicines are natural products and are therefore safe also contribute to their popularity (Abas, 2001; Niggemann and Gruber, 2003). Although, the fact remains that the safety of plant materials can only be established upon proper scientific studies.

In Malaysia, the government realizes and draws attention to the huge contribution of herbal medicines both in the traditional and complementary medical systems. The government has in stages actively providing the direction for the country in relation to the implementation of strategies, formulation of action plans and programmes, research and development related to herbs. All efforts are now focused towards establishing herbal industry at international level. This becomes one of the main agenda in the eighth Malaysian Development Plan (Ministry of Agriculture Malaysia, 2000; Ministry of Health Malaysia, 2000). These are expected to help spearhead, improve and sustain the herbal industry as an important economic contributor to the nation's coffers.

1.3.1 Safety issue of herbal medicine

Safety is one of the consumer's basic rights. It is defined as the right to be protected against the marketing of goods or provisions of services that are hazardous to health and life (Mohan, 2001). Although there has been a huge escalation in the use of herbs in our country and the world, the scientific input to backup their usage are still lacking. The quality, safety and efficacy of these products remain the main issues of relevance (Erah, 2002; Barnes, 2003). The concern for toxic side effects, their efficacy and limitations must be given serious attention and should not be taken for granted just because they are natural.

Normally medicinal herbs contain potent, bioactive compounds. Many of their pharmacological effects remain unidentified (Abas, 2001; Zakiah, 2001; Barnes, 2003). In fact, their chemical constituents are far from being studied thoroughly. This situation gets complicated when herbal preparations are mixed with other known active ingredients (Erah, 2002) and when they are used as a complement to conventional medicine. As known widely, the preparation and formulation of herbal remedies is different to that of pharmaceutical synthetic medicine. Even if they are now available in the forms of capsule, pills and oral dosage forms but they are not scientifically prescribed by regulatory bodies and the information on their efficacy, appropriate dosages and drug interactions are poorly understood (Abas, 2001; Barnes, 2003). Therefore, they should be used with caution and while they are relatively safe when taken properly as preventive agents and for the promotion of health rather than strictly for healing.

Scientific evidence to justify their safety is much awaited (Abas, 2001; Mohsin, 2005).

Apart from the above, most of herbal preparation is not properly standardised by scientific accredited methods. Standardisation of these products is of particular importance to ensure their therapeutic efficacy (Azizol and Rasadah, 2000). Most of the herbal products available in Malaysia are not assessed for purity and consistency of active compound and this led to misidentification, substitution and adulteration. These could be due to the addition of incorrect species, altered mixtures of species, intentional or unintentional inclusion of allergens, pollen, insect parts, heavy metals such as lead, mercury and arsenic (Seeff, 2009) and scheduled poisons (drugs) (Abas, 2001). Therefore, a proper approach that allows highly precise standardisation both chemically and biologically need to be put forward (Hylands, 2001). Meanwhile, detailed toxicity and safety assessments of medicinal products and subsequently the clinical trial must be performed to assure their safety and efficacy for human consumption.

As the years go by, concern and awareness of any hazardous effects derived from the utilization of herbs among consumers and clinical practitioners are rising. This is a positive scenario where it will encourage the production of safer and high quality herbal products. As emphasized in the WHO guidelines, herbal medicine should be required to undergo toxicology testing in order to verify their safety when used on the human (World Health Organization, 1998). Since 1992, Malaysia through its Drug Control Authority (DCA) acting via its

secretariat, the NPCB has implemented regulatory measures to ensure the quality and safety of any traditional products sent to them for approval and registration. The registered products falling into the list of items under this regulation are expected to be prepared in a scientific manner and comply with the Good Manufacturing Practice (GMP) requirements. Nonetheless, such products are only labelled as cures for general illness and not for treatment of specific disease due to the lack of scientific data to support the claims of their efficacy (Zakiah, 1999).

The dearth of detailed scientific information on the safety and efficacy of herbal products are one of the major problem faced in our country. Indeed, knowledge of their pharmacological properties and chemical constituents are very limited in the literature. The pace of scientific advancement shows an imbalance with the trend of herbal use. Hypothesis, assumptions and claims are definitely not good enough for these products to gain support and acceptance amongst professionals. The importance of the need for scientific research to be carried out by trained scientists is a fact that cannot be denied. The application of science and technology is vital indeed in the mission of transforming ambiguous traditional preparations into modern authenticated pharmaceutical products. Furthermore, systematic documentation and scientific explanation for their properties as medicinals, are urgently needed and are of the utmost importance in order to provide us with valuable and appropriate references on local herbs.

1.3.2 *Labisia pumila* (Blume) Fern.-Vill

One of the most popular herbs that is receiving high demand by the local populace in Malaysia is *Labisia pumila* or locally known as Kacip Fatimah (KF). The term KF is used to describe the plant *L. pumila* in general. There are at least four known varieties of *L. pumila* found in Malaysia (Stone, 1988; Mohd Noh *et al.*, 2002). Though, only three of them are popularly used by the Malays and these are *L. pumila var. pumila* (LPP), *L. pumila var. alata* (LPA) and *L. pumila var. lanceolata* (LPL) (Stone, 1988).

If Tongkat Ali (*Eurycoma longifolia*) is supposed to benefit the male species, *L. pumila* is closely associated to women and therefore thought to benefit the female. It has been utilized by the Malays and aboriginal women of reproductive age for numerous purposes. These include the balancing of bodily function, promotion of women's health and as a sexual stimulant. According to elderly Malays, this herb is a compulsory ingredient in most of the traditional herbal preparation since it is believed that *L. pumila* could improve the effectiveness of the other herbal products (Zainon, 2004). Traditionally, water decoctions of the root or whole plant have been given to pregnant woman prior to childbirth for the main purpose of inducing and facilitating labour (Stone, 1988; Jamia *et al.*, 1999a; Jaganath and Ng, 2000). It is also used as a postpartum medication (Stone, 1988) in the form of mixed preparation to help in the recovery of the post-gravid uterus. It is thought that the herb could normalise the size and function of this organ in a shorter period than the average of six weeks seen in normal mothers (Muhamad and Mustafa, 1994; Yoga, 2003). Further, *L. pumila* is reported to extend fertility, to regularize blood

circulation and to help regain body strength of postpartum mothers (Muhamad and Mustafa, 1994). Its other folkloric uses include treatment of dysentery, flatulence, dysmenorrhoea, gonorrhoea, rheumatism, haemorrhoid and help to firm and tone the abdominal muscles (Jamia *et al.*, 1999b; Jaganath and Ng, 2000). Other than that, many believe that this herb is able to enhance libido (Asiah *et al.*, 2007), alleviate fatigue and promote emotional well being. Thus, it is always recommended to women who have less interest in sex. Furthermore, it is also claimed as a phytoestrogen and therefore may assist women in achieving fuller breasts and can tighten vaginal muscles (Yoga, 2003; Ayida *et al.*, 2006).

The popularity of *L. pumila* is manifested by the emergence of many commercial products in the Malaysian market. These come in many forms including capsules, tablets, canned drinks and instant tea or coffee containing this herb. These products are claimed to contain the ground or extracted parts of the plant (Houghton *et al.*, 1999; Wan Nazaimoon, 2002). Moreover, these products are widely advertised by various advertising media. In fact, the popularity of *L. pumila* is not only amongst Malays but other races as well. Nevertheless up to the year 2002, there were no standardised preparation or scientific data authenticating or evaluating the quality, safety and efficacy of this herb (Houghton *et al.*, 1999). In addition, little is known of the chemical constituents of this plant.

Recognizing the importance and commercial potential of *L. pumila* as a high quality herbal product or nutraceutical, the Government of Malaysia had

identified this herb as one of the priority species for a multi-disciplinary scientific research. The research started in 2002, as part of the National Natural Product Biotechnology Programme and funded by the National Biotechnology Directorate under the Ministry of Science, Technology and Innovation (MOSTI). Funding allocations are from the Ministry's Intensive Research on Priority Areas of the eighth Malaysian Development Plan. Several universities and research institutions, i.e. Institute for Medical Research (IMR), Forest Research Institute of Malaysia (FRIM), Universiti Sains Malaysia (USM), Universiti Kebangsaan Malaysia (UKM), Universiti Malaysia Sabah (UMS) and so forth are involved in this research. The groups' objectives are summarised into five main efforts:

i) Sourcing and propagation of *L. pumila* - FRIM, UMS

ii) Standardisation of crude and formulated *L. pumila* - UKM

iii) Estrogenic and androgenic activities of *L. pumila* - IMR, HUKM

iv) General and reproductive toxicology of *L. pumila* - IMR, USM

v) Clinical trials (phase 1, 2, 3) on *L. pumila* - IMR, HUSM, HUKM, MOH, UH, HKL, Ipoh Hospital.

(Wan Nazaimoon, 2002; IMR, 2003)

The present study is the effort of the fourth group above and is intended to complement the overall documentation of *L. pumila.*

1.4 Scope of the study

The present study was designed to assess the possible reproductive, developmental and female toxicities of LPA using animal model. The LPA chosen is the most commonly used by the Malay community in the country (Jamia *et al.*, 2004) and can be easily obtained throughout the region. The type and method of extract preparation as well as the route of administration in this study were applied based on the traditional ways. This is to mimic the human practices as close as possible and to induce similar effects in the animal model. The decision to undertake this study is due to the fact that this herb is popular and has been used as a natural remedy for female reproductive problems. Couple to the above information, the lack of data on its safety make the intention of this study all the more important.

A set of study comprising of a preliminary experiment and four main studies were conducted in rats, as the best animal model for this study. Prior to the commencement of the main study, a preliminary evaluation of teratogenicity was conducted using self-prepared, unstandardised aqueous extract of LPA. The main studies were subsequently performed as described below using commercially prepared standard aqueous extract of LPA, registered with the National Pharmaceutical Control Bureau (NPCB) as the trade name of Biolabisia®. The studies are listed as follows:

(a) "Evaluation of the Teratogenicity of Biolabisia® in Rats" (hereinafter referred as "Teratogenic study"). This study was designed to screen the teratogenic potential of the herb when it is administered during the organogenesis period.

(b) "Evaluation of the Female Reproductive Toxicity of Biolabisia® in Rats" (hereinafter referred as "Reproductive toxicity study"). This study was designed to screen the effects of the herb on reproductive function and to mimic the traditional consumption of *L. pumila* during the reproductive period, at the end of pregnancy (Stone, 1988; Jaganath and Ng, 2000) and during confinement and breastfeeding (Stone, 1988).

(c) "Evaluation on the Effects of Biolabisia® on Fertility and Implantation in Rats" (hereinafter referred as "Fertility study"). The design of this study was to evaluate the effects of the herb on initiation of pregnancy and to make a representation of the period where usage of *L. pumila* in human continued into the early pregnancy period while the pregnant mother is not aware of the pregnancy.

(d) "Evaluation of the Female Toxicity of Biolabisia® in Rats" (hereinafter referred as "Female toxicity study"). This study was designed to evaluate the effects of the herb during non-pregnancy state and to reflect on conditions where *L. pumila* is consumed by women during the peri menopausal period and in between pregnancies for energy gain and for the sustenance of their reproductive health.

The results obtained from these experiments are expected to provide useful information on the type and degree of toxicities of *L. pumila* on reproductive system in rodent. The collection of data in this study is meant to provide some basis for the development of the safety profile of this herb and thereby help to alleviate concerns over its possible toxicity. Further, studies using a second animal species, preferably non-rodent like rabbits for evaluation

of teratogenicity (ICH-S5A, 1994; OECD, 2001; Janer *et al.*, 2008) and monkeys for female toxicity study (Chahoud, 2004), need to be undertaken in order to complete the investigation of reproductive toxicity of *L. pumila* in animal models.

1.4.1 Test procedures and guidelines used

The designs and methods used in performing the female reproductive and developmental toxicity studies as described in this thesis were adapted from current internationally accepted guidelines (OECD, 1983; ICH Steering Committee, 1993; WHO Regional Office for the Western Pacific, 1993; Manson and Kang, 1994a; U.S. Food and Drug Administration, 2000b; U.S. Food and Drug Administration, 2000a; Christian, 2001c; OECD, 2001). These guidelines were efforts of the World Health Organization (WHO), Organization for Economic and Co-operation and Development (OECD), United States Food and Drug Administration (U.S FDA) and International Conference on Harmonization (ICH). Basically, all organizations address a similar standard guideline. Even though each guideline is presented in its own way and approaches, all aspects of reproductive and developmental toxicity testing are covered. These include various study designs and evaluation endpoints as summarized within the guidelines. Information of each organization and regulatory guidelines will be elaborated in Chapter Two (subchapter 2.5) of the current thesis.

1.5 Objectives

1.5.1 General objectives

The general objectives of the present study were to evaluate the potential effects of Biolabisia® on female reproductive system and development of progenies in rats. This study is a part of the ultimate goal in an effort towards establishing reliable safety profiles for the herb using animal model (rodent).

1.5.2 Specific objectives

The specific objectives of this study are listed below:

i. To prepare the aqueous extract of LPA and to determine the appropriate dose range required for the elucidation of the potential toxicity of the extract on female reproductive system in rats through a preliminary study.

ii. To evaluate the teratogenic potential of commercially prepared LPA (Biolabisia®) in pregnant rats by means of the Teratogenic study.

iii. To evaluate the effects of Biolabisia® on reproductive toxicity of pregnant rats via the Reproductive toxicity study.

iv. To assess the effects of Biolabisia® on fertility and progression of implantation in pregnant rats by means of the Fertility study.

v. To assess the potential female toxicity of Biolabisia® in non-pregnant rats via the Female toxicity study.

The research strategy of this study is indicated in Figure 1.1.

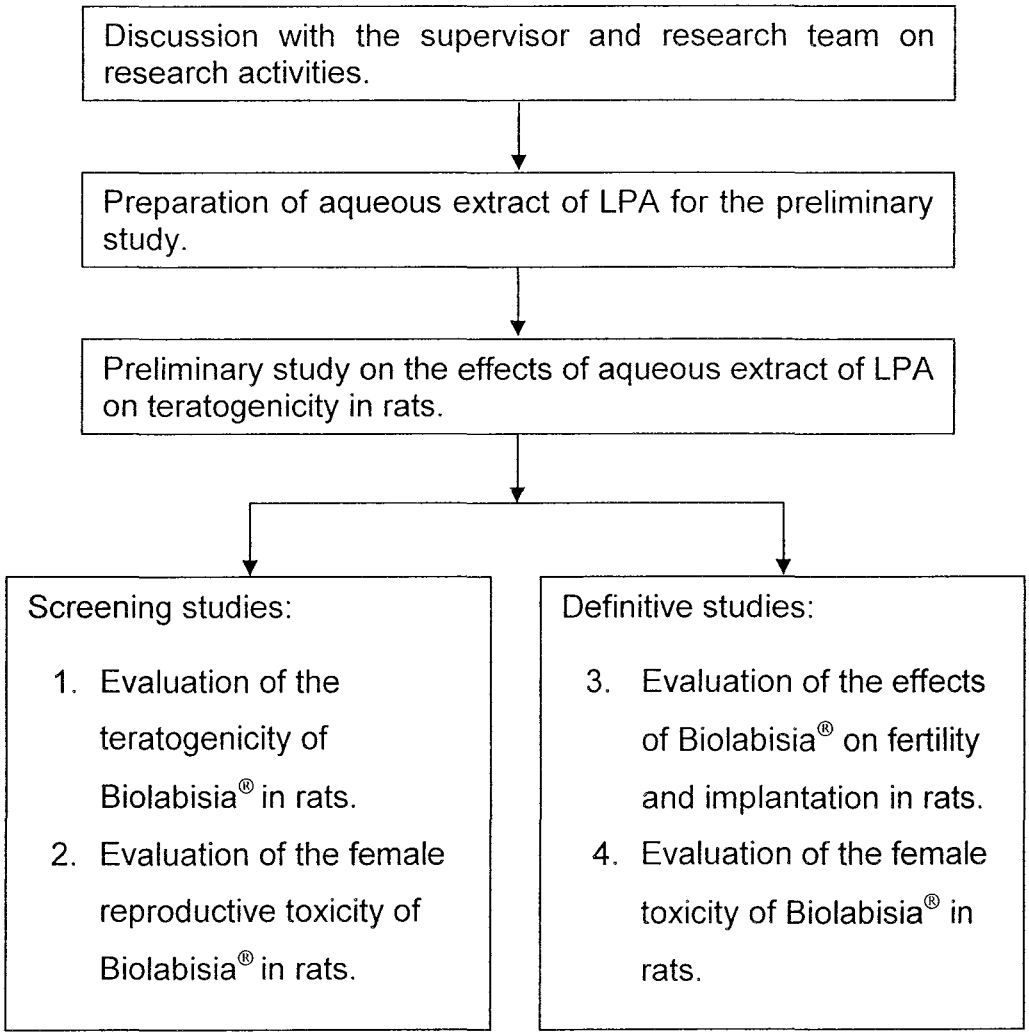

Figure 1.1 Flowchart of the study indicating important research activities

1.6 Importance of the study

The evolution of scientific methodologies in herbal research uncovers huge potential of developing medicinal herbs into therapeutic agents and other health related chemical entities. Concurrently, the present research step up emphasizes quality and safety issues of Biolabisia® that are paramount in ensuring its safety. This research is expected to help establish relevant safety profile and provide assessment of the potential toxicities of this herb during its development as a herbal medicinal product.

Data regarding the adverse effects of *L. pumila* in human has not been reported while no scientific work was ever attempted to investigate the toxicology of this popular herb in the female reproductive system. The limited scientific literature pertaining to its quality, safety and efficacy enhance the urgency of conducting this present research. Information gathered could be utilized as an initial scientific platform for the scientific community in particular, as well as for the general public in general. Further, this research can be a basis for the initiation of other related works investigating the potential toxicological effects of other herbal preparations on female reproductive system. This is especially pertinent for the Malaysian scenario.

Apart from expanding new knowledge regarding this herb, the present research can also indirectly enlighten consumers about *L. pumila* and help to alleviate fear or apprehension in using Biolabisia® as an alternative to the synthetically produced pharmaceutical drugs. This is particularly so in relation to the promotion of women's health. The confidence and support from the public

and professionals can inspire greater awareness and therefore help to improve the herbal market on one hand while creating a stronger public demand for safe products. This can also be a driving force for the development of newer safer herbal medicines from the relatively rich, unexplored plant resources of Malaysia.

Alternatively, the science of reproductive and developmental toxicology applied in this study can also help in ensuring better understanding among Malaysian researchers, while the need to establish adequate number of expertise in this field is also becoming more urgent in Malaysia. In essence, the development of new knowledge concerning potential hazards of consumer products like herbal preparation will ultimately help protect the well-being of the public via improved public health delivery.

CHAPTER TWO

LITERATURE REVIEW

2.1 Medicinal plants in Malaysia

The home to the world's richest biodiversity is widely recognized as being located in the tropical regions (Bodeker, 2000). Nearly 35,000 to 70,000 species out of the estimated 422,000 flowering plant species in the world are used for medicinal purposes. Out of this, at least 6,500 plant species had been reported to have therapeutic properties are found in Asia. This makes Asia as one of the largest biodiversity region of the world. Six of the world's 18 megabiodiversity hot spots are situated in Asia. These include the Eastern Himalaya, North Borneo, Peninsular Malaysia, Sri Lanka, Philippines and the Western Ghats of South India. All these areas are known as the concentrated area of flora species richness (Handa, 2005).

Malaysia is blessed with exceedingly rich and diverse bioresources potentials. In fact, the Malaysian tropical rain forest that evolved over 100 million years is one of the oldest and complex biological ecosystems in the globe (Suhaimi *et al.*, 2001). These natural assets feed various industries and have become the provider of natural resources chemical entities and biomass for herbal, pharmaceutical, nutraceutical, pure phytochemical, cosmeceutical, agrochemical industries et cetera.

A figure by EarthTrends Country Profiles (2003) indicated that a total number of known higher plants in Malaysia from year 1992 to 2002 is 15,500 species. Bidin and Latiff (1995) and Parris and Latiff (1997) reported that the flora of Malaysia is estimated to number around 15,000 species of flowering plants and more than 1,170 species of ferns and fern allies. Peninsular Malaysia alone is host to approximately 7539 species from 1510 genera of flowering plants, 632 of ferns and fern-allies species and 27 of gymnosperms species (Bidin and Latiff, 1995; Turner, 1995). Of these, about 1,200 species or 8% of the total flora has been used in various traditional medicinal practices (Soepadmo, 1999). However, these numbers do not include the species found in East Malaysia, which is believed to have some unique species only found in that region. Sadly there is no known published exhaustive checklist of the medicinal plants found in Sabah and Sarawak (Soepadmo, 1999; Kamarudin and Latiff, 2002).

Since the past decades, the rural communities in Malaysia have been proud of the role and contribution of medicinal plants as a source of their traditional care systems, sustenance as well as in the production of herbal medicinal products. It is estimated that 17.1% and 29.6% of Malaysian utilised medicinal plants to treat their health problems and for health maintenance respectively (Azriani *et al.*, 2008a). Various groups such as Malay, Chinese, Indian, the aborigines (Orang Asli) and other ethnic groups of Sabah and Sarawak (Kadazan, Dusun, Iban, Bajau and so forth) have been identified as the major consumers of medicinal plants (Mohd Azmi and Norini, 2001). Plant

parts used for remedies include the roots, leaves, barks, seeds, flowers and fruits (Haliza, 1994; Mohd Azmi and Norini, 2001).

The indigenous knowledge on medicine is mainly obtained through heritage where the information is being passed over from generation to the next generation (Halijah *et al.*, 2005) through verbal or practices. Sometimes, the knowledge is acquired from the traditional healers through special asceticism (Haliza, 2000). However, the spread of information was slow and private among small groups of people, which makes it difficult until now to find any manuscript written by these traditional practitioners regarding the utilization of medicinal plants. The Malay traditional healers are more comfortable to claim that their knowledge are exclusive for their use or to be past down to members of their family only (Mohsin, 2006). However, there is one Malay historical manuscript namely the "Tajul Muluk" which describes ingredients of Malay traditional medicine used by the ancient kings. But there is no thorough scientific investigation of its contents nor any academic discourse as to how far it had been referred to by other traditional practitioners in this country (Siti Amrah, 2004).

According to Kamarudin and Latiff (2002), for more than 50 years, the books by Burkill and associates such as the "Malay Village Medicine" by Burkill and Haniff (year 1930) and "A Dictionary of the Economic Products of the Malay Peninsula" by Burkill (year 1935) were the only published reference available on Malaysian medicinal plants in this region. Since then, not much studies or new data collection were carried out. However, in the mid-1970s there has been a

resurgence of interest amongst young academics and researchers at the UKM, Universiti Malaya (UM) and local research institutions on medicinal plants. Realizing the importance of scientific research in this field, a multidiscipline research team namely "Kumpulan Penyelidikan Ubatan Tradisional" or "KUBATRA" in short, was subsequently set up in UKM in the early 1980s (Kamarudin and Latiff, 2002).

KUBATRA has inspired the formation of other research groups, which consisted of researchers and scientists from other universities. In 1985, "Kumpulan Penyelidik Sebatian Semulajadi Malaysia" was set up and subsequently developed into an established formal team namely "Persatuan Sebatian Semulajadi Malaysia" in 1997. This organization spearheaded and coordinated many annual research activities related to medicinal plants with multidisciplinary approaches towards utilization, conservation and development of safe, efficacious and quality products derived from medicinal plants. The research activities included seminar, workshop, conference, forum and publication that involved local and international researchers, academicians, government, private sector, entrepreneurs and policy makers (Siti Amrah, 2004).

Presently, the government has taken many steps to encourage advance scientific research, standardisation and introduction of new technology in the quality control procedures related to medicinal plants. These efforts were promoted while not ignoring aspects of conservation, good methodology, cultivation and legislation relevant to the development of the herbal and