EFFECT OF ROOT EXTRACTS OF EURYCOMA LONGIFOLIA JACK ON RABBIT PENILE ERECTION AND UNDERLYING MECHANISM OF ACTION

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by

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Dedicated;

"To my lovely family without whom life would be meaningless"

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LIST OF ABBREVIATIONS

5’GMP	5’ guanosine monophosphate
5-HT	5-Hydroxytryptamine / Serotonin
ACN	Acetonitrile
AOT	Acute oral toxicity
AQUEL	Aqueous extract of *E. longifolia* roots
AUC	Area under curve
ALX	Alloxan monohydrate
BPH	Benign prostatic hyperplasia
BSA	Bovine serum albumin
CBB	Coomassie brilliant blue dye
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
DL	Detection limit
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
FBG	Fasting Blood Glucose
ECL Substrate	Enhanced chemiluminescence substrate

ED	Erectile dysfunction
EL	Eurycomanol
ELISA	Enzyme-linked Immunosorbent Assay
E. longifolia	*Eurycoma longifolia*
EN	Eurycomanone
eNOS	Endothelial nitric oxide synthase
ETHEL	Ethanol extract of *E. longifolia* roots
GADPH	Glyceraldehyde 3-phosphate dehydrogenase
GTP	Guanosine triphosphate
HRP	Horseradish peroxidase
ICH	International community for harmonization
IgG	Immunoglobulin G
LD_{50}	Median lethal dose 50
LC-MS	Liquid chromatography mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantitation
METEL	Methanol extract of *E. longifolia* roots
MWCO	Molecular weight cut-off
NO	Nitric oxide
NOS	Nitric oxide synthase

nNOS	Neuronal nitric oxide synthase
OECD	Organization for economic and co-operation and development
PBS	Phosphate buffered saline
PDE	Phosphodiesterase enzyme
PDE-5	Phosphodiesterase 5 enzyme
PKG	Protein kinase G
PSA	Prostate specific antigen
PTFE	Polytetrafluoroethylene
QL	Quantitation limit
RNA	Ribonucleic acid
RP-HPLC	Reversed-phase high performance liquid chromatography
RCF	Relative centrifugal force
RSD/S_{rel}	Relative standard deviation
Sarkosyl	Sodium lauroyl sarcosinate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SNP	Sodium nitroprusside
StAR	Steroidogenic acute regulatory protein
STZ	Streptozotocin (Streptozocin)
TEA	Tetraethylammonium
TEMED	Tetramethylethylenediamine

THAM	Tris (hydroxymethyl)aminomethane
TMB	Tetramethylbenzidine
Tris-HCl	Tris Hydrochloride
WB	Western blot

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KESAN EKSTRAK AKAR *EURYCOMA LONGIFOLIA* JACK KEATAS EREKSI ZAKAR ARNAB DAN ASAS MEKANISME TINDAKANNYA

ABSTRAK

Objektif kajian ini adalah untuk menilai kesan ekstrak hidro-alkohol akar *Eurycoma longifolia* Jack kepada ereksi zakar dan asas mekanisme tindakannya. Pengekstrakan akar *E. longifolia* Jack menggunakan metanol, etanol, dan air sebagai pelarut menghasilkan tiga ekstrak iaitu; METEL, ETHEL dan AQUEL, masing-masing. Kajian ketoksikan akut pada tikus jantan mendapati bahawa $LD_{50} \geq 2000$ mg/kg bagi kedua-dua METEL dan ETHEL dan ≥ 5000 mg/kg bagi AQUEL. Pemberian oral perencat PDE5, sildenafil pada 1.5, 3, 15, dan 30 mg/kg pada arnab menyebabkan ereksi zakar sebanyak 1.6 ± 0.4, 3.1 ± 0.3, 8.6 ± 1.1, dan 10.9 ± 3.0 mm, masing-masing. Daripada tiga ekstrak, hanya METEL 500 dan 1000 mg/kg menyebabkan ereksi zakar masing-masing 4.9 ± 1.4 dan 2.9 ± 0.9 mm dimana dos terakhir telah mencapai kesan toksik. Penderma nitrik oksida (NO), natrium nitroprusida (0.2 mg/kg, i.v.), dengan ketara mempotensiasi ($P<0.001$) ereksi aruhan sildenafil lebih tinggi dari METEL (50 mg / kg, i.v.; $P<0.05$). Pemberian METEL (200 mg/kg, p.o.) dan testosteron enantat (30 mg / kg, s.c.) selama enam minggu meningkatkan dengan ketara paras testosteron pada kedua-dua tikus dan arnab normal ($P<0.001$-0.01). Begitu juga, paras NO telah meningkat dengan ketara pada arnab normal dan diabetik oleh kedua-dua rawatan ($P <0.001$-0.05). Di samping itu, berkurangannya tindak balas arnab diabetik kepada sildenafil telah diterbalikkan dengan ketara oleh kedua-dua rawatan testosteron enantat dan METEL selama enam minggu. Kajian ekspresi protein menunjukkan bahawa kedua-dua ekspresi nNOS dan protein PDE5 telah berkurangan pada arnab diabetik namun diterbalikkan oleh

pemberian selama enam bulan METEL dan testosteron. Analisis HPLC mendapati bahawa METEL, ETHEL dan AQUEL mengandungi 20.04 µg/mL, 19.79 µg/mL dan 21.71 µg/mL urokomanon (EN) masing-masing. Oleh itu, ereksi itu tidak telah mungkin disebabkan oleh EN, suatu kuasinoid walaupun kuasinoid telah dibuktikan terlibat dalam spermatogensis dan pengeluaran testosteron. Kesimpulannya, pemberian akut ekstrak *E. longifolia* sendirian memberikan aktiviti ereksi zakar yang sangat kecil dan hanya pada dos yang menghampiri kesan toksik. Walau bagaimanapun, sama seperti testosteron, selepas pemberian kronik ia boleh digunakan untuk meningkatkan ereksi mati pucuk separa seperti yang berlaku pada pesakit diabetik.

EFFECT OF ROOT EXTRACTS OF *EURYCOMA LONGIFOLIA* JACK ON RABBIT PENILE ERECTION AND THE UNDERLYING MECHANISM OF ACTION

ABSTRACT

The objective of this study was to assess the effect of hydro-alcoholic root extracts of *Eurycoma longifolia* Jack on penile erection and its underlying mechanisms. Extraction of roots of *E. longifolia* Jack using methanol, ethanol, and water as solvent produced three extracts namely; METEL, ETHEL, and AQUEL, respectively. Acute oral toxicity in male rats found LD_{50} of ≥ 2000 mg/kg for both METEL and ETHEL and ≥ 5000 mg/kg for AQUEL. Oral administration of a PDE5 inhibitor, sildenafil at 1.5, 3, 15, and 30 mg/kg in rabbit caused penile erections of 1.6 ± 0.4, 3.1 ± 0.3, 8.6 ± 1.1, and 10.9 ± 3.0 mm, respectively. Out of the three extracts, only METEL 500 and 1000 mg/kg caused penile erections of 4.9 ± 1.4 and 2.9 ± 0.9 mm respectively, with the latter reached toxicity level already. Nitric oxide (NO) donor, sodium nitroprusside (0.2 mg/kg, i.v.) significantly potentiated ($p < 0.001$) sildenafil-induced erection higher than METEL (50 mg/kg, i.v.; $p < 0.05$). Six week daily administration of METEL (200 mg/kg, p.o.) and testosterone enanthate (30 mg/kg, s.c.) significantly increased the testosterone level in both normal rats and rabbits ($p < 0.001 – 0.01$). Similarly, NO level was significantly elevated in normal and diabetic rabbits with both treatments ($p < 0.001 – 0.05$). In addition, diminished responsiveness to sildenafil in diabetic rabbits was significantly reversed by both testosterone enanthate and METEL after six weeks treatments. Protein expression studies showed that both nNOS as well as PDE5 proteins were reduced in diabetic rabbits which was reversed by both METEL and testosterone administration. HPLC

analysis found that METEL, ETHEL, and AQUEL contain 20.04 µg/mL, 19.79 µg/mL, and 21.71 µg/mL, of eurœcomanone (EN), respectively. Therefore, the erection could not have been induced by EN – a potent quassinoid, although quassinoids have been implicated in the spermatogensis and testosterone production. In conclusion, acute administration of *E. longifolia* extract on its own has very little penile erection activity and only at near toxic doses. However, similar to testosterone, after chronic use it may improve partial erectile dysfunction such as in diabetic patient.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

