PRELIMINARY INVESTIGATION ON EFFECTS OF BURANTASHI EXTRACT ON LIPOPROTEINS OF ALBINO MALE AND FEMALE WHISTAR RATS

BY

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CERTIFICATION

This is to certify that this project "The Preliminary Investigation on Effect of Burantashi Extract on LipoProteins Of Albino Male and Female Whistar Rats" was undertaken by Miss Etiani Jennifer of the Department of Biochemistry, Caritas University Amorji-Nike Emene, Enugu state Nigeria.

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DEDICATION

This project is dedicated to the Almighty God, the foundation of Wisdom, knowledge and understanding, for the gift of life.

I also dedicate this work to my beloved family, for being there for me and with me through the good and bad times.

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I am most grateful to the Almighty God who has been my most gracious guardian and source of strength throughout this course.

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ABSTRACT

This work was carried out to investigate the effects of Burrantashi extracts on the lipoproteins Burantashi is a popular seasoning agent to barbecued meat (Suya) in Nigeria. Found in the northern parts of the country. Lipoproteins are the principal steroid or fat that is synthesized in the liver or intestines of animals. Erectile dysfunction (ED) is defined as the consistent or recurrent inability of a man to attain or maintain penile erection, sufficient for sexual activity (2^{nd} international consultation on sexual dysfunction Paris, June 28^{th} –July 1^{st} 2003). Following the discovery and introduction of burantashi research on the mechanism underlying penile erection, had an enormous boost and many preclinical and clinical papers have been published in the last five years on the peripheral regulation of, and the mediators involved in human penile erection. The most widely accepted risk factors for e.g. are discussed. The research is focused on human data and the safety and effectiveness of burantashi stem as a phosphodiesterase – 5- inhibitors (PDEs) used to treat erectile dysfunction.

CHAPTER ONE

INTRODUCTION

Erectile dysfunction, ED, is a sexual dysfunction that affects the reproductive systems of both men and women. By definition according to National Institute of Health consensus Development Panel on impotence (1993), in Males, it is a sexual dysfunction characterized with the inability to develop or maintain an erection of the penis sufficient for satisfactory sexual performance. It is also known as Male impotence or Baby D syndrome, while in women, according to American Psychiatric Association (1994), it is characterized with the persistent or recurrent inability to attain, or maintain until completion of the sexual activity, an adequate Lubrication- Swelling response that otherwise is present during female sexual arousal and sexual activity is thus prevented. Hence, it is called Women impotence or female erectile dysfunction.

The word impotence may also be used to describe other problems that may interfere with sexual intercourse and reproduction, such as lack of Sexual Desire and problems with ejaculation or orgasm. Using the term "erectile dysfunction," however makes it clear that those other problems are not involved (NIH, 2005).

An erection occurs as a hydraulic effect due to blood entering and being retained in sponge-like bodies within the penis and clitoris. The process is most often than not initiated as a result of sexual arousal, when signals are transmitted from the brain to nerves in the pelvis.

Erectile dysfunction is, therefore indicated when an erection is consistently difficult or impossible to produce, despite arousal (Laumann et al., 1999).

1.1 PREVALENCE OF ERECTILE DYSFUNCTION IN WOMEN

Erectile dysfunction which is known as Female erection dysfunction in women occurs in about 43% of American Women (NIH Consensus Conference, 1993). And this medical Condition is a persistent or recurrent inability to attain or maintain clitoral erection until completion of the sexual activity, an adequate Lubrication –Swelling response that is normally present during Female sexual arousal and sexual activity is therefore, absent. The individual having the condition is said to experience frigidity (American Psychiatric Association, 1994). Again,

According to Otubu et al. (1998) about 8.7% of Women suffer from this very condition in the United States while between 35.3 - 40%, according to Adequnloye (2002) and Eze (1994) of Women in Nigeria suffer from this condition. Spector and Carey (1994) reported 5-10% in the United States.

In addition, Female erectile dysfunction occurs at any age but majorly in old age. Hence, the most significant age related change is menopause (Karen, 2000) and (Rod et al., 2005). However, erectile dysfunction may be caused by diabetes, atherosclerosis, hormonal imbalances, neurological problems etc. (Organic causes) or stress, depression etc.

Because treating the underlying causes (Organic or Psychological), the first line treatment of ED consists of a trial of PDES inhibitor (the first of which was Sildenafil or Viagra). In some cases, treatment can involve prostag-Landin tablets in the Urethra, intravenous injection with a fine needle into the penis or clitoris that causes swelling of Penis or Clitoris Pump or Vascular surgery, estrogen replacement therapy for the women etc.

Although there are various methods and techniques that are used to treat this very condition, however, for the purpose of this project, the treatment is restricted to *Yohimbe*, an extract from *Pausinystalia yohimbe.*

1.2 PREVALENCE OF ERECTILE DYSFUNCTION IN MEN

Erectile dysfunction, ED, varies in severity; some men have a total inability to achieve an erection, others have inconsistent ability to achieve an erection, and still others can sustain only brief erection. The variation in severity of erectile dysfunction makes estimating its frequency difficult.

Many men also are reluctant to discuss erectile dysfunction with their doctors, and thus, the condition is under-diagnosed. Nevertheless, experts have estimated that ED affects 30 million men in the United States. Again, according to the statistical research carried out by Adegunloye (2002) and Eze (1994) respectively in Nigeria, results shows that about 23-26.4% of men suffer from this condition while according to Spector and Carey (1999) discovered that about 4-9% of men suffer from the condition in the United States.

While erectile dysfunction can occur at any age, it is uncommon among young men and more common in the elderly. By the age of 45, most men have experienced erectile dysfunction at least some of the time. According to the Massachusetts Male Aging Study, complete impotence increases from 5% among Men 40 years of age to 15% among Men 70 years and older. Population studies conducted in the Netherlands found out that some degree of ED occurred in 20% of Men between 50 - 54 and in 50% of men between ages 70 - 78. In 1998, the National Ambulatory Medical care Survey counted 1,520,000 Doctor Offices visited for ED.

1.3 OBJECTIVE STUDY AND AIMS

This project focuses to give a clear picture of the effect on erectile tissues of the Penis, Clitoris of both Men and Women.

1.4 NITRIC OXIDE-CYCLIC GMP PATHWAY WITH SOME EMPHASIS ON CAVERNOSAL CONTRACTILITY

Nitric Oxide (NO) is formed from the conversion of L- arginine by nitric oxide synthase (NOS), endothelial (eNOS), and inducible (iNOS). nNOS is expressed in penile neurons innervating the corpus Cavernosum, and eNOS protein expression has been identified primarily in both Cavernosal Smooth Muscle and endothelium. NO is released from nerve endings and endothelial cells and stimulates the activity of soluble guanylate cyclase (sGC), leading to an increase in cyclic guanosine- 3',5',- Monophosphate (cGMP) and, finally, to Calcuim depletion from the cytosolic space and Cavernous Smooth muscle relaxation. The effect of cGMP are mediated by cGMP dependent Protein Kinase, cGMP-gated ion channels, and cGMP-regulated Phosphodiesterases (PDE). Thus, cGMP effect depends on the expression of a Cell-Specific cGMP-receptor protein in a given cell type. Numerous systemic vasculature diseases that cause erectile dysfunction (ED) are highly associated with endothelial dysfunction, which has been shown to contribute to decrease erectile function in men and a number of animal models of penile erection. Based on the increasing knowledge of intracellular signal propagation in cavernous smooth muscle tone regulation, selective PDE inhibitors have recently been introduced in the treatment of ED. Phosphodiesterase-5 (PDE5) inactivates cGMP, which terminates NO-cGMP-mediated SMooth Muscle relaxation. Inhibition of PDE5 is expected to enhance penile erection by preventing cGMP

degradation. Development of pharmacologic agents with this effect has closely paralleled the emerging science.
(International Journal of impotence Research (2004)). Nitric oxide (NO) was first described by Stuehr and Marletta (1985) as a product of activated murine machrophages. Also, the substance known as endothelium- derived relaxing factor (EDRF), described by Furchgott and Zawadzki (1980), has been identified as NO.

Soluble guanylate cyclase (sGC), responsible for the enzymatic conversion of guanosine -5- triphosphate (GTP) to cyclic guanosine -3'5'- monophosphate (cGMP), was first identified as a constituent of mammalian cells almost three decades ago. No and cGMP together comprise an especially wide-ranging signals transduction system when one considers the many roles of cGMP in physiological regulation, including smooth muscle relaxation, visual transduction, intestinal ion transport, and platelet function.

Erectile dysfunction (ED) is defined as the constituent inability to achieve or maintain an erection sufficient for satisfactory sexual performance and is considered to be a natural process of ageing. Studies have shown that ED is caused by inadequate relaxing of the corpus cavernosum with defeat in NO production.

It is clear that NO is the predominant neurotransmitter responsible for cavernasal Smooth muscle relaxation and hence penile erection. Its action is medicated through the generation of the second messenger cGMP. Neutrally, derived NO has been established as a mediator of smooth muscle relaxation in the penis and it is thought that constitutive forms of nitric oxide synthase (NOS) work to mediate the convesion of GTP to the intracellular second messenger cGMP in smooth muscle cells. An increase in cGMP modulates cellular events, such as relaxation of smooth muscle cells.

This review will describe current knowledge of cellular events involved in cavernosal relaxation and the range of putative factors involved in NO-mediated relaxation.

1.5 SYNTHESIS OF Nitric Oxide (NO).

Recent observation suggest that the main site of NO biosythesis in human corpus cavernosum is within the terminal branches of cavernosal nerves supplying the erectile tissue. It is strongly suggested that NO released from nonadrenergic – noncholinergic (NANC) neurons increases the production of cGMP, which in turn relaxes the cavernous smooth muscle. Endothelial –derived NO plays a major role in the penis. Some suggest that NO is highly labile, therefore it cannot be stored as a preformed neurotransmitter. Other proerectile mediators, such as acetylcholine, calci-tonin gene related peptide (CGRP) or substance P, act via endothelialcells by prompting the synthesis and release of NO by these cells, (Bivalacqua et al., 2001). Found in their study that in vivo adenoviral gene transfer of CGRP in combination with adrenomedullin (ADM) or prostaglandin E1(PGEI) induce penile erection by activating different receptors.

The combination of molecular oxygen and the amino acid arginine in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and NO synthase, (NOS) yields citruline nitrogen of L- arginine. L- citrulline can be converted by arginine synthase (AS) to form L-arginine, the precursor for NO. Each of these enzymes, co-factors, or transport systems could be an eventual target of pharmacologic intervention in the NO cascade.

Oral administration of L-arginine in high doses seems to cause significant subjective improvement in sexual function in men with Organic

ED only if they have decreased production of plasma and urine nitrite and nitrates, which are stable metabolites of NO. There are at least three isoform of NOS (neuronal, endothelial, and macrophage). A constitutive form of NOS is found in endothelial and neurons, and is calcium dependent. The constitutive NOS-3, whereas the constitute NOS found in neutral and epithelial tissue has been named NOS-1. An inducible form of NOS, now designated iNOS, is calcium independent. It is induced within 4-24h of the appropriate stimulus and can produce NO in a 100-fold greater amount than can constitutive NOS.

Neutral NOS has multiple regulator sites, including binding sites for nicotinamide adenine dinucleotide phosphate (NADPH), Flavin adenine dinucleotide (FAD), and flavin Monoucleotide (FMN). All of these are (O factors for the synthesis of NO. these cofactors bind to a reductase domain to process election transfer. This is then linked to heme and tetrahydrobiopterin (BH_4) - containing catalytic oxygenenase domain by calcium-calmodulin complex (figure 2).

The complete enzyme converts L-arginine to L- citrulline and NO in the presence of molecular Oxygen. In addition to the various protein modules or domains of neuronal NOS, which are involved in electron transfer, substrate binding, oxygen activation and calcium binding, a four amino –acid motif (glycine- Leucine-glycine- Phenylalanine, GLGF) has been identified in amino terminal region of NOS-1. Although the function of this amino-acid motif in NOS-2 has not been established, a study on other proteins containing this motif indicates that it may serve to target proteins to specific sites in the cell. nNOS has a recognition site for calmodulin that is also present in eNOS and macrophages NOS. The constitutive isoforms are generally regulated by Ca^{2+} -calmodulin, whereas inducible forms are not.

nNOS in the penis is expressed primarily as a variant of the brain form of nNOS and has been termed PnNOS. It has an additional 102-bp alternative exon located between exons 16 and 17. The function of this additional coding region is unknown. PnNOS is thought to be responsible for trigging the nitregic mechanism responsible for cavernosal relaxation. A similar variant, nNO-SU is present in the neuromuscular plates of skeletal muscles, including the perineal muscles involved in erectile rigidity and ejaculation in rats. The control of NO synthesis in the Cavernosal nerve, whether due to sexual stimulation emanating.

Centrally, from the brain, or peripherally by means of the dorsal nerve spinal reflex is assumed to be exerted through the activation of PnNOS activity. This mechanism occurs mainly by Ca2+ binding to calmodulin by means of Ca^{2+} flux through the N-methyl-D-aspartate receptor (NMDAR). Both the NMDAR and inhibitors of nNOS activity, such as protein inhibitors of nNOS activity, such as protein inhibitors of NOS(PIN) and carboxy terminal POZ Ligand of nNOS (CAPON), also bind to nNOS .

The nitrognic activation of penile erection is not restricted to peripheral nerves of the corpora cavernosa but is also dependent on central nervous system (CNS) regulated.

It was found that PnNOS, the brain type nNOS, and PIN were expressed in the hypothalamus in contrast, NMDAR1-T was expressed only in the penis, whereas the brain –type- NMDARI was present in the brain and sacral spinal cord and not in the Penis. PnNOS was found in the media preoptic area, posterior magnocellular, and the Parvocellular regions of paraventriccular nucleus, Supraoptic nucleus, septohypothalamic nucleus, medial septum, Cortex, and in some of the nNOS staining neurone through the brain. It was absent in organum vasculosum of the lamina terminalis.

PIN staining was present in neurons of the medial septum and cortex, but not in the supraoptic nucleus septohypothalamic nucleus or organum vasculosym of the Laminal terminals.

Inhibitors of NOS are substrate analogues of L-arginine, such as N-Monomethyl -L- arginine (L- NMMA), nitro-L- arginine methyl ester (L-NAME). and N-amino –L- arginine.

Drugs that inhibit the dephosphorylation of eNOS might alleviate ED. eNOS abnormalities may play a role in diabetic ED. Hyperglycemia decreases NO production by eNOS via O-Linked glycossylation of eNOS at the targets S1177 in hyperglycemic cell culture conditions and in animal models of diabetes. ED in diabetes is associated with peripheral nerve damage but may involve diminished endothelial-production of NO as well. Numerous systemic vasculature, diseases (hypertension, atherosclerosis, hyperoholesterolemia, diabetes mellitus, etc) that cause ED are highly associated with endothelial dysfunction, which has been shown to contribute to decreased erectile function in men and a number of animal models of penile erection.

The activity of nNOS is controlled by a number of mechanisms. A balance of various inhibitory and stimulatory transcription factors determines gene transcription of the enzyme. Enzyme activity can be halted by phosphorylation by a cyclic adenosine Monophosphate (cAMP) – dependent protein kinase (PKA) or cGMP- dependent protein kinase (PKG), providing a negative feed back loop. The enzyme is activated by increased intracellular calcium, which binds to calmodulin to form the essential cofactor.

It is also likely that co- transmitters influence nNOS activity perhaps by altering calcium concentration by activation of prejunctional receptors. VIP

is a probable stimulatory co-transmitter, while noradrenaline acting on x-2 adrenoceptors inhibits NO formation.

