EFFECT OF LAURIC ACID ON ERECTILE AND TESTICULAR DYSFUNCTION IN DIABETIC MALE WISTAR RATS

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIRMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN HUMAN PHYSIOLOGY.

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DECLARATION

I declare that, the work in this thesis entitled "**Effect of Lauric Acid on Erectile and Testicular Dysfunction in Diabetic Male Wistar Rats**" was carried out by me in the Department of Human Physiology, Ahamdu Bello University Zaria**.** The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Makinde Vincent, OLUBIYI ………………… ……………………..

Signature Date

CERTIFICATION

This thesis entitled **"Effect of Lauric Acid on Erectile and Testicular Dsyfunction in Diabetic Male Wistar Rats"** meets the regulations governing the award of the degree of Doctor of Philosophy of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literacy presentation.

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Chairman, Supervisory Committee

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Member, Supervisory Committee

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PROF. M. U. KAWU
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PROF. S.A. ABDULLAHI
Dean, School of Postgraduate Studies

DEDICATION

This thesis is dedicated to my Lord and Saviour Jesus Christ, and my family.

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ABSTRACT

Diabetes mellitus is a risk factor for reproductive and sexual dysfunction. Standard medications are not completely satisfactory, hence the consideration of natural products as alternative therapy. Lauric acid is the most abundant constituent of coconut oil, which is renowned for its therapeutic properties. This study sought to investigate the influence of lauric acid on testicular and erectile function in diabetic male Wistar rats. Seventy male rats (150-200 g) were divided into seven groups of ten each. Group 1 (NC): Normal control. Group 2 (DM-UT): Diabetic untreated. Group 3 (DM + LA 90): Diabetic treated with Lauric acid (90 mg/Kg). Group 4 (DM + LA 180): Diabetic treated with lauric acid (180 mg/Kg). Group 5 (DM + LA 360): Diabetic treated with lauric acid (360 mg/Kg). Group 6 (DM + CO): Diabetic treated with coconut oil (1.42ml/kg). Group 7 (DM + Sild): Diabetic treated with sildenafil (20 mg/Kg). Diabetes was induced by an intraperitoneal injection of streptozotocin (65 mg/Kg). Treatments were administered orally for four weeks after which the animals were euthanized. Gonadosomatic index, semen analysis, testicular histology and serum testosterone assay were then carried out. The corpus cavernosum was also extracted and contractile responses to phenylephrine and KCl; relaxant responses to acetylcholine and sodium nitroprusside (SNP); relaxation response to lauric acid in the presence of L-NAME; and contraction response to Ca^{2+} in the presence of lauric acid were evaluated. Gonadosomatic index in NC and DM + CO rats was significantly higher ($p < 0.05$) compared to the other groups. Serum testosterone in all groups were respectively lower ($p < 0.05$) compared to NC. Sperm count in NC and DM + CO rats respectively, were significantly higher ($p < 0.05$) compared to the other groups. Percentage of motile sperm cells in NC and DM + CO groups were significantly higher ($p < 0.05$) compared to the other groups: The percentage of progressively motile sperm

cells in all the groups were significantly lower ($p < 0.05$) compared to NC. The percentage of progressive motile sperm cells in DM + LA 90 and DM + CO were significantly higher ($p < 0.05$) compared to DM-UT. The percentage of normal sperm cells in NC rats was significantly higher ($p < 0.05$) compared to all the other treatment groups. The percentage of normal sperm cells in DM + LA 90, DM + LA 360, DM + CO and DM + Sild were significantly higher ($p < 0.05$) compared to DM-UT. The percentage of viable sperm cells in NC and DM + CO rats respectively, were significantly higher ($p < 0.05$) compared to other groups. Testicular histology showed degradation in DM-UT group and was considerably improved only in DM + LA 360 and DM + CO. Percentage contraction to KCl was significantly higher ($p < 0.05$) in DM-UT and DM + CO rats respectively compared to the NC rats. The percentage relaxation of phenylephrine pre-contracted tissues to acetylcholine was significantly higher ($p < 0.05$) in NC and DM + Sild rats compared to the other groups. The percentage relaxation of KCl pre-contracted tissues to acetylcholine was significantly higher ($p < 0.05$) in NC and DM + LA 90 rats compared to the other groups. The percentage relaxation of phenylephrine pre-contracted tissues to sodium nitroprusside (SNP) was significantly lower ($p < 0.05$) in DM-UT, DM + LA 360 and DM + CO rats compared to the NC rats. The percentage relaxation of KCl pre-contracted tissues to SNP was significantly lower ($p < 0.05$) only in DM-UT rats compared to the NC rats. The percentage relaxation of phenylephrine pre-contracted corpus cavernosal tissues to lauric acid was significantly higher ($p < 0.05$) in the presence of L-NAME compared to the control. The percentage relaxation of KCl pre-contracted cavernosal tissues to cumulative concentrations of lauric acid was significantly lower ($p < 0.05$) in the presence of L-NAME compared to the control. It was therefore concluded that, lauric acid rescued some but not all testicular dysfunctions in diabetic male rats. Lauric acid also improved erectile function in diabetic rats by improving the corpus cavernosum smooth muscle relaxation.