Penile erection is an important physiological phenomenon which is vital for a successful penetration during intercourse. A normal erection in a male adult usually involves a set of complex neurovascular events that give rise to increased supply of arterial blood into sinusoidal spaces in the penis in response to supraspinal responses (Andersson, 2011). Several distinct neurotransmitters as well as mediators regulate penile erection. A solid erection is necessary for a successful penetration of vaginal cavity during sexual contact.

Normal penile erection is usually affected by several risk factors such as aging, smoking, cardiovascular diseases, neurological diseases/disorders, endocrine defects, certain medications, hormonal abnormalities, and radical prostatectomy. These, individually or collectively, lead to endothelial dysfunction in general which results in the failure of smooth muscle relaxation during erection. Endothelial insufficiency then gives rise to erectile dysfunction (ED) in men (de Mendonça, 2012).

The fact that ED is not a life-threatening condition makes it difficult to estimate its incidence as many men with ED do not seek treatment (Melman, 1999). Findings of a study conducted in 1290 men of 40-70 years old between 1987 and 1989 indicated an overall prevalence of ED of 52% (Feldman, 1994). Elsewhere, a relatively recent report suggested a prevalence rate of ED of 18.4% in men aged 20 years and above which were mainly age-related (Selvin, 2007).

Since its discovery as a substance capable of inducing penile erection in male, Sildenafil – a potent inhibitor of phosphodiesterase-5 (PDE5) enzyme, has played important role in treating ED in men by enhancing the erectile capacity of the penis. Few other PDE5 inhibitors such as vardenafil, tadalafil, and mirodenafil have also been introduced to improve penile erection in men with ED. Despite this, satisfactory erections are not produced in men with severe diabetes or cardiovascular diseases by these agents. However, side effects associated with long-term use of these agents have limited their use in the treatment of ED (Raina, 2003, McMurray, 2007).

Generally, androgens play vital role in the growth and development of sexual organs as well as in regulating various metabolic functions in the body. Also, erectile function is largely influenced by sexual hormones predominantly by androgens and the deficiency of which may lead to impotence in men (Smith, 2001). Adequate production of androgens mainly testosterone is essential in the preservation of healthy and functional erectile function in men in order to enhance libido and sexual performance. Besides, they indirectly regulate the expression of several proteins viz. neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS), and PDE5 which are key mediators involved in the stimulation and maintenance of penile erection (Traish, 2005, Zhang, 2005). Androgen deprivation results in hypoandrogenism (male hypogonadism) which may arise as a complication of genetic and/or autoimmune abnormalities, systemic diseases, tumor, radiation, injury, aging, infection, and medical or surgical castration in men (Seftel, 2006, Sterling, 2015).

Testosterone replacement therapy or gonadotropin releasing hormones (GnRHs) have been prescribed to correct low testosterone levels and hypogonadism which occurs mainly in middle-aged and old men or those with ED (Sterling, 2015).

Also, some investigations have suggested the concomitant use of testosterone and PDE5 inhibitors which have been proven to be effective in managing erectile dysfunction (Alhathal, 2012). However, this is associated with higher risk of hematocrit and prostate cancer in middle-aged & old men (Calof, 2005, Marks, 2006).

For ages, men have used herbs with potentials to treat sexual dysfunction (impotence). Traditional remedies prepared from various herbs like *Tribulus terrestris, Myristica fragrans, Psoralea coryifolia, Eurycoma longifolia*, *Satureja khuzestanica, Fadogia agrestis, Panax ginseng, Avena sativa, Butea frondosa, Withania somnifera* (Ashwagandha), *Lepidium meyenii, Phoenix dactylifera, Montanoa tomentosa, Chione venosa, Ruta chalepensis, Eriosema kraussianum,* and *Gingko biloba* have shown promising outcomes in managing infertility and sexual impairments in various animal models (Malviya, 2011).

Similarly, there are numerous reports recognizing aphrodisiac potentials of *E. longifolia* root extracts in human as well as rodents. So far, much of the pharmacological investigations pertaining to aphrodisiac effect of *E. longifolia* have mainly focused on the assessment of spermatogenesis and steroidogenesis in normal and/or castrated animal models. Besides, there are several other studies which have evaluated penile reflexes, sexual motivation, mating behaviour, mount frequency, orientation activity, ejaculation frequency, intromission frequency, sexual performance, sexual quality, post-ejaculatory interval, pro-androgenic effect, and copulatory behaviour in rodents mainly rats (Wizneh, 2014). Despite this, there has been no investigation which would evaluate the effect of *E. longifolia* root extract on penile erection and its underlying mechanism of action in both normal and disease-

induced ED models. Moreover, influence of *E. longifolia* on mediators of penile erection such as nNOS, eNOS, and PDE5 which are indirectly regulated by testosterone have not been studied so far.

Based on the above discussions, this study was undertaken to explore the impact of short- and long-term administration of hydro-alcoholic root extracts of *E. longifolia* on penile erection and the possible underlying mechanism of action in both normal and diabetes-induced ED in animal models. Additionally, characterization of the bioactive extract/s by high performance liquid chromatography were executed to standardize these through detection and quantification of the most active constituent.

1.2 Research Objectives

1) To perform hydro-alcoholic extraction of roots of *Eurycoma longifolia* Jack.
2) To carry out acute oral toxicity of hydro-alcoholic extracts of *Eurycoma longifolia* Jack in adult male Sprague Dawley rats.
3) To study the effect of acute administration of root extracts of *Eurycoma longifolia* on penile erection in conscious adult male New Zealand white rabbits.
4) To investigate the effect of root extracts of *Eurycoma longifolia* on serum testosterone level in normal and streptozotocin-induced diabetic male SD rats.
5) To study the effect of chronic administration of bioactive extracts of *Eurycoma longifolia* on penile erection in normal and alloxan-induced diabetic adult male New Zealand white rabbits.
6) To estimate the testosterone and nitric oxide levels in the serum samples of normal and diabetic rabbits following chronic administration of methanol extract (METEL) of *Eurycoma longifolia*.
7) To study the effect of chronic administration of methanol extract (METEL) of *Eurycoma longifolia* on the expression of PDE5, nNOS, and eNOS proteins in penile protein samples of normal and diabetic rabbits through SDS-PAGE and western blot analysis.
8) To detect and quantitate eurycomanone (EN) in the bioactive extracts of *Eurycoma longifolia* by means of reversed-phase high performance liquid chromatography technique.

