CHAPTER II

REVIEW OF RELATED LITERATURE AND RESEARCH

Kwaao Khruea

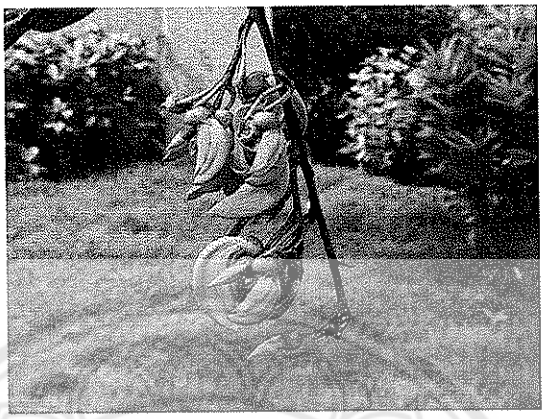
Kwaao Khruea is a native herbal plant found in deep forests of the northern region of Thailand. It has been well known to local people for many years due to its distinguished properties and efficacy as in using as Thai traditional medicine. According to its classification, there are 3 varieties of Kwaao Khruea which are beneficial and can be used for medicinal, food supplementary and cosmetic purpose. These varieties are White Kwaao Khruea (*Pueraria mirifica*), Red Kwaao Khruea (*Butea superba*) and Black Kwaao Khruea (*Mucuna macrocarpa*). White Kwaao khruea is a Thai phytoestrogen-rich plant that is postulated to have effects on reproductive organs [12]. When given orally, it can produce several effects on reproductive functions such as induction of uterine growth, vaginal cornification, and suppression of gonadotropin levels in both female and male rats [13]. It was shown that White Kwaao Khruea could modulate proliferation of MCF-7, an ER positive human mammary adenocarcinoma cells [14]. Red Kwaao Khruea and Black Kwaao Khruea have been traditionally consumed in Thai males for rejuvenate as well as maintain sexual performance such as prevent erectile dysfunction [10]. Although, Red Kwaao Khruea is the most widely used very few of scientific reports on the biological activities of this plant have been available.

Butea superba

Butea superba is a plant in the Family *Papilionaceae* [8, 9] (Figure 1) which is found in deciduous forest in Thailand, especially in the northern and eastern regions. The plant has a unique characteristic of being a crawler that wraps around large trees. The flowers are of a yellowish orange color. The long tuber roots are under the ground and different in sizes. Its appearance is similar to a yam root. It is reproduced through

seeds and the propagation of its tuber root. Its sap is red when the root is cut [9]. It is, therefore, called Kwaao Khruea Daeng or Red Kwaao Khruea.

(A)



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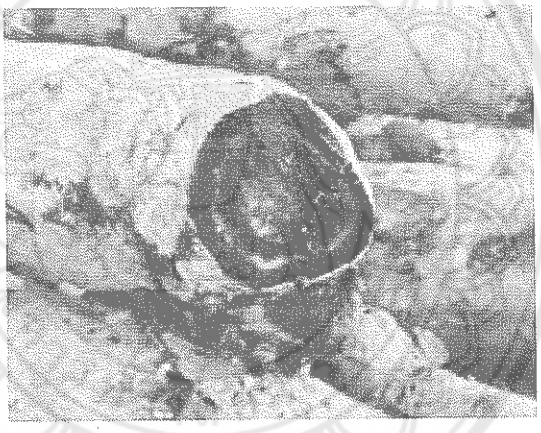

(C)

Figure 1 The picture of *B. superba* yellowish orange color flowers (A), tuber root (B), and stem (C).

1. Chemical constituents of *B. superba*

Active components found in the tuberous roots of Thai *B. superba* have been isolated and identified into 5 groups as following: carboxylic acids, flavonoid (3,7,5'-trihydroxy-4'-methoxyflavone), flavonoid glycoside (3,5'-dihydroxy-4'-methoxyflavone-7-0-β-D-glucopyranoside), sterol compounds (β-sitosterol, campesterol and stigmasterol) and sterol glycosides (β-sitosteryl-3-O-β-D-glucopyranoside and stigmasteryl-3-O-β-D-glucopyranoside) [15].

2. Traditional use of *B. superba*

The tuber and stem of *B. superba* have been used for physical and mental strength and for prevention of age-related health problems [10]. It is believed to give power and increase male sexual performance. In Thai traditional medicine, *B. superba* is a rejuvenating herb for men. Its use is, therefore, increasing particularly in Thai men. According to traditional use, recommended amount taken for individual with body weight of 50 kg is approximate to a pepper-sized seed, which is about 50 mg/day [10].

3. Pharmacological activities

Antioxidative activity

The ethanolic extract of *B. superba* exhibits the antioxidative activity by ABTS technique [16]. Recently, Nuengchamnong et al (2005) developed the method for separation and identification of anti-oxidant compounds in the extract of *B. superba* by using reverse-phase HPLC coupled on-line to ESI-MS and a DPPH-based assay. It was found that the anti-oxidant compounds in *B. superba* are procyanidin B2, (-)-epicatechin and procyanidin B5 [17].

Antimicrobial activity

The 3,5,7,3',4'-pentahydroxy-8-methoxy-flavonol-3-O-beta-D-xylopyranosyl (1-->2) alpha-L-rhamnopyranoside, flavonol glycoside isolated from the stems of *B. superba*, exhibits antimicrobial activity against plant pathogenic fungi including, *Trich viride*, *Aspergillus fumigatus*, *A. niger*, *A. terreus*, *Penicillium exansum*, *Helmitnhosporium oryzae*, *Botxitis cinerea*, *Rhizopus oligosporus*, *R. chinensis*,

Kelbsiella pneumoniae, *Fusearium moniliforme* and gram-positive bacteria including *Streplococcus pyogenes*, *Staphylococcus aureus*, *Bacillus subtillis* as well as gram-negative bactera including *Eschericia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* [18].

Effect on male reproductive system

Flavonoid and flavonoid glycoside isolated from *B. superba* inhibit cAMP phosphodiesterase that are important for controlling several diseases including penile erection [11].

In addition, rats orally received dried powder and ethanolic extract from roots of *B. superba* for 21 days showed the significant increase in libido, sperm density, relative weight of reproductive organs and the size of penis during erection. On the other hand, rats received both extracts for 42 days showed that the relative weight of seminal vesicle and the length of penis were decreased [19].

The clinical trial study on erectile dysfunction in Thai males receiving the powder of *B. superba* for 3 months showed that 82.4% of ED patients improved the erectile function after taking *B. superba*. There was no alteration of the hematology and blood chemistry within 3 months of treatment [20].

Anti-proliferative effect

B. superba alcoholic extract reveals no proliferative and anti-proliferative effects on the growth of MCF-7, estrogen receptor positive human mammalian carcinoma cells at 10, 100 and 1000 μg/ml with an ED_{50} value of 370.91 μg/ml [14].