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ABBREVIATIONS

Ach	Acetylcholine
AGE	Advanced glycation end product
Ca^{2+}	Calcium ion
$CaCl_2$	Calcium chloride
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CO_2	Carbondioxide
DAG	Diacylglycerol
DHT	Dihydrotestosterone
DM + CO	Diabetic treated with coconut oil
DM + LA 360	Diabetic treated with lauric acid at 360 mg/Kg
DM + LA 90	Diabetic treated with lauric acid at 90 mg/Kg
DM + Sild	Diabetic treated with Sildenafil at 20mg/Kg
DM-UT + LA 180	Diabetic treated with lauric acid at 180 mg/Kg
DM-UT	Diabetic untreated
DNA	Deoxyribonucleic acid
ED	Erectile dysfunction
EGTA	N,N-ethylene glycol tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
GSI	Gonadosomatic index
GTP	Guanosine triphosphate
H and E	Hematoxylin and Eosin
HbA1c	Glycated haemoglobin
HDL	High Density Lipoprotein

HIV	Human Immunodeficieny Virus
IGT	Impaired Glucose Tolerance
IIEF	International Index of Erectile Function
IP_3	Inositol triphosphate
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
L2	Second lumbar vertebra
LH	Luteinizing hormone
L-NAME	N-nitro-L-arginine methyl ester
MCFA	Medium Chain Fatty Acids
$MgSO_4$	Magnesium sulfate
MLCK	Myosin light chain kinase
MLCP	Myosin light chain phosphatase
NaCl	Sodium chloride
$NaHCO_3$	Sodim bicarbonate
NANC	Non-adrenergic non-cholinergic nerves
NC	Normal control
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
O_2	Oxygen
ODT	Orally disintegrating tablet
PDE	Phosphodiesterase
PE	Phenylephrine
PGE1	Prostaglandin E1
PKG	Protein kinase G
S.E.M.	Standard error mean

S2	Second sacral vertebra
S4	Fourth sacral vertebra
SNP	Sodium nitroprusside
T9	Ninth thoracic vertebra
VED	Vasculogenic erectile dysfunction
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycaemia, caused by deficiency of insulin production by the pancreas or inefficient action of the insulin produced (WHO, 2019). Due to the chronic hyperglycaemia, diabetes is associated with a number of complications across the body systems such as retinopathy, neuropathy, nephropathy and cardiovascular dysfunction (Punthakee *et al.* 2018)

Diabetes mellitus has also been shown to have a negative impact on sexual and reproductive function. Diabetes has been associated with or is a risk factor for erectile dysfunction. Neuropathy and endothelial dysfunction associated with diabetes are implicated as the major causes of erectile dysfunction in diabetes (Eulises *et al.,* 2012). Other reproductive dysfunction associated with diabetes mellitus include: testicular damage, reduced testosterone level (Mohamed *et al*., 2018) and reduced semen and sperm quality (Zhu *et al*., 2017). It has also been reported that reduced testosterone levels due to diabetes can also lead to erectile dysfunction (Bokarica, 2015).