CHAPTER 2: LITERATURE REVIEW

2.1 Penile Erection

In a normal healthy adult an erection involves a set of complex neurovascular process which arises in response to several supraspinal stimuli (Andersson, 2011). A normal penile erection consists of some individually distinct components as sexual desire (libido), penile tumescence (erection), the climax of sexual excitement (orgasm), seminal discharge (ejaculation), and penile detumescence (refractory period) (Milhoua, 2006). The term "erection" itself is defined as "the state marked by firm turgid form and erect position of a previously flaccid bodily part containing cavernous tissue when that tissue becomes dilated with blood" (Merriam-Webster, 2014b). In other words, penile erection is the state of engorgement of smooth muscles of corpora cavernosa (CC) due to augmented blood flow via dilated blood vessels in the penis. It is the ultimate consequence of a spinal response to central and autonomic neural and/or humoral processes. Penile erection ensues when the influx of blood to the corpora cavernosa outweighs its outflow and therefore causes the sinusoidal enlargement. Meanwhile, the emissary mechanism is repressed to prevent drainage of blood out of penis (Lue, 2000). A firm erection is desirable to penetrate the vaginal cavity during sexual intercourse.

2.1.1 Male Reproductive System

The building blocks of male reproductive system comprises internal and external genital structures which include spermatic cord, scrotum, testes, epididymis, vas deferens, prostate gland, paradidymis, ejaculatory ducts, seminal vesicles,

bulbourethral glands, and penis (Standring, 2008). These play essential role in facilitating a normal erectile function in men through multifaceted neurovascular mechanisms.

2.1.1(a) Penis

Penis is the male sexual organ for intercourse that is present in a continuum from flaccid to fully rigid state (Milhoua, 2006). Structurally it looks like a cylindrical tissue anchored to the inferior pubic rami. Essentially, it consists of an attached root (radix) in the perineum and a free hanging body known as corpus. It is a muscular body that plays major role in delivering semen into the vagina during sexual intercourse and also in urinal discharge (Standring, 2008).

2.1.1(a)(i) Skin

The penis skin is a thin layer which wraps the entire penile tissue. Over the corona of penis, it forms a layer covering the penis glans which is known as prepuce. Proximal to the penis orifice, it forms the frenulum right below the neck of the penis.

2.1.1(a)(ii) Root

Two crura of the penis and bulb form the root of the penis. The crura – rounded elongated structures enclosed by ischiocavernosus and strongly attached to the ischiopubic rami, are connected to the pubic arch while the bulb is attached to the perineal membrane (Standring, 2008).

2.1.1(a)(iii) Body

Two erectile tissue masses namely corpora cavernosa and the median corpus spongiosum which are the continuation of the crura and bulb of the penis respectively, shape the main body of the penis. See Figure 2.1. These tissues are filled with blood during penile erection. A flaccid penis is cylindrical in shape but changes to triangular form when erect (Standring, 2008).

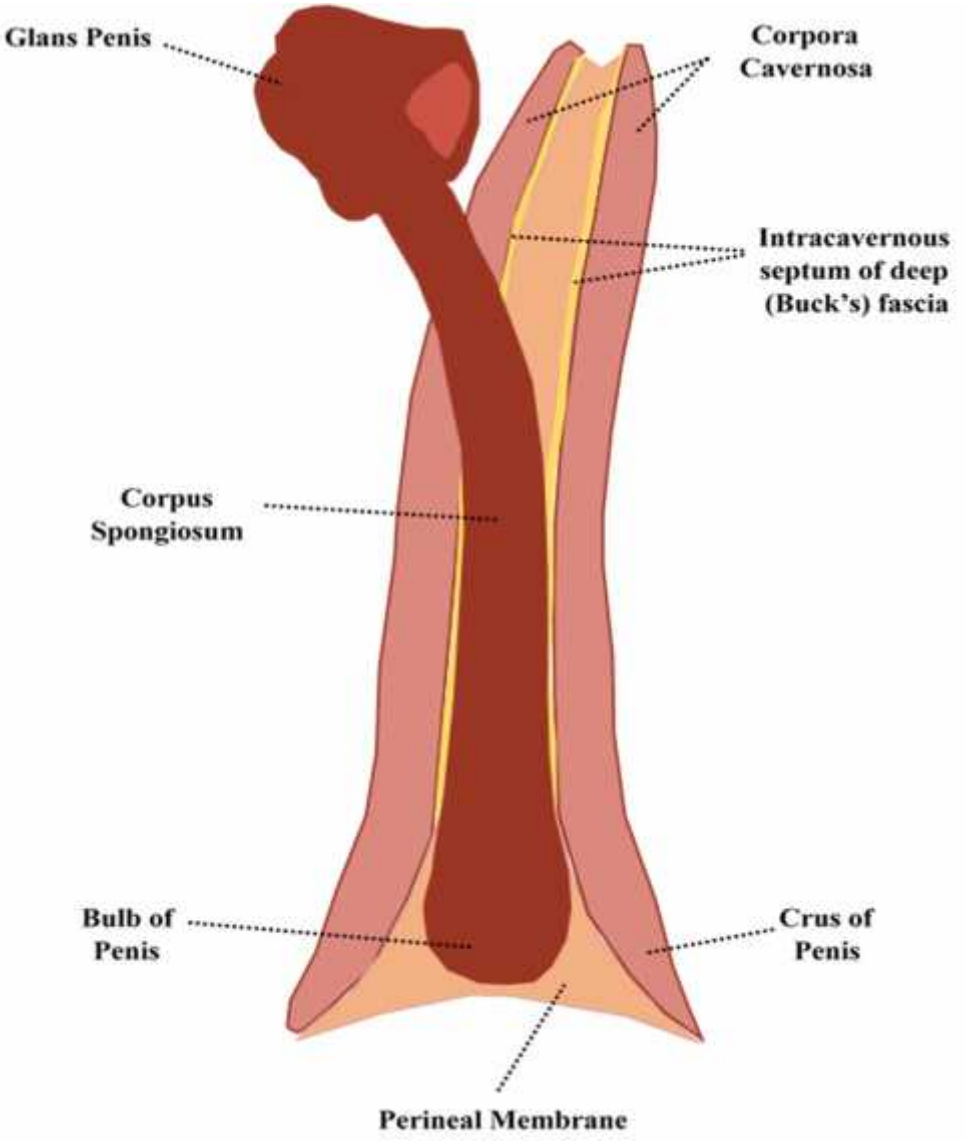

Figure 2.1 Anatomical illustration of human penis body.

2.1.1(a)(iv) Corpora Cavernosa

There are two corpus cavernous tissues running parallel to each other on the dorsal surface of the penis above corpus spongiosum which are analogous cylindrical erectile tissues enveloped in tunica albuginea. These are separated by a wide median septum on urethral surface and a narrower one at the dorsal surface. They originate from the crura of the penis CC, run along the length of the penis, and end distally within the proximal part of the glans penis. Sinusoidal spaces within CC are dilated with blood during the course of erection by cavernous arteries which run the length of the CC (Standring, 2008). See Figure 2.2.