Immunomodulating activity

Immunomodulating activities of *B. superba* extracts were tested for in *vitro* phagocytotic response of mouse macrophages and proliferative assay of mouse lymphochytes from spleen, bone marrow and thymocytes. It was shown that the extract of *B. superba* has a tendency of in *vitro* immunomodulating activity which can be further developed as active constituents in nutraceutical products [21].

Acetylcholinesterase inhibitory activity

The methanolic extracts of *B. superba* were tested for acetylcholinesterase (AChE) inhibitory activity using Ellman's colorimetric method in 96-welled microplate. The extract of *B. superba* at the concentration of 0.1 mg/ml showed 55.87 ± 5.83 % inhibition on AChE activity [22].

4. Toxicity study of *B. superba*

Subchronic toxicity of *B. superba* was studied in rats orally receiving the powder of *B. superba* at the dose of 0, 10, 100, 150 and 200 mg/kg BW/day for 3 months. At the dose of 200 mg/kg BW/day, it was shown that the neutrophil content was decreased but eosinophil was increased. Both neutrophils and eosinophils have the responsibility in immune response. In addition, the level of alkaline phosphatase (ALP) and aspartate aminotransferase (AST) were significantly increased in rats treated with *B. superba* at the dose of 150 mg/kgBW/day. The level of LH and testosterone were also decreased in rats receiving *B. superba* [23]. Moreover, oral administrations of *B. superba* suspension dose of 1,000 mg/kg BW/day for 9 weeks also induced the formation of micronuclei in polychromatic erythrocytes without any effect on male reproduction or abnormality in fetus [24].

The chronic toxicity of *B. superba* was investigated in Wistar rats orally receiving the powder of *B. superba* for 6 months at the doses of 10, 100, 250 and 1000 mg/kg BW/ day. The hematological parameters were significantly higher in the groups of rats treated with *B. superba* at the dose of 100 mg/kgBW/day and higher. The biochemical and histopathological data from the rats treated with *B. superba* at the dose of 250 mg/kgBW/day revealed that the liver and its function were significantly changed. The higher levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin and BUN as well as the formation of micronuclei in polychromatic erythrocytes were detected in rats receiving the powder of *B. superba* at the dose of 1,000 mg/kg BW/day for 9 wks [25].

Penile erection

1. Anatomy of penis

Human erectile cavernous tissue is composed of two dorsal corpora cavernosa and a single ventral corpus spongiosum, which also contains the penile urethra (Figure 2). The main function of corpora cavernosa is erection of the penis. Anatomically, the erectile tissue of the corpus cavernosum and corpus spongiosum are composed of numbers of cavernous spaces separated by trabeculae. The trabeculae contain a smooth muscle. The surfaces of the trabeculae are covered with endothelial cells as found in blood vessels [26].

The blood supply of the penis comes from the branches of aorta to the internal and external iliac artery, and finally a pudendal artery. The penis is supplied by branches of the internal pudendal arteries such as dorsal arteries supply the fibrous tissue around the corpora and the penile skin, deep arteries supply the erectile tissue. Venous drainage of the penis is more varied and complex than the arterial supply. Blood from the cavernous spaces is drained into the deep dorsal vein of penis and joins the prostatic venous plexus. Blood from the superficial fascia covering of the penis drains into the superficial dorsal vein and the superficial external pudendal vein [26].

2. Physiological control of penile erection

Erection occurs via coordinated of psychological, neuronal, hormonal, vascular, and cavernous smooth muscle systems. Normal erectile and ejaculatory function are controlled by both central nervous system and its peripheral connection [29].

2.1 Central pathway

The first regulatory pathway of penile erection is the central nervous system. Regions that control the erection include the anterior part of the cingulated gyrus, preoptic region, lateral hypothalamus and tegmentum. The regions of the brain that modulate the psychogenic component of erection are the thalamic nuclei, the rhinencephalon, and the limbic structures, with integration of these various areas

occurring in the preoptic anterior hypothalamic area. Input from the brain involves descending spinal pathways and is sent through both lumbar sympathetic and sacral parasympathetic outflows to the penis [29]. Anti-erection sympathetic efferent pathways arise in paravertebral sympathetic chain ganglia and course to the penis primarily through hypogastric and pudendal nerves [30, 31].

2.2 Peripheral pathway

The erection of penis is regulated by both autonomic (sympathetic and parasympathetic) and somatic (sensory and motor). The cavernosal nerve is derived from the pelvic ganglionic plexus located retroperitoneally near the rectum. Both sympathetic and parasympathetic nervous system supply input to this plexus which enter the corpus cavernosum to regulate blood flow to the penis during penile erection and detumescence [30, 31] The somatic pudendal nerves are primarily responsible for sensation and the contraction of the bulbocavernosus and ischiocavernosus muscles.

2.2.1 Autonomic pathway

Sympathetic nerves originate from the low thoracic and upper lumbar regions of the spinal cord [31]. They condense into the superior hypogastric plexus located inferior to the aortic bifurcation. Sympathetic fibers leave the hypogastric plexus as the right and left hypogastric nerves. The hypogastric nerves fuse distally prior to entering the pelvic plexus. Nerve fibers leaving the pelvic plexus innervate not only the penis but also the pelvic viscera including the bladder, seminal vesicles, prostate and rectum. The cavernous nerves leave the pelvic plexus, pass between the rectum and urethra, and enter the dorsalmedial aspect of the corpora cavernosa and supply autonomic input to the penis.

The parasympathetic input to the pelvic plexus is derived from nerves whose cell bodies are located in the sacral spinal cord (S2-4). These parasympathetic fibers form the pelvic nerve that courses in the endopelvic fascia before reaching the pelvic plexus, where they are joined by the sympathetic nerves from the superior hypogastric plexus [31].

Stimulation of the pelvic plexus and the cavernous nerves induces erection, whereas stimulation of the hypogastric nerve or the sympathetic trunk causes detumescence. Cavernous nerves release neurotransmitters that are capable of relaxing the cavernous smooth muscle. These transmitters include nitric oxide (NO) and vasoactive intestinal peptide (VIP). NO is a free radical and therefore highly reactive and chemically unstable molecule. It is also produced by certain animal and plant cells from an amino acid, L-arginine, by NO synthase (NOS) [32, 33, 34]. NOS is classified into 3 subtypes: endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS). Both constitutive and inducible NOS isoforms are produced in the cavernosal tissues. Constitutive NOS is produced by the endothelial cells and the nerve terminals, whereas inducible NOS appears to be produced by the corporeal smooth muscle cells only [32]. It crosses the plasma membrane of cells and targets on the guanylate cyclase enzyme, producing a conformational change in the molecule that increases its activity. The activated guanylate cyclase catalyzes the conversion of guanosine triphosphate (GTP) to cGMP. The accumulation of cGMP induces a loss of contractile tone of penile smooth muscle leading to an increase in blood flow to the cavernous body. In mammals, NO is a major mediator of the penile erection by relaxing penile smooth muscle fibers and arteries. NO is released by the endothelial cells of the penile arteries, nonadrenergic/noncholinergic neurons of the lacunar spaces, and by postganglionic parasympathetic nerve endings running in the penis itself. NO is also involved in the nonadrenergic/noncholinergic neurotransmission leading to smooth muscle relaxation in the corpus cavernosum. It induces a reduction of cytosolic free Ca^{2+}. The other induction pathway of relaxation and erection is mediated by vasoactive intestinal peptide (VIP). The VIP receptors in the cavernous body are coupled to specific proteins that stimulate catalytic activity of adenylate cyclase with formation of cAMP. VIP and NO may function as co-transmitters [35].