Erectile dysfunction (ED) is the continual incapacity to achieve and/or maintain sufficient penile erection necessary to complete a satisfactory sexual activity (Mcmahon, 2014). It is reported to affect about 3–76.5% men globally and this wide range is due to differences in geographical region and ages of the population study (Kessler *et al.,* 2019). The prevalence of erectile dysfunction among the male population in Nigeria was shown to be 58.9% (Oyelade *et al.,* 2016). Erection is triggered by tactile or psychic sexual stimulation, which causes impulses to be sent through the parasympathetic nervous system and the nitrergic nerves (non-adrenergic non-cholinergic nerve fibres) to the erectile tissue of the penis. The parasympathetic nerve fibres release acetylcholine, which acts on the endothelium of the corpus cavernosum and penile vessels to release nitric oxide (NO). The nitrergic nerves directly release NO (Sáenz de Tejada, 2002). The NO causes an increase in the production of cGMP, which stimulates a cascade of other processes that eventually lead to the relaxation of the smooth muscles of the corpus cavernosum and dilation of the penile arteries, thus leading to erection (Hidalgo-Tamola & Chitaley, 2009). Erection is terminated by the reduction in the parasympathetic impulses and release of norephinephrine by the sympathetic nerves, causing contraction and detumescence, returning the penis to the flaccid state. (Hidalgo-Tamola & Chitaley, 2009).

Contraction of smooth muscles is generally controlled by Ca^{2+} sensitivity. Stimulation of adrenergic receptors found in penile tissues by norepinephrine causes the influx of Ca^{2+} into the smooth muscle cell, coupled with an increase in Ca^{2+} sensitisation. Inhibition of the above processes of contraction is a target of pharmacological intervention for erectile dysfunction. The level of cGMP is increased by the action of NO and has been shown to inhibit Ca^{2+} influx. This reduces contraction of the penile smooth muscles resulting in erection (Andersson, 2011).

Infertility has been defined as "the failure to achieve clinical pregnancy after 12 months or more of regular unprotected sexual intercourse" (WHO, 2020). Semen analysis is a common clinical practice in evaluating fertility in men. Insufficient concentration of sperm cells in the semen or impairment in the structure or function of the sperm cells usually indicates infertility (Sharma *et al*, 2021).

Sperm cells are produced and mature in the seminiferous tubules of the testes before being stored in the epididymis. Testosterone is produced mainly in the interstitial

(Leydig) cells of the testis (Durairajanayagam & Rengan, 2015). Testosterone facilitates spermatogenesis and also plays a great role in the regulation of male sexual and reproductive functions (Corona *et al.*, 2009). Diabetes has been associated with reduction in the production of sperm cells and testosterone, consequently leading to further impairment in sexual function (Ghanim *et al.*, 2018).

Sildenafil, which is a Phosphodiesterase 5 (PDE5) inhibitor, is commonly prescribed as an oral treatment for erectile dysfunction. However, it is associated with some side effects and shown not be effective in treating erectile dysfunction in diabetic patients (Güven, 2020)

Over the years, long before the advent of orthodox medicine, plant products have been used as therapy for various ailments. Plant products are more accessible, affordable and have fewer side effects compared to orthodox drugs. Therefore, scientific research is now being drawn to these therapeutic plant products and by-products in a bid to elucidate their mechanism of action. In addition, the active components of these plant products and their physiological roles are being investigated in a bid to create standard therapeutic products (Sharma *et al*, 2020).

Lauric acid is the major fatty acid in coconut oil, constituting about 50% of the total fatty acid composition and considering lauric acid makes up a large portion of the constituents of coconut oil, it is hypothesized to contribute greatly to the physiologic effects of coconut oil (Dayrit, 2015). Coconut oil, a common plant product locally consumed, is renowned throughout history for its medicinal and nutritional value. It has been shown to reduce oxidative stress, improve lipid profile, possess antidiabetic properties (Iranloye *et al.*, 2013), and improve testicular function and semen quality

(Ogedengbe *et al*., 2016). However, there is dearth of scientific information available about the effect of lauric acid on male sexual and reproductive functions.

1.1 Statement of the Research Problem

Diabetes mellitus is one of the major global health challenges affecting about 422 million adults globally and is estimated to double by 2025 with greater threat to developing and underdeveloped countries (WHO, 2016).

Diabetes mellitus is a risk factor for sexual and reproductive dysfunction. Diabetes has been shown to cause impairment in semen and sperm quality, testicular dysfunction, reduced testosterone synthesis, degeneration of spermatogenic cells and consequently infertility in male diabetics (Jangir and Jain, 2014)

Diabetes is also linked to erectile dysfunction (Asafu-Adjei and Gittens, 2020). Erectile dysfunction has also been shown to be more prevalent among diabetic patients than the general population. In Africa, about 71% of diabetics are affected with erectile dysfunction (Shiferaw *et al*., 2020). In addition, according to a study carried out by Ugwu *et al*., (2016) in Ile Ife, Nigeria, about half of the diabetic population in Ile Ife were affected with erectile dysfunction.