1.6 INACTIVATION

NO is inactivated by heme and the free radical, superoxide .thus scavengers of superoxide anion such as superoxide dismutase (SOD) may protect NO, enhancing its potency and prolong its duration of action. Conversely, interaction of NO with super oxide may generate the potent tissue damaging moiety, peroxynitrite (ON00-1), which has a high affinity for sulfhydryl groups and thus inactives several key sulphydryl-bearing-enzymes. This effect of perotynitrite is regulated by the cellular content of glutathione.

Khan et al., (2001) found that NO- and electrical field stimulated (EFS) - mediated cavernosal smooth muscle relaxation is impaired in a rabbit of diabetes but SOD significantly reversed the impaired relaxation. Manipulation of physiological NO concentration is unlikely to give physiological benefits in ED, since higher levels will predispose to toxic effects NO availability may be increased by the use of the enzyme superoxide dismutase (SOD), which causes decreased levels of superoxide anion.

1.7 **The NO receptor: Soluble guanylate cyclase**

Soluble GC is a heme- containing protein found in the cytosolic fraction of virualy all mammalian cells. With the highest concentrations found in the lungs and brain. Several isoforms of sGC have been Cloned and characterized. Originally sGC was purified (to apparent homogeiniety) from bovine and rat lung and shown to exist as a heterodimer, consisting of 82

Koa (rat) or 73Koa (bovine) and 70Koasubunits, termed x, and β1respectively. Further subunits termed x1 and β2 have also been identified from the human foetal brain (82Koa) and rat kidney (76Koa), respectively, GUCIA2; the gene coding for the x2-Subunit,has been localized to position q21-q22 on the human chromosome 11.

Soluble GC is a heterodimer with at least three functional domains for each subunit (figure 3). These domains are a heme binding domain, dimerization domain, and catalytic domain. The N- terminal portion of each subunit constitutes a heme-binding domainand represents the least conserved region of the protein; it is the heme moiety that confers the NO-sensitivity of the enzyme. Heme- reconstituted more NO sensitive than an equivalent protein containing 1 mole heme per dimmer.

Oxidation of the heme group to a ferric state results in less of the enzyme activity; thus reducing agents such as thiols or ascorbate enhances enzyme activation and thereby facilitating the reaction between NO and (ferrous) heme. On the other hand, oxidizing agents such as Methylene blue inhibit enzyme activation (thiols may also facilitate enzyme activation by forming S- nitrosothiols with NO released from nitrovasodilator drugs).

The heme moiety is bound to the enzyme protein via an –imidazole, axial Ligand shown by point mutation to be provided by his 105 in the B1-Subunit. At the C-terminus of each subunit is a catalytic domain that exhibits a high degree of homology, both between sGC monomers and the C-terminal regions of particulate GC and AC (udenylate cyclase). Interveining between the heme binding and catalytic regions is a dimerization domain that is thought to mediate the subunit association to form heterodimers, which is obligatory for catalytic activity.

Binding of NO to the heme-iron of sGC results in the formation of a pentacordinate nitrosyl- heme complex, which breaks the breaks the bond to the bond to the axial histidine and activate the enzyme.

In addition to iron, sGC possesses a second metal ion, copper which is also thought to function as a cofactor for enzyme activity. Free copper ions inhibit purified sGC activity by reducing Vmax, although the potency of NO-stimulation is unaffected. Activation of sGC can be achieved satisfactory with NO donors, such as glycerol trinitrate nitroprusside, or S-nitrosothiols. Agents like methylene blue and LY83583 (6-anilinoginoline – 5,8-quinoline) can be utilized for inhibition of the enzyme. Both compounds have been shown to release superoxide in aqueos solution and a significant component of their activity may therefore be via inactivation of NO.

Due to the ubiquitous nature of the NO-sGC-cGMP pathway, signal transduction by sGC also has profound pathophysiological significance for example septic shock and migraine may be due to overactivity of the pathway and impotence, hypertention, and asthma as a result of underactivity.

1.8 Intracellular cyclic GMP receptor proteins

Cyclic GMP interacts with three types of intracellular receptor proteins: cGMP-dependent protein kinases (PKGs), cGMP-regulated ion channels and cGMP-regulated cyclic nucleotide phosphodiesterases (PDEs).

This means that cGMP alter cell function through mechanism not directly related to protein phosphorylation.

Two general classes of cGMP kinases exist in vertebrate cells: a type 1 and a type 11 form. The type 1 cGMP kinase is more abundant and widely distributed and has been isolated from vascular and other tissues while the ype 11 form has been detected in vertebrate intestinal epithelial cells.

Cyclic GMP kinase are found in a number of different cells but are most abundant in three cell types in vertebrates smooth muscle, platelet and cerebellum. The calcium-sensitizing Rho-A/Rho-kinase pathway may play a synergistic role in cavernosal vasoconstriction to maintain penile flaccidity. Rho-kinase is known to inhibit MLCP and to directly phosphorylate myosin light-chain (in solution), altogether resulting in a net increase in activated myosin and the promotion of cellular contraction. (Chitaley et al., 2001) found that Rho-kinase antagonism stimulates rat penile erection independently of NO (Mills et al., 2002) in their study support the hypothesis that NO inhibits Rho-kinase-induced cavernosal vasoconstriction during erection. These initial findings introduce a novel potential therapeutic approach for the treatment of ED.

The mechanisms by which cGMP kinase act are still not understood. Findings from several Laboratories have indicated that one effect of cGMP kinase is stimulation of a Ca^{2+}- pumping ATPase, an action that would be predicted to lower $[Ca^{2+}]$ in smooth muscle cells activated with contractile agonists or by depolarization. The generation of PKGs by cGMP leads to a number of events that decrease $[Ca^{2+}]$. It has been shown to phosphorylate and therefore inhibit the inositol 1,4,5- triphosphate [IPs] receptor on the sarcopasmic reticulum, thus preventing calcium release from the store. In addition, PKG increases activity of plasma and sarcolemmal (mediated via the regulatory protein, phospholamban) cation-atpase pumps encouraging sequestration of calcium into stores and out of the cell.

nNOS and eNOS are activated by calcium entry into the cell, binding to calmodium associated with the enzymes. Whereas physiologic penile erection lasts several minutes, the calcium dependent activation of nNOS or eNOS is quite transient. Recently, several groups showed that the phosphotidylinositol 3-kinase (P13- kinase) pathway that activates the serine/threonine protein kinase (also known as PKB) causes direct phosporylation of eNOS, reducing the enzyme's calcium requirement and causing increased production of NO. This pathway is responsible for both shear stress and growth –factor enhancement of blood flow that can last for hours. Finding of Hurt et al., support a model in which rapid brief activation of neuronal NOS initiates of Ca^{2+}-ATPase by the stimulation of phosphatidylinositol -4-phosphate (PIP) formation by cGMP kinase and phosphorylation of 240-KDA protein that mediates the activation of Ca^{2+} -ATPase by cGMP kinase. PKG may catalyze the phosphorylation of phisphatidylinositol kinase. Leading to the formation of PIP and the activation of Ca^{2+} -ATPase by the Lipid. The role of the 240-KDa protein is unknown. It is possible that this protein is a component of the cytoskeleton that is involved in the recruitment of additional Ca^{2+} -ATPase molecules from internal stores to the plasma membrane.

Regulation of phosphodiesterase (PDE) activity is an important component of control of cGMP concentration and hence activity of the NO-cGMP pathway. Mammalian PDEs comprise 11 identified families (PDE2-PDE11) and their isoforms,which are distinguished by their substrate specificities and tssue concentration.

To date, five of these 11 isoenzymes (PDE1,2,3,4, and 5) have been proven to be of pharmacological relevance. Currently, the

presence of mRNAs specific for 14 different human phosphodiesterase isoforms in humans cavernous tissue was shown by means of RT-PCR and Nothan blot analysis. The expression of the following genes were detected in human cavernous tissue: PDE1A, PDE1B, PDE2A, and PDE10A, which hydrolyze both cAMP and cGMP; the cAMP specific PDES, PDE3A, PDE4A-D, PDE7A, and PDE8A, and the cGMP-specific PDEs and PDE5A and PDE9A. The molecular identification of PDE isoenzymes was paralled by efforts to detect and characterize the hydrolyzing activities of PDE proteins expressed in human penile erectile tissue. Based on the result s of organ bat studies on the effects of various PDE inhibitors (papaverine, guazinone, mitrinone, rolipram, and zaprinast) in the adrenergic tension of isolated human corpous cavernosum, street and co-workers concluded that cavernous smooth muscle tone is mainly regulated by cAMP and that cGMP –inhibited PDEsis of major importance in the control of cAMP turnover, while others postulated that cGMP-specific PDEs is the predominant- isoenzyme in the degradation of cyclic nucleotide Monophosphate (cNMP in the corpus cavernosum. Nevertheless, both conclusions are supported by the efficacy of intracavernous milrinone and orally administered sildenafil to induce penile erection sufficient for sexual intercourse. Accordingly, drugs that inhibit PDEs can enhance and prolong the smooth muscle relaxant effects of the NO-cGMP cascade in the corpus cavernosum, thereby potentiating penile erection. The prototype of this now therapeutic class of PDEs inhibitors is sildenafil, which was approved for treatment of ED in 1998. Tadalafil and vardenafil are new agents in this class.

Silidenafil is more selective for PDEs than for other PDEs: >80-fold more than for PDE1:> 1000- fold more than for PDE2 to PDE4i and about 10-fold more than for PDE6, an enzyme found in the retina. The lower selectivity of sildenafil for PDE5 over photoreceptor PDE6 may account for the color visual disturbances observed with increasing frequency with larger doses or higher plasma levels of sildenafil. In vitro studies with tadalafil have demonstrated a 710000-fold greater selectivity for PDE5 versus PDE1 to PDE4 and PDE7 to PDE10, as well as approximately 700-fold greater selectivity for PDE5 than for PDE6. Vardenafil is also selective for PDE5 in vitro and more selective for PDE5 than for PDE1 to PDE4.

It appears that no single mechanism explains all the effects of cGMP on relaxation in the variety of systems examined. The advantage for intracellular signaling is that elevation in cGMP and activation of PKG promote rapid and efficient phosphorylation of substrates in response to signals such as NO.

CHAPTER TWO

LITERATURE REVIEW ON ERECTILE DYSFUNCTION

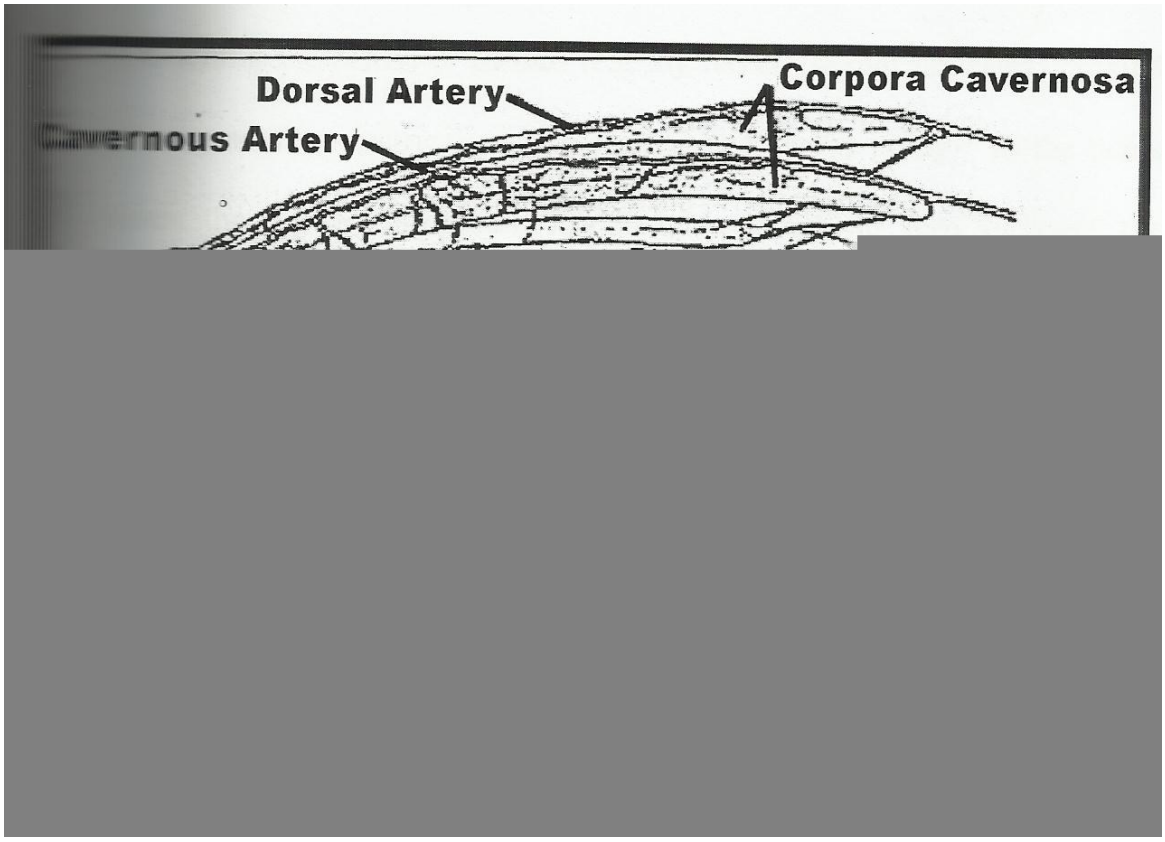


Figure 2: **Penis Anatomy**

2.1 Normal Penis Anatomy

The penis contains two chambers called the corpora cavernosa, which run the length of the upper side of the penis and another chamber called the corpus spongiosum, forms the ventral portion of the penis over the distal end of the penis. The spongy urethra passes through the corpus spongiosum penetrates the glana penis and opens as the external urethra orifice. The urethra is the channel for urine and ejaculate. Filling the corpora cavernosa is a spongy tissue, spaces, veins and arteries. A membrane called the tunica albuginea surrounds the corpora cavernosa. Veins located in the tunica albuginea drain blood out of the penis.

2.2 How Erection Occurs In Men

Erection begins with sexual stimulation. Sexual stimulation can be tactile (e.g. by sexual fantasies). Sexual stimulation generates electrical impulses along the nerves going to the penis and causes the nerves to release nitric oxide which in turn increases the production of cyclic GMP (cGMP) in the smooth muscle cells of the corpora cavernosa. The cGMP causes the smooth muscles of the corpora carvernosa to relax and allow rapid blood flow into the penis. The in-coming blood fills the corpora cavernosa, making the penis expand.

2.3 How Erection Is Sustained

The pressure from the expanding penis compresses the veins (blood vessel that drain the blood out of the penis) in the tunica albuginea, helping to trap the blood in the corpora cavernosa, thereby sustaining erection. Erection is reversed when cGMP levels in the corpora cavernosa to contract, stopping the inflow of blood and opening veins that drain blood away from the penis. The levels of the cGMP in the corpora cavernosa fall because it is destroyed by an enzyme called phosphodiesterase type 5 (PDE5) (NKUD1C, 2003).

2.4 Causes of ED in Males

The ability to achieve and sustain erection generally requires:

1: A healthy nervous system that conducts nerve impulses in the brain, spinal column, penis.

2: Healthy arteries in and near the corpora cavernosa

3: Healthy smooth Muscles and Fibrous tissues within the corpora cavernosa, and

4: Adequate levels of nitric oxide in the penis and clitoris (American Psychiatric Association, 1994) and (NKUDIC, 2003).

Note that the cause of ED in males could be

(a) Physical or

(b) Psychogenic

2.5 Physical Causes Of ED in Males:

- **Diabetes Mellitus:**

According to the Canadian Diabetes Association (CDA) erectile dysfunction, ED, is common for men who have diabetes. Often, it's the first symptom that men may notice and the one that may lead them to the doctor in the first place. Only after they have sought medical help for ED do they also receive a diagnosis of diabetes.

Erectile dysfunction tends to develop 10-15 years earlier in diabetic Men than among non-diabetic Men. In a population study of Men with type 1 diabetes for more than 10 years, ED was reported by 55% of Men between the ages of 50 to 60 years. The increased risk of ED among Men with diabetes Mellitus may be due to the earlier on set and greater severity of atherosclerosis that narrows the arteries and thereby reduces the delivery of blood to the penis. When insufficient blood is delivered to the penis, it is not possible to achieve an erection.

Diabetes Mellitus also causes erectile dysfunction by damaging both Sensory and autonomic nerves, a condition called diabetic neuropathy. Smoking cigarette, obesity, poor control of blood glucose levels and having diabetes mellitus for a long time further increases the risk of ED in diabetes,

in addition to atherosclerosis and /or neuropathy causing ED in which the compliance of the muscle in the corpora cavernsa is decreased and clinically this present as inability to maintain the erection (Canadian Diabetes Association, 2006).

- **Hormonal Imbalances**

Imbalances of hormones, such as thyroid hormones, prolactin, and testosterone can affect a man's response to sexual stimulation. These imbalances can be the result of a tumor of the pituitary gland, kidney disease, liver disease or hormonal treatment of prostate cancer (John, 2007)

- **Cardiovascular Disease**

The most common cause of cardiovascular disease in the United States is atherosclerosis, the narrowing and hardening of arteries that reduces blood flow. Atherosclerosis typically affects arteries throughout the body and is aggrevated by hypertension, high blood Cholesterol level, cigarette smoking, and diabetes mellitus. When coronary arteries (arteries that supply blood to the heart, muscles) are narrowed by atherosclerosis, heart attacks and angina occur. When cerebral arteries (arteries that supply blood to the heart) are narrowed by atherosis, stroke occurs (NKUDIC 2003) in this vein, when arteries that supply blood to the penis and pelvic organs are narrowed by atherosclerosis, insufficient blood is delivered to the penis to achieve an erection. There is a close correlation between the severity of atherosclerosis in the coronary artery and erectile dysfunction than men with mild or no coronary artery atherosclerosis. Some doctors suggest that men with new onset erectile dysfunction should be evaluated for silent coronary artery disease (advanced coronary artery atherosclerosis that has not yet caused angina or heart (NKUDIC,2003).

- **Tobacco, Alcohol or drug use:**

All of these substances can damage a person's blood vessels and/or restrict blood flow to the penis; causing ED in people with arteriosclerosis. Marijuana, heroin, corcaine and alcohol abuse contribute to erectile dysfunction. Alcoholism, in addition to causing nerve damage can lead to atrophy of the testicles and lower testosterone levels (John, 2007).

- **Nerve or Spinal Cord Damage**

Damage to the spinal cord and nerves in the pelvis can cause erectile dysfunction. Nerve damage can be due to disease, trauma, or surgical procedures e.g. injury to the pelvic nerves from prostrate surgery, multiple sclerosis, Alzheimer's disease, parkinson's disease, peyronies disease and long term diabetes mellitus (health wise, 2006).

- **Hypertension (High Blood Pressure)**

Patients with essential hypertension or arteriosclerosis have increased risk of developing erectile dysfunction. Essential hypertension is the most form of hypertension: it is called essential hypertension because it is caused by another disease, for instance, by kidney disease (Kendric et al., 2005). It is not clearly known how essential hypertension has been found to have low production of nitric oxide by the arteries of the body, including the arteries in the penis. Scientists now suspect that the decreased levels of nitric oxide in patients with essential hypertension may contribute to erectile dysfunction (Kenedric et al., 2005).

- **Medications**

Many common medicines produce ED as a side effect. Medicines that can cause erectile dysfunction include many used to treat high blood

pressure, anti-histamines, antidepressants, tranquilizer and appetite suppressants. Examples of common medicine that can cause ED include beta blockers such as propanol, hydrochlorothiazide, digoxin, indomethacin etc (NKUDIC 2003).

- **Venous Leak:**

If the veins in the penis cannot prevent blood from leaving the penis during an erection, an erection cannot be maintained. This is known as Venous Leak and can be a result of injury or disease (Kendric et al., 2005).

- **Surgery:**

Surgery performed to treat disease such as prostrate cancer and bladder cancer often require the removal of nerves and tissues around the affected area which can lead to ED. Some of these surgeries result in only temporary problems (Lasting 6-18 months) which in others result is a permanent damage to the nerves and tissues around the penis and require treatment in order for an erection to be achieved (James, 2010).

- **Prostrate Cancer:**

Prostrate cancer does not cause ED on its own but treatment (radiation, hormonal manipulation or surgery to remove the cancer) can lead to erectile problems (John, 2007).

- **Ageing:**

There are two reasons why older men are more likely to experience erectile dysfunction than younger men. Firstly, older men are more likely to develop disease (such as heart attacks, angina, strokes, diabetes mellitus and high blood pressure that are associated with ED. Secondly the aging process alone can cause erectile dysfunction in some men; primarily by decreasing the compliance of the tissues in the corpora cavernosa, although it has been suggested, but not proven that there is also decreased production of nitric

oxide in the nerves that innervate the corporal smooth muscle within the penis (NKUDIC, 2003).

- **Sexually Transmitted Disease:**

Sexually transmitted disease which is also known as veneral disease play also a role in erectile ED. STD's such as gonorrhea, syphilis, staphylovoccus Aureus, genital herpes etc(Hashmi, 1998).

2.6 Psychological Causes Of Erectile Dysfunction in Males

Psychological factors are responsible for about 10%-20% of all causes of ED. It is often a secondary reaction to an underlying physical cause. In some cause, the psychological effects of ED may stem from childhood abuse or sexual trauma. However, the most common psychological causes of ED includes:

Stress: this can be job-related, Money-related, or the result of marital problems will happen again. This can lead to 'performance anxiety' or a fear of sexual failure and consistently cause ED.

Guilt: a man may feel guilty that he is not satisfying his partner.

Depression: a common cause of ED, depression affects a person physically and psychologically: Depression can cause ED even when a man is completely comfortable in sexual situation. Drugs used to treat depression may also cause.

Low Self-Esteem: this can be due to prior episodes of ED (thus a feeling of inadequacy) or can be the result of other issues unrelated to sexual performance.

Indifference: this may also come as a result of age and a subsequent loss of interest in sex, be the result of medications or stem from problems in a couples relationship. All men at one time or another will experience ED.

Only if the problem becomes persistent, occurs more than 50% of the time, or becomes a source of distress for you or your partner should you be concerned and consider seeking medical advice and treatment. For men whose erectile dysfunction is caused by psychological problems, therapy may be needed (Traish et al., 1999).

2.7 Diagnosis Of Erectile Dysfunction

A diagnosis of erectile dysfunction is made in men, who have repeated inability to achieve and/or maintain an erection for satisfactory sexual performance for at least 3months. Candid communication between the patient and the doctor is important in establishing the diagnosis of ED, assessing its severity, and determining the cause. During patients interviews, the doctor may ask any of the following questions:

(a) Is the patient suffering from ED or from loss of Libido or disorder of ejaculation?

(b) Is ED due to psychological or physical factors? Healthy Men have involuntry erections in the early morning and during REM stage (a stage in the sleep cycle with rapid eye movements). Men with psychologenic ED (ED due to psychological factors such as stress and anxiety rather than physical factors) usually maintain these involuntary erections. Men with physical causes of ED (for example atherosclerosis, smoking and diabetes) usually do not have these involuntary erections.

(c) Are there physical causes of ED? A prior history of cigarette smoking, heart attacks, strokes, and poor circulation in the extremities suggests atherosclerosis as the cause of the ED. Diminished sensation of the penis and the testicles, bladder dysfunction and decreased sweating in the lower extremities suggest diabetic nerve damage. Loss of sexual desire and drive,

lack of sexual fantasies, gynecomastia (enlargement of breasts), and diminished facial hair suggest low testosterone levels.

(d) Is the patient taking medications that can contribute to erectile dysfunction?

The physical examination can reveal clues for physical causes of ED. For example, if the penis does respond as expected to touching, a problem in the nervous system may be the cause. Small testicles, lack of facial hair and enlarged breast can point to hormonal problems such as hypogonadism with low testosterone levels. A reduced flow of blood as a result of atherosclerosis can sometimes be diagnosed by finding diminished arterial pulses in the legs or listening with a stethoscope for bruits (the sound of blood flowing through narrowed arteries). Unusual characteristics of the penis itself could suggest the root of the ED, for example, bending of the penis during erection could be the result of peyronnies disease.

Common Laboratory Tests to evaluate ED include:

a. **Complete Blood count (CBC):** this is to check if the individualhas low blood count against any form of anaemia.
b. **Urinalysis:** an abnormal urinalysis may be a sign of diabetes mellitus and kidney damage.
c. **Lipd Profile:** high levels of LDL cholesterol (bad cholesterol) in the blood promote atherosclerosis.
d. **Blood Glucose Levels:** abnormally high blood glucose levels may be a sign of diabetes mellitus.
e. **Serum Creatinine:** an abnormal serum creatinine may be the result of kidney damage due to diabetes.
f. **Total Testosterone Levels:** blood samples for total testosterone levels should be obtained in the early morning (before 8a.m) because

of wide Fluctuations in the testosterone levels throughout the day. A low testosterone levels suggest hypogonadism. Measurement of bio-available measurement testosterone may be a better measurement that total testosterone, especially in abose men and women with liver disease, but measurement of bio-available testosterone is not widely available.

g. PSA Levels: (Prostate Specific antigen) blood cancer is important before starting testosterone treatment, since testosterone can aggravate prostate cancer

Monitoring erections that occurs during sleep (noctural penile tumescence, NPT) can help distinguish between ED of psychological and physical causes. If noctural erections do not occur, and then the cause of ED is likely to be physical rather than psychological, however, tests of noctural erections are not completely reliable. Scientists have not standardized the tests and have not determined in whom they should be done.

2.8 LITERATURE REVIEW ON FEMALE ERECTILE DYSFUNCTION:

Female erectile dysfunction which is a sexual impotence, occurs when a woman is unable to attain and maintain a complete erection of her clitoris through orgasm (MC vary, 2008).

2.9 NORMAL ANATOMY OF THE FEMALE EXTERNAL GENITALIA:

The external female genitalia also referred to as the vulva or the pudendum: consists of the vestibute and its surrounding structures. The vestibule is the space into which the vagina opens posteriorly and urethra

opens anteriorly. A pair of thin, longitudinal skin folds called the labia minora form a border on each side of the vestibule. A small erectile structure called the clitoris is located in the anterior margin of the vestibule. Anteriorly, the Labia minora unite over the clitoris to form a fold of skin called the prepuce (Rod et al., 2008).

The clitoris is usually less than 2cm in length and consists of a shafts and a distal glans. Well supplied with sensory receptors, it initiates and intensifies levels of sexual tension. The clitoris contains two erectile structures, the corpora cavernosa, each of which expands at the base end of the clitoris to form the crus of the clitoris and attaches the clitoris to the coxal bones. The corpora cavernosa of the clitoris are comparable to that of the penis and they become engorged with blood as a result of sexual excitement in most women, this engorgement results in an increase in diameter, but not the length of the clitoris. With increased diameter, the clitoris makes better contacts with the prepuce and the surrounding tissues and is more easily stimulated.

Erectile tissue that corresponds to the corpus spongiosum of the male lies deep into and on the lateral margins of the vestibular floor on each side of the vaginal orifice. Each erectile body is called bulb of the vestibule like other erectile tissues, it becomes engorged with blood and more sensitive during sexual arousal. Expansion of the bulbs causes narrowing of the vaginal orifice and produce better contacts of the vagina with the penis during intercourse (Rod et al., 2008).

On each side of the vestibule, between the vaginal opening and Labia minora, is an opening of the duct greater vestibular glands. Additional small mucous gland, the lesser vestibular glands, paraurethral glands, are located

near the clitoris. They produce a lubricating fluid that helps to maintain the moistness of the vestibule.

Laternal to the Labia minora are two prominent rounded folds of skin called the Labia majora. Subcutaneous fat is primarily responsible for the prominence of the Labia majora. The two Labia majora unite anteriorly in an elevation over the symphysics pubis called the Mon's Pubis. The lateral surfaces of the Labia majora and the surface of the Mons pubis are covered coarse hair. The media surface are covered numerous sebaceous and sweat gland.

The space between the Labia majora is called the pudendal cleft. Most of the Labia majora are in contact with each other across the midline closing the pudendal cleft and concealing the deeper structures within the vestibule (Philip et al., 2008).

2.10 Causes of ED in Females

Note that just like in males the causes of ED in females could also be physical or psychogenic. Other causes may result due to any of the following factors:

- Low Concentration Of Estrogen:

Estrogen plays a significant role in regulating female sexual performance. Estradiol levels affect cell throughout the peripheral and central nervous system and influence nerve transmission. A decline in serum estrogen levels results in the thinning of the vaginal mucosal epithelium and atrophy of vaginal wall smooth muscle. Decreased estrogen levels also results in a less acidic environment in the vaginal canal. This can ultimately lead to vaginal infection, urinary tract infections and incontinence as well as complaints of sexual dysfunction (Shen et al., 1999).

Estrogen also have vaso- protective and vaso-dilatory effects which result in increased vaginal, clitoral and urethra arterial flow resulting in maintainance of the females sexual response by preventing atherosclerotic compromise to pelvic arteries and arterioles (Sherwin et al., 1995).

With estrogen in menopause and decline in circulatory estrogen levels, a majority of women experience some degree of change in sexual function. Common sexual complaints include loss of desire, decreased frequency of sexual activity, painful intercourse, diminished sexual responsiveness, difficult achieving orgasm, and decreased genital sensation. Masters and Johnson first published the findings of the physiologic changes occurring in menopausal women that related to sexual function 1966. We have since learned that symptoms related to alterations in genital sensation and blood flow are, in part, secondary to declining estrogen levels and that there is direct correlation between presence of sexual complaints and levels of Estradiol below 50pg/cm3 (Shen et al., 1999).

- Low Concentration Of Testosterone:

Low testosterone levels are also associated with a decline in sexual arousal, genital sensation, libido and orgasm. This can be accompanied by loss of hair, vaginal mucosal thinning and overall diminished sense of well being (Sarreh, 1998) and (Davis, 2000).

Therapeutic success with testosterone for inhibited desire in naturally menopausal women has been reported using a testosterone pellet (Tarcan et al., 1990).

- Vascular Insufficient Syndrome:

The recently named clitoral and vaginal vascular-insufficient syndromes are directly related to direct diminished genital blood flow secondary to atherosclerosis of the iliohypogastric/pudendal arterial bed (Goldstein et al.,

1998). Although other underlying conditions either physchological or physiological/ organic may also manifest as decreased vaginal and clitorial engorgement, arterial insufficiency is one etiology that should be considered. Diminished pelvic blood flow secondary to aortoiliac or atherosclerotic disease leads to vaginal wall and clitoral smooth muscle fibrosis. This can ultimately result in symptoms of vaginal dryness and dyspareunia. Histomorphometric evaluation of clitoral Cavernosal artery wall thickening, loss of corporal smooth muscle and increase in collagen deposition. In human clitoral tissue, there is a similar loss of corporal smooth muscle with replacement by fibrous connective tissue in association with atherosclerosis of clitoral-cavernosal arteries (Park et al., 1998). While the precise mechanism is unknown, it is possible that the atherosclerotic changes that occur in clitoral vascular and trabecular smooth muscle interfere with normal relaxation and dilation responses to sexual responses. Aside from atherosclerotic disease, alterations in circulatory estrogen levels associated with menopause contribute to age –associated changes in vaginal and clitoral smooth muscles. In addition, any traumatic injury to the iliohypogastric/ pudendal arterial bed from pelvic fracture, blunt trauma, surgical disruption, or chronic perineal pressure from bicycle riding, for instance, can result in diminished vaginal and clitoral blood flow and complaints of sexual dysfunction (Goldstein et al., 1998).

- Neurogenic Health Conditions:

The same neurogenic etiologies that cause erectile dysfunction in men can also cause sexual dysfunction in women.

These includes:

a. Spinal cord injury or disease of the central or peripheral nervous system including diabetes and

b. Complete upper motor neuron injuries affecting sacral spinal segments.

Women with incomplete injuries retain that capacity for psychogenic arousal and vaginal lubrication (Traish et al., 1999). With regard to orgasm, women with spinal cord injury have significantly more difficulty achieving orgasm than normal controls. The effect of specific spinal cord injuries on female sexual response as well as the role of vasoactive pharmacotherapy in this population are being investigated.

- Sexually Transmitted Diseases:

Sexually transmited disease caused by microorganism such as Neisseria gonorrhea, Chlamydia trichomonas, Staphylococcus aureus e t c, can also cause erectile dysfunction (John, (Ed), 2007).

2.11 PSYCHOLOGICAL CAUSES OF ED IN FEMALES

In women, despite the presence or absence of organic disease, emotional and relational issues significantly, affect sexual around. Issues such as self-esteem, body image and the quality of the relationship with her partner can all affect her ability to respond sexually. In addition, depression and other psychological and mood disorders are associated with female sexual dysfunction. Further more, the medications commonly used to treat depressions can significantly affect the female sexual response. The most frequently used medications for uncomplicated depression are serotonin re-uptake inhibitors (SSR1). Women receiving these medications often complain of decreased desire, decreased arousal, decreased genital sensation and difficulty achieving orgasm. Several studies have recently been published documentary improvement of SSR1- induced sexual dysfunction in women with Sildenafil (John, 2007).

2.12 MECHANISM OF ACTION BURANTASHI

The mechanism of action of Sildenafil citrate involves the release of nitric oxide (NO) in the corpus cavernosum of the enzyme guanylate cyclase, which results in increased levels of cyclic guanosine monophosphate (cGMP), leading to smooth muscle relaxation (Vasodilation) of the intimal-cushions of the helicine arteries, resulting in increased in flow of blood and an erection (Webb et al., 1999). Robert F. Furchgott won the Nobel prize in physiology or medicine in 1998 for his discovery and analysis of endothelium- derived relaxing factor, a key part of the NO mechanism of action.

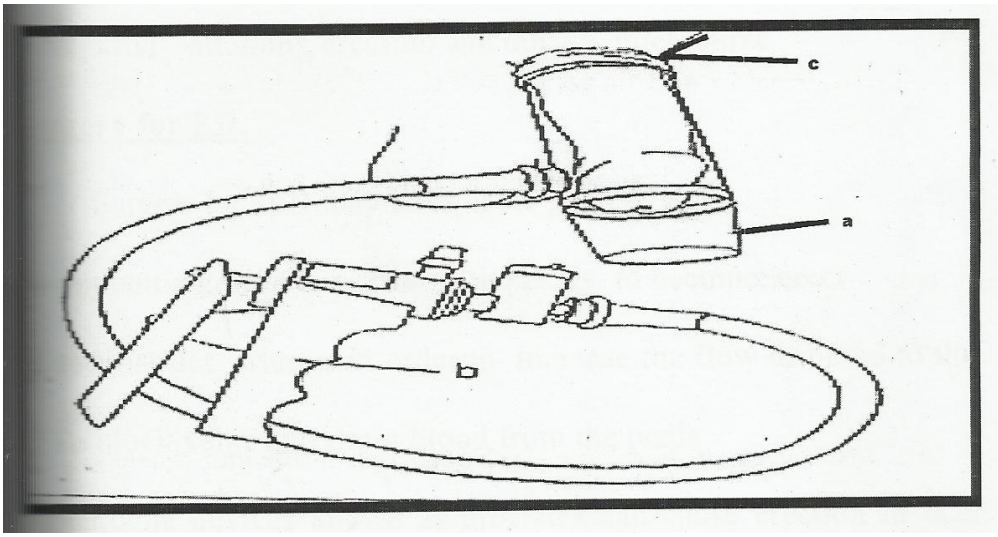
Burantashi is a potent and a selective inhibitor of cGMP specific phosphodiesterase type 5 (PDE5), which is responsible for degradation of cGMP in the corpus cavernosum. The molecular structure of burantashi is similar to that of cGMP and acts as a competitive binding agent of PDE5 in the corpus cavernosum, resulting in more cGMP and better erections (Revill, 2003). Without sexual stimulation, and therefore lack of activation of the NO/cGMP system, burantashi should not cause an erection. Others drugs that operate by the same mechanism include tadalafil citrate (Cialis) and vardenafil hypochloride (Levitra). Although Sildenafil (Viagra), Vardenafil (Levitra), and tadalafil (Cialis) all work by inhibiting PDE5, tadalafils pharmacologic distinction is its longer half-Life (17.5 hours) compared to Viagra (4.0-5.0 hours) and Levitra (4.0-5.0 hours) resulting in longer duration of action and so partly responsible for the weekend pill sobriquet (Revill, 2003).

(1) Vaccum Devices:

What are Vaccum devices?

Mechanical vaccum devices cause erections by creating a vaccum around the penis that draws blood into the penis, engorging it and expanding it. The devices have three components:

Diagram a



a. A plastic cylinder in which the penis is placed,
b. A pump, which draws air out of the cylinder, and
c. An elastic band, which is placed around the base of the penis, to maintain the erection after the cylinder is removed and during intercourse by preventing blood from flowing back into the body.

One variation of the vaccum idevice involves a semi-rigid rubber sheath that is placed on the penis and remains there after attaining erection and during intercourse.

Surgery for ED:

Surgery for ED may have as its goal;

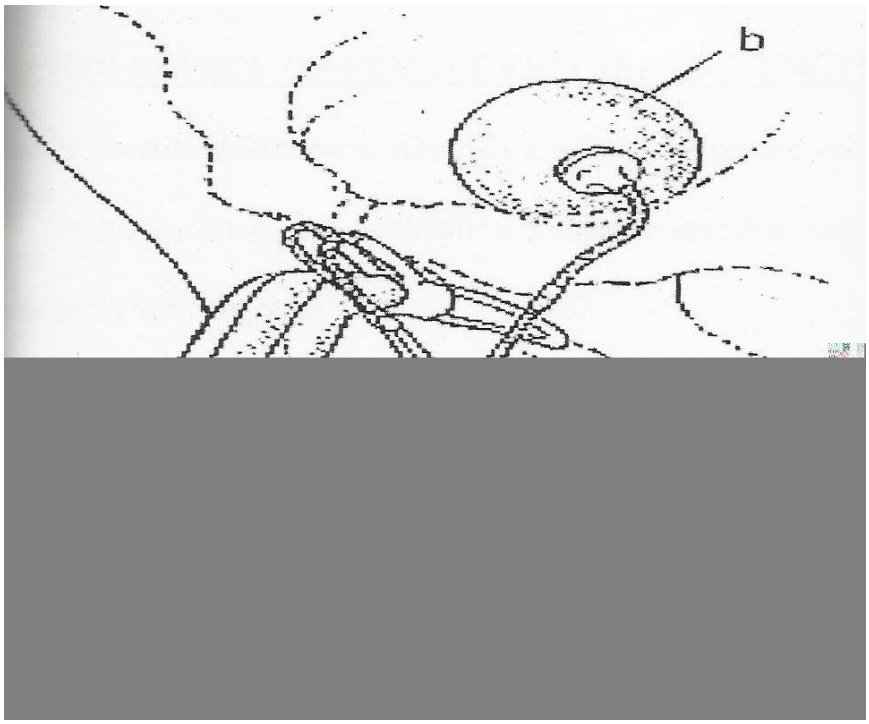
1. To implant a device that causes the penis to become erect.
2. To reconstruct arteries in order to increase the flow of blood to the penis

Or

3. To block veins that drain blood from the penis. Implantable devices known as prosthesis can cause erections in many men with impotence.

Malleable implants usually consists of paired rods, which are inserted surgically into the corpora cavernosa, the twin chambers running the length of the penis. The user manually adjusts the position of the penis and, therefore, the rods. Adjustment does affect the width or length of the penis.

Diagram b



(a) implanted in a scrotum. The pump causes fluid to flow from a reservoir

(b) Residing in the lower pelvis to two cylinders

(c) Residing in the penis. The cylinder expands to create the erection

An inflatable implant consists of paired cylinders, which are surgically inserted inside the penis and can be expanded using pressurized fluid. Tubes connect the cylinders to a fluid reservoir and pump, which also are surgically implanted. The patient inflates the cylinders by pressing on the small pump, located under the skin in the scrotum. Inflatable implants can expand the length and width of the penis somewhat. They also leave the penis in a more natural state when not inflated.

Surgery to repair arteries can reduce impotence caused by obstructions that block the flow of blood to the penis. The best candidates for such surgery are young men with discrete blockage of an artery because of a physical injury to the pubic area or a fracture of the pelvis. The procedure is less successful in older men with widespread blockage of arteries.

1. A tablet called herbal v-x which is a plant-based natural tablet which contains muira puama and Gingko Biloba has also had some success in improving libido. Muira puama is a root and bark extract used to strengthen the nervous system and Gingko Biloba is a herb made from tree leaves which increases the flow of blood to the sex organs (Perry, 2010).

2.13 TAXONOMY

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnolioopsida

Order : Rubiales

Family : Rubiaceae

Genus : Pausinystalia

Species : Yohimbe

Botanical Name : *Pausinystalia yohimbe*

(Oliver – Beyer, 1986)

Common Names:

English : Yohimbe

Yoruba : Idagbon

Itausa : Burantashi

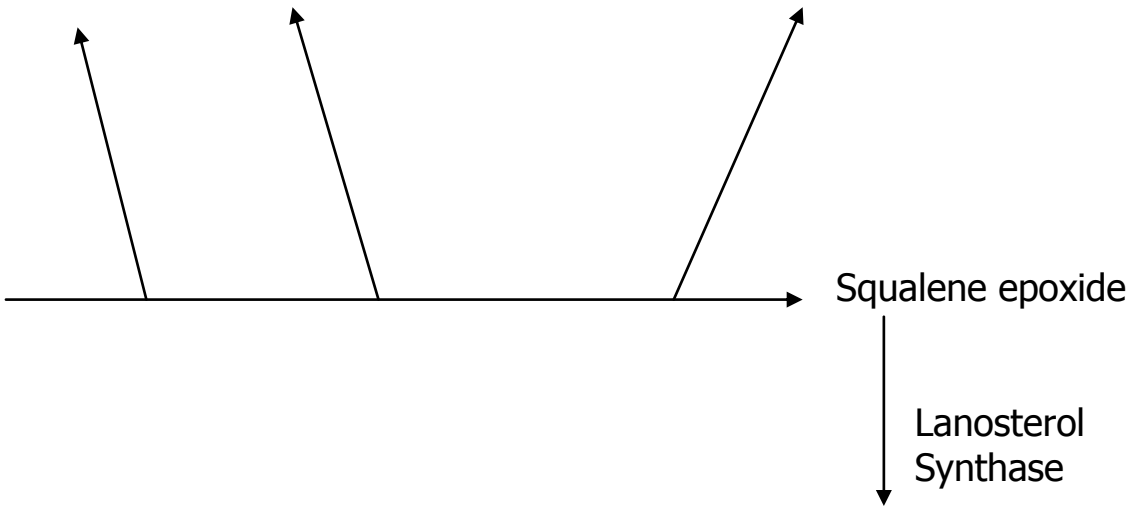
Trade Name : Yohimbe

2.14 CHOLESTEROL

The name cholesterol originates from the Greek "Chole" (bile) and "Stereos" (solid), and the chemical suffix "OL" for an alcohol. Francois poulletier delasalle first indentified cholesterol in solid form in gall stones in 1769. However, it was only in 1815 that chemist Eugene Cheverul named the compound "Cholesterine (Olson, 1998).

Cholesterol is the principal steroid of fat thatis synthesized in the liver or intestines of animals. However, small quantities can be synthesized in eukaryotes such as fungi, and plants. It is almost completely absent among prokaryotes, including bacteria (Pearson, et al., 2003). Cholesterol produces hormones and cell membrane and is transported in the blood plasma of all animals. It is required to establish proper membrane permeability and fluidity. Biochemistry, cholesterol is of significant importance because it is a precursor of a large number of important steroid which includes; bile acids,

adrenocortical hormones, D-vitamins, cardiac glycosides, sex hormones, sitosterols of the plants kingdom, and some alkaloids. It is the best known as steroid because of its association with atherosclerosis and heart diseases. Although, cholesterol is important and necessary for mammal, high levels of it in the blood can clog arteries and are potentially linked to diseases associated with the cardiovascular system. (National Health Service, 2010). Cholesterol is amphiphatic with a polar head group at c-3 (the hydroxyl group) and a non-polar hydrocarbon body (the steoid nucleus and the hydrocarbon side chain at c-17), about as long as 16 carbon fatty acids in its extended form. It has the molecular formular C27 H46O, with a molar mass of 386.65g/mol. Its physical appearance is in the form of white crystalline powder (safety MSDs data for Cholesterol, 2007), it has a density of 1.052g/cm3, melting point of 148-150°C (safety MSDs data for cholesterol, 2007), and boiling point of 360°C. Its solubility in water is 0.095mg11(30°C) and has a standard state at 25°C, 100kpa. Sterols are structural lipids present in the membranes of most eukaryotic cells. The characteristic structure of this fifth group of membrane lipids in the steroid nucleus consisting of four fused rings, three with six carbons, and one with five carbons. The steroid nucleus is almost planar and is relatively rigid. The fused rings do not rotate about c-c bonds (Garrett, et al., 1999).



Formation of Cholesterol from Squalene.

Cholesterol is doubtless the most publicized lipid, notorious because of the strong correction between high levels of cholesterol in the body (hypercholesterolemia), and the incidence of human cardio-vascular disease (Craford, 2003). Cholesterol is very essential in many animals, including humans, but it is not required in the mammalian diet. All cells can synthesize it from simple precursors.

Cholesterol Structure (Voet and Voet, 2004)

A little more than half the cholesterol of the body arises by synthesis (about 700mg11), and the remainder is provided by the average diet. The liver and intestine account for approximately 10% each of total synthesis in humans (Garrett, et al., 1999). Virtually, all tissue containing nucleated cells are capable of cholesterol synthesis which occurs in the endoplasmic reticulum (ER) and the cytosol. The structure of this 27-carbon compound suggests a complex biosynthetic pathway, but all of its carbon atoms are the source of all carbon atoms in cholesterol which involved many biochemical reactions.

2.15 DIETARY SOURCE AND EFFECT OF DIET IN CHOLESTEROL LEVEL

Animal fats are complete mixture of triglycerides, with lesser amounts of phospholipids and cholesterol. As a consequence all foods containing animal fat contain cholesterol to varying extents (Christie, and William, 2003)

Investigations indicate that a diet rich in animal facts tends to raise the level cholesterol and the related fats and lipids in the blood (Encyclopedia, 2006). Major dietary sources of cholesterol include cheese, egg yolks, beef, pork, poultry, and shrimp (USDA National Nutrient Database, 2008). Human breast milk also contains significant qualities of cholesterol (Jensen, et al., 1978). Evidence strongly indicates that people with such high levels are more likely to develop atherosclerosis and heart attacks than those with lower levels. The amount of cholesterol present in plant-based sources (USDA) National Nutrient Database 2008, Behrman, and Gopalan, 2005).

In addition plant product such as flax seed and peanuts contain cholesterol –like compounds called phytosterol, which are suggested to help lower serum cholesterol levels (Ostlund, et al., 2003). Total fat intake, and high intake of saturated fat, trans fat, (American Journal of Clinical Nutrients) and calories in excess of body requirement, pays a larger role in the elevation of the blood cholesterol than intake of cholesterol itself (Crowford, 2003). Saturated fat is present in full fat dairy products, animal fats, and several types of oil and chocolate.

Saturated Polyunsaturated and monounsaturated fats are thought to raise, lower and have no effect on serum cholesterol respectively. Trans fats are typically derived from the partial hydrogenation of unsaturated fats and do not occur in significant amounts in nature. Trans fat is most often encountered in margarine and hydrogenated vegetable fat, and consequently in many fast foods, snack foods and fried or baked goods.

2.16 FUNCTIONS OF CHOLESTEROL IN THE BODY

- Cholesterol is required to build and maintain membrane by modulating membrane fluidity over rangr of physiological temperature.
- Cholesterol reduces the permeability of the plasma membrane to protein and sodium ions.
- Within the cell membrane, cholesterol function in intracellular transport, cell signal and nerve conduction.
- Cholesterol assists in the formation of lipid raft in the plasma membrane
- Cholesterol converts the bile in the liver and store in the gall bladder

It is also important precursor molecule for the synthesis of vitamin D and steroids hormones including adrenal gland, hormone cortis and aldosterone as well as sex hormone – progesterone, estrogens and testosterone and their derivatives. Cholesterol synthesis can be turned off or inhibited when cholesterol level are high. In addition to providing a soluble means for transporting cholesterol through the blood, lipoproteins have cell-targetting signals that direct the lipid they carry to certain tissues for this reason; there are several types of lipoprotein within blood called in order of increasing density. Chylomicrons, VLDL, IDL, LDL, and HDL.

The more cholesterol and less protein a lipoprotein has, the denser it is. The cholesterol within all the various lipoprotein is identical, although some cholesterol is carried as the free alcohol and some is carried as fatty acyl esters referred to as cholesterol esters. However, the different lipoproteins contain apolipoprotein which serves as ligand for specific receptor on cell

membrane (Olson, 1998). Cholesterol provides good number of vital functions in the body, the body makes its own cholesterol in the liver, because it is steroid and it is a necessary component of the cell membrane it is possible for more cholesterol to come from endogenous source than from the diet, the body packages this cholesterol for transport in the blood stream through several classes of lipoproteins like LDL, and VLOL, which serves as transport of lipids in the blood (Campbell and Farrell, 2008).

2.17 LIPOPROTEIN METABOLISM

It transports hydrophobic fats in plasma. The major lipoprotein circulating in the blood are chylomicrons, VLDL, LDLs, and HDLs. IDLs are derived from VLDLs in the formation of LDLs. Fatty acids are important cellular fuels and are stored as triacylglycerols in adipose tissue principally as triacyglycerol in chylomicrons and VLDLs. In adipose tissue, chylomicrons are rapidly degraded, and the remnant particles re-enter the circulation and are taken up by the liver. VLDLs are degraded in adipose tissue to LDLs which then circulates as the major transport lipoprotein for cholesterol. HDLs are lipoprotein that cotinously circulate, the contain enzymes “cholesterol acyltransferase” that converts free cholesterol to cholestery esters.

Cholesterol esters are transferred out of HDL by cholesteryl ester transfer protein (CETP). CETP promotes the transfer of cholesteryl esters to VLDL and LDL in exchange for triacylglycerol. In this way, CETP enables HDL to transport more cholesteryl esters derived from the LCAT reaction.

Cholesterol Cholesterol ester

Phosphotidyl Choline Lysophosphatidyl choline

(Lecithin)

2.18 Reaction Catalyzed by LCAT

Lipoprotein lipase is an extracellular enzyme that hydrolyzes triacylglycerol into 2-monoacylglycerol and two fatty acids. The fatty acids then enter the cell passively down a concentration gradient.

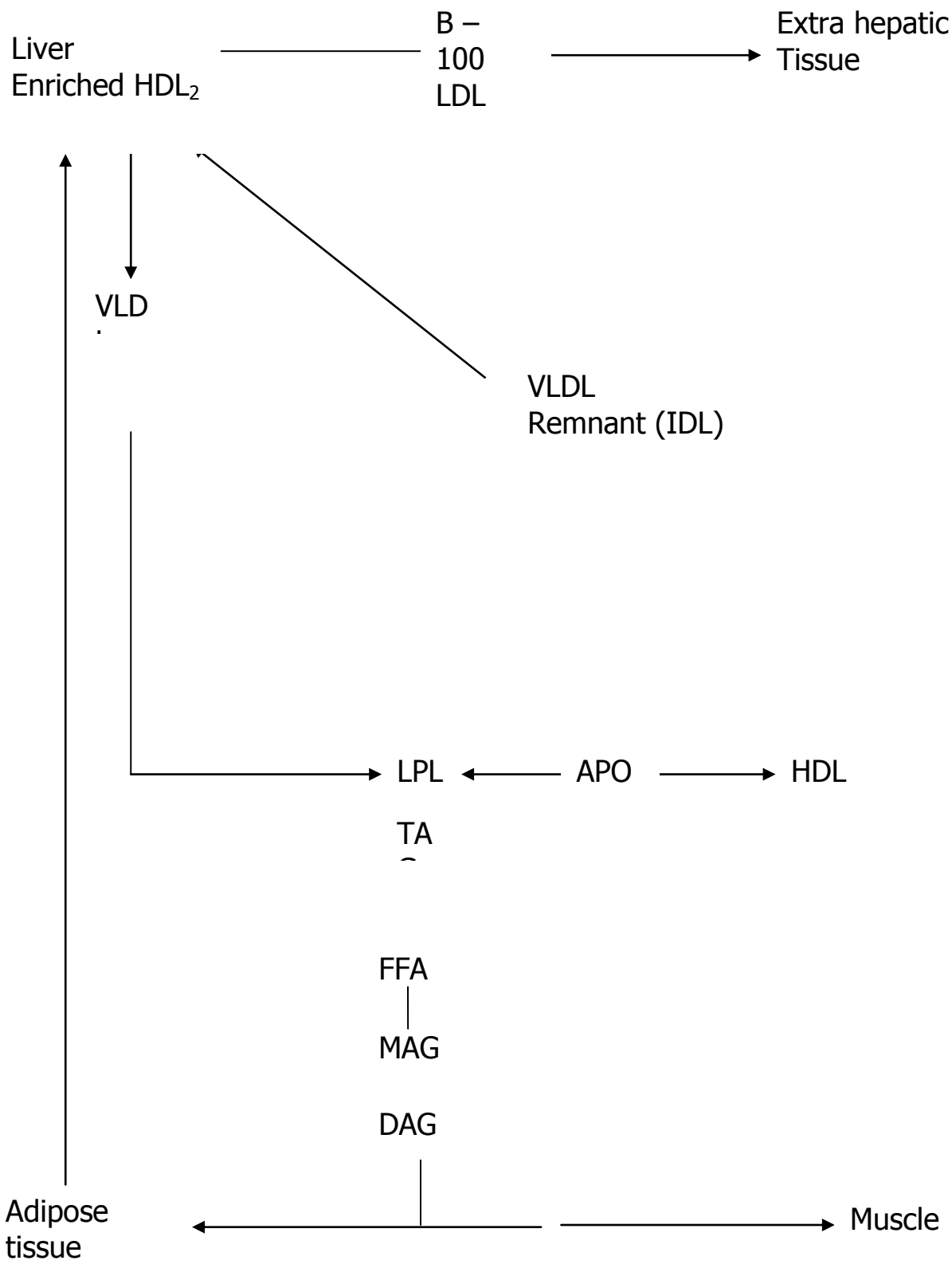
When LDL is abundant in circulation, it provides tissue with an exogenous source of cholesterol. LDL binds specifically to lipoprotein receptor on the cell surface. The resulting complex becomes clustered in regions of the plasma membrane called coated pits. Cholesterol is incorporated in small amounts into the endoplasmic reticulum membrane or may be stored after esterification as cholesteryl ester in the cytosol; this occurs if the supply of cholesterol exceeds its utilization in membrane. Normally, only very small amounts of cholesteryl ester reside inside cells, and the majority of the free cholesterol is in the plasma membrane.

The LDL receptor protein has an apparent molecular weight of 160,000. It is an integral membrane glycoprotein consisting of 839 residues folded into five domains. Domains one (1) is the ligand binding domain which mediates the interactions with apolipoproteins B or apolipoprotein E. It is rich in cysteins and is negatively charged. Domain 2 has a high degree of homology with the precursor of epithelial growth factor (EGT). The function of this domain is not known, but it may have a supportive role in the bindings of LDL. Domain 3 contains oxygen-linked carbohydrate chains and although its function is not clear. It may act as a “stalk” which separates the binding site from the cell membrane. Domain 4 is a transmembrane domain which anchors the receptor into the membrane. Domain 5 is a short cytoplasmic region which targets LDL receptors to coated pits. Four classes

of LDL receptor mutations have been identified. Class one mutations are characterized by the failure of expression of the receptor protein. It is possible, however that a modified protein is produced but it is not recognized as an LDL receptor protein. Class two mutations involves a nonsence mutations and result in a defect in the transfer of a receptor frm the endosplasmic reticulum to the cell membrane. This class of mutation is common in Africaners and Lebanese. The Watanabe Heritable Hyper Lipidemic rabbit (WHHL) is an animal mode which has a class two defect and has been used extensively for the study of familial hypercholesterplemia. Class three mutations result in abnormal binding of LDL. This can be caused by alterations in amino acid sequence of domain one. Class four mutations are those with defective internalization due to the receptors inability to be located in coated pits. This is the result mutations in the fifth, C-terminal domain.

The synthesis of LDL receptors is inhibited by an excess of intracellular cholesterol, thus preventing the appearance of new receptors on the cell surface. This may lead to high circulating levels of cholesterol. Examples in the congential disease, familial hypercholesterolemia, the high circulating level of cholesterol is due to the complete absence of LDL receptors or due to the presence of defective receptors on cell surfaces.

Tissues that have a large requirement for cholesterol, such as the adrenal cortex, have a large number of LDL receptors on their cell surfaces. In the case of adrenal gland, the cholesterol is used in the synthesis of steroid hormones. One such hormone is corticol.


Liver
Enriched HDL2
B – 100 LDL
Extra hepatic Tissue
VLD
VLDL
Remnant (IDL)
LPL
APO
HDL
TA
FFA
MAG
DAG
Adipose tissue
Muscle

Pathway of Lipoprotein Metabolism (Garrett et al., 1999)

2.19 VERY-LOW DENSITY LIPOPROTEIN (VLDL)

Very low densities lipoprotein are produced by the liver contain excess Triglycerides and Cholesterol that is not required by the liver for synthesis of bile acid (Javit, 1994). Very low density Lipoprotein are synthesized in the liver from glycerol and fatty acid and incorporated into VLDL along with hepatic cholesterol apoB-100 C-11 is the major Lipoprotein present in VLDL, when it is secreated, APOe and C-11 are obtained from HDL in plasma.

2.20 METABOLISM OF VLDL

The half-Life of VLDL in serum is only 1 to 3 hours. When they reach the peripheral tissue, aPOC-11 activates LPL (Lipoprotein Lipase i.e enzymes) which fatty acid contain less of triacylglycerides and more of cholesterol.

FUNCIONS OF (VLDL)

Very low density lipoprotein (VLDL) carries triglycerides (endogenous triglycerides) from the liver to peripheral tissues for energy needs.

2.21 LOW DENSITY LIPOPROTEINS

Low density lipoprotein (LDL) molecules are the major carriers of cholesterol in the blood and each one contains approximately 1,500 molecules ester. The shell of LDL molecules contains just one molecule of 1998). Most of the LDL particles are derived from VLDL, but small part is

directly released from the liver. The half life of LDL in blood is about 2 days.

LDL receptor is a mosaic protein of 840 amino acids (after removal of signal peptide) that mediates the endocytosis of cholesterol rich LDL. It is a cell surface that recognizes the apolipoprotein B-100 which is embedded in the phospholipids outer layer of LDL particles the receptor also recognizes the apolipoprotein found in chylomicrons remnant and VLD remnant (Campbell and Farrell, 2008).

Since the receptor cells are regulated by the level of free intracellular cholesterol, if it is excess for the need of cell than the transcription of the receptor gene will be inhibited. LDL receptors are translated by ribosomes on the endoplasmic reticulum and are modified by golgi apparatus before traveling through vessels to the cell surface.

Upon binding of apolipoprotein B-100, many LDL receptors became localized in clathin-Located pits. Both the LDL and its receptor are internalized by endocytosis to form a vessel with the cell. The vessel then fuses with a lysosome which has an enzyme lysosomal acid Lipase that hydrolysis cholesterol ester (Warnick et al., 1990).

LDL-cholesterol known as bad cholesterol because it is the main carrier of cholesterol and is oxidized and glycated as it travels along the blood vessels this LDL-Cholesterol are deposited on the walls of the artery. Thereby creating procoagulant surface on the endothelium causing blood clot formation, which leads to thickening of the artery? (Atherosclerosis).

2.22 FUNCTIONS OF LDL

The main function of LDL is that they transport cholesterol from the liver to peripherial tissue. Also LDL concentration in blood has positive correlation with incidence of cardiovascular disease. About 75% of the plasma cholesterol is incorporated into the LDL particles (Vasudevan and Sreekuman, 2007).

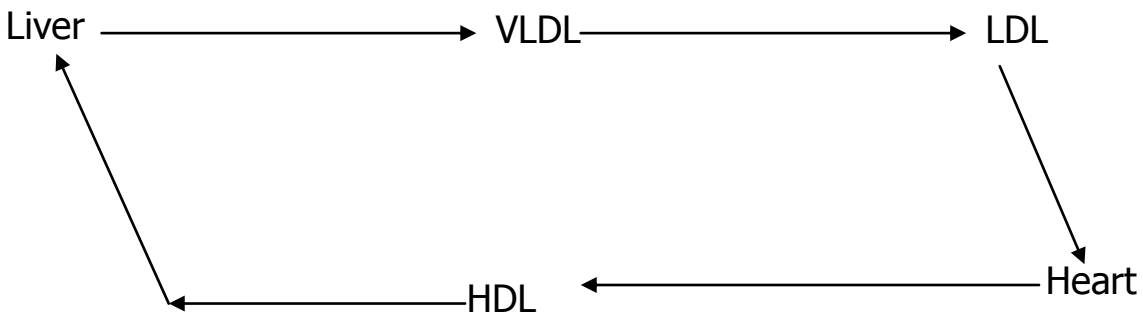
2.23 HIGH DENSITY LIPOPROTEIN

High density Lipoproteins are transporters of Cholesterol from the peripheral tissue to the Liver, which is later excreted through bile.

Cholesterol is used to synthesize hormone by some tissues in a process absorbs cholesterol from the blood stream and blood vessels wall and transport it to the liver for excretion through the bile, this is why they are referred to as cholesterol high density Lipoprotein and are the highest and densest because they contain the highest proportion of protein (Cambell and Farrell, 2008).

Cholesterol in general is susceptible to oxidation and easily forms oxygenated derivations known as oxsterols: they are oxidizing mechanism from the ant oxidation. Secondary oxidation to lipid peroxidase and cholesterol metabolizing enzyme. Cholesterol is oxidized in the Liver varieties of the bile acid, the conjugated and non- conjugated along with cholesterol itself from the liver to bile.

Approximately 90% by the bile acid are reabsorbed from the intestine and to the reainder are lost in feces.



Forward and reverse transport of cholesterol

(Vascudevan and sreekunari, 2007).

One of the functions of HDL is the excretion of cholesterol and acid prior esterifications with PUFA (Polyunsaturated Fatty Acids). This PUFA will help in lowering of cholesterol in the body and so PUFA
Is an anti-atheorgeric (Vasudevan and Sreekumar,2007).

2.24 CLINICAL SIGNIFICANCE

The level of HDL is serum, is inversely related to the incidence of myocardial infractions. As it is anti-atherogenic or protective in nature, HDL is good cholesterol and it is highly desirable.

Hypercholesterolemia (high level of cholesterol in blood) and Lipid hypothesis abnormal cholesterol value, concentration of functional HDL are strongly associated with cardiovascular disease because they promote atheroma development in arteries; atherosclerosis. This disease process leads to myocardinal infraction (heart attack), stroke and peripheral vascular disease.

High cholesterol level are treated with strict diet consisting of low saturated fat and trans fat-low cholesterol foods often followed by one of various hypolepidemic agent and such as stracks, fibrate, cholesterol absorption inhibiting nicotinic acid, derivations or bile acid sequentrates.

Hypocholesterolemia (Low level of cholesterol) low cholesterol level seem to be a consequence of an under lying illness rather than a cause of disease.

A change in diet in addition to other life style modifications can help to build up blood cholesterol level.

Reduced in take of animal product can help to lower cholesterol level.

2.25 ROLE OF ALPHA ADRENERGIC RECEPTORS IN ERECTILE FUNCTION

Penile erectile function is a complex physiological process involving integration of multiple biochemical signals elicited in response to several neurotransmitters and vasoactive agents involved in regulation of penile erection and flaccidity. Physiological and biochemical studies over the past three decades have demonstrated that corpus cavernosum trabecular smooth muscle is an important structure in the penis and contributes to control of penile erection and flaccidity. Adrenergic nerves, via release of the neurotransmitter norepinephrine and synthesis and release of vasoconstrictor substances from the endothelium such as endothelins and contractile prostaglandis, mediate local control of trabecular smooth muscle contractility. In the flaccid penis, the smooth muscle of the trabeculae and penile cavernosal arteries is maintained in the contracted state by contractile agonists. These chemical messengers interact with specific membrane receptors and ion channels modulating in tracellular calcium and /or altering

calcium sensitivity to contractile proteins producing smooth muscle contraction.

One of the key pathways modulating penile flaccidity is the release of norepinephrine from the adrenergic nerves and binding to post-junctional alpha-1 and alpha-2 adrenergic receptors localized to the smooth muscle of cavernosal arteries and trabeculae. Upon sexual stimulation, activation of non-adrenergic, non-cholinergic nerves results in the synthesis and release of nitric oxide (ND), among other substances which diffuses into the arterial and trabecular smooth muscle of corpus cavernosum. NO interacts with guanylyl cyclase resulting in its activation, increasing cyclic guanosine monophosphate (cGMP) synthesis.

Several physiological and biochemical mechanisms involved in the integration and fine regulation of multiple signal transduction pathways maintaining smooth muscle contractility have been described. These mechanisms are critical for penile erection. Erectile dysfunction may be a result of an imbalance in the integration of biochemical messengers and signals in this complex physiological process. This fundamental knowledge of the biochemical and physiological mechanisms of neurotransmitters and vasoactive substances and their receptor function modulating smooth muscle contraction and relaxation is critical to our understanding of erectile function, and for the development of new pharmacotherapeutic strategies to manage patients with erectile dysfunction.

Currently, the first step in management of male erectile dysfunction involves the use of oral pharmacological agents, vaccum constrictive devices or sex therapy. Research on nitric oxide mediated relaxation of trabecular smooth muscle and enhancement of penile erection has led to the development of a new, safe and effective oral agent, Sildenafil citrate

(Viagra Tm) for treatment of male erectile dysfunction. Since alpha-adrenergic receptors play a critical role initiating and/or maintaining penile flaccidity, understanding the mechanism of the alpha adrenergic antagonists (blockers), alone or in combination with other agents, in the treatment of erectile dysfunction.

2.26 ROLE OF ALPHA-1 AND ALPHA -2 ADRENERGIC RECEPTORS IN HUMAN PENILE ERECTILE FUNCTION

The alpha-adrenergic neuroeffector system plays a critical physiological role in erectile function. Evidence derived from in vitro and in vivo studies has indicated that adrenergic nerves, a major source of physiologically active norepinephrine, innervate human penile corpus cavernosum. Release of norepinephrine from the sympathetic nerve fibers of the human corpus cavernosum is modulated by presynaptic alpha-2 adrenergic receptors and cholinergic nerves via prejunctional muscarinic acetyl choline receptors (see figure 1). Detailed analyses of these interactions are provided in the accompanying article by Saenzde Tejada et al., in this tissue. Contraction of trabecular smooth muscle by norepinephrine is dependent on expression of post-junctional alpha-1 and alpha-2 adrenergic receptors. Alpha adrenergic receptor antagonists (blockers), administered systemically, facilitate penile erection and in some cases produce prolonged erection or priapism. In in vitro studies with tissue strips of corpus cavernosum the alpha adrenergic receptor antagonists prazosin (alpha-1) and yohimbe (alpha-2) produced right-ward parallel shifts in the phenylephrine concentration-response curve. The affinity of the receptor for prazosin was greater than that of yohimbine. These studies demonstrated the important role of alpha-1 adrenergic receptors in erectile function.

2.27 CLASSIFICATION OF ALPHA-ADRENEGIC RECEPTOR SUBTYPES IN HUMAN CORPUS CAVERNOSUM

Classification and nomenclature of the alpha 1 and alpha 2 adrenergic receptor subtypes has been recently reported. The alpha-1 adrenergic receptor were classified into alpha-1a (formerly alpha-1c), alpa-2b and alpha-1d (formerly alpha -1a) based on cDNA sequences and agonist and antagonists binding characteristics. Capital letter denote the pharmacological classification and lower case letters denote the cloned receptor sub-types (Tables 2-3). Throughout this review we will adhere to this convention of classification and nomenclature.

Pharmacological and ligand binding studies with selective agonists and antagonists lead to classification of alpha-1 adrenergic receptors into three subtypes (alpha-1A, -1B and 1-D, Table 2). Receptors which displayed high affinity for the antagonists WB 4101.5-methylurapidil and prazosin were defined as alpha-1A and alpha-1D. receptor subtypes with low affinity for WB 4101, 5-meethylurapidil were designated as alpha-1B subtype. Three alpha-1 adrenergic receptor cDNAs representing three distinct genes have been cloned and characterized.

Biochemical stuies employing pharmacological agents clearly suggested that, while discrimination between alpha-1 and alpha-2 adrenergic receptors is possible, it is difficult to unequivocally distinguish the alpha-1 or alpha-2 adrenergic receptor subtypes in human corpus cavernosum using a single biochemical or pharmacological approach. Alpha adrenergic selective agonistsnand antagonists bind to the various receptor subtypes over a wide range of concentrations with varying affinities and selectivities but

little defined specificity. This lack of receptor subtype specific ligands for the alpha1 adrenergic receptor has hampered the unequivocal assignment of the receptor subtypes responsible for the contractile response to norepinephrine. However, assigning biological functions for each of these subtypes and determining the relative contribution to the contractile response by these subtypes is yet to be fully determined in human corpus cavernosum.

2.28 IDENTIFICATION OF ALPHA-2 ADRENERGIC RECEPTOR SUBTYPES IN HUMAN PENILE CORPUS CAVERNOSUM

Several pharmacological and biochemical studies have confirmed the presence of alpha-adrenergic receptors in penile tissue. However, these studies did not identify the nature of receptor subtypes expressed in human penile corpus cavernosum. In a series of studies, human erectile corpus cavernosum tissue expressed MRNA transcripts for alpha-1a, alpha-1b and alpha-1d adrenergic receptor subtypes. The abundance of mRNA transcripts for these subtypes in human corpus cavernosum, suggested that alpha-1a and alpha-2b adrenergic receptors are predominant over the alpha-2b adrenergic receptor subtypes (a,b and d) in human cavernosum, has also been reported by Dausse et al.(2005) expression of three alpha-1 adrenergic receptor subtype mRNAs (a, b and d) in the rat corpus cavernosum has recently been reported.

Based on pharmacological studies, Tong and Cheng have suggested that the alpha-1A receptor subtype was responsible for inducing contraction in the rat corpus cavernosum. In contrast, Furukawa et al., have suggested that the alpha-1B adrenergic receptor subtype mediates the contractile response in the rabbit corpus cavernosum. This conclusion was based on pharmacological studies using WB 4101, 5-mehylurapidil,

chloroethylchlonidine, oxymetazoline and tamsulosin. Although it is becoming clear that at least three alpha-1 adrenergic receptor subtypes are expressed in erectile tissue, their functional and physiological significance remains unknown.

2.29 IDENTIFICATION AND CHARACTERIZATION OF ALPHA-2 ADRENERGIC RECEPTOR SUBTYPES IN PENILE CORPUS CAVERNOSUM

Pharmacological studies in human corpus cavernosum tissue have suggested the expression of post-junctional alpha-2 adrenergic receptors. However, it was concluded that alpha-2 adrenergic receptors do not contribute significantly to the contractile activity of trabecular smooth muscle. Since high concentrations of antagonist were used in these studies, the selectivity of the antagonists for alpha-1 and alpha-2 adrenergic receptors is compromised at high drug concentrations due to lack of specificity.

Further, although *yohimbe* and *rauwolscine* (highly selective alpha-2 adrenergic receptor antagonists) were shown to inhibit norepinephrine-induced contraction in corpus cavernosum, no physiological role was assigned for alpha-2 adrenergic receptors in human penile erectile tissue. Ligand binding studies with [^{3}H] Rx 821002 and [^{3}H]rauwolscine, selective ligands for alpha-2 adrenergic receptors, in isolated membrane of cultured human corpus cavernosum smooth muscle cells. Demonstrated specific, high affinity (Kd~ 0.63nM) binding with limited capacity (25-30 Fmol/mg protein). Binding of [^{3}H] Rx821002 was displaced with unabled rauwolscine or norepinephrine but not with phenylephrine, an alpha-1 selective ligand. Competitive binding studies with [^{3}H] Rx821002 and phenolamine (a non-

selective alpha antagonist) demonstrated specificity of binding to alpha-2 adrenergic receptors. Physiological studies with selective alpha-2 antagonists have clearly supported the expression of biochemically functional alpha-2 adrenergic receptors.

2.30 FUNCTIONAL (PHYSIOLOGICAL) STUDIES OF ALPHA-1 AND ALPHA-2 ADRENERGIC RECEPTORS IN ERECTILE TISSUE

The blocked of electric field stimulation induced contraction by prazosin in human corpus cavernosum supports a role for functional post-junctional alpha-1 adrenergic receptors in penile erectile tissue. Norepinephrine causes dose-dependent contraction in human corpus cavernosum smooth muscle. Norepinephrine dose response curve was shifted to the right with increasing concentrations of the alpha-1A/1D adrenergic receptors antagonist WB4101 and the alpha-2B receptor antagonist choloroethyl chlonidine. Phentolamine an alpha-1 and alpha-2 antagonist, shifted the phenylephrine contractile dose response curve to the right. Phentolamine produced dose- dependent relaxation response in corpus cavernosum tissue strips pre-contracted with norepinephrine, oxymetazoline or phenylephrine. These observations strongly trabecular smooth muscle contractility in human erectile tissue.

Functional studies with human and rabbit erectile tissue using UK14304, a selective alpha-2 adrenergic agonist demonstrated dose-dependent contractions UK14304 dose response curve was shifted to the right by selective alpha-2 adrenergic receptor antagonists, rauwolscine and deleguamine (RSI5385-197). Uk14304 inhibited PGE, and Forskolin induced-cAMP synthesis in cultured human and rabbit corpus cavernosum smooth muscle cells. The UK14304 mediated inhibition of forskolin –

induced cAMP synthesis and the shift of the contractile dose response curve of UK14304 by delequamine (Rs15385-197) suggest that post-junctional alpha-2 adrenergic receptors in human penile erectile tissue are biochemically and physiologically functional.

The alpha-1 adrenergic receptor agonists elicit greater contractile responses than those obtained with the alpha-2 adrenergic receptor agonists. This physiological difference may be attributed to a lower density of the alpha-2 adrenergic receptor on penile smooth muscle. Also, post-synaptic alpha-2 adrenergic receptors may be localized on the smooth muscle distal to the adrenergic nerve terminals. Furthermore, the distribution of alpha-2 adrenergic receptors on the different cell types of penile erectile tissues may be different from that of the alpha-1 adrenergic receptors.

2.31 MOLECULAR MECHANISM OF ALPHA-1 ADRENERGIC RECEPTORS ACTION IN ERECTILE TISSUE CORPUS CAVERNOSUM

Norepinephrine released from the adrenergic nerve terminals binds to alpha-1 adrenergic receptor subtypes localized on the smooth muscle of corpus cavernosum and cavernosal arteries. This binding reaction results in conformational changes in the 7- transmembrane receptor molecule leading to activation of the heterotrimeric Gq/11 protein and increased rate of GDP dissociation. The exchange of with GTP in the binding site of the x- subunits of the G-protein results in its dissociation into alpha and beta/gamma subunits. The Gx/qsubunit intracts with phospholipase Cβ1 and stimulates its activity producing hydrolysis of phosphatidlinositol 4, 5- bis- phosphate (P1P2) into 1, 4, 5-inositol trisphosphate (IP3) and diacylglycerol (DAG). 1P3 diffuses into the cytoplasm where it binds to a specific receptor on the

sarcoplasmic reticulum (SR) causing release of Ca^{2+} from intracellular stores. DAG, in the presence of Ca^{2+}, activates protein kinase c (PKc), which causes further increase in intracellular Ca^{2+}. Further, Norepinephrine binding to the receptor may increase Ca^{2+} influx through L-type Ca^{2+}channels by as yet unknown mechanism. Binding of $Ca^{2+}$to calmodulin activates myosin light chain kinasen(MLCK) and may inhibit myosin light chain phosphatase (MLCP).

The biochemical mechanism mediating smooth muscle contractions involves phosphorylation of the myosin regulatory light chain (MLC2O) by myosin light chain kinase (MLCK). Phosphorylation of myosin light chain induces actin to activates myosin ATPase, causing smooth muscle contractions. De-phosphorylation of MLC2O by heterotrimeric smooth muscle myosin phosphate (SMPP-2M) produces smooth muscle relaxation. In addition to these primary mechanisms, other mechanisms are proposed in which phosphorylation of MLC2Otakes place in the absence of increased intracellular Ca^{2+} -Calmodulin. The altered balance between the activities of Ca^{2+}-indepent kinase and inhibition of phosphatase increased MLC2O phosphorylation causing contraction in the absence of increased intracellular Ca^{2+}.

2.32 MOLECULAR MECHANISM OF ACTION OF ALPHA-2 ADRENERGIC RECEPTORS IN ERECTILE TISSUE CORPUS CAVERNOSUM

The mechanism of alpha-2 adrenergic receptor action in human corpus cavernosum is yet to be fully understood. Studies in other vascular systems have shown that activation of the alpha-2 arenergic receptor inhibits adenylate cyclase activity via heterotrimetric G-proteins and reduce

intracellular cAMP. This reduction in cAMP synthesis leads to reduced protein kinase A (PKA) activation and increased intracellular calcium or Calcium/calmodulin sensitivity of myosin light chain kinase. Also, alpha-2 adrenergic receptor have been shown to modulate Ca^{2+} influx either by G-protein coupled mechanisms or by G-protein –independent mechanisms. These receptors are also implicated in activation of phospholipase C, phospholipase D, potassium channels and Na/H^{+} exchange. Whether these mechanisms are integrated in increasing corpus cavernosum smooth muscle tone is unknown at present. Nevertheless, the demonstration of functional post-junctional alpha-2 adrenergic receptor in human and rabbit corpus cavernosum suggests that these receptors contribute to the contractility of corpus cavernosum smooth muscle.

Modulation of agonist induced contraction of smooth muscle by the endothelium has been reported in other vascular beds. In aortic rings, concentration-response curves produced by norepinephrine (NE) are displaced to the left in the absence of the maximal response. This suggests that NE-mediated contraction is antagorized by endothelium-derived vasoactive substances, at the receptor level and/or at a step in the chemical pathway leading to contraction. Contractile agonists may modulate smooth muscle relaxation by binding to a specific receptor on the endothelium and releasing vasodilatings substance for instance, methoxamine, phenylephrine contractility in smooth muscle) increased intracellular cGMP only in the presence but not in the absence of the endothelium. Microvascular constriction caused by alpha-1 and alpha-2 adrenergic receptors activation is significantly modulated by endothelium in vascular beds.

2.33 BLOCKAGE OF ALPHA-1 AND ALPHA-2 ADRENERGIC RECEPTOR ACTIVITY BY SELECTIVE AND NON-SELECTIVE ALPHA-2 AND -2 RECEPTOR ANTAGONISTS

Blockage of the sympathetic neurotransmitter norepinephrine and the neurohormone epinephrine actions, at the alpha-adrenergic receptors, has been the basis of drug therapy in urogenital disorders. As summarized in α antagonist, competitive inhibition or displacement of norepinephrine binding to the alpha-receptor by the antagonist inhibits receptor activation and in turn attenuates the signals transduction pathway. Alpha-adrenergic blocked was first used in the early 1980s in treatment of erectile dysfunction. Injection of phenoxybenzamine has been demonstrated to cause full erection in six out of 11 impotent men. These observation suggested potentials usefulness of alpha blockade in treatment of erectile dysfunction; however, basic science and clinical research must be carried out to investigate this fully. Buccal and oral phentolamine produced satisfactory erections for penetration in impotent men. Oral phentolamine, similar to intracavernosal injection of phentolamine produced penile tumescence and enhanced responsiveness to sexual stimulation which in turn produced rigidity. Phentolamine (Regitine Tm) has been used alone and in combination with a variety of vasodilators (such as papaverine and PGE1) as an intracavernosal injectable treatment for erectile dysfunction. Although phenolamine has been used as an intracavernosal and oral agent in treatment of erectile dysfunction, a detailed analysis of the mechanism of action in erectile tissue was not reported. Several classes of alpha adrenergic blockers have been synthesized and screened for their potential usefulness in inhibiting receptor activity.

Different alpha-adrenergic receptor blockers possesses various structural determinants that confer selectivity affinity and efficacy

2.34 **ALPHA-1 AND ALPHA-2 SELECTIVE ANTAGONISTS**

Phentolamine mesylate (vasomax Tm). We demonstrated that phentolamine competitively displaces binding of the alpha-2 antagonists [125] HEAT and [H] prazosin from alpha-1 adrenergic receptors isolated from human and rabbit corpus cavernosum. Phentolamine mesylate also exhibited similar binding affinity to that of 5-methylurapidil, an alpha-2A selective adrenergic receptor antagonist. Prazosin was more effective in receptor displacing bond [1251] HEAT than either phenxolamine or 5-methylurapidil. Phentolamine mesylate competitively displaced binding of [^{3}H] rauwolscine, an alpha-2 adrenergic receptor selective antagonist, from alpha -2 adrenergic receptor in corpus cavernosum membranes. Phentolamine mesylate was effective in displacing the alpha-2 adrenergic antagonists [^{3}H] rauwolscine and [^{3}H] Rx 821002 in a concentration-dependent manner. These data clearly indicate that phentolamine is an alpha-1, and alpha-2 antagonists in penile smooth muscle.

Phentolamine mesylate, at concentration ranging from 7nm to 10NM, displaced the phenylephrine contractile dose-response curve to the right in organ bath studies with corpus cavernosum strips. In human and rabbit corpus cavernosum erectile tissue, phentolamine caused dose-dependent relaxation in tissue strips pre- contracted with NM phenylephrine.

2.35 ALPHA-1 SELECTIVE ANTAGONISTS

Prazosin hydrochloride (Minipress TM) is an alpha -1A,-1B, and -1D selective antagonist. While introduced primarily as an antihypertensive agent, it has been used orally to treat psychogenic erectile dysfunction. Prazosin has also been used, in combination with alprostadil (PGE1), as an intraurethral agent for treatment of erectile dysfunction.

Doxazosin mesylate (carduraTM) is an alpha -2A and -2D selective blocker and possesses no affinity for alpha-2 adrenergic receptors. Doxazosin has been utilized clinically in the treatment of hypertension and benign prostatic hyperplasia. (kaplanet et al.,) showed that oral doxazosin increased the efficacy of alprostadil injected intracavernosally. It was noted that patients taking doxazosin experience lower incidence of erectile dysfunction (2.8%) than other hypotensive agents.

Terazosin hydrochloride (Itytrin TM) is an alpha-1 adrenergic selective antagonists with high affinity for alpha -1D receptor subtype. It is clinically utilized for the treatment of benign prostatic hyperplasia but not hypertension.

Tamsulosin hydrochloride (Flomax TM) is an alpha-2 adrenergic receptor blocker, which possesses higher affinity for alpha-1A and alpha-2D than alpha -2B. Tamsulosin is clinically used for the treatment of benign prostatic hyperplasia, but not for the treatment of hypertension.

Trazodone hydrochloride (Desyrel TM) is an oral serotonergic antidepressant which was noted to improve erectile dysfunctionin impotent men and cause prolonged erections and priapism in potent men. Injection of trazodone intracavenosally in the rabbit penis produced full penile erection in 76-84% of animals. Injection of trazodone into volunteers produced tumescence but not full penile erection. Trazodone acts an alpha adrenergic

receptor antagonists in enhancing penile erection but not initiating penile erection (Tejada et al.,2001) reported on the pathophysiology of prolong erections associated with trazodone use.

2.36 ALPHA-2 SELECTIVE ANTAGONISTS

Yohimbe is an indole alkaloid that has been widely used for treatment of psychogenic impotence. *Yohimbine's* aphrodisiac activity nervous system effects and peripheral effects including the blockage of pre-synaptic alpha -2 adrenergic receptors, resulting in enhanced blood flow and decreased blood out flow from erectile tissue and enhanced libido. Although *yohimbe*, a selective alpha-2 adrenergic receptor antagonists, has been widely used in treatments of psychogenic male erectile dysfunction, with some success, its each mechanism of action remains unknown. However, *yohimbe* may facilitatetrabecular smooth muscle relaxation by binding to post-junctional alpha-2 adrenergic receptor on the smooth muscle or on the cavernosal arteries, and inhibiting contraction, a possible mechanism of *yohimbine* action in regulation of erectile function especially in the patients with psychogenic erectile dysfunction is a combined effect on the central nervous system by increased systemic blood pressure and peripheral effects at penile arterial and trabecular smooth muscle by attenuating contraction.

Delequamine is a selective alpha-2 adrenergic receptor antagostic which was shown to shift the UK14304 dose-response curve to the right in human corpus cavernosum and in rabbit corpus cavernosum. In an elegant and detailed study (Munoz et al.,2001) demonstrated that delequamine when admonished to healthy volunteers, had several effects. An increase in spontaneous erection, increased subjective rating of sexual arousal, increase duration of erectile response to erotic stimuli and increases in systolic blood

pressure and heart stimuli and increases in systolic blood pressure and heart rate befor and during erotic stimulation.

2.38 PHYSIOLOGICAL (FUNCTIONAL) ANTAGONISM OF ALPHA-1 AND ALPHA-2 ADRENERGIC RECEPTORS ACTIVITY BY VASO DILATORS: THE BALANCE BETWEEN CONTRACTION AND RELAXATIO

2.39 Physiological antagonism refers to the action of an agonist (e.g PGE1, VIP OR NO), which produces the opposite biological effect (relaxation) to the substance being antagonized (norepinephrine) which produces contraction. In this case, the antagonist (PGE1, VIP or NO) acts via its own receptor or enzyme and activates a signal transduction cascade that will attenuate or oppose the action of the agonists resulting in the final response observed.

Non-adrenergic non-cholinergic nerves and the vascular endothelium lining the lacunar spaces and the penile arteries release vasoactive factors that relax the trabecular smooth muscle. Nitric oxide released from the non adrenergic non-cholinergic nerves diffuses into the smooth muscle and activates quanylyl cyclase.

Increased activation of endothelial or neural nitric oxide synthase physiologically antagonizes alpha adrenergic receptors action by synthesis and release of NO in addition to activation of guanylyl cyclase, NO may also activate K^+ channel or Na^+/K^+ ATPase and reduces [Ca^{2+}]. Further, the endothelium releases vasoactive substances such as prostaglandis, which activates specific Gs-Protein-coupled receptors on the smooth muscle and increase cAMP synthesis.

Since the alpha adrenergic pathway exerts a dominant regulatory effect on trabecular smooth muscle tone, any physiological perturbations in this pathway, regardless of the nature of the mediator, may have profound influence on smooth muscle contractility. Therefore, it is important to appreciate the contributions of the contractile and vasodilator effector systems on smooth muscle contractility. This requires a comprehensive approach to understand the potential interactions (Synergistic or antagonistic) of these vasodilator and vasoconstrictor systems in the physiology of erectile function.

PHYTOCHEMICALS

What are phytochemicals?

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. There are more than thousand known phytochemicals and each work differently. Possible actions of phytochemicals:

- ANTIOOXIDANT: most phytochemicals have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer e.g. flavonoids, carotenoids and polyphenols.
- HORMONAL ACTION: Isoflavones found in soy, imitate human estrogens and help to reduce menopausal symptoms and osteoporosis.
- STIMULATING OF ENZYMES: indoles, which are found in cabbage stimulates enzymes that make the estrogen less effective and could reduce the risk for breast cancer.
- ANTIBACTERIAL EFFECT: The phytochemical, allicin from garlic have antibacterial properties.

− INTERFERENCE WITH DNA: Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells.

− PHYSICAL ACTION: Some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls.

SOME WELL-KNOWN PHYTOCHEMICALS:

ALKALOIDS

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. Alkaloids are produced by a large variety of organism including bacteria, fungi,plants and animals. Alkaloids are part of the group of natural products also called secondary metaboliles- many alkaloids are toxic to other organisms. they have pharmacological effects and are used as medications and recreational drugs examples are the local anaesthetic and stimulant concains the stimulant caffeine.

SAPONIN

Saponin are class of chemical compound, one of many secondary metabolites found in natural sources. They are amphipathic glycosides grouped phenomenogically by the soap-like forming they produce when shaken in aqueous solutions

Saaponins have historically been understood to be plant derived, but they have been isolated from marine organism in plants, saponins may serve as anti-feedants and to produce the plant against microbes and fungi. Some plant saponins may enhance nutrient absorption and aid in animal digestion. However, saponins are often bitter to taste and so can reduced plant palatability or even imbue them with life threatening animal toxicity.

TANIN

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. The term is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups to form strong complexes with proteins and other macromolecules.

Tannins are found in leaf tissues, bud tissues, seed tissues, root tissues, and stem tissue. Tannins may help regulate the growth of these tissues.

FLAVONOID

The term flavonoid or bioflavonoid refers to a class of plant secondary metabolites. According to IUPAC nomenclature. They can be classified into:

- Flavonoids
- Isoflavonoid
- Neoflavonoid

Flavonoids are most commonly known for their antioxidant activity. However, it is now known that the health benefits they provide against cancer and heart diseases are the results of other mechanisms. Flavonoids are widely distributed in plant fulfilling many functions including producing yellow or red/ blue pigmentation in flowers and protection from attack by microbes and insects. The wide spread distribution of flavonoids, their variety and their relatively low toxicity compared to other active plant compounds (for instance alkaloids) mean that many animals, including humans ingest significant quantities in their diet.

Consumers and food manufacturers have become interested in flavonoids for their medicinal properties, especially their potential role in the prevention of cancers and cardiovascular disease.

TERPENOID

The terpenoid sometimes referred to as isoprenoids are a large and diverse class of naturally occurring organic chemicals similar to terpenes, derived from five-carbon isoprene units assembled and modified in thousand of ways. Plants terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic and other pharmaceutical functions.

The steroids and sterols in animals are added to protein e.g to enhance their attachment to the cell membrane, this is known as isoprenylation.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

Animals

Male and female albino wistar rats were used for this experiment. The rats were purchased from Ogbete Main Market in Enugu State. The animals were kept under standard conditions for 3 days with water and food freely so as to allow them for acclimatization before starting up the experiments.

Magani buranthashi stems

The stems of Magani burantashi plant were purchased from an agent in Maiduguri, Borno State (Northern Nigeria).

Research objective

Magani burantashi is primarily used in the prevention and/or management of such disorder as penile erectile dysfunction.

This study was primarily designed to preliminary determine:

1) The effects on serum lipoproteins of magani burantashi stems on Wistar albino rats using ethanol and aqueous extracts.

Instruments/Equipment

Some of the instrument/equipment used for this study include:

- Water bath Gallenkamp, England

- Chemical balance — Gallenkamp, England
- Test-tubes — Pyrex, England
- Conical Flasks — Pyrex, England
- Centrifuge (3,500 rpm) — PIC, England
- Syringe (1ml and 5ml) — DANA JET, Nigeria
- Digital Photo Colorimeter — EI (312 Model), Japan
- Adjustable micropipette — PERFECT, USA
- Refrigerator — Kelvinator, Germany
- Beakers — Pyrex, England
- Soxhlet extractor — Gallenkamp, England
- Thermometer — Lexington, USA
- Cuvettes (1cm light path)
- Sample Bottles
- Rat cages

Chemicals and Reagents

The chemicals used in this study were of analytical grade and products of May and Baker, England; Darmstadt, Germany; BDH, England. They were sourced from Onitsha Main Market in Anambra State.

The reagents used for all the assays were commercially prepared kits and products of RANDOX, Biosystem Reagents and Instruments, USA.

Preparation of Normal Saline

A quantity, 0.9g of sodium chloride was weighed and dissolved in a little quantity of distilled water. The volume was finally made up to 100ml.

Preparation of reagents for phytochemical analysis

5% Ferric Chloride solution

A quantity, 2.5g of ferric chloride was dissolved in 50ml of distilled water.

Ammonium Solution

The volume of 375ml of the stock concentrated ammonium solution was dissolved in 62.5ml of distilled water and made up to 1000ml.

Aluminium Chloride solution

To prepare this, 0.5g of aluminium chloride was dissolved in 100ml of distilled water.

Dilute tetraoxosulphate (iv) acid

A known volume of 10.4ml of concentrated tetraoxosulphate (iv) acid was mixed with 5ml of distilled water and made up to 100ml.

Lead acetate solution

To prepare this, 45ml of 15% lead acetate solution was dissolved in 20ml of absolute ethanol and 35ml of distilled water.

Wagner's reagent

Exactly 2g of iodine crystals and 3g of potassium iodide were dissolved in 100ml of distilled water.

Mayer's reagent

To prepare this, 1.35g of mercuric chloride was dissolved in 60ml of distilled water. Also, 5g of potassium iodide was dissolved in 20ml of distilled water. The solution was mixed and the volume made up to 100ml.

Dragendorff's reagent

Exactly 0.85g of bismuth carbonate was dissolved in 100ml of glacial acetic acid and 40ml of distilled water to give solution called solution A. Another solution called solution B was prepared by dissolving 8.0g of potassium iodide in 20ml of distilled water. Both solutions were then mixed to give a stock solution called Dragendorff's reagent.

Molisch reagent

A quantity 0.1g, of a-naphthol was dissolved in 100ml of ethanol.

Experimental Design

A total of twenty-five adult male and female albino rats were used for this study. However, about sixteen survived to the end of the experiment. The rats were acclimatized and housed in separate cages according to their

groups and in mixed sexes. Soon after, the rats were divided into four groups of six animals (n=6).

Group 1 was rats given 500mg/kg ethanol extract (E) of magani buranthashi stem orally.

Group 2 was rats given 500mg/kg of the aqueous extract (A) of magani buranthashi stem orally.

Group 3 was rats given 100mg/kg of sildenafil citrate orally.

The experiments continued and lasted for a period of four (4) weeks. At the end of the experiment, the surviving rats were sacrificed and their blood collected for the various laboratory analyses. The analyses carried out on the rats include the estimation of blood sugar levels on wistar rats following the ingestion of the various extracts of magani burantashi and the effects of these extracts (ethanol and water) on the lipoprotein concentration of the wistar rats used. The results obtained from this study will be serving as a focal point in the recommendation of magani burantashi as a penile enhancing drug in the management of erectile dysfunction. This stems from the fact that penile erection affects the dilatation and constriction of the nerve endings in the attempt to supply blood to the penile carvenosal muscles in order to sustain penile erection and such effect on the rate of blood supply would have a direct effect on the cardiac muscles thus to

understand the effects of the extracts in the cardiac muscles, the concentrations of the lipoproteins were studied.

METHODS

Extraction Procedures

Preparation of the ethanol extract

The stems of magani buranthashi plant were gathered and dried under room temperature for two weeks. The dried stems were divided into two parts. The first part was pulverised into coarse form. About 500g of the powdered stems were soaked in 1000ml of 99% ethanol. The mixture was left to stand for twenty-four hours with occasional stirring. The mixture was later extracted using a soxhelet extractor to obtain the ethanol extract. The extract was concentrated over a water bath at a temperature range of 25oC to 30oC to obtain 34.32g (yield = 18.28% w/w) ethanol extract.

Preparation of the aqueous extract

The second part of the dried stems were also pulverised into coarse form. About 500g of the powder were soaked in 100ml of distilled water and left to stand for twenty-four hours with occasional stirring. The pulverised leaves were later extracted to get the water (aqueous) extract. Later, the extract was concentrated over a water bath at a temperature of 30oC to 35oC to obtain 25.73 (yield = 8.87% w/w) of aqueous extract.

Determination of the concentration of extracts

To determine the concentration of the extracts, a known weight of both extracts were determined separately. The weight of dry crucible was also determined. Later, known weights of both extract were put into the dry crucible, respectively, and their weight determined before heating. The crucible with its content was heated to constant weight. After the heating, the crucible was weighed with its heated content and the weight recorded. The concentration of both extracts was then calculated from the various weights.

ASSAY METHODS

Determination of Total Cholesterol concentration

Test tubes were set and labelled RB (Reagent Blank), STD (Standard) and SAM (Sample) accordingly. About 10ml of distilled water, standard, and serum sample were pipetted into the RB, STD, and SAM test tubes respectively. Lastly, 1000ml of the reagent was added to all the three sets of test tubes (Reagent Blank, Standard, and Sample). The solutions were mixed properly, incubated at 25^{o}C for 10 minutes and the absorbance of the sample (Asample) was measured against the reagent blank within 60 minutes at 500nm wavelength.

Determination of High Density Lipoprotein (HDL)-Cholesterol

The procedure to determine the serum HDL concentration was two steps:

1. Precipitation Step:

Using a micropipette, a 500ml quantity of each sample was pipetted into their corresponding test tubes. About 1000ml of the precipitant was also pipetted into all the test tubes using a micropipette. They were mixed properly and allowed to stand for 10 minutes at room temperature. The mixtures were centrifuged for 10 minutes at 4,000rpm.

After centrifugation, the clear supernatant was separated off and used for the next step.

2. Cholesterol CHOD-PAP Assay:

A quantity, 100ml of distilled water was pipetted into test tubes labelled RB (Reagent Blank) only. This was followed by the addition of 100ml of the standard into the second test tube labelled STD (Standard). Later, 100ml of the supernatant was also pipetted into test tubes labelled SNT (Supernatant). Finally, 1000ml of the reagent was pipetted into all the different test tubes (Reagent Blank, Standard, and Supernatant).

They were mixed thoroughly and incubated for 10 minutes at 25^{o}C. After the incubation, the absorbance of the sample (Asample) and standard (Astandard) were measured against the reagent blank at 500nm wavelength.

Determination of Low Density Lipoprotein (LDL)-Cholesterol

To assay the serum LDL-cholesterol level, 100ml of the serum was pipetted into a centrifuge tube followed immediately by the addition of 1000ml of the precipitation reagent. They were mixed thoroughly and allowed to stand for 10 minutes at 25^{o}C. The mixture was then centrifuged for 15 minutes at 3500rpm. The supernatant was collected and its cholesterol concentration determined within an hour of centrifugation.

Distilled water of about 50ml was pipetted into test tubes labelled RB (Reagent Blank). This was followed by 50ml each of the standard solution and the supernatant which was pipetted into test tubes labelled STD (Standard) and SNT (Supernatant), respectively. Finally, 1000ml of the reagent solution was pipetted into all the different test tubes (Reagent Blank, Standard, and Supernatant).

They were mixed and incubated for 10 minutes at 25^{o}C. After incubation, the absorbance of the sample (Asample) and standard (Astandard) against the reagent blank was measured at 500nm wavelength.

Determination of Triacylglycerol concentration

To assay for serum triacylglycerol, test tubes were set accordingly: RB (Reagent Blank), STD (Standard), and SAM (Sample). Ten micro-litres each of the sample and standard were pipetted into test tubes labelled SAM and STD, respectively. Later, 1000ml of the reagent was pipetted into all the test tubes: Reagent Blank, Standard, and Sample. The test tube contents were mixed thoroughly and incubated for 10 minutes at 25^{o}C.

The absorbance of the Sample (Asample) and Standard (Astandard) were measured against the reagent blank at 500nm wavelength.

PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical analysis involved tests for the presence or absence of the following constituents: alkaloids, acidity, carbohydrates, fats and oil, proteins, glycosides, reducing sugar, flavonoids, terpenoids, steroids, resins, tannins, and saponins.

Test for Alkaloids (General Tests)

About 2ml of 5% tetraoxosulphate (iv) acid in 50% ethanol was added to 5ml of the methanol and water extracts, respectively. The different mixtures were brought to heat on a boiling water bath for 10 minutes. They were cooled and filtered.

To 2ml of each filtrate was added few drops of:

Mayer's Reagent (Potassium mercuric iodide solution)

Dragendorff's Reagent (Bismuth potassium iodide solution)

Wagner's Reagent (Iodine in potassium iodide solution)

Picric acid solution (1%)

The remaining filtrates were placed separately in 100ml separator funnels and made alkaline with dilute ammonia solution. The aqueous alkaline solution, of each extract, was separated and extracted with two 5ml portions of dilute sulphuric acid. Both extracts were tested with a few drops of Mayer's, Wagner's, and Dragendorff's reagents. Alkaloids showed up as milky precipitate with one drop of Mayer's reagent and a reddish brown precipitate with one drop of Wagner's reagent.

Test for Acidity

About 0.1g of both extracts were placed in clean dry test tube and sufficient water poured into the mixtures. The mixtures were warmed in a hot water bath and allowed to cool. A wet blue litmus paper was dipped into each of the mixture and the colour change observed. A colour change to red indicated acidity.

Test for Carbohydrate (Molisch's Test)

One gram of each extract (methanol and water extracts) was boiled with 2ml of distilled water and then filtered. Concentrated sulphuric acid

was gently poured down the sides of each test tube to form a lower layer. A purple interface of ring indicated the presence of carbohydrates.

Test for Fats and Oils

One gram of each extract was pressed between a clean filter paper. The filter paper was observed for translucency which indicated the presence of oils in the extracts.

Test for Proteins (Million's Test)

Two drops of Million's Reagent were added, respectively, to both extracts in a test tube. The formation of white precipitate indicated the presence of proteins.

Test for Glycosides (Fehling's Test)

About 5ml of a mixture of equal parts of Fehling Solutions I and II were added to about 5ml of each extract and then heated on a water bath for 5 minutes. A brick red precipitate showed the presence of reducing sugar.

Test for Reducing Sugars (Fehling's Test)

About 1g of both extracts were shaken vigorously with 5ml of distilled water and later filtered. The filtrate was used for the Fehling's test.

Fehling's Test: To 1ml portion of the filtrate were added equal volumes of Fehling's Solutions I and II and boiled on a water bath for few minutes. A brick red precipitate indicated the presence of reducing sugars.

Test for Saponins (Fehling's Method)

About 20ml of water was poured into 0.25g of both extracts in a 100ml beaker and boiled gently on a hot water bath for 2 minutes. Both mixtures were respectively filtered out and allowed to cool. The filtrates were used for the Fehling's test. A reddish precipitate indicated the presence of Saponins.

Test for Tannins (Ferric Chloride Method)

One gram of both extracts was boiled respectively with 50ml of distilled water. Each was filtered and the filtrate used for the test. To about 3 ml of the respective filtrate, few drops of ferric chloride solution were added. A greenish black precipitate indicated the presence of tannins.

Test for Flavonoids (Ammonium Test Method)

About 10ml of ethylacetate were added to 0.2g of both extracts. Both mixtures were treated on a water bath for 3 minutes. Each mixture was cooled, filtered and the filtrate used for the ammonium test.

Ammonium Test: A quantity, 4ml of each of the filtrates were shaken with 1ml of ammonia solution. The layers were allowed to separate and the yellow colour in the ammoniacal layer indicated the presence of flavonoids.

Test for Resins (Precipitation Test)

About 0.2g of both extracts was washed with about 15ml of 95% ethanol and the mixture poured into 20ml distilled water in a beaker. The formation of a precipitate indicated the presence of resins.

Test for Steroids and Terpenoids

About 9ml of ethanol was poured into 1g of the extract. It was refluxed for a few minutes and then filtered. The filtrate was concentrated to 2.5ml on a boiling water bath and 5ml of hot water was added. The mixture was allowed to stand for 1 hour and the waxy matter filtered off. The filtrate was extracted with 2.5ml chloroform using a separating funnel.

Later 1ml of concentrated sulphuric acid was poured into about 0.5ml of the chloroform extract in a test tube. The appearance of a reddish-brown interface showed the presence of steroids.

Another 0.5ml of the chloroform extract was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10 minutes on a water bath. A grey colour indicated the presence of terpenoids.

CHAPTER FOUR

RESULTS

YIELD OF THE EXTRACTS

As shown in Table 3.1, extracting 500g of the stems of magani burantashi gave 34.32g of the ethanol extract and 25.73g of the aqueous extract. Their extractive yields are 18.28% w/w and 8.87% w/w respectively.

Table 3.1: Extract yield of ethanol extract and aqueous extract

Weight of plant material (g)	Total weight of extract (g)	Extractive yield (%w/w)
500	34.32 ethanol	18.28
500	25.73 aqueous	8.87

PHYTOCHEMICAL DATA

Phytochemical analysis showed that the aqueous extract tested positive to carbohydrates, proteins, glycosides, reducing sugars, flavonoids, terpenoids, steroids, resins, tannins, and saponins while the ethanol extract gave positive reactions to alkaloids, carbohydrates, glycosides, reducing sugars, flavonoids, terpenoids, steroids, resins, and tannins (Table 3.2).

Table 3.2: Phytochemical properties of extracts

Constituents	Aqueous extract	Methanol extract
Alkaloids	-	++
Carbohydrates	+++	+++
Fats and Oils	-	-
Proteins	++	-
Glycosides	++	+++
Reducing Sugar	++	++
Flavonoids	+++	++
Terpenoids	+	++
Steroids	++	++
Resins	+++	+++
Tannins	++	++
Saponins	+	-

Effect of extracts on cholesterol level of rats

Extract	Dose (mg/kg)	Cholesterol Concentration (mg/dl)			
		1	2	3	4
Ethanol	500	43.98 ± 1.01	35.19 ± 1.13	31.43 ± 2.14	25.63 ± 3.79
Aqueous	500	45.31 ± 1.32	40.35 ± 1.64	37.19 ± 2.44	32.86 ± 3.64
Control	100	41.76 ± 4.01	45.11 ± 3.25	46.03 ± 2.80	63.03 ± 1.39

Total cholesterol levels of rats treated with the ethanol extract were significantly ($P<0.05$) lower than those of the aqueous rats.

Effect of extracts on LDL level of rats

Extract	Dose (mg/kg)	Low Density Lipoprotein Concentration (mg/dl)			
		1	2	3	4
Ethanol	500	58.80 ± 1.31	56.76 ± 1.81	53.90 ± 2.98	51.57 ± 3.01
Aqueous	500	59.57 ± 1.75	58.20 ± 1.95	56.20 ± 2.00	53.83 ± 2.04
Control	100	65.14 ± 3.54	69.20 ± 3.05	71.70 ± 2.71	80.45 ± 2.03

The low density lipoprotein LDL levels of rats treated with the ethanol and aqueous extracts of magani burantashi stems were not-significantly ($P>0.05$) affected when compared to those of the control rats.

Effects of extracts on HDL of rats

Extract	Dose (mg/kg)	High Density Lipoprotein Concentration (mg/dl)			
		1	2	3	4
Ethanol	500	30.58 ± 3.01	44.17 ± 2.96	62.01 ± 2.21	68.41 ± 1.72
Aqueous	500	38.22 ± 2.98	47.81 ± 2.46	53.33 ± 1.89	57.71 ± 1.03
Control	100	52.80 ± 1.25	43.19 ± 1.95	34.49 ± 2.85	25.13 ± 3.47

HDL levels of rats treated with the ethanol extract did not significantly ($P>0.05$) increase more than those of the control rats.

Effect of extracts on triacylglycerol level on diabetic rats

Extract	Dose (mg/kg)	Triacylglycerol Concentration (mg/dl) in weeks			
		1	2	3	4
Ethanol	500	92.78 ± 1.48	88.64 ± 1.93	81.26 ± 2.86	74.38 ± 3.20
Aqueous	500	94.11 ± 1.63	90.76 ± 2.04	87.96 ± 2.81	85.10 ± 3.37
Control	100	94.43 ± 3.74	110.27 ± 3.44	119.87 ± 2.97	124.53 ± 2.41

Serum triacylglycerol TG level was significantly ($P<0.05$) increased in wistar rats compared to the control rats. Triacylglycerol levels of rats treated with both extracts decreased significantly ($P<0.05$) in the treated animals. The results show that both extracts have effects on the serum triacylglycerol level in wistar rats ($P<0.05$).

CHAPTER FIVE

DISCUSION

The magani burantashi extracted has long been used as an erectile enhancing compound hence the need to study it effect on the maintenance of the intensity of the heart. The blood lipoproteins are very vital in the causation of plagues which, when untreated causes CVDS (cardio vascular disorders). Any food or drugs that increase the deposition of such plagues increase the disposition to CVDS.

CONCLUSION

In this study, the ethanol extract of magni burantashi stems were seen to have significantly maintained the cholesterol level within range when compared with the rats given only sildenafil citrate. It implies that there is and no significant effect of Burantashi extracts on the cholesterol levels. Meanwhile when compared among the extracts, ethanol and aqueos extracts showed slight significant different.

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