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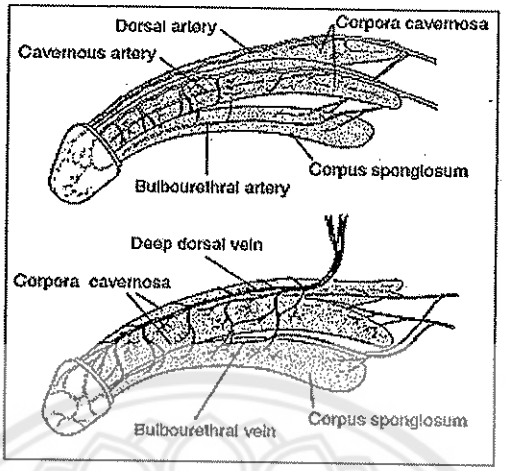

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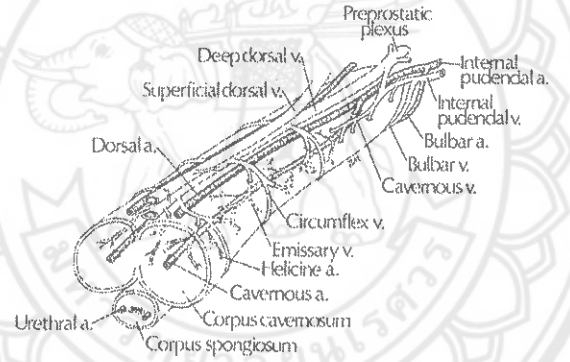

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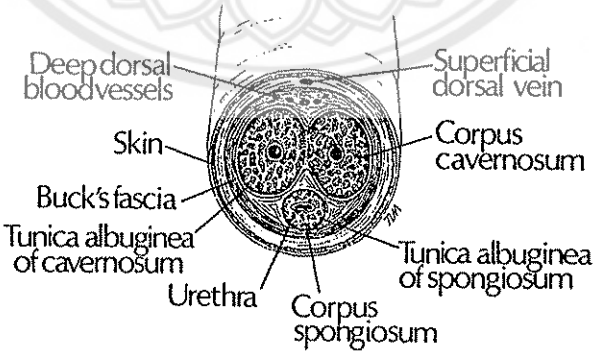

Figure 2 Anatomy of penis. (A) Arteries (top) and veins (bottom) penetrate the long, filled cavities running the length of the penis, the corpora cavernosa and the corpous spongiosum. (B) Arterial supply and venous drainage of the human penis. (C) Cross section of the human penis [27, 28].

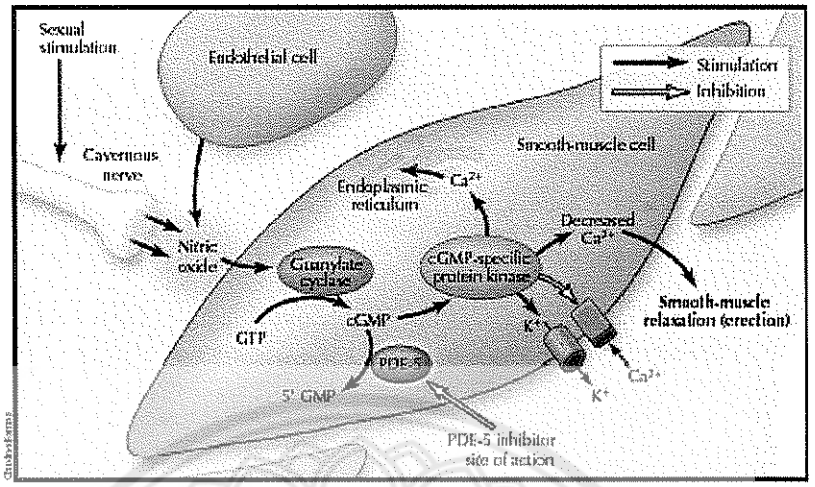

Figure 3 Cellular perspective of the erection pathway.

2.2.2.2.2 Somatic pathway

The somatosensory pathway originates from the sensory receptors in the penile skin, glans penis, urethra and within the corpus cavernosum. The local stimulus of the ejaculatory response involves the dorsal nerves of the penis. Genital skin afferents pass through the sensory division of the pudendal nerve. Afferent sensory stimuli from the seminal vesicle and prostate pass through to the cord by means of the pelvic splanchnic nerves. In human glans penis, there are numerous free nerve endings and corpuscular receptors. The free nerve endings are derived from thin myelinated and unmyelinated C fibers and are different from other cutaneous area in the body [30]. The nerve fibers from the receptors are joined to form bundles of the dorsal nerve of the penis, which join other nerves to become the pudendal nerve. Activation of these sensory receptors sends messages of pain, temperature, and touch via the dorsal nerve of the penis and pudendal nerves, spinal cord, and spinothalamic tract to the thalamus and sensory cortex for sensory perception. Recent evidences have clearly demonstrated that the dorsal nerve of the penis is a mixed nerve with both somatic and autonomic components that enable to regulate both erectile and ejaculatory functions.

somatomotor penile innervation. These nerves pass through in the sacral nerves to the pudendal nerve to innervate the ischiocavernosus and bulbocavernosus muscles. Contraction of the ischiocavernosus muscles produces the rigid erection phase. Rhythmic contraction of the bulbocavernosus muscle is necessary for ejaculation [30].