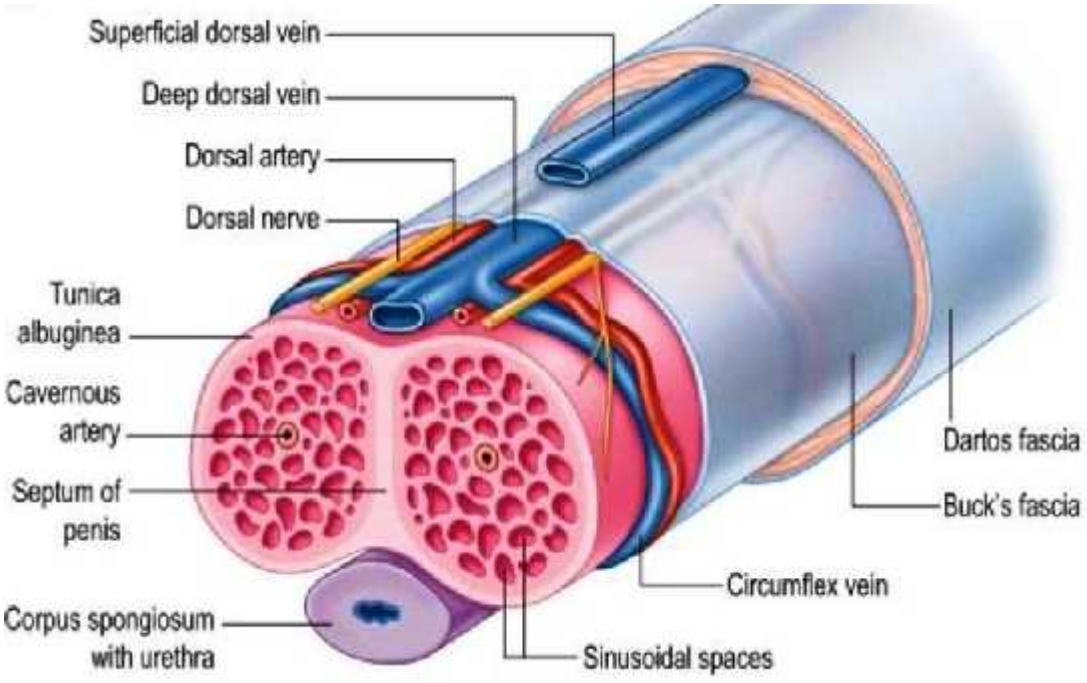

Figure 2.2 Cross section of the body of penis (Standring, 2008).

2.1.1(a)(v) Corpus Spongiosum

A spongy-like cylindrical tissue through which urethra passes and is surrounded by tunica albuginea. It borders the central groove on the urethral surface of the conjoined CC and ends at glans penis. The urethra within corpus spongiosum ends at the external urethral orifice (meatus) of glans penis which facilitates urinal and seminal discharge. Blood supply via bulbourethral and to some extent dorsal arteries during an erection results in the elongation of its sponge-like structure (Milhoua, 2006)

2.1.1(a)(vi) Penile Fascia

Penile fascia are of two types; superficial and deep fascia. Superficial penile fascia, also known as “Dartos fascia” is made of loose connective tissue without fat underneath which superficial dorsal vein of the penis extends along the penile shaft. The deep penile fascia also known as Buck’s fascia is rather compressed to shape a discrete strong sheath which covers both CC and corpus spongiosum. In addition, it accommodates deep dorsal vein, dorsal arteries, and dorsal nerves (Standring, 2008).

2.1.1(a)(vii) Suspensory Ligaments of Penis

Composed mostly of elastin fibers, suspensory ligaments provide support to penis body during erection and flaccid state. Fundiform suspensory ligament springs from the lowest part of the linea alba and splits into two fasciculi which encircles the body of the penis. The triangular suspensory ligament however is situated deeper to the fundiform ligaments which extends from the pubic symphysis and unites at the base of the penis before septum of the scrotum (Standring, 2008).

2.1.1(a)(viii) Penile Vasculature

Arterial supply of the penis

Arterial blood supply of penis is mainly attributed to the internal pudendal artery which ascends from internal iliac artery. It further branches off into dorsal, cavernosal and bulbourethral arteries which deliver arterial blood into various tissues of the penis. Dorsal or superficial penile arteries which are the terminal branch of the internal pudendal artery end at glans penis and prepuce by travelling between crus penis and pubic symphysis, and then by penetrating through suspensory ligaments of the penis. These lie deep to Buck's fascia and give rise to circumflex arteries that run laterally along the shaft of the penis and which predominantly supply tunica albuginea and to some extent corpus spongiosum. Cavernous artery also known as deep artery of the penis is the other terminal branch of the internal pudendal artery. It penetrates tunica albuginea, enters the crus penis at the penile hilum, and runs medially along the length of the corpus cavernosum. Cavernous arteries within CC give rise to two types of arteries; outer capillaries and inner helicine arteries. The outer capillaries play important role in penile nutrition during the flaccid state and supplying the smooth muscle and nerve fibers whereas the latter help in penile elongation and erection without compromising blood flow. Bulbourethral artery which is a short artery of large caliber also originates from internal pudendal artery. See Figure 2.3. It supplies both bulbourethral gland of the penis and corpus spongiosum (Benoit, 1987, Banya, 1989, Breza, 1989, Montorsi, 1998, Standring, 2008).

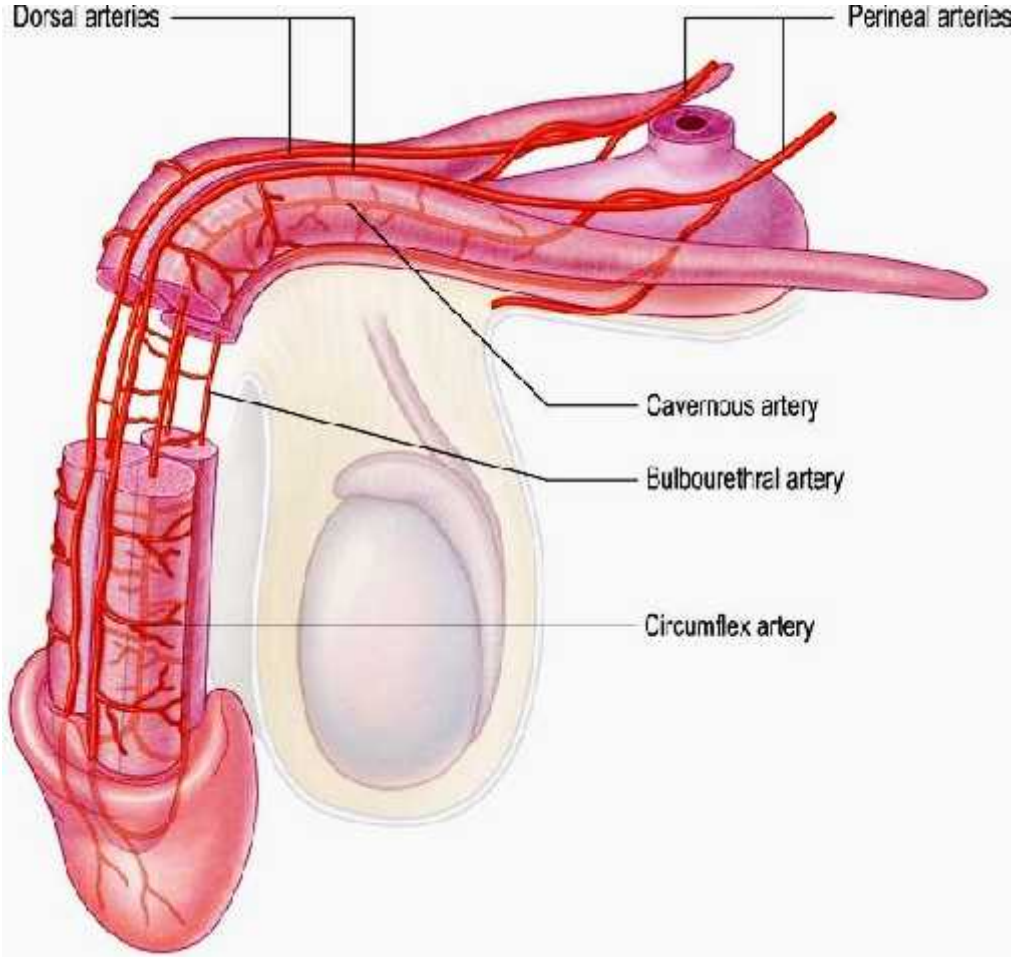

Figure 2.3 Arterial supply of the penis (Standring, 2008).

Venous supply of penis

Three sets of veins; superficial, intermediate, and deep facilitate venous emptying of penile tissues. See Appendix 2.1. The superficial dorsal veins mainly draw off blood from the skin and subcutaneous tissues superficial to Buck's fascia which subsequently drains into external pudendal vein. The intermediate set of veins which empty the glans penis are located deep to Buck's fascia. These form a retrococcal plexus that drain into deep dorsal vein which ultimately drains into Santorini's plexus (periprostatic plexus). Both the CC and corpus spongiosum are drained via deep venous system. Emissary veins of the middle and distal penis formed through

coalescence of postcavernous venules unite to shape circumflex veins which empty into deep dorsal vein. The emissary veins of the proximal penis however give rise to cavernous vein which drains into internal pudendal vein (Fournier, 1987, Aboseif, 1989, Fuchs, 1989, Lue, 1996, Moscovici, 1999).

Lymphatic drainage

Penile lymph vessels that accompany the external pudendal blood vessels to the superficial inguinal nodes are responsible for the drainage of penile skin. Lymph vessels that drain the glans penis enter the external iliac nodes whereas the lymph vessels of urethral and erectile tissues drain into internal iliac nodes (Standring, 2008).

2.1.1(a)(ix) Penile innervation

Neural regulation of erectile function in male is mediated by the autonomic nervous system (ANS) through sympathetic and parasympathetic input. The penis is innervated with nerve fibers stemming from T11 – L2 segments of thoracolumbar (sympathetic) and S2 – S4 segments of sacral (parasympathetic) spinal cord. See Figure 2.4. Stimuli originating from parasympathetic source trigger penile erection whereas sympathetic outflow prompts penile contraction and flaccidity. Neural stimulus instigates the release of key neurotransmitters; Acetylcholine (ACh; parasympathetic), noradrenaline (NA; sympathetic), and nitric oxide (NO; non-adrenergic non-cholinergic (NANC)) which play distinct role during erectile function (Taub, 1993). The penis is innervated with three types of nerve fibers: dorsal, perineal, and cavernousal. Dorsal and perineal nerves both ascend from pudendal nerves. The dorsal fibers supply sensation to penile skin by traveling within Buck's fascia along with dorsal arteries and veins while the perineal nerves innervate bulbospongiosus

muscle, ventral shaft skin, and the frenulum (Brooks, 2007). On the other hand, cavernous nerves arising from autonomic pelvic plexus travel along the periprostatic neurovascular bundle and penetrate the cavernous tissue underneath the pubic arch (Akman, 2001, Yucel, 2003). Cavernous nerve which contains both parasympathetic and sympathetic components divides into two nerve fibers: lesser cavernous nerve and greater cavernous nerve. The former innervates the urethral and spongiosus erectile tissues whereas the latter enters CC about the vessels in the hilum of the penis (Lepor, 1985, Lue, 1984).

2.1.1(b) Scrotum

The scrotum is a cutaneous fibromuscular sac hanging below the pubic symphysis between the thighs which is house to testicles, epididymis, paradidymis, vas deferens, and Spermatic cord. It comprises of skin, superficial (Dartos) fascia, external spermatic fascia, cremaster muscle and fascia, internal spermatic fascia, and parietal layer of tunica vaginalis. A cutaneous raphe divides the scrotum into two halves; left and right. Presence of a longer spermatic cord on the left side of the scrotum makes it seem slightly lower than the right side.

2.1.1(b)(i) Testes

Testes are the primary reproductive gonads of the male which are ovoid in shape and are responsible for both spermatogenesis and androgen production mainly testosterone. They are components of both reproductive and endocrine system. The average weight of testes differs among individuals which is from 10.5 – 14 g; the average dimensions are; 4 – 5 cm in length, 2.5 cm in breadth, and 3 cm in anterioposterior diameter. Within the scrotum the testes are suspended by Dartos

muscle and spermatic cords. The right testis is usually positioned slightly higher than left testis within the scrotum. Each testis is supplied by three coats which are, from inside outwards, the tunica vasculosa, tunica albuginea, and tunica vaginalis (Standring, 2008).

2.1.1(b)(ii) Epididymis

The epididymis is invested by tunica vaginalis and is responsible for the storage of the sperms and its transportation from the testes. It is positioned posterio-lateral to the testis and consists of three parts: globus major (head), corpus (body), and cauda/globus minor (tail). A deep depression known as the sinus epididymis is present laterally between the testis and the epididymis (Standring, 2008).

2.1.1(b)(iii) Vas deferens

The vas deferens (ductus deferens) arises as a muscular conduit at the end of the epididymis is of 45 cm long which carries sperm to the ejaculatory ducts. It is the direct continuation of the canal of the epididymis. It is quite tortuous in the beginning and straightens as it rises the posterior part of the testis (Standring, 2008).

2.1.1(b)(iv) Paradidymis

Paradidymis is a vestigial body which is found above the epididymal head and anteriorly in the spermatic cord is a collection of circuitous tubules. Generally, it is believed to be a remnant of the mesonephros which is covered by the ciliated columnar epithelial tissue (Standring, 2008).

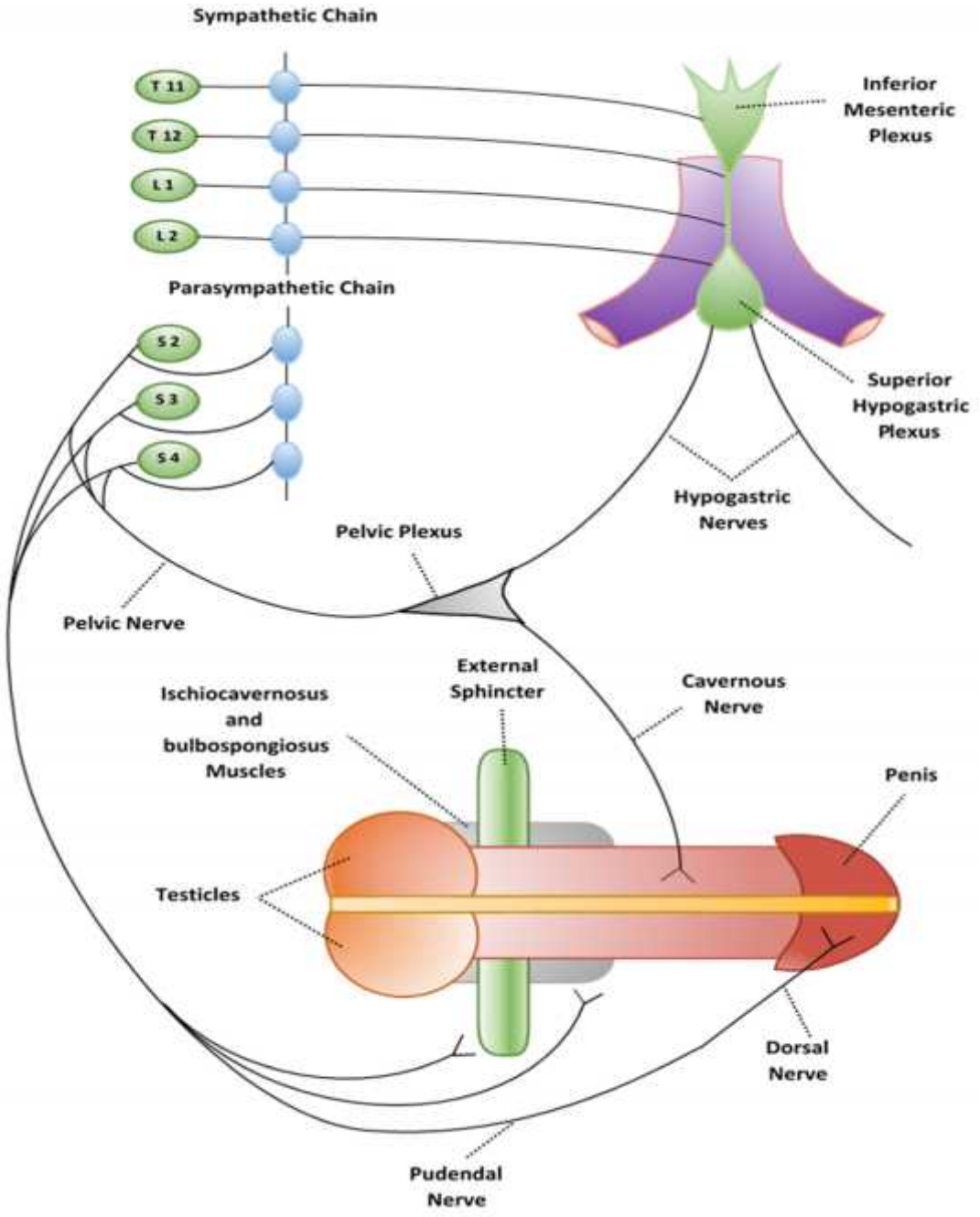

Figure 2.4 Schematic representation of the penile innervation.

2.1.1(b)(v) Spermatic Cord

As the name indicated the spermatic cord is a cord-like structure which consists of (a) vas deferens, (b) the testicular artery and pampiniform plexus of veins, (c) the lymph vessels and nerves of the penis and epididymis, and (d) remains of the processus vaginalis. It stretches from the testis all the way to the deep inguinal ring. Three sheaths of tubular in nature viz. the external spermatic fascia, the cremasteric muscle and fascia, and internal spermatic fascia enclose the spermatic cord (Standring, 2008).

2.1.1(c) Ejaculatory ducts

The merging of the duct of seminal vesicle with the ampulla of the ductus deferens establish the ejaculatory duct on either side. Length of each ejaculatory duct is less than an inch (about 2 cm) which begins at the bottom of the prostate and travels anterioinferiorly between its median and left/right lobes. The ducts pass through the prostate and open into the urethra at the colliculus seminalis/verumontanum by slit-like apertures. They facilitate the ejaculation of semen which passes through prostate into urethra during ejaculation process (Martin, 1951, Standring, 2008).

2.1.1(d) Seminal vesicles

Seminal vesicles (seminal glands/vesicular glands) are a pair of hollow sacculated organs which lie inferiorly to the bladder and in front of the rectum. Each vesicle is about 3 – 4 mm in diameter which look slightly pyramidal in shape. Basically, a seminal vesicle is a single twisted duct with non-uniform diverticula. A coiled tube measures approximately 2 inches in length whereas its unravelled length is ranges from 10 – 15 cm (Martin, 1951). A substantial percentage of the seminal fluid

(70 – 85%) is secreted by seminal vesicular glands which is alkaline in nature (Kierszenbaum, 2011).

2.1.1(e) Bulbourethral glands

The bulbourethral glands (*glandulae bulbourethrales*) are two tiny, spherical, and to some extent lobulated masses of a yellow color. Each gland measures about 1 cm (roughly the size of a pea) in diameter which is surrounded by transverse fibers of the sphincter urethrae. These are exocrine glands which sit lateral to the membranous part of the urethra between the sheets of the fascia of the urogenital diaphragm. Bulbourethral glands produce a mucus-like fluid called as pre-ejaculate during sexual arousal which makes the urethra more hospitable for the sperm to pass through by neutralizing its acidic environment (Gray, 1972, Britannica, 2014).

2.1.1(f) Prostate gland

The prostate is a partly glandular, partly muscular reproductive organ of male which if of dark red-brown color. It is a firm structure surrounded by a dense fascia sheath which is placed within the pelvic cavity behind the pubic symphysis. Prostate is about the size and shape of a chestnut. It has an apex that faces downwards, an upward looking base, a posterior surface, a pair of lateral surfaces, and an anterior surface. Prostate secretes a slightly alkaline fluid that is milky/white in appearance to nourish the semen and to help enhance the lifespan of spermatozoa by augmenting its motility in acidic environment of vaginal tract. Prostatic fluid along with fluid from seminal vesicles and spermatozoa constitute approximately 30% of the semen volume (Standring, 2008).

2.1.2 Mechanisms of Penile Erection and Contraction

Penile erection and contraction are neurovascular phenomenona involving nerve fibers, blood vessels and tissue cavities, and smooth muscle cells of the target organ. Penile erection is a process that is mainly mediated through spinal response which comprises of various central and peripheral mechanisms. Olfactory, imaginative, visual, and tactile stimuli which originate from central nervous system (CNS) as well as peripheral nervous system (PNS) can trigger release of certain neurotransmitters that promote smooth muscle relaxation. These facilitate increase inflow of oxygenated blood into corporeal smooth muscle which results in the sinusoids to be stretched and expanded to accommodate more blood to produce erection. Relaxation and elongation of corporeal smooth muscles compress the surrounding internal venules to hinder blood outflow. Therefore, allowing the intracavernosal pressure to rise to mean systolic pressure to cause penile rigidity (Rehman, 2001, Andersson, 2011).

Relaxation of smooth muscle cell is mainly brought about by the action of neurotransmitter NO. Following the excitatory signals from the supraspinal region parasympathetic cholinergic nerve endings within corpus cavernosum (CC) release ACh which promotes the formation and release of NO mediated by endothelial nitric oxide synthase (eNOS) from endothelial cells. Meanwhile, non-adrenergic non-cholinergic (NANC) nerves in CC also release NO via the action of neuronal nitric oxide synthase (nNOS). NO then diffuses into the smooth cell and activates the soluble guanylyl cyclase (sGC) which facilitates the conversion of guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP) which in turn activates cGMP-specific protein kinase (PKG). In the meantime, prostaglandin E_1 (PGE_1) activates

enzyme adenylyl cyclase (AC) by binding to G-protein-coupled-receptor (GPCR) promoting the formation of cyclic adenosine monophosphate (cAMP) which in turn activates cAMP-specific protein kinase (PKA). Activation of both PKG and PKA causes reduction in intracellular Ca^{+2} levels by (1) inhibiting its influx, (2) stimulating its uptake by endoplasmic reticulum (ER), and (3) activating the efflux of K^{+}. Subsequently, myosin head detaches from actin triggering the relaxation of smooth muscle cells which leads to increased inflow of blood and ultimately penile erection (Lue, 2000, Corona, 2010, Andersson, 2011). See Figure 2.5.

On the other hand, penile detumescence (flaccidity) or contraction occurs mainly as a consequence of stimulation by sympathetic nervous system in the penile tissues. Release of anti-erectile neurotransmitters such as norepinephrine (NE) from adrenergic nerve endings in penile tissues stimulate the release of calcium ions and hence promotes contraction of the smooth muscle cells. Meanwhile, NE triggers the activation of Rho-A/Rho-kinase pathway which results in calcium sensitization leading to smooth muscle cell contraction and penile flaccidity (Andersson, 2011). NE released from adrenergic nerve endings of sympathetic nervous system binds to α1 receptor in the smooth muscle cell membrane, stimulating both phospholipase C (PLC) and guanine nucleotide exchange factors (GEFs). Stimulated GEFs facilitate the activation of Rho-A signaling pathway by converting inactive Rho-A GDP into its active form Rho-A GTP. This in turn activates Rho-associated protein kinase (ROCK) protein which facilitates the phosphorylation of myosin light chain leading to the contraction of smooth muscle cells. Meanwhile, activation of PLC generates inositol triphosphate (IP_3) which triggers the augmentation of intracellular Ca^{+2} concentration. This is followed by formation of calcium – calmodulin complex which causes the phosphorylation of myosin light chain by stimulating the action of myosin light chain

kinase (MLCK) leading to the contraction of penile tissues (Lue, 2000, Corona, 2010).

See Figure 2.6.

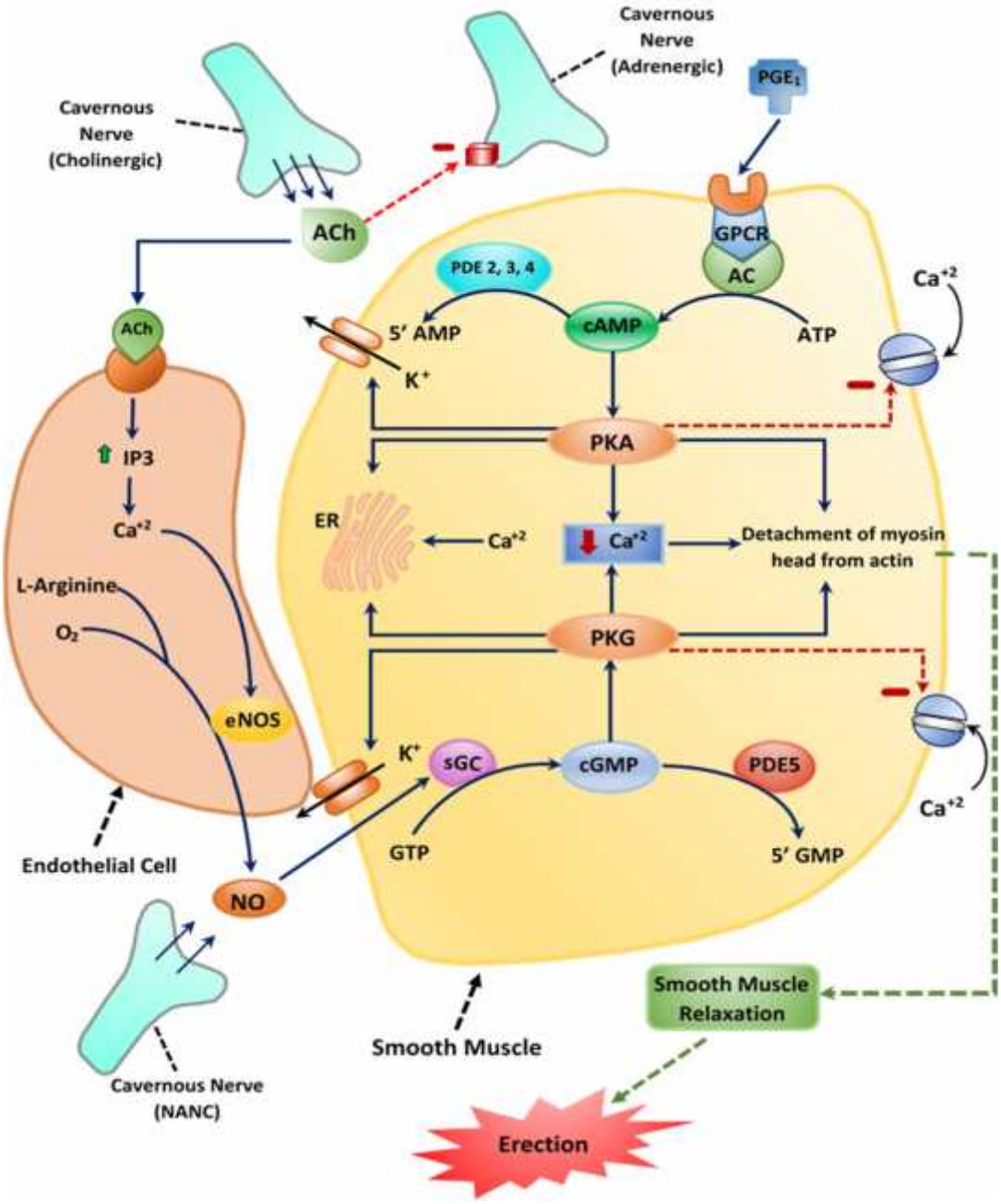

Figure 2.5 Molecular mechanism of penile smooth muscle relaxation. ACh; acetylcholine, PGE_1; prostaglandin E_1, AC; adenylyl cyclase, GPCR; G-protein coupled receptor, cAMP; cyclic adenosine monophosphate, cGMP; cyclic guanosine monophosphate, ER; endoplasmic reticulum, ATP; adenosine triphosphate, 5'AMP; 5'adenosine monophosphate, PKA; cAMP-specific protein kinase, PKG; cGMP-specific protein kinase, sGC; soluble guanylyl cyclase, PDE; phosphodiesterase enzyme, eNOS; endothelial nitric oxide, IP3; inositol triphosphate, NO; nitric oxide, GTP; guanosine triphosphate, 5'GMP; 5'guanosine monophosphate.O_2; oxygen, Ca^{+2}; calcium ions, K^+; potassium ions. (Adopted from Lasker *et al.* 2006).

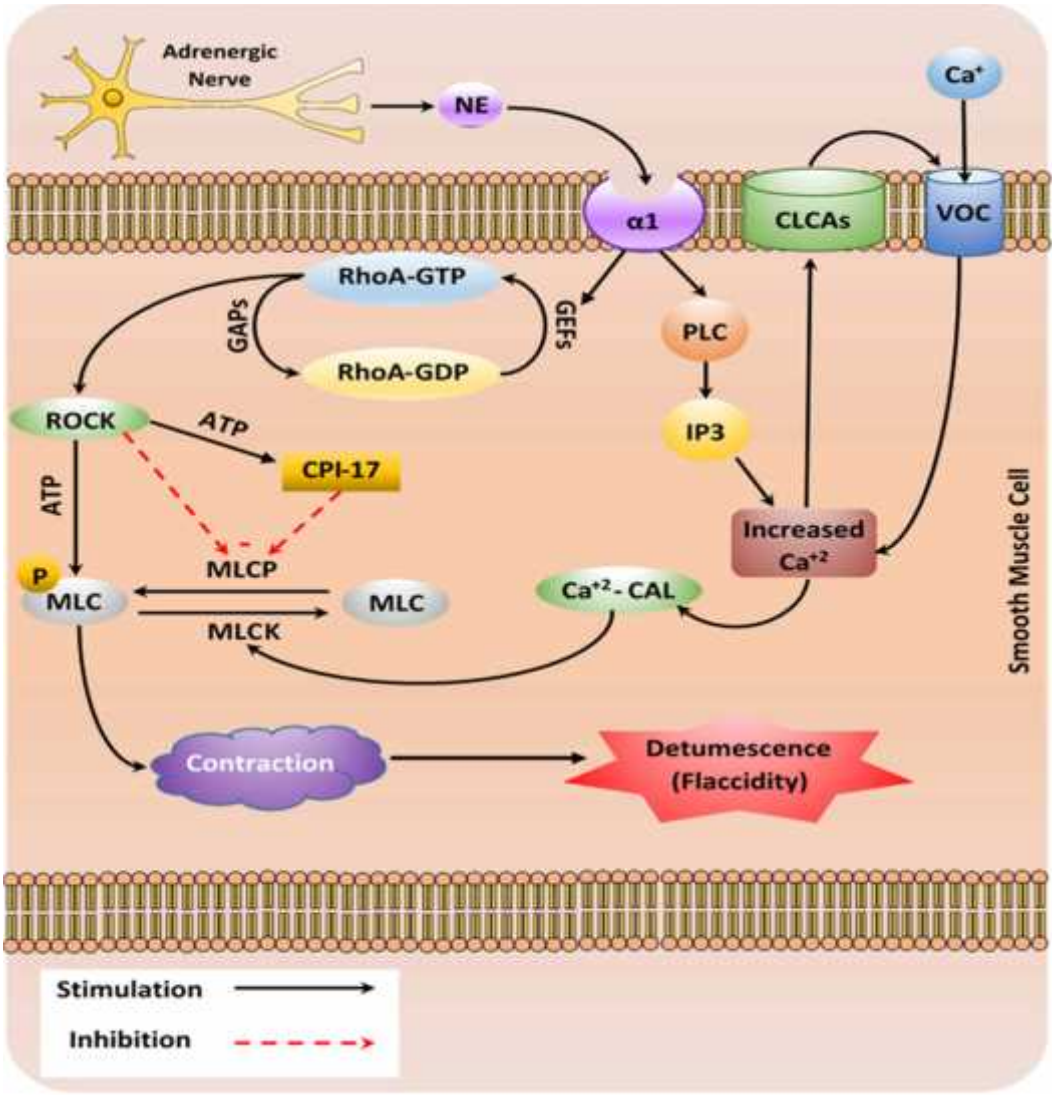

Figure 2.6 Molecular mechanism of smooth muscle contraction of the penis. NE; norepinephrine, CLCA; calcium-activated chloride channels, VOC; voltage operated channel, PLC; phospholipase C, IP3; inositol triphosphate, CAL; calmodulin, GEFs; Guanine nucleotide exchange factors, GAPs; GTPase-activating proteins, ROCK; Rho-associated protein kinase, Rho-A; ras-homolog gene family member A, MLC; myosin light chain, MLCK; myosin light chain kinase, MLCP; myosin light chain phosphatase, CPI-17; C-kinase-activated protein phosphatase-1 inhibitor-17. (Adopted from Corona *et al*. 2010).

2.1.2(a) Central Regulation of Penile Erection

Mechanism of central regulation of erectile function involves both spinal and supraspinal pathways. Findings of earlier investigations suggest that the male sexual arousal is largely regulated by the limbic system and hypothalamus under central supraspinal systems. Also, some key structures viz. paraventricular nucleus (PVN),

medial preoptic area (MPOA), medial amygdala, the periaqueductal gray, and ventral tegmentum play major role in the central management of male sexual function. Moreover, studies have recommended that the electrical stimulation of the MPOA and PVN or even the hippocampal formation gives rise to penile erection (Giuliano, 2000a, b, Andersson, 2011, Melis, 2011). Various neurotransmitters and neuropeptides; excitatory or inhibitory, regulate penile erection among which 5-HT (5-hydroxytryptamine/serotonin), oxytocin, dopamine (DA), nitric oxide (NO), excitatory amino acids, adrenocorticotropin/α-melanocortin stimulating hormone (α-MSH), and opioid peptides are well recognized. These either facilitate or inhibit penile erection by acting in different regions of the brain with the PVN being major one (Melis, 2011, Andersson, 2011).

2.1.2(a)(i) Proerectile Mediators

Certain central neurotransmitters such as dopamine, oxytocin, excitatory amino acids, adrenocorticotropins/melanocortins, hezarelin analog peptides, pro-VGF nerve growth factor inducible (VGF) derived peptides, cannabinoids, NO, prolactin, androgens have demonstrated facilitative effects on male sexual functions in general in number of animal models. Injection of several dopaminergic agonists have shown to induce penile erection in mammals following systemic administration (Hull, 2004, Melis, 1995). A similar proerectile effect was observed when oxytocin was injected into the PVN or other extrahypothalamic regions of the brain which was suggested to be due to the presence of a group of oxytocinergic neurons (Argiolas, 2004, Baskerville, 2008, Melis, 2011). Besides, evidence from several anial studies revealed that the injection of excitatory amino acids; NMDA (Argiolas, 2005, Melis, 1994), hexarelin analog peptides (Melis, 2001, Argiolas, 2005), pro-VGF-derived peptides

(Argiolas, 2005), nitric oxide (Argiolas, 1994), and adrenocorticotropins/melanocortins (Bertolini, 1975, Wessells, 2005, King, 2007) agonists and cannabinoids antagonist (Melis, 2004) into the PVN induced penile erection via diverse mechanisms. Androgens, in particular testosterone have been shown to have important role in regulating erectile function (both centrally and peripherally) absence of which causes a decline in erectile and ejaculatory functions in male (Traish, 2007, Buvat, 2010). Short-term central prolactin treatment is associated with stimulatory effect on sexual behavior in rodents (Cruz-Casallas, 1999).

2.1.2(a)(ii) Anti-erectile Mediators

Reports suggest that anti-erectile central neurotransmitters like serotonin/5-HT, γ-amino butyric acid (GABA), opioid peptides, and prolactin as well as NE to certain extent suppress the sexual function in male. Serotonin being the most distinguished of all is thought to have a general inhibitory effect on male sexual activities which comprises both sympathetic and parasympathetic, and somatic outflow pathways (Hull, 2004, Marson, 1992, Bitran, 1987). Generally, hyperprolactinemia is linked with depressant effect on sexual behavior and reduction in sexual potency as well as sexual reflexes in male. Moreover, long-term central prolactin treatment is associated with inhibitory effect on the sexual behavior (Drago, 1984, Cruz-Casallas, 1999, Rehman, 2000, Kruger, 2005). Studies on the role of the GABA in erectile function have underlined the general inhibitory action of this neurotransmitter in the pathways involved in penile erection (de Groat, 1993, Melis, 2002). However, a study in male rats revealed that the activation of $GABA_A$ subtype receptor reduces copulatory activity while its $GABA_B$ subtype receptor stimulation