ANIMAL STUDIES ON MALE FERTILITY ENHANCING PROPERTIES OF PLANTS IN MALAYSIA: A REVIEW OF THE PAST 16 YEARS

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INTRODUCTION

For centuries, people have been practising phytomedicine in treating ailments or reducing risk of suffering certain diseases. It is considered as a part of an ancient medicine with interest in it becomes rapidly escalating in this modern era. Expansive and prescribed medicine is not the only true path to treat various illnesses. Without disclaiming the modern medicine, herbs can be used as an alternative or a combined therapy with conventional medication. Phytomedicine or ethnomedicine is not a new field in the world of research. It has been started long ago since the ancient times. As we realized the expensive modern medication is not the only mean to treat illness, interest in the discovery of remedy through natural or conventional products has become more escalating.

In the field of andrology, there is a need and an urgency to discover alternative treatment for fertility issue seems the male reproductive capacity recorded to be reduced globally. The impairment in fertility will affect personal, biological and social activities of a man. Development of modern drugs that are now available in the market to overcome infertility had been taking place in the past few years. As defined by World Health Organization (WHO) (2010), infertility is a state of inability to have conception after regular sexual activity within 12 months. Numerous drugs are available such as clomiphene, tamoxifen, anastrozole and aromatase inhibitor. However, some of these drugs fail to achieve normal sperm parameters. A prospective study conducted by Nada (2014) indicated that 6 months duration of tamoxifen treatment failed to show significant improvement in sperm motility. Therefore, it is a great concern has been brought into attention to look for another alternative in curing male sexual problems with minimal or zero side effects. It is noted that male fertility becomes one of the paramount issue to be highlighted in the development of phytomedicine. The use of plant in medicine or ethnomedicine is now globally accepted and it has to be scientifically validated. Malaysia, with the magnificent number of biodiversity, should uphold the in-depth study of ethnomedicine. The enthusiasm in practising herbal medicine should not be neglecting the evidence-based medicinal approach. Animal study and properly designed human trials should be worked out and encouraged to determine the efficacy and safety of potential phytotherapies. Thus, the traditional approach of herbal medicine can be translated into properly justified method of treating illness and improving human wellness.

This review summarizes the local studies on fertility enhancing plants with emphasize to the part of plant have been used, type of extract prepared, animal model used and fertility parameters used. The literature covered is of 16 years from 2000 to 2016 for around 13 plants possessed promising findings in male fertility activity (Table 1). Numerous recent studies show medicinal plant potency in improving male fertility on animal.

Andrographis paniculata

Andrographis paniculata has been used for centuries as a medicinal herb for the treatment of various ailments. Treatment of 50% of ethanolic extract *A. paniculata* was significantly increased serum testosterone level, while FSH and LH concentrations remained statistically unchanged at any of the dose levels. Increased in testosterone

level could indicate the beneficial use of this plant as fertility enhancer (Dasuki, Wan, Hasnan, Siti Amrah, & D'Souza, 2015).

Chlorophytum brovilianum

Chlorophytum borivilianum or safed musli is from the family Lilliaceae. 60 days administration of its dried roots aqueous extract able to increase sperm count in both the treated groups, 125 mg/kg and 250 mg/kg in a dose dependent pattern (Kenjale, Shah, & Sathaye, 2008). Its aqueous roots extract also successfully restored the sperm count and endogenous antioxidative enzymes in streptozotocin-induced diabetic rats. All the oxidative stress parameters also reduced in treated rats (Giribabu, Kumar, Rekha, Muniandy, & Salleh, 2014).

Eurycoma longifolia

Eurycoma longifolia or commonly known as Tongkat Ali in Malaysia from the Simaroubaceae family is a symbol of man's strength as it is claimed by Malaysian men to increase sexual activity and virility. A study conducted in 2000 indicated the extensive growth of both ventral prostate and seminal vesicle in all treated rats with 200, 400 and 800 mg/kg butanol, methanol, water and chloroform fractions (Ang, Cheang, & Ahmad Pauzi, 2000). The treatment of aqueous extract of *Eurycoma longifolia*for 28 days indicated an improvement in the sperm count, motility and viability in all treated rats. However, no evident changes found in sperm morphology (Mahanem, Abu Hassan Shaari, & Lukman, 2004). A study of standardized methanol extract containing 13-alpha(21)-epoxyeurycomanone, eurycomanone, 13-alpha,21-dihydroeurycomanone,eurycomanol increased sperm count, plasma and testicular testosterone level and spermatocytes in the seminiferous tubules and the Leydig cells appeared normal (Chan, Low, Teh, & Das, 2009).An aqueous extract of *Eurycoma longifolia* capable of reversing the effects of estrogen by increasing spermatogenesis and sperm counts in rats after fourteen consecutive days of treatment (Norhazlina, Norfilza, Wan Nurul Heriza, & Das, 2010).