2.3 Disorder of penile erection

2.3.1 Epidemiology of erectile dysfunction

ED is a common problem and serious public health problem affecting the quality of life in both physical and psychological conditions of men worldwide. The incidence of ED increases strongly with age, especially after age of 60 years. Twenty six percent of men with mild ED will develop to moderate or severe ED [4]. The Massachusetts Male Aging Study (MMAS) and a Brazilian study revealed that the incidence of moderate or complete ED was 26 cases per 1000 person-years. The incidence of ED nearly doubled with each decade of life [36]. An increased incidence of ED associated with age, the incidence of ED was 7%in the American male aged 18-29 years and 18% in those aged 50-59 years [36]. A number of chronic diseases may contribute to the pathogenesis of ED [37]. Diabetes mellitus, heart disease and cerebrovascular disease enhance the development of ED [36, 38]. In the Multi-National Survey of aging male, a study of 14,000 men aged 50-80 years in 7 countries (United States, United Kingdom, France, Germany, Italy, Spain, and the Netherlands), the severity of high blood pressure was strongly associated with the frequency and severity of sexual problems. The association between depression and ED has been reported [36]. Other risk factors include trauma, irradiation or pelvic region survey, use of drugs, chronic renal failure also show a strong association with ED. An epidemiology study in Thailand investigated ED in a nationwide representative sample of 1,250 urban Thai men aged between 40 and 70 years. The estimation of overall prevalence of ED among the people living in urban areas was 37.5 %. This proportion consisted of 19.1 % of male with mild dysfunction, while about 13.7 % and 4.7 % of the samples had moderate and severe dysfunction, respectively. Bangkok had the lowest prevalence comparing with all

four regions while the North had the highest level. In addition, the prevalence estimation for smaller provinces was 36.4 % and 46.4 % for larger provinces. Moreover, the prevalence of ED rapidly increased from age group 40-49 to 60-70 years [2].

2.3.2 Causes of erectile dysfunction

ED is resulted from a number of causes. Although in the past ED was believed to primarily have a psychologic origin, it has now been shown that the majority of cases (>70%) have a physiologic origin. Depressive symptoms could contribute to the increased prevalence of ED [36]. The physiological etiology may be vascular, neurogenic, hormonal, mixed etiologies, or related to cavernosal abnormalities, cavernosal or local nerve damage, or drugs. Vascular disease is a common cause of ED, and persistent hypertension results in damage to the vascular system [38]. Smoking is a key risk factor of ED [39]. Other causes are high cholesterol diet, arteriosclerosis, congestive heart failure, and diabetes. Neurological diseases may result in ED. Spinal cord injury can interrupt the sympathetic and parasympathetic pathways that are essential to erectile function. Neurological dysfunction caused by stroke, multiple sclerosis, head injury, Parkinson's disease or Alzheimer's disease may also cause ED [36]. Structural deformities (such as peyronies and epispadias) may contribute to ED [40]. An interruption in the nerves related to injury (cycling, horseback riding) or iatrogenic causes (radical prostatectomy, revascularization surgery, renal transplant, pelvic irradiation) may cause ED [36]. Hormone deficiency may account for a small percentage of ED cases. The decreased level of free serum testosterone and the concentration of sex hormone binding globulin could be associated with loss of libido and reduced frequency of erection. Testosterone, LH, and prolactin levels can be assessed [36]. Certain drugs have been associated with ED including alcohol, anti-androgens, estrogens, anticholinergics agents, antidepressants, psychotropics, some antihypertensive agents (beta blockers, sympatholytics), nicotine, cocaine, histamine 2 receptor blockers, ketoconazole, lipid-lowering agents, marijuana, narcotics, cytotoxic drugs, spironolactone and other diuretics [41].

2.3.3 Diagnosis of erectile dysfunction

The appropriate evaluation of all patients with ED should include a history, a physical examination, basic laboratory studies, and occasional vascular testing. All informations are helpful in evaluating the cause of ED [42].

2.3.3.1 History

The possible factors contributing to causing ED are usually obtained from the history. A sexual history and information of child illnesses, current illnesses and medications are essential. Specifically, attention should be focused on vascular, neurological, endocrinological and psychological problems that may correspond to the risk factors for ED [36]. The most common causes of ED are systemic illness such as diabetes mellitus, arterial occlusive disease, and neurological disorder [36]. The cause of ED in diabetes may be neurogenic, vasculogenic or a combination of these factors. A history of chemotherapy or radiotherapy for any malignancies is also important because spermatogenesis is usually impaired [43]. The patient who has had a retroperitoneal lymph node dissection with interruption of the sympathetic nodal chain or its peripheral long nerve may show either aspermia [44]. A history of all medications is essential in the evaluation of patients with impotence since many drugs may be associated with ED [42]. Additionally, exogenous androgenic steroids actually depress gonadotropin secretion and suppress normal spermatogenesis. In addition, assessment of the patient's psychological status is also important. Most ED comes from psychological etiology [42]. Sexual assessment is quite difficult for interviewing since sex is obviously a highly emotional, private subject for most people. Thus, it may be difficult to get all necessary information from patients and their couples. Nowadays, there are a number of validated questionnaires available to obtain information about patient's sexual function. The International Index of Erectile Function (IIEF) has been widely used and certified in several languages (Appendix A) [45]. This questionnaire asks about frequency of a variety of sexual activities, sexual desire, feeling about sex, feelings towards the mate, etc. These questionnaires are useful for clinician to define the patients with ED and also available monitoring change following treatment.

2.3.3.2 Physical examination

Physical examination of the patients with ED should include a complete evaluation [42]. Any factor that affects overall health can be responsible for abnormalities in sperm production. Examination of external genitalia is important to distinguish between congenital or acquired abnormalities of the penis. Abnormalities in penile angulation can result in improper position of the ejaculate within the vagina. Decrease in testicular size is often associated with impaired spermatogenesis. The presence of small testes and reduced secondary sexual characteristics may suggest hypogonadism. Examination of the peritesticular area is also important. Epididymal induration, irregularity, and cystic changes should be assessed. The engorgement of the pampiniform plexus should be identified, since a varicocele can cause abnormalities of gonad function [46]. A full neurological examination is important, especially the sacral spinal outflow.

2.3.3.3 Laboratory test

Laboratory tests should be begun after history-taking and the physical examination is completed. Baseline haematological and biochemical screens are necessary. Plasma glucose will represent for diabetes mellitus. The liver function test is useful for detecting hepatic impairment which may be associated with increased serum estrogen levels and a reduced plasma testosterone. An evaluation of the endocrine status of the reproductive hormonal axis (hypothalamus, pituitary, and testis) is an essential in the evaluation of men with azoospermia and severe oligospermia. The most common endocrinopathy causing impotence is diabetes mellitus. A prevalence study showed that more than 50% of patients with diabetes mellitus suffer from sexual dysfunction [47]. Endocrinopathies have been estimated for 5-35% of cases of impotence.

2.3.3.4 Pharmacological test

Intracorporeal injection of pharmacoactive agents have been used to test the venous system of the penis [48]. Initial studies, injection with normal saline could be perfused into the cavernous bodies to achieve and maintain an

artificial erection. The erection was observed the flow or measured the intracavernosal pressure. Differentiation between initiation flows and maintenance flows could be measured and cavernosography could be performed if an artificial erection was inadequate. The pharmacological substances used to induce erectile function including papaverine, phentolamine and prostaglandin E1 [49]. Pericavernosal structures such as the cavernous muscle and perineal muscles are not activated during these studies. Doppler evaluation further adds to the accurate diagnosis of vascular abnormalities of the penis. The complication of pharmacological test is prolonged erection.

