APHRODISIAC ACTIVITY AND SCREENING FOR ACUTE TOXICITY OF ASIAN SWAMP EEL, *Monopterus albus* EXTRACTS ON MALE MICE

BY

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ABSTRACT

Monopterus albus is one of the terrestrial animal sources that is widely consumed by the local people, who believe in the medicinal properties offered by the species. However, there is not many research has been done on the species to prove the claims. Therefore, this study was conducted to evaluate the potential of *M. albus* extracts as an aphrodisiac and to characterize the components of the extracts. This study comprises of three phases of study: aphrodisiac, fertility and acute toxicity test. Two types of extraction were used; aqueous and lipid. Prior to treatment, the extracts were analysed for the mineral contents and fatty acid analysis using inductively coupled plasma optical emission spectrometry (ICP-OES) and Gas Chromatography Mass Spectrometry (GC-MS), respectively. Then, the sexual behaviour was observed in eight groups of ICR male mice with four animals in each group (n = 4). The doses used for both extracts were 50, 100 and 200 mg/kg. Negative control group received 0.9% saline, meanwhile positive control group received 5 mg/kg Sildenafil citrate or known as Viagra®. After an hour administration (*i.p.*) of the respective doses, the observation of sexual behaviour was conducted. The male mouse was paired with a female mouse for three hours to determine the effect of extracts on sexual behaviour. In addition, mating assessment also was carried out at similar dose level. On the sixth day, the copulatory plug was observed on the female mice used to confirm the presence of ejaculation. Afterwards, the male mice were slaughtered to evaluate the sperm count and motility as well as testicular histology. Another group of male mice were used for the analysis of testosterone levels. After that, toxicity test was conducted to determine the effect of a single dose of lipid extract in rats. The rats administered orally with the doses of 5, 50, 300 and 2000 mg/kg of *M. albus* lipid extract and control group received 0.9% saline. The survival and toxicity signs were observed for 14 days. Besides that, the blood analysis and histopathological examination of liver and kidney tissues also were investigated. From the analysis of free fatty acid, it shows that lipid extracts of *M. albus* contained higher level of arachidonic acid (AA), the essential fatty acid which influences the reproduction. The results obtained shows that all doses of lipid extract has significantly increased ($p \leq 0.05$) the mounting frequency (MF) and sperm count as compared to negative control. Meanwhile, in aqueous extract, only the dose of 200 mg/kg shows significant difference of MF but no improvement in sperm count. In the analysis of testosterone levels, only the dose of 200 mg/kg lipid extract and positive control group showed tendency to increase the hormone levels in treated mice. Whereas, in the toxicity study, the haematological, biochemical and histological analyses showed no significant difference ($p \geq 0.05$) as compared to the control. The treated rats showed no acute signs of toxicity and no mortality was observed. Therefore, the *M. albus* was safe to be consumed by the people and has great potential as an aphrodisiac agent. Thus, the study suggests that *M. albus* lipid extract could be used as an alternative source for sexual activity in men.

خلاصة البحث

إن حيوان منوبتوس ألبوس (*Monopterus albus*) نوع من الأسماك التتنفس في الهواء وتستهلك على نطاق واسع من قبل السكان المحليين، الذين يؤمنون بالخصائص الطبية لهذا النواع. ومع ذلك فليس هنالك أبحاث معتبرة أجريت على هذا النوع لإثبات تلك المزاعم. ولذلك، فإن هذه الدراسة تهدف إلى تقييم مستخلصات *M. albus* كمنشط جنسي ولوصف مكونات المستخلصات. وتتألف هذه الدراسة من ثلاث مراحل: أولها، اختبار اثارة الشهوة الجنسية والخصوبة واختبار السمية الحادة. تم استخدام نوعين من المستخلصات المائي والدهني قبل العلاج. وقد تم تحليل المستخلصات عن المحتويات المعدنية وتحليل الأحماض الدهنية باستخدام الحث بالإضافة إلى البلازما البصرية مطيافية الانبعاث (ICP-OES) والغاز اللوني الطيفي الكتلي (GC-MS) ، على التوالي. لوحظ أن السلوك الجنسي في ثماني مجموعات من ذكور الفئران مع أربعة حيوانات في كل مجموعة (ن = 4). وكانت الجرعات المستخدمة لكلا من المستخلصات 50 و 100 و 200 ملغم / كغم. تلقت مجموعة المراقبة السلبية 0.9٪ من المحلول الملحي، تلقت مجموعة المراقبة الإيجابية في هذه الأثناء 5 ملغ / كغ فياغرا سيترات أو المعروفة باسم .®Viagra بعد ادارة ساعة (*i.p.*)من تناول الجرعات، أجريت مراقبة السلوك الجنسي. وكانت تقترن ذكور الفئران مع إناث الفئران لمدة ثلاث ساعات لتحديد أثر المستخلصات على السلوك الجنسي. وبالإضافة إلى ذلك، تم إجراء تقييم التزاوج على مستوى جرعة مماثلة. في اليوم السادس، وقد لوحظ تكون copulatory plug في إناث الفئران والتي تستخدم لتأكيد وجود القذف. بعد ذلك، تم ذبح ذكور الفئران لتقييم عدد الحيوانات المنوية وحركتها وكذلك الأنسجة الخصية. واستخدمت مجموعة أخرى من ذكور الفئران لتحليل مستويات هرمون التستوستيرون. بعد ذلك، تم إجراء اختبار السمية لتحديد تأثير جرعة واحدة من المستخلص الدهني في الفئران. تم اعطاء الفئران عن طريق الفم جرعة من 5، 50، 300 و 2000 ملغ / كغ من المستخلص الدهني من *M. albus* وتلقت المجموعة الضابطة المحلول الملحي 0.9٪. وقد لوحظت علامات بقاء وسمية لمدة 14 يوما. وبالاضافة الى ذلك، تم التحقيق في تحليل الدم وفحص أنسجة الكبد وأنسجة الكلى. من خلال تحليل الأحماض الدهنية الحرة ثبت أن المستخلص الدهني الوارد من *M. albus* له مستوى مفعول أعلى من حمض الأراكيدونيك (AA) ، والأحماض الدهنية الأساسية التي تؤثر على الإنجاب. من النتائج المتحصل عليها تبين أن كل جرعة من المستخلص الدهني ازداد بشكل ملحوظ (ص ≤ 0.05) على وتيرة متصاعدة وعدد الحيوانات المنوية بالمقارنة مع المجموعة الضابطة السلبية. وفي الوقت نفسه، في المستخلص المائي، جرعة 200 ملغ / كغ تظهر اختلافا كبيرا في MF ولكن ليس هناك أي تحسن في عدد الحيوانات المنوية. في تحليل مستويات هرمون التستوستيرون، في جرعة 200 ملغم / كغم من المستخلص الدهني والمجموعة الضابطة الإيجابية أظهرتا اتجاها لزيادة مستويات الهرمون في الفئران التي عولجت. في حين، في دراسة السمية، وأمراض الدم، أظهرت التحاليل البيوكيميائية والنسيجية أنه لا يوجد فرق كبير ($p \leq 0.05$) بالمقارنة مع المجموعة الضابطة. لم نظهر الفئران أي علامات حادة السمية و لم يلاحظ أي وفيات. ولذلك، كان *M. albus* آمن إلى أن يستهلك من قبل الشعوب ولديه إمكانات كبيرة كمثير للشهوة الجنسية. وهكذا، ترى الدراسة أن المستخلص الدهني ل *M. albus* يمكن أن يستخدم كمصدر بديل للنشاط الجنسي لدى الرجال.

APPROVAL PAGE

I certify that I have read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences.

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DECLARATION

I hereby declare that this thesis 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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To my beloved family, may they always be blessed by Allah S.W.T.

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In the name of Allah the Most Beneficient and the Most Merciful

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AA	Arachidonic acid
ADH	Andiuretic hormone
AP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate aminotransferase
BF_3-CH_3OH	Boron trifluoride methanolic sodium hydroxide
BHT	Butylated hyroxytoluene
$CHCl_3$	Chloroform
CH_3OH	Methanol
$C_{15}H_{24}O$	Butylated hydroxytoluene
CK	Creatine kinase
CO_2	Carbon dioxide
DHA	Docosahexaenoic acid
EDTA	Ethylenediaminetetraacetic acid
EPA	Eicosapentaenoic acid
GCMS	Gas chromatography mass spectrophometer
GGT/ɣ-GT	Gamma-glutamyl transferase
HDL	High-density lipoprotein
HB	Haemoglobin
HCT	Haematocrit
HCl	Hydrochloric acid
IF	Intromission frequency
IL	Intromission latency
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MF	Mounting frequency
ML	Mounting latency
MUFA	Monounsaturated fatty acid
NA	Not applicable
NaCl	Sodium chloride
Na_2SO_4	Sodium sulphate
NaOH	Sodium hydroxide
$(NH_4)_2SO_4$	Ammonium sulphate
PCV	Packed cell volume
PUFA	Polyunsaturated fatty acid
RBC	Red blood cell
S.E.M	Standard Error Mean
SFA	Saturated fatty acid
UV	Ultraviolet
WBC	White blood cell

LIST OF SYMBOLS

%	percentage
°C	degree celcius
b.w.	body weight
cm	centimetre
fL	fluid ounce
ɣ	gamma
g	gram
hr	hour
i.p.	intraperitoneal
kJ	kilojoules
kg	kilogram
L	litre
µg	microgram
µL	microliter
µmol	miromolar
mbar	millibars
mg	milligram
mL	mililitre
min	minute
mmol	milimolar
ω	omega
p	probability
rpm	revolutions per minute
sec	seconds
w/w	weight/weight

CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

Sexual activity is vital component of a normal and healthy lifestyle. The sex is the most intimate, imperative and integral part of every individual and can be a source of pleasure and fulfillment. The basic and fundamental purpose of sex and sexuality is the continuation of progeny. However, the problems in sexuality may disturbed interpersonal relationship and even can cause divorce in married couples. The main sexual problems are related to sexual desire and sexual dysfunction (Gonzales, Cordova, Vega, Chung, Villena and Gonez, 2003). It was estimated approximately 30% of couples infertility and sexual dysfunction are due to male factors (Isidori, Pozza, Gianfrilli and Isidori, 2006). According to Schwartz and Kloner (2011), sexual dysfunction is a serious medical and social symptom that occurs in 10% - 52% of men and 25% - 63% of women. Male sexual dysfunction, which includes erectile dysfunction (ED), affects 34.8% of men aged between 40 - 70 years (Schwartz and Kloner, 2011). Epidemiological studies have demonstrated a high prevalence and incidence of ED in developed countries (Hatzimouraditis, Amar, Eardley, Giuliano, Hatzichristou and Montorsi, 2010), and therefore it is consider being an important health problem.

Erectile dysfunction defined as the persistent or recurrent inability to achieve or maintain a penile erection of sufficient for sexual performance (Bella, Lee, Carrier, Benard and Brock, 2015). The major causes of ED include obesity, diabetes mellitus, hypertension, cardiovascular disease, increasing age and non-healthy lifestyle such as

smoking, drug abuse, stress and unhealthy food habits. There are many alternative ways for the treatment of ED including psychotherapy, surgery, mechanical devices, drugs and oral phosphodiesterase type 5 inhibitors (PDE 5). Since 1998, Viagra® (Sildenafil citrate), was the first and the most favorable PDE 5 inhibitor that have been demonstrated to be effective in treating ED (Jackson, Gillies and Osterloh, 2005). Viagra® will increase the level of cyclic Guanosine Mono-Phosphate (cGMP) in the corpus cavernosal smooth muscle cells, leading to facilitation and prolongation of penile erection (Meinhardt, Kropman and Vermeji., 1999). However, in some men, the oral prescription drug is not compatible and the satisfaction is below the expectation. Nevertheless, the intake of Viagra ® caused certain side effects to the patients such as headache, flushing, dyspepsia, nasal congestion, dizziness and sometimes can cause blurred vision (Schiff and Mulhall, 2005). According to Hatzimouraditis et al. (2010), the intake of prescription drug should be considered with timing or frequency of intercourse and interactions with food or alcohol, for effective results in sexual activity.

Therefore, there is a demand to look for traditional medicine as opposed to conventional medicine which offers least invasive and successful treatment in sexual dysfunction. Since ancient times, a large number of plants have been tested throughout the world for the possible aphrodisiac to relieve sexual dysfunction and enhance fertility properties (Kabbashi, Dafaalla, Hassan, Idris, Abdoun and Modawe, 2016). The medicinal plants are extensively used as aphrodisiac that provide nutritional value, thereby, improving sexual performance and *libido* (Yakubu, Akanji and Oladiji, 2007). Aphrodisiac is defined as a substance in a form of food or drug that is capable to enhance sexual activity and help in fertility. It can be categorized

according to their mode of action into three types: those that increase libido (sexual desire), potency (effectiveness of erection) and sexual pleasure (Sandroni, 2001).

Plant materials such as *Myristica fragrans, Tribulus terrestris, Asparagus racemosus, Ginkgo biloba* and *Eurycoma longifolia* have been used as a male aphrodisiac remedy (Patel, Kumar, Prasad and Hemalatha, 2011). Those plants have been tested for their aphrodisiac activity *in vivo, in vitro* and human clinical trials to support the claims. Besides these plants, some marine resources are also known to have aphrodisiac value. This includes sea cucumber, *Holothuria scabra* (Nurjanah, Gumbira-Sa'id, Suprihatin and Riani, 2008), oyster, *Crassostrea iredalei* (Ridzwan, Hanani, Siti Norshuhada, Farah Hanis and Aileen, 2013) and sea slug, *Aplysia dactylomela* (Ridzwan, Noor Atika Elliyana, Farah Hanis and Jamaluddin, 2014). According to Wang and Croll (2006), several types of marine resources such as octopus, sea hare, soft-shell clam and oyster contains high level of steroid.

Previous studies have indicated that sex steroids involved in the male hormone testosterone secretion. It is able to stimulate hypoactive sexual desire in men (Matsumoto, 1994; Seidman, 2000). Therefore, many people prefer to use natural sources of therapy because they believe the possible bioactive aphrodisiacs such as steroid were derived from the animals. Steroids hormone plays an important role in sexual behaviour. For example, steroid sandfish extract (*Holothuria scabra*) able to enrich testosterone and cholesterol concentration in blood serum and fastening secondary reproduction characteristics in male chick (Riani, Gumbira-Said, Syamsu, Kustiariyah, Kaseno and Cordova, 2013). Thus, sandfish steroid has potential to be used as an aphrodisiac for men.

Currently, one of terrestrial resources that aroused interest in Asian countries is rice field eel, *Monopterus albus* or locally known as 'belut sawah'. Ancient herbal

and traditional medical records show that eel has been traditionally used to treat bronchitis, tuberculosis, anxiety and for life longevity. Studies on this species is limited to its fatty acid composition (Razak, Basri, Dzulkefly, Razak and Salleh, 2001), heavy metals uptake by *M. albus* (Sow, Ismail and Zulkifli, 2012), antibacterial, antifungal and antiproliferative of *M. albus* (Nik Mohd Ikhram and Ridzwan, 2013; Atif, Zahri, Esa, Zilfalil, Rao and Nordin, 2015) and eel skin gelatin as alternative to bovine gelatin (Nurul and Sarbon, 2015). However, there is no study on aphrodisiac properties of this eel, except, Chan and Philips (1969), reported gonad of *M. albus* produced sex steroid which is commonly found in most other vertebrate animals. Nowadays, not only by the Chinese, Japanese and Koreans but also by the Malays who recognize eel as an aphrodisiac. But, to this day, the claims of benefit values from *M. albus* were not yet widely discovered. Thus, this study was conducted to assess the potential of *M. albus* extract in inducing sexual activity and fertility in male mice.

1.2 SIGNIFICANCE OF STUDY

There are a number of conventional medical treatments which act as sex stimulant and enhancing the sexual desire and activity in men and women. Although the use of medicine has shown the significant improvement in treating sexual dysfunction, it also caused severe unwanted side effects. The use of synthetic aphrodisiac will dilate blood vessel in other parts of body and causing headache and fainting in patient. Therefore, people nowadays intended to look for safe and effective natural resources as opposed to synthetic medicine. Interestingly, people from many countries like to eat eel, but it has been a long period before it was discovered where eels came from. Traditionally, eel has always been viewed as a health enhancing food and even Malays currently consume eel especially, ricefield eel as an aphrodisiac.

Medical research had found that oily fish like eel contains high amounts of Omega-3 and Omega-6 polyunsaturated fatty acids (PUFA) which comprises mainly of eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) (Razak et al., 2001; Suseno, Saraswati, Hayati and Izaki, 2014). Dietary intake of these PUFA is necessary since human cannot synthesis these fatty acids because lack of fatty acid desaturase enzymes. The PUFA, therefore, need to be provided in the diet as these PUFA are essential for numerous processes include promoting reproduction. Therefore, the present study will determine the nutritional value presence in the lipid extraction of *M. albus* and reveal the association between the extraction and its effect on sexual behaviour in male mice. In addition, the toxicity effect of the extraction also was conducted in this study. It is hoped that the findings from the study may provide scientific evident for the role of *M. albus* in aphrodisiac as acclaimed by the locals.

1.3 OBJECTIVE

1.3.1 General Objective

The overall objectives of this research project is to evaluate aphrodisiac activity as well as fertility potential on male animals, to characterize the *M. albus* extracts and its toxicity level.

1.3.2 Specific Objectives

1. To evaluate the effects of *M. albus* aqueous and lipid extracts on aphrodisiac activity of male mice.
2. To evaluate the effective extract on the male mice fertility.
3. To identify the components of the most effective extract.
4. To identify the toxicity level of the effective extract.

CHAPTER TWO

LITERATURE REVIEW

2.1 ASIAN SWAMP EEL; *Monopterus albus*

2.1.1 Taxonomy and Distribution of Asian swamp eel

Asian swamp eel, *Monopterus albus* (Zuiew, 1793) belongs to the family synbranchidae of the order synbranchiformes (Rosen and Greenwood, 1976). It is commonly known as rice eel, rice field eel, rice paddy eel or 'belut sawah'. A brief taxonomy classifying rice field eel are as follows:

Kingdom : Animalia

Phylum : Chordata

Class : Actinopterygii

Order : Synbranchiformes

Family : Synbranchidae

Genus : *Monopterus*

Species : *M. albus*

The Asian swamp eel is native to the tropical and subtropical areas of northern India and Burma to China, Thailand, Philipines, Malaysia, Indonesia, and possibly north eastern Australia (Collins, Trexler, Nico, and Rawlings, 2002). In the early 1990s, it was found in several freshwater in North America including Hawaii, Florida and Georgia due to an aquarium release or a fish farm escaped (Fuller, Nico and Williams, 1999).