A standardized extract of methanol/aqueous resulted in four fractions and eurycomanone is one of the fractions. Microscopic analysis of the rat testis following treatment with eurycomanone showed significant increase in the number of spermatocytes and round spermatids. The plasma testosterone, FSH and LH were recorded to be high as compared to control (Low, Das, & Chan, 2013). Prolonged treatment (28 days) of aqueous extract of *Eurycoma longifolia* improved the sperm count, motility, viability and histology of the testis as compared to short period of treatment (14 days) (Amal Salem Farag & Mahanem, 2013).

Ficus deltoidea

Among Malaysian, *Ficus deltoidea* is popularly known as mas cotek, serapat angina and telinga beruk. It has been used as a food supplement. Four weeks treatment of aqueous and methanol extract of *Ficus deltoidea* leaves elevated the testosterone level, sperm count and motility in alloxan-induced diabetic rats (Nurdiana, Mohd Idzham, Zanariah, & Mohd Luqman Hakim, 2011; Samsulrizal, Awang, MohdNajib, Idzham, & Zarin, 2011).

Gynura procumbens

Gynura procumbens is a plant under family Asteraceae and can be found in China, Africa and Southeast Asia. This plant was recorded to protect the male reproductive system from toxicity caused by diabetis. Sperm count, motility, viability and testicular lactate dehydrogenase activity were increased in rats treated with 100 mg/kg body weight *Gynura procumbens* aqueous extract (Hakim, Halimah, & Mahanem, 2008).

Hibiscus sabdariffa

Hibiscus sabdariffa or roselle is a shrub tree under family Malvaceae. Its calyx extract is famously used as beverages in many tropical and sub-tropical countries. A study was conducted on streptozotocin-induced diabetic rats reported improvement in sperm quality and follicular stimulating hormone but no improvement in testosterone and luteinizing level (Muhd Hanis, Siti Balkis, Mohamad, & Jamaludin, 2012).

Hylocereus costaricensis

Red pitaya or dragon fruit is fruit of*Hylocereus costaricensis* and native plant of the tropical forest regions in Mexico and South America. Farid and Mahanem (2012) stated that oral administration of *Hylocereus costaricensis*ethanol extractin ICR mice for 25 days improved sperm parameters and testicular histology. Epididymal sperm count was found to be highest in 500 mg/kg body weight extract while 1000 mg/kg successfully increased sperm viability and its production rate. Testicular histology in 500 mg/kg body weight indicated high spermatogenic activity as compared to 1000 mg/kg body weight and control (Farid & Mahanem, 2010).

Lunasia amara

Lunasia amara or sanrego is classified under the family Rutaceae. Its used was documented in traditional medicine by local people of Sabah, Philipines and Indonesia. A study showed that treatment with 60 mg/kg body weight of *Lunasia amara* aqueous extract for 42 days increased the sperm count, motility, viability, testosterone level and testicular antioxidant enzyme activities (Nor-Raidah & Mahanem, 2015).

Mitragyna speciosa

A great interest has been given to this plant recently following the discovery of its bioactivity. It is known as ketum in Malaysia and local plant of Southeast Asian region. Its leaves exhibit an opium-like effect could lead to addiction. There was a study investigated the potential of *Mitragyna speciosa* leaves methanol extract on male mice fertility in period of 14 days oral gavage. As compared to fertility drug,clomiphene (25 mg/70kg), the treated groups (50, 100 and 200 mg/kg body weight) clearly increased the sperm concentration. However, the sperm motility was reduced and abnormal sperm morphology was more apparent (Mohamad Syamsudin, Wan Mastura, Arifah, & Fuzina, 2011).