2.3.3.5 Vascular evaluation of ED

Color Duplex Doppler evaluation is a non-invasive method for estimating venous functions in patients with adequate arterial blood supply to the penis [50]. The increase in diastolic velocity is measured after administration of pharmacologically active agents to the penis. The drainage from the deep dorsal vein before initiation of erection, during the initiation phase, and after erection has occurred. If diastolic velocity is adequate and maximal arterial inflow is obtained. Color Doppler can be used to visualize the initial increase in venous outflow followed by rapid loss of venous outflow and decrease in deep dorsal vein diameter. This method is used to differentiate patients with venous abnormalities from patients with arterial abnormalities in combination with pharmacological injection therapy with papaverine, phentolamine or prostaglandin E1.

2.3.3.6 Neurogenic evaluation of ED

Sacral autonomic innervations and higher centers which modulate basic sexual reflexes are essential for penile erection during sexual intercourse. Damages to these pathways may result in impotence. Damage may occur as part of a generalized neurological disorder, so that ED may either be an isolated symptom or part of a widespread symptom complex. Several methods have been developed to identify neurological disease in patients with ED. Among them, the widely used method is bulbocavernous reflex test (BCR) which is the contraction reflex of the bulbocavernosus muscle produced by compress the glans penis [51]. These sacral

reflexes may be used to assess the integrity of both the afferent and efferent neurons and also the central connections within the sacral cord.

3. Treatment of erectile dysfunction

3.1 Medical Therapy

Drugs used in the treatment of male erectile dysfunction can be classified into there groups according to their administration routes: oral drug therapy, intracavernosal therapy and topical drug therapy.

3.1.1 Oral drug therapy

α-adrenoceptor Antagonists

Yohimbine

Yohimbine is an indole alkaloid derived from the bark of yohimbine tree (*Pausinystalia yohimbe*) in Africa. It has long been used in the treatment of ED in men. Yohimbine is pharmacologically characterized as an α-2-adrenergic receptor antagonist with activity in the central and peripheral nervous system [52]. Its activity is also mediated by blocking presynaptic α-2- adrenergic receptors in adrenergic nerve terminals distributed in penile nerve [52]. Activation of α-2- adrenergic receptors located in the CNS results in inhibition of sympathetic tone and decrease of blood pressure. These receptors act as a negative feedback mechanism by inhibiting the release of norpeinephrine. Thus, yohimbine acts by increasing in both sympathetic nerve action and in norepinephrine action [52]. A blockade of presynaptic α-2-adrenergic receptors by yohimbine subsequently enhances NO release from the penile nerves and induces penile erection [52]. It has been found that yohimbine acts as an α-2-adrenergic receptor antagonist in medial preoptic area and other hypothalamic areas associated with libido and penile erection [53]. Human studies have shown that yohimbine has a positive effect on male sexual performance over placebo [54, 55, 56]. Yohimbine's action on α-adrenergic receptor is not limited to erectile function but generalize effect on other organs [57]. Side effects include nausea, insomnia, nervousness, and dizziness. Large dose of yohimbine can increase blood pressure and heart rate.

Phentolamine

Phentolamine is an α-1 and α-2 adrenoreceptor antagonist [58]. Blocking of both α-1 and α-2 adrenoreceptors within the cavernosal smooth muscle and on the arteriolar smooth muscle of the penis increases sympathetic outflow and release of noradrenaline, resulting in cavernosal smooth muscle relaxation and arteriolar dilation [59]. Side effects include nasal congestion, headache, low blood pressure and nausea.

PDE inhibitors

Sildenafil citrate

Sildenafil citrate (Viagra ®) enhances penile responses to sexual stimulation via selective inhibition of phosphodiesterase-5, the predominant isozyme metabolizing cyclic GMP in the corpus cavernosum and allowing higher local concentration of cGMP within the corpus cavernosum [60, 61, 62]. Normal penile erection depends on the relaxation of smooth muscles in the corpora cavernosa. In response to sexual stimuli, cavernous nerves and endothelial cells release nitric oxide, which stimulates the formation of cGMP by activated guanylate cyclase [34]. Sildenafil enhances cGMP accumulation drive with NO in the corpus cavernosum and promote vasorelaxation [61, 63]. The most common side effects include headache, flushing and dyspepsia. It has been reported that some men have vision problems, including color differentiation and blurriness. Men with recent history of cardiovascular risks such as myocardial infarction, cerebrovascular accident, unstable angina or cardiac failure are absolutely contraindicated to take sildenafil [64].

Dopaminergic agents

Apomorphine

Apomorphine is dopaminergic agonist synthesized from morphine but its pharmacological is little similar to morphine [65]. It acts specificly with a direct central D1 and D2 dopamine receptor. Central dopaminergic pathways play a key role in the penile erection, mainly the central dopaminergic neurons with comprise the

hypothalamic system with projections to the medial preoptic area (MPOA) and paraventricular nucleus (PVN) [28]. The MPOA and PVN nuclei play a critical role in sexual behavior and related sexual responsiveness because lesions of these areas abolish male sexual behavior [28]. It is administered via mucosal membranes or other possible routes including intranasal, sublingual, and rectal. Apomorphine, acts directly on the brain, the erection response is similar to physiological erection [66]. It has been reported that 67% of psychogenic ED patients achieved favorable results when taking apomorphine [67]. The adverse effects of apomorphine include persistent yawning, nausea, vomiting and hypotension, but sublingual sustained-release tablets minimize these side effects [67].

Nitric oxide donor

L-Arginine

L-Arginine is a precursor of nitric oxide. A previous study showed that long term oral administration of L-arginine improved the erectile response to electrical stimulation of the cavernosal nerve and up-regulated penile NOS activity in the aging rat [68]. In a double-blind, randomized, placebo-controlled study, oral administration of high-dose (5 g/day) L-arginine was effective in patients with organic ED only if they had decreased NO release or production [69]. L-arginine would improve erectile function in patients with ED associated with increase in plasma levels of the endogenous NOS inhibitor, seen in renal failure, diabetes mellitus, hypertension and hypercholesterolemia. L-arginine is suggested to use for the treatment of the ED patients with secondary defective NO production [70].

3.1.2 Intracavernosal therapy

PDE inhibitors

Papaverine hydrochloride

Papaverine is a benzylquinoline synthesized from tyrosine. It functions as a phosphodiesterase inhibitor resulting in accumulation of both intracellular cAMP and cGMP levels and corporal smooth muscle relaxation [71].

Papaverine, *in vitro*, relaxes the penile arteries, the cavernous sinusoids, and the penile veins. Long term injection of papaverine might induce corporal fibrosis [72].

Prostaglandins

Prostaglandin E1 (Alprostadil)

Alprostadil, a synthetic prostaglandin E1, is the vasoactive agent used for treatment ED via injection intracavernously or intraurethrally [73, 74]. PGE1 is used to treat ED with some etiologies such as neurogenic, psychogenic and mild arteriogenic [73]. In a clinical trial, 40 to 70% of patients with ED responded to intracavernosal injection of PGE1 [73]. The major adverse effect with the use of intracavernous PGE1 is painful at the injection site. Pain persists throughout erection but does not appear to cause prolonged erection as it is rapidly metabolized in the cavernous tissue [73]. Thus, transurethral PGE1 has been used instead to deliver drug into the penis with less painful. Intratranurethal PGE1 is absorbed from the urethra and transported throughout the erectile tissue by the vessels between the corpus spongiosum of the urethra and the corpora cavernosa [74].

Phentolamine

Phentolamine is an antagonist of α 1- and α 2- adrenoceptor found in the cavernosal smooth muscle and on the arteriolar smooth muscle of the penis [58]. Its plasma half-life is about 20-30 minutes [75]. Intracavernous injection of phentolamine alone does not give a full and rigid erection. Therefore, it is used in combination with papaverine and alprostadil [76]. The most common adverse side-effect is systemic hypotension and tachycardia [76].

Vasoactive intestinal peptide (VIP)

VIP is widely distributed in male and female urogenital tracts as well as the central and peripheral nervous systems [77]. VIP has been shown to produce a wide range of effects including vasodilation, inhibition of contractile activity in many types of smooth muscle, stimulation of cardiac contractility and stimulation of many

exocrine secretions [78]. It stimulates adenylate cyclase and the formation of cyclic AMP [79]. It has been reported that the level of VIP and distribution of VIP nerve fibers in human cavernous tissue were lower in men with organic ED than in men with psychogenic ED or in a control group [80]. From this result the reduction of VIP may play a key role in the development of impotence. In animal studies, intracavernous injection of VIP induced penile erection, increased arterial flow, decreased venous flow and induced sinusoidal relaxation [81]. However, it failed to produce a rigid penile erection both in men with ED and healthy volunteers. VIP is recommended to use in the combination with phentolamine to give a better erectile response [82].

Moxisylyte (thymoxamine)

Moxisylate is a competitive α1-receptor antagonist [83]. In the treatment of ED, intracavernous injection of moxisylate induces an adequate erection for intercourse in most patients. *In vitro*, moxisylate showed a dose-dependent relaxation of a norepinephrine-induced contraction of human corpus cavernosum strip [84]. Moxisylate is less effective than alprostadil and papaverine but it has been shown to have a lower rate of adverse effects compared to papaverine and alprostadil [85, 86]. It has been suggested to use as a first-line treatment for ED before alternative to alprostadil or papaverine, due to its fewer side effects [85].

Calcitonin Gene-Related Peptide (CGRP)

CGRP, 37-amino acid peptide, is a potent vasodilator [87]. It relaxes the smooth muscle cells of the corpora cavernosa by inducing hyperpolarization via K^+ channel opening, and activation of adenylyl cyclase with subsequent increase in intracellular cAMP [88]. Intracavernosal injection of CGRP in ED patients increases the penile arterial inflow, cavernous smooth muscle relaxation, cavernous outflow occlusion, and in erectile responses in dose-dependent manner [89]. The combination of CGRP with PGE1 has been used as an intracavernous combination therapy to improve the treatment of ED [90].

Linsidomine chlorhydrate (SIN-1)

SIN-1, an active metabolite of the antianginal drug molsidomine, acts as a NO donor [91]. Intracavernosal injection of SIN-1 induces an erectile response by increasing the arterial inflow and relaxing cavernous smooth muscle in a dose-dependent manner [92].

3.1.3 Topical drug treatment

Nitroglycerine

Nitroglycerin (glyceryl trinitrate), a nitric oxide donor, causes the relaxation of vascular smooth muscle by stimulating soluble guanylate cyclase via enzymatic release of NO [93]. It was found to relax isolated strips of human corpus cavernosum [94]. Topical application of nitroglycerin to the penis or the perineum has been shown to induce penile erection [95]. Side effects include hypotension and severe headache.

Papaverine

Papaverine is an opium derivative that increases intracellular cAMP via nonselective inhibition of phosphodiesterases. In this way, papaverine alters the membrane calcium channel function and increases calcium effluxs from cells, resulting in a decline of intracellular calcium levels and subsequent smooth muscle cell relaxation [71]. Papaverine relaxes all components of the penile erectile system, i.e. the cavernous sinusoids and the penile veins. Papaverine gel applied topically to the penis has been shown to increase penile blood flow [96]. It is successful in the treatment of ED patients from spinal cord injuries [96].

Minoxidil

Minoxidil is a vasodilator widely used in alopecia androgenetica treatment [97]. The active metabolites of minoxidil act directly as vasodilator of the arterial smooth muscle and resulting in a reduction in peripheral resistance and cavernosal muscle relaxation [98]. It acts by promoting the veno-

occlusive mechanism. In comparison with nitroglycerin, minoxidil was shown to be more effective in producing erectile rigidity [99].

Prostaglandin E1 (Alprostadil)

Topical alprostadil (PGE1) has also been used in the treatment of ED because it initiates the hemodynamic events of a natural erection [100]. PGE1 relaxes smooth muscle of the corpus cavernosa because it increases intracellular concentrations of cAMP and has a short half-life. PGE1 interacts with specific membrane bound receptors that stimulate adenylate cyclase and elevate intracellular cAMP leading to the activation of protein kinase and smooth muscle relaxation. In treating impotence PGE1 induces penile erection by relaxing trabecular smooth muscle and dilating cavernosal arteries and their branches. Kim and McVary concluded that topical PGE1 appeared to be safe and well tolerated after application to the genitals and significantly increased blood flow in the penis [101].

3.1.4 Non-medical therapy

Psychosexual therapy

Psychogenic influences are the most likely causes of intermittent ED in men. Psychosexual counseling may be helpful to such kind of the patients in relieving their anxiety and stress. This therapy can be used together with pharmacological treatment [102].

Device therapy

The principle of vacuum constriction device (VCD) is a combination of vacuum and tension. The basic components of this device include a vacuum chamber, negative pressure pump and tension band. The penis is inserted into the proper size of the chamber. Then a proper tension band is placed around the base of the chamber. The vacuum chamber is placed over the flaccid penis and an air-tight seal obtained. By activating the pump, negative pressure is created within the vacuum chamber which draws blood into the penis to produce either erectile expansion or an

erection-like state [103]. Possible adverse effects of this device are penile irritation, penile enlargement and vaginal discomfort.

Hormonal treatment

Androgen deficiency is the most common cause of endocrinological abnormality in ED patients. The androgen replacement therapy in these patients is used for maintaining serum testosterone in the normal range that enhances sexual behavior [104]. Therefore, testosterone treatment is used to treat ED patients with diagnosed hypogonadism [104]. Testosterone should not be used in eugonadal men with erectile dysfunction as it may enhance prostatic hyperplasia or promote the growth of occult prostate cancer [105].

Experimental models of ED study

1. In *vitro* models

The principal mechanism of penile erection involves the relaxation of arterial and trabecular smooth muscle in the corpus cavernosum. Penile erection is controlled by the autonomic innervation to the penis [29, 30]. Parasympathetic pathways are the main proerectile pathways. On the other hand, sympathetic pathways play a major role in flaccidity and detumescence [30]. In the flaccid state, noradrenaline, released by postganglionic sympathetic fibers and acting at α_1-adrenergic receptors present on the smooth muscle of the cavernous arteries and of the corpus cavernosum, leading to contract of penile. Moreover, the relaxation of erectile arteries and veins induced by nonadrenergic, noncholinergic nerve stimulation is mainly due to nitric oxide (NO) released from perivascular nerve ending. NO induces vasorelaxation by activating soluble guanylate cyclase and increasing the level of cyclic guanosine monophosphate (cGMP) within vascular smooth muscle [31]. It has been applied all knowledge to study various neurotransmitters and vasoactive factors involved in this process in *vitro* model.

The isolated corpus cavernosal tissue has been used to study the mechanism of either contractile response or relaxing agents [106, 107]. Strips of smooth

muscle isolated from rabbit or rat corpus cavernosum are mounted in an organ bath. The strips of muscle are allowed to equilibrate in physiologic salt solution. The muscle is pre-contracted with either contraction agents such as phenylephrine and potassium chloride. Then, the relaxation of the muscle is measured after the addition of test substances. Relaxation of the cavernosum is considered a positive result for the test substance. Tension is measured with isometric force transducers, and muscle relaxation is expressed as the percent decrease of precontraction induced by the contracting agent [106].

The advantages of this method are easy in preparation, minimal equipment requirements, and obtaining the reproducible result.

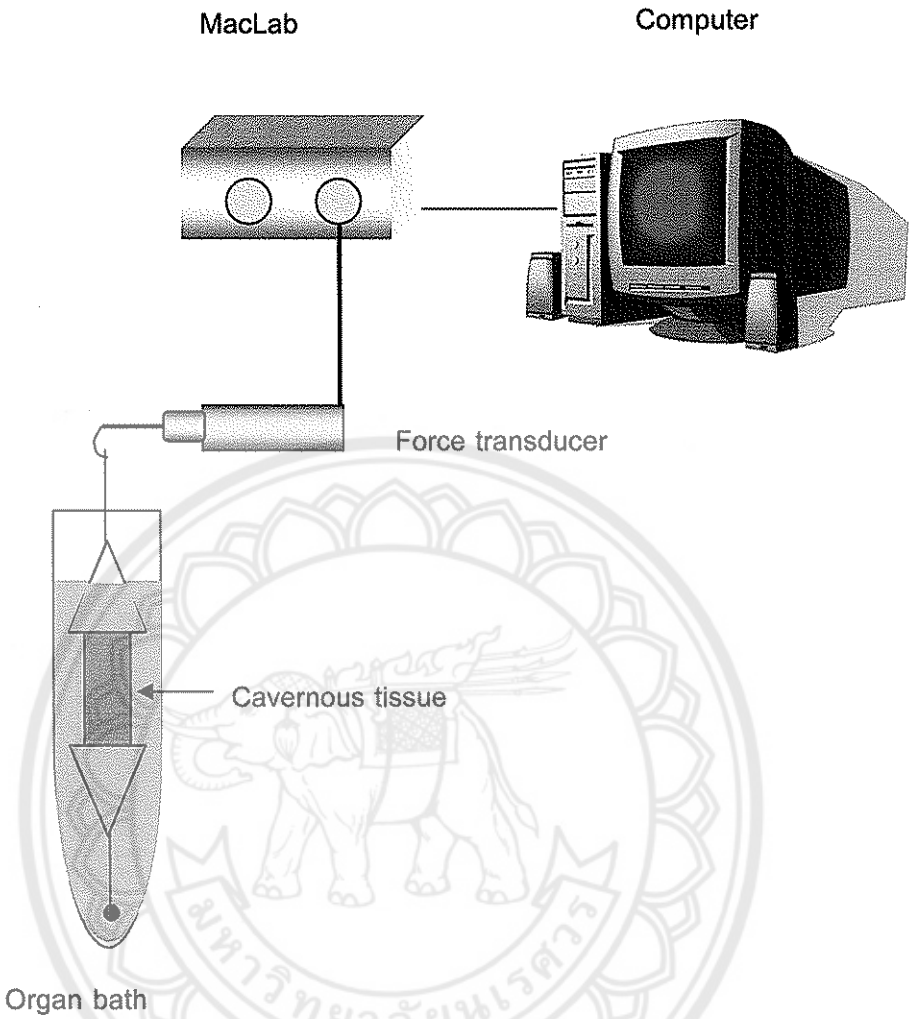

Figure 4 Diagram of the isolated cavernous tissues preparation in organ bath used to study of muscle tension

2. In *vivo* models

The roles of autonomic and somatic neural pathways involved in the control of penile erectile tissue are investigated in an in *vivo* rat model. Monitoring of intracavernosal pressure (ICP) have been developed and used as a standard method for evaluating the mechanism of erectile function. Animals used for the ICP study include monkeys, dogs, rabbits and rats [108]. During erection, there is an increasing in arterial blood flow, sinusoidal relaxation, and venous resistance, resulting in turgidity of the corpora cavernosa and corpus spongiosum. Subsequent contraction of the bulbocavernosus and ischiocavernousus muscle either spontaneously or reflexively compress the proximal corpora muscles, culminating in cavernosal rigidity and further engorgement of the glans penis, rapidly increase in ICP. It may reach a value of 10-20 mmHg below the systolic blood pressure [108, 109, 110]. In anesthetized animals, penile erection can be obtained by cavernous nerve or pelvic nerve stimulation. It obtains an increase of ICP whose amplitude is depend on both the stimulation applied and blood pressure (BP).

This technique has been modified to measure the ICP in a rat model for investigating erectile physiology, monitoring the hemodynamics in the corpus cavernosum and femoral artery after electrical stimulation of the carvernous nerve (Figure 5) [108]. These studies offer an advantage in analyzing the effects of test compounds on ICP and BP. Monitoring of ICP and BP are carried out in anesthetized rats by dissecting lower abdominal midline for isolation of the carvernous nerve and corpus cavernosum. Femoral arterial and cavernosal cannulae are connected to pressure transducers and amplified to monitor mean arterial pressure (MAP) and intracavernous pressure (ICP) which are expressed in mmHg (Figure 6).

Assessment of sperm function

Important parameters used to evaluate sperm are sperm concentration, semen volume, number of sperm in the ejaculate, progressive motility, percentage of viable cells, and sperm morphology [111]. Alterations in these parameters reflect the pathological of testicular function. The concentration of spermatozoa in the semen is an important clinical predictor of male infertility. An evaluation of motility is the most widely used test because it is simple, quick, and inexpensive [112]. It is a good indicator of the intactness of the membrane and functionality. The flagella activity of the sperm cell is needed for normal transport through the female reproductive tract and to reach the oviduct for fertilization. Sperm morphology is another interesting parameter and appears to be related to fertility. The variation in morphologic characteristics of the sperm indicates an insult to the testis such as viral infection, heat, or radiation.

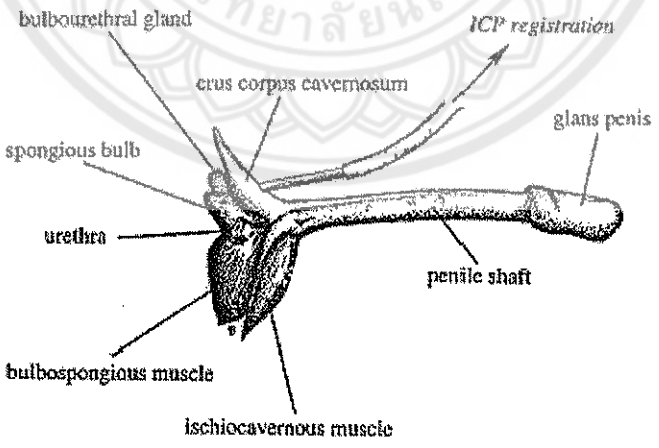

Figure 5 Technical landmarks for the registration of intracavernous pressure in *vivo*.

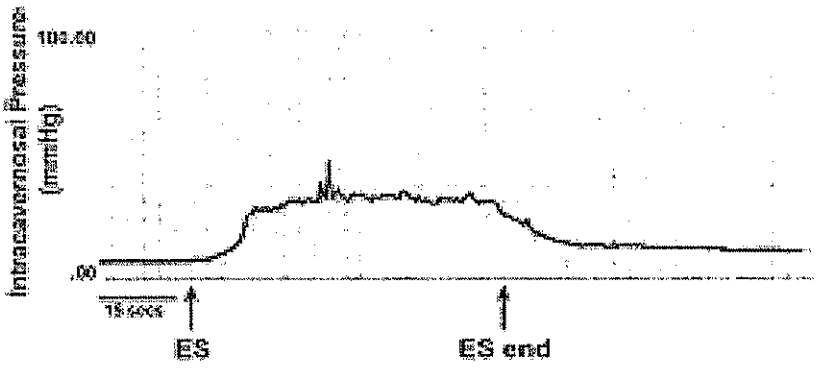

Figure 6 Tracing showing the changes in the intracavernosal pressure following cavernous nerve electrical stimulation (ES). ES indicates electrical stimulation started; ES end, electrical stimulation terminated.

Toxicity study

Most toxicity study conducted in animal is designed to obtain biological information indicative of toxicity that is reproducible, reliable, and dose-related as well as extended to the assessment of health risks to human [113]. Test substances are typically administered by the expected route of human exposure. The specific design of toxicity studies should be tests for toxicity prior to human clinical investigations as part of the non-clinical laboratory tests of pharmaceuticals. Animal test results often represent the only means by which toxicity in human can be effectively predicted. Toxicity study is classified into three types according to the length of the treatments: acute, subchronic and chronic toxicity studies.

1. Acute toxicity study

Acute toxicity study is conducted in animals to evaluate the adverse effects occurring within a short time of administration of a single dose of substance or multiple doses given within a 24 hour. Adverse effects are any effects that result in functional impairment or biochemical lesions that may affect the functions of organs. Death is a

major concern in the acute exposure. Then, the survived animals are observed for their abnormality symptoms such as changes in behavior, respiration, cutaneous effects, including some associated with the autonomic nervous system, sensory nervous system responses, gastrointestinal effects, cyanosis and coma. Each animal is observed for a minimum of 24 hours and daily thereafter for 14 days. At the end of 14 days, all the surviving animals are sacrificed. The organs and tissues are examined for morphological changes related with pathological disease. The toxicity of test substances are determined and expressed as median lethal dose (LD_{50}). LD_{50} is defined as the estimating dose that results in the death of 50% of the animals exposed to the test compound under the same condition. The LD_{50} is used to compare the relative toxicity of an un known compound in terms of the toxicity associated with other agents tested in the same species. For selecting the starting dose to determine the LD_{50} values of substances, several protocols have been used to set up acute toxicity study such as limit test, fixed-dose procedure, toxic class method and up-and down- method. The single dose administered to the animals provides useful information on pharmacokinetics of test substances such as the rate of disposition, biotransformation, and elimination of test substances [113, 114].

2. Subchronic toxicity study

Subchronic toxicity results from repeated exposure to test substances for several weeks or months. This is a common human exposure pattern for some pharmaceuticals and environmental agents. The animals will be treated with test substances daily for 90 day. This study requires three doses of a test substance and control. The highest dose of the test substances is below the LD_{50} values. It will provide the dose-effect relationship between biochemical, physiological, and morphological effects over the dosage range and the duration of administration of the test compounds. Parameters monitored in this study include daily observations for clinical signs of toxicity and mortality; weekly physical examinations; body weight and feed consumption; and measurement of serum biochemistry enzymes. The tissues and organs of the treated

and control animals are examined for morphological changes. Finally, the histopathology of each organ is observed for tissue damages [113].

3. Chronic toxicity study

Chronic toxicity study is performed according to international guidelines to detect any effects on health and tumor incidence of a test substance chronically given to the animals at different doses. Chronic toxicity represents cumulative damage to specific organ systems and takes 6 months to 2 years to become a recognizable clinical disease. This study requires three doses of a test substance, varying from a non-toxic low dose to a dose that is higher than the expected therapeutic dose and a control group similar to subchronic toxicity study. Prolonged treatment can demonstrate the carcinogenic effect of test substances. Parameters monitored in this study include observation for moribundity and mortality; weekly physical examinations; body weight as the indicator of growth and development and feed consumption; and measurement of serum biochemistry enzymes that reflect liver function test throughout the period of test substances administration. The carcinogenicity test determines the number of various types of tumors; the number of tumor-bearing animals; the number of tumors in each animal; and onset of tumors whenever determined. All dead as well as the remaining animals are sacrificed for pathological observation. In addition, reproductive test are conducted to detect alterations in the reproductive cycle and teratogenic effects [113].