Infertility has been reported to affect 48 million couples and 186 million persons worldwide (WHO, 2020). Although largely overlooked, 20-30% of the cases of infertility in couples is attributed solely to the male factor (Babakhanzadeh *et al*., 2020)

Erectile dysfunction has a prevalence of about 152 million globally and is projected to be almost double by 2025 (Masuku *et al*., 2020a). Studies by Adebusoye *et al* (2012) and Oyelade *et al* (2016) reported a prevalence of about 55.1% and 58.9%, respectively among the adult population in Nigeria.

The use of standard drugs like Sildenafil for the treatment of erectile dysfunction has been reported to have side effects like headache, flushing and visual impairment (Masuku *et al*., 2020b). Also, diabetics with erectile dysfunction have been reported to exhibit resistance to these orthodox drugs (Phé & Rouprêt, 2012). In addition, although there are conflicting reports on the impact of sildenafil on fertility, it was shown to have a negative impact on sperm count and sperm morphology (Al-fartosi, 2009), cause premature acrosome reaction (Glenn *et al*., 2007) and reduce sperm motility (Sousa *et al*., 2014).

1.2 Justification

The prevalence of diabetes has been shown to be on the rise even among the younger population (Lascar *et al*., 2018). There have been reports showing the association between diabetes and the rise in the prevalence of infertility among young men in their active reproductive age (Rato *et al*., 2019). Capogrosso *et al*., (2013) reported that, about 25% of men under the age of 40 were affected with erectile dysfunction. These statistics suggest a threat to sexual and reproductive health not just among the aged but even the young and sexually active population.

Medical research is recently being focused on plant products to evaluate their possible therapeutic effect in various disease conditions (Kamisah, *et al*., 2016). These plant products can also serve as nutritional remedies, which are well tolerated by patients (Cicero and Colletti, 2015). Extracts from plants offer a cheaper, safer, affordable and readily affordable alternative therapy, and studies are being carried out on the physiologic activities of their active constituents in a bid to create standard therapeutic products (Modak *et al*., 2007).

Lauric acid makes up a large portion of the constituents of coconut oil, which is largely consumed in the tropics. Coconut oil has been reported to have anti-diabetic properties (Iranloye *et al.*, 2013), and ameliorative effect on testicular dysfunction (Ogedengbe *et al.*, 2016). Majority of the physiologic properties of coconut oil have been hypothetically attributed to lauric acid (Dayrit, 2015).

Lauric acid has also been specifically reported to have vaso-relaxant and antioxidant properties (Alves *et al.*, 2017) and these properties can favour amelioration of sexual dysfunction in diabetic conditions. However, to the best of my knowledge, there is a dearth of scientific information on the effects on erectile and testicular function in diabetic states.

1.3 Aim and Objectives

1.3.1 Aim of the study

The aim of this study was to investigate the effects of lauric acid on erectile and testicular function in diabetic male Wistar rats.

1.3.2 Objectives of the study

The specific objectives of the study were to evaluate the effect of lauric acid on the following parameters in diabetic male Wistar rats:

i. Gonadosomatic index
ii. Serum testosterone level
iii. Sperm parameters: sperm count, sperm motility, sperm viability and sperm morphology
iv. Histology of the testes
v. Adrenergic mediated contraction of the corpus cavernosal smooth muscle
vi. Depolarization-mediated contraction of the corpus cavernosal smooth muscle
vii. Endothelium-dependent relaxation of the corpus cavernosal smooth muscle
viii. Influence of nitric oxide in the relaxation of corpus cavernosum
ix. Calcium sensitivity in corpus cavernosal smooth muscle

1.4 Research Hypothesis

Lauric acid has no effect on erectile and testicular functions in diabetic male rats

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Erectile Function

It has been an age-long believe that an erect penis symbolizes masculinity and bravery, thus, erectile dysfunction has a negative psychosocial effect on men and thus, negatively affects the quality of life (Moser, 2000)

In the course of history, a number of hypothesis have been made about the cause of erection of the penis; from assertions made by Hippocrates, the father of medicine (460 - 370BC), to Aristotle (384 – 322 BC) and a host of other medical scientists and philosophers in the middle ages (van Driel, 2015). It was not until around the 15th century that the penis was first comprehensively studied by Leonardo Da Vinci. He first made his observation from the penis of criminals executed by hanging (Belt, 1965). In addition, he had the privilege of thoroughly studying the penis of free cadavers at an infirmary in Florentine (Morris, 1986). He thus, made the novel discovery that erection is caused by the engorgement of the penis with blood. He proposed that the penis operated of its own free will; its owner cannot make it erect or limp at will and the penis erects liberally while its owner is sleeping. However, over the years, a substantial number of studies have shown that the penis does not as it were have its own mind but is controlled by the central nervous system (Goldstein, 2000).