Nigella sativa

Nigella Sativa seeds are frequently used in folk medicine in the Middle East and some Asian countries for the promotion of good health and treatment of many ailments. It

is commonly known as black seed or black cumin and from Ranunculaceae family.A study was conducted using an active constituent of *Nigella sativa* named thymoquinone. Thymoquinone successfully inhibited the reproductive toxicity caused by chemotherapeutic drug, cyclophosphamide (200 mg/kg body weight). Administration of thymoquinone (10 mg/kg body weight) on alternate days for 53 days in male mice increased seminiferous tubules integrity and reduced total number of DNA-damaged cells (Kamarzaman, Sha, & Rahman, 2013) and increased sperm count despite no improvement in testicular histology (Suzanah, Noor Faridah, Shaik Sadak, & Saheera, 2013). Another study on reproductive protective effect of *Nigella sativa* showed that the extract able to reverse the detrimental effect on the sperm concentration, viability, and motility due to lead exposure (Assi et al., 2016).

Phaleria macrocarpa

Phaleria macrocarpa or mahkota dewa is a native plant of Papua, Indonesia. It has been planted widely due to its beneficial effects on health. In fact, there is a claim this plant capable of increase sexual libido and strength. Earlier study of *Phaleria macrocarpa* on fertility was conducted by Parhizkar, Maryam Jamielah, & Mohammad Aziz (2013). Aqueous extract (240 mg/kg body weight) of *Phaleria macrocarpa* significantly increased sperm viability without altering the sperm motility and morphology after 7 weeks treatment (Parhizkar, Maryam Jamielah, & Mohammad Aziz, 2013). Similar study was conducted had reported an increase in the number of spermatogonia cell and the thickness of seminiferous tubules of male rats (Parhizkar, Suriani, & Mohammad Aziz, 2014).

Plantago major

This plant is called "ekor anjing" by Malaysian. The used of this plant as traditional remedies were documented by numerous folk medicines including Spanish, Mexican and native people in Brazil. Aqueous extract of its seed (30, 60, 100 and 200 mg/kg body weight) elevated the sperm count after 20 days oral gavage. The increasing trend in testosterone level was insignificant (Noor, Juing, Chee, Kueh, & Zolkepli, 2000).

Species name	Part used	Type of plant extract/ active constituents	Animal models	Fertility Parameters	Reference
Eurycoma longifolia	Root	Butanol, methanol, water and chloroform fractions	Rat	Promoted growth of ventral prostate and seminal vesicle	(Ang, Cheang, & Ahmad Pauzi, 2000)
		Aqueous	Rat	Improvement in the sperm count, motility and viability	(Mahanem, Abu Hassan Shaari, & Lukman, 2004)
		Methanol/ 13-alpha(21)-epoxyeurycomanone, eurycomanone, 13-alpha,21-dihydroeurycomanone,eurycomanol	Rat	Increased sperm count Plasma and testicular testosterone were significantly increased Spermatocytes in the seminiferous tubules and the Leydig cells appeared normal	(Chan, Low, Teh, & Das, 2009)
		Aqueous	Rat	Significantly higher sperm counts and sperm motility	(Norhazlina et al., 2010)
		Methanol, aqueous/ eurycomanone	Rat	Increased in the number of spermatocytes and round spermatids Increased plasma testosterone, FSH and LH	(Low, Das, & Chan, 2013)
		Aqueous	Rat	Increased the sperm count, motility, viability and histology of the testis	(Amal Salem Farag & Mahanem, 2013)
Plantago major	Seed	Aqueous	Rat	Increased sperm concentration and testosterone level	(Noor et al., 2000)
Gynura procumbens	Leaf	Aqueous	Rat	Increased sperm count and motility Sperm mortality decreased Testicular LDH increased	(Hakim et al., 2008)

Hylocereus costaricensis	Fruit	95% ethanol	Mice	Increased sperm count, sperm viability High density of sperm in seminiferous tubules.	(Farid & Mahanem, 2010)
Ficus deltoidea	Leaf	Aqueous, ethanol	Rat	Improved the testosterone level, sperm count and motility Reduced sperm abnormalities	(Nurdiana et al., 2011)
Mitragyna speciosa	Leaf	Methanol	Mice	Increasedin the number of sperm count	(Mohamad Syamsudin et al., 2011)
Hibiscus sabdariffa	Calyx	Aqueous	Rat	High sperm concentrations and sperm motility Low sperm abnormality Plasma follicle-stimulating hormone level elevated No alteration in plasma testosterone and luteinizing hormone level	(Muhd Hanis, Siti Balkis, Mohamad, & Jamaludin, 2012)
Phaleria macrocarpa	Fruit	Aqueous	Rat	Increased sperm viability without changing the sperm motility and morphology	(Parhizkar et al., 2013)
				Increased the number of cell and the thickness of seminiferous tubules	(Parhizkar et al., 2014)
Chlorophytum borivilianum	Root	Aqueous	Rat	Increased sperm count Reduced percentage of sperm abnormality	(Giribabu et al., 2014)
Nigella sativa	Seed	Oil	Rat	Improvement in histology and function of both prostate gland and seminal vesicle	(Lina, Hashida, & Eliza, 2014)
				Increase sperm quality and showed better testis histological features	(Ping, Noor Hashida, & Durriyyah Sharifah, 2014)

		Thymoquinone	Mice	Significant difference in testicular weight Significant difference in sperm motility Improvement in the histology of the testes	(Suzanah, Norul Athirah, Shaik Sadak, & Saheera, 2014)
		Water suspension	Rat	Improved in sperm concentration, viability, and motility	(Assi et al., 2016)
Andrographis paniculata	Leaf	50% ethanol	Rat	Increasedserum testosterone level	(Dasuki, Wan, Hasnan, Siti Amrah, & D'Souza, 2015)
Lunasia amara	Stem	Aqueous	Rat	Sperm count, motility, viability, testosterone level and testicular antioxidant enzyme activities increased Increasedspermatozoa density	(Nor-Raidah & Mahanem, 2015)

Discussion

In Malaysia, the most studied plant as fertility enhancer was *Eurycoma longifolia*. It is widely known that *Eurycoma longifolia* or "Tongkat Ali" is highly regarded as male sexual enhancer. The progression of Tongkat Ali's research up to clinical trial or human study is an impressive breakthrough in the Malaysian's herbal medicine. The used of rat as animal model found to be more prominent. Some studies had shown a positive effect of plant in spermatogenesis which is a vital element in male fertility. Sperm quality is the key of male fertility apart from androgen level. A comprehensive analysis on each parameters associated with male fertility enhancement to ensure the data and findings are properly justified.

It is clearly that the profound animal study of the effect of plants towards male reproductive system will support the use and the effectiveness of the medicinal plants. It is a great loss if their potentials are neglected without proper study and analysis. More studies should be conducted to understand the action mechanism, its active components as well as their molecular aspect. The development of the current methodologies able to provide comprehensive view on the therapeutic value, efficacy as well as its possible mechanism of action. It is crucial to have robust and valid methods in assessing the improvement of sperm function and its fertilizing ability. There are several parameters can be used to evaluate the fertility promoting ability of any compound or natural product. These parameters encompass the overall fertility modalities including sperm quality, hormonal concentration, testicular and accessory organs histology and weight, seminal fluid content and deoxyribonucleic acid (DNA) integrity. Some of the studies equip their analysis with oxidative stress and antioxidant level assays.

Is it worth to continue the use of animal study in searching for male fertility enhancer? It is a continuous debate among the researchers regarding this issue. With the development of *in vitro* technique (Mocé & Graham, 2008) as an alternative in evaluating the fertility improvement properties of a plant, it can be a driving factor to develop more research without animal involvement which can be considered as an ethical-wise. However, it has to be agreed that *in vivo* data solely cannot ultimately conclude the ability or potential of any herb in promoting fertility. Reproductive system is a complex body system and influenced by various factors especially hormonal regulation. Therefore, the deep understanding of herbal product in hormonal mechanism and spermatogenesis is vital. This can be achieved via properly designed of animal study. It is fair to conclude that both *in vivo* and *in vitro* study are complementary and each of them has their role or ability in assessing natural product on male fertility.Although extrapolations from animal data to clinical study has its limitations, they can sometimes be suggestive. The aspects of quality, efficacy and safety should be in place to ensure the progress of medicinal plant research with regard to male fertility.

Conclusion

Management of male infertility using herbal remedies is useful because of long cultural history of utilization and the current renewed interest in natural products to sustain health globally. Comprehensive research on the efficacy and safety of herbal

approach for the management of male infertility is demanded as a way of appreciating the values and roles of traditional medical knowledge in health care provision. The screening of medicinal plants with fertility enhancing effect should be in line with the further isolation and identification of active constituents from plants.Thus, the rising of herbal medicine in improving male fertility in Malaysia is significant and may bring a dynamic change in the modern world.

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