Penile erection is the outcome of neurovascular and cavernosal smooth muscle processes with some psychological and hormonal influences. Any event that favours the relaxation and vasodilation over the contraction and vasoconstriction of smooth muscles of the cavernosum and the associated blood vessels favours erectile function (El-sakka & Lue, 2004).

2.1.1 Functional anatomy of erection

2.1.1.1 *Basic structure*

The penis is made up of three erectile tissues viz; a pair of corpus cavernosum (also referred to as corpora bodies) and an unsegmented corpus spongiosum. These erectile tissues make up the body of the penis are each bound by tunica albuginea and then all enveloped by layers of deep and superficial fascia and then the skin (Onofrei *et al.,* 2016)

The corpora cavernosa are positioned at the dorsal parts of the penile body. The partition between the copora bodies is incomplete; this ensures unanimity in their vascular activities. They are filled with sinusoids (vascular spaces) each bound by trabecular smooth muscles, they also contain the cavernous nerves and helicine arteries (Awad *et al*., 2011). The tunica albuginea of the corpus cavernosum is largely made up of collagen fibres and a little bit of elastic fibres (Bitsch, *et al.*, 1990). It has an an outer longitudinal and inner circular layer respectively (Andersson & Wagner, 1995). The corpora bodies are the chief erectile tissues involved in penile erection as it engulfs the deep penile artery. Dilation of this artery causes the engorgement of the sinusoids with blood occurs in response to sexual stimulus causing turgidity and erection of the penis (Awad *et al*., 2011).

The corpus spongiosum is found ventral to the corporal bodies. It houses the urethra which passes through it and enlarges at the proximal end and at the distal ends to form the urethral bulb and glans penis respectively. It has a similar functional structure to the copora bodies however it has a thinner tunica albuginea (Nunes & Webb, 2012), which contains larger sinusoids, is composed of majorly elastic fibres and has only one layer which is circular (Bitsch *et al*., 1990).

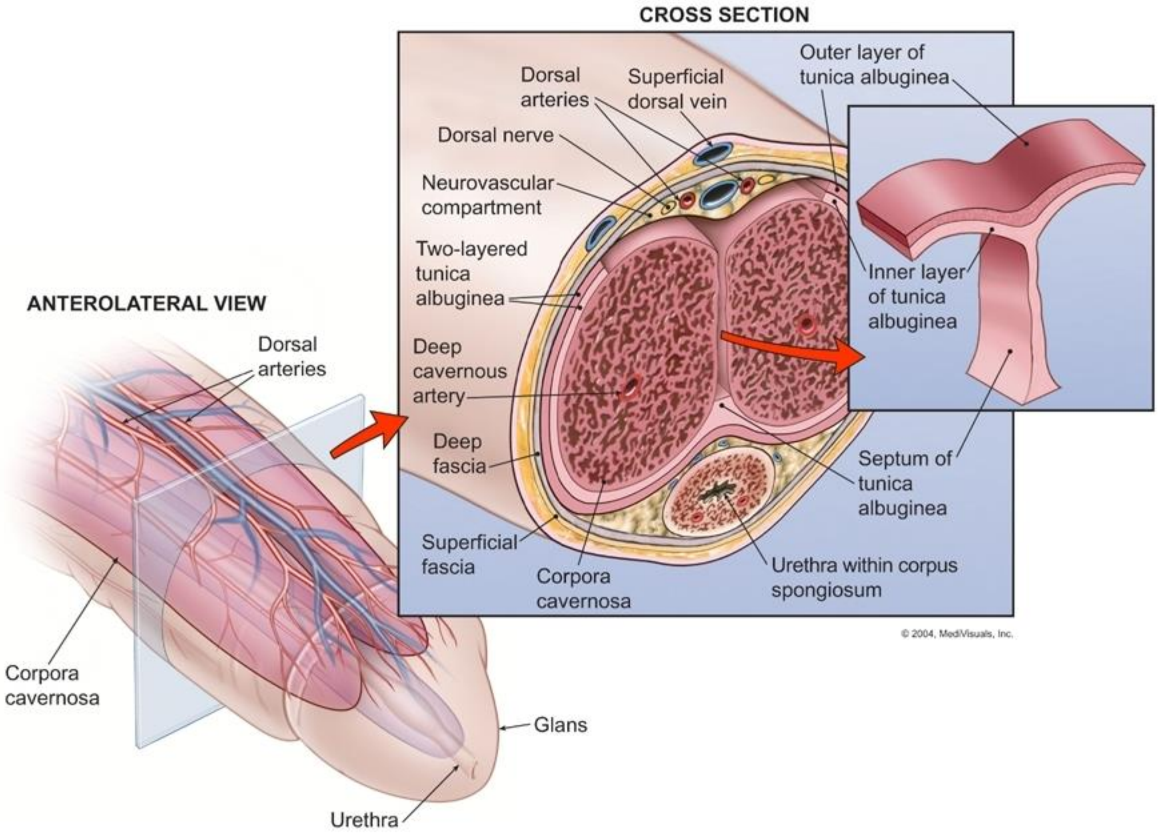

Figure 2.1: Cross section of the penis (MediVisuals Inc, 2009)

2.1.1.2 *Nervous supply*

The penis is innervated by autonomic (parasympathetic and sympathetic) and somatic nerves. The parasympathetic nerves start off as preganglionic fibres from S2, S3 and S4 section of the spinal cord, and run through the pelvic nerves to synapse in the pelvic plexuses (also called inferior hypogastric plexuses) which also contain the sympathetic nerves (El-sakka and Lue, 2004)

The sympathetic nerves start as preganglionic fibres from T9 – L2 segments of the spinal cord and then courses through to synapse in the sympathetic chain ganglia. Postganglionic fibres arise and travel to the superior hypogastric plexus and then travel to the pelvic plexus through the hypogastric nerves (Dean and Lue, 2005).

The cavernous nerves; which are made up of parasympathetic and sympathetic nerves, branch off the pelvic plexus to directly innervate the penis and influence flaccidity and erection (El-Sakaa and Lue, 2004).

The somatic pathway of erection is highlighted as follows: sexual sensation is picked up by free nerve endings and corpuscular receptors of the penis. This impulse is conveyed by the dorsal nerve of the penis to the pudendal nerve, which carries the impulses to the spinal cord at the level of S2-S4. Motor afferents course out from the spinal cord coursing through the pudendal nerves to act on the ischiocavernous and the bulbocavernosus muscles surrounding the penis (Auffenberg *et al.*, 2016).

2.1.1.3 *Blood supply*

The major arterial supply to the penis comes from the deep penile (cavernosal), bulbourethral and dorsal arteries which are all branches of the internal pudendal artery. The deep and dorsal arteries provide blood to the corpus cavernosum. The deep penile artery is embedded in and runs longitudinally and centrally along the length of the corpus cavernosum. It branches to form hecline arteries, which directly supply blood to the sinusoids, and this is important to the process of erection (Jung, *et al.,* 2014). The dorsal arteries are located below the deep fascia and run side by side with the dorsal nerves and the deep dorsal vein. It supplies blood to the bulb of the penis causing its enlargement during erection. The bulbourethral arteries supply blood to the corpus spongiosum and the urethra (Auffenberg *et al.*, 2016).

The human penile venous system consists of the superficial, intermediate and deep veins. The superficial veins are located dorsolaterally between the deep and superficial fascia of the penis. They drain blood from the penile skin and prepuce and merge to form the superficial dorsal vein, which then drains into the superficial external pudendal veins and then into the great saphenous veins (Ellsworth and Gest, 2015).

The intermediate system is located beneath the deep fascia and it is made up of the deep dorsal and circumflex veins. They receive blood from the glans, corpus spongiosum and a part of the corpus cavernosa. The circumflex veins are located laterally surrounding the coporal bodies. The deep dorsal vein is situated between the two corpora cavernosa. The emissary veins of the corpora cavernosa and the emissary veins drain into the deep dorsal vein and then into the prostatic plexus (Ellsworth and Gest, 2015).

2.1.2 Mechanisms of erection

2.1.2.1 *Vascular dynamics of erection*

In the flaccid state, blood flow to the penis is impeded by the tonically contracted smooth muscles of the corpus cavernosum and the blood vessels. This blood supply is inadequate to cause erection but just enough to cater for the basic nutritional and physiologic demands (Dean and Lue, 2005). Sympathetic impulses and vasoconstrictors (e.g. endothelin) released by the endothelium keep these smooth muscles tonically contracted (El-Sakaa and Lue, 2004).

For erection to occur, there is dilation of the penile arteries and arterioles, thus increasing blood flow. Increased blood supply to the sinusoids causes them to expand and blood is trapped in them. Expansion of the penile arteries and sinusoids; which squeeze the veins against the tunica albuginea coupled with the extension of the tunica to its limit causes the compression of the emissary veins between its outer and inner layers thus obstructing the flow of blood out of the veins. These results in increased intracavernosal pressure up to 100 mm Hg, causing the turdgidity and bulging of the penis and is referred to as the full erection phase. Contraction of the Ischiocavernous muscles further raises the intracavernous pressure beyond 100 mm Hg by a supplementary constriction of the cavernosal veins. This phase is termed the rigid erection phase (El-Sakaa and Lue, 2004).

In the process of erection, pressure in the corpus spongiosum and glans penis is about 30-50% of that in the corpus cavernosum even though they both have an increase in blood supply. This is due to the thinner or almost non-existent tunica albuginea surrounding the spongiousm and glans, respectively, resulting in an incomplete occlusion of the penile veins. The corpus spongiosum thus, contribute very little to erection and serves as an arteriovenous shunt especially during the full erection phase. However, just like in the corpus cavernosum, during the rigid erection phase, the ischiocavernosus and bulbocavernosus muscles compact the spongiosum and the veins thus, causing increased tumescence and pressure in the spongiosum and glans (Dean and Lue, 2005).

After the process of erection, the penis returns to the flaccid state and this is referred to as detumescence. First, there is a brief rise in intracorporeal pressure. This is due to initiation of contraction of the smooth muscles, while the veins are still closed. Thereafter, the veins are gradually being reopened, thus there is a gradual decline in intracorporal pressure. At the last phase of detumescence, the veins are fully opened and there is maximum outflow thus, a rapid decrease in pressure thereby completely terminating erection (El-Sakaa and Lue, 2004).

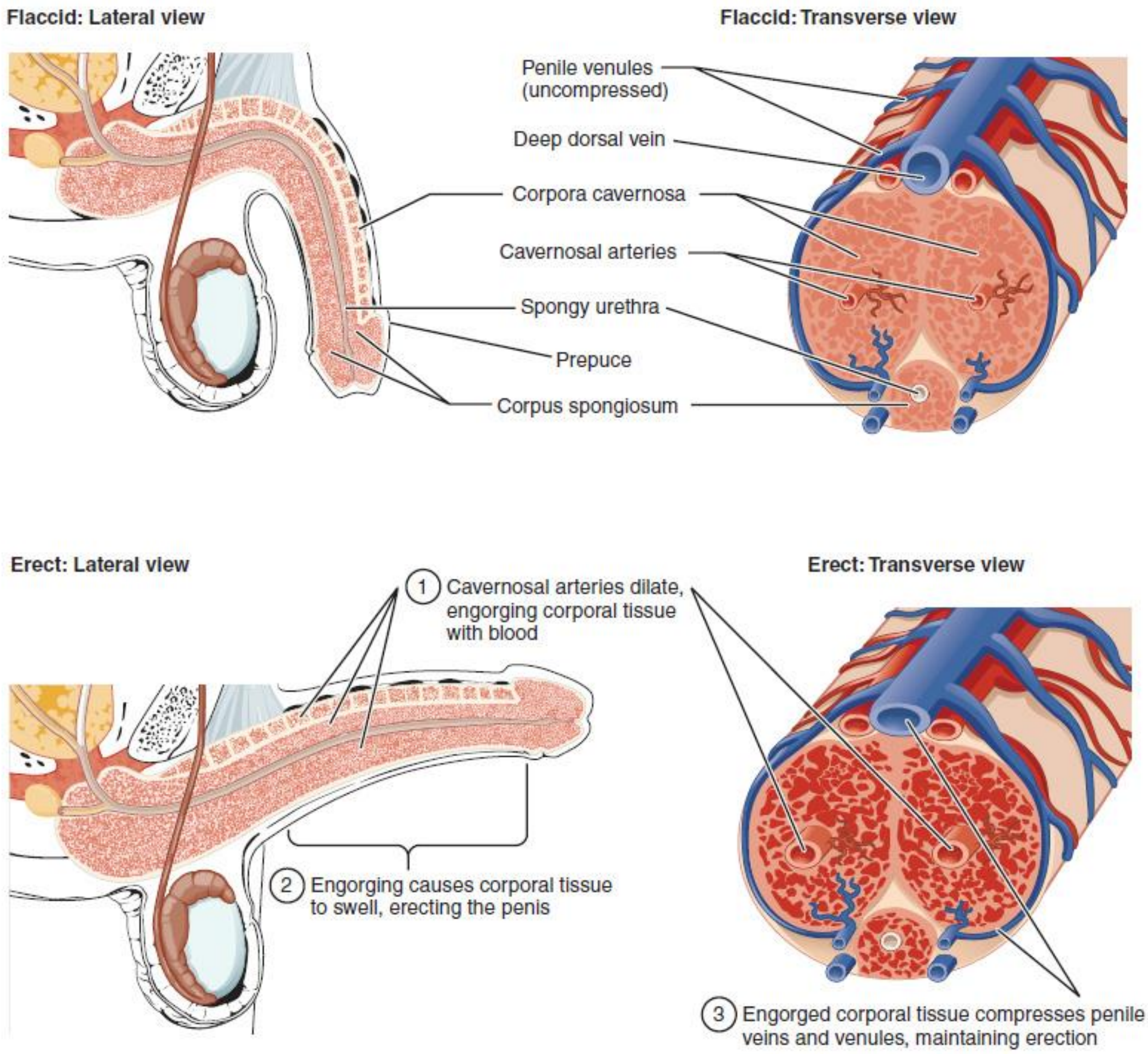

Figure 2.2: Illustration of the erectile tissues of the penis during the flaccid and erect phase (Douglas College Human Anatomy & Physiology II, 2017)

2.1.2.2 Neurophysiology of erection

In the flaccid penis, sympathetic nerve impulses cause a tonic contraction of the smooth muscles. Sexual stimuli excites the parasympathetic and non-adrenergic non-cholinergic nerves (NANC), causing the release of acetylcholine and nitric oxide, respectively. Also, stimulation of the parasympathetic nervous system causes the suppression of the release of norepinephrine from the sympathetic nerves eventually leading to cavernosal relaxation and vasodilation of the penile arteries and arterioles thus, leading to erection. Decrease in parasympathetic impulses leads to reduced suppression of sympathetic nerve impulses which then causes an increased production of norepinephrine which then causes the contraction of smooth muscles of the penis, thus bringing about detumescence (Nunes & Webb, 2012).

The role of the central nervous system in the regulation of erection is complex and even with appreciable progress in various scientific studies, it yet to be fully elucidated. The influence of the central nervous system involves spinal cord and supra-spinal centres (Andersson, 2011)

Involvement of the supra-spinal centres is not well understood however, the limbic system and the hypothalamus have been shown to play a major role. The type of erection that originates from the supra-spinal centres is referred to as psychogenic erection and it is kick-started by psychic, visual, olfactory and tactile stimulus (Melis & Argiolas, 2011).

The sacral segments of the spinal cord receive and processes inputs from the supra-spinal centres and the periphery. The spinal cord receives afferents from the penis after a direct tactile stimulation of the penis. Efferent from the sacral spinal cord segments travels via parasympathetic nerves to cause erection. This sacral spinal reflex leads to erection and it is referred to as reflexive erection. Reflexive erection can occur even without supra-spinal inputs (Auffenberg *et al.*, 2016).

The spinal cord also serves as the link between the supraspinal centres and the periphery in the local initiation of the erection in the penis. The sacral spinal cord is important in the processesing of psychogenic stimuli from the higher centres. In fact, psychogenic erection is greatly impeded without the sacral segments of the spinal cord (Auffenberg *et al.,* 2016).

2.1.2.3 *Cellular and molecular mechanisms of erection*

The flaccidity of the penis is caused and sustained by norepinephrine released by the sympathetic nerves and also by vasoconstrictor substances produced by the endothelium of the smooth muscles of the penis e.g. endothelin, and prostaglandin $F_{2\alpha}$, etc (Andersson, 2011). These substances bind to their appropriate receptors which are attached to G-proteins. These G-proteins consequently stimulate the synthesis of inositol triphosphate (IP_3) and diacylglycerol (DAG). The IP_3 acts on the endoplasmic reticulum to initiate the opening of Ca^{2+} stores within the cell, while DAG activates protein kinase C which now causes the opening of Calcium channels in the cell membrane allowing the influx of Ca^{2+} into the cell. Thus IP_3 and DAG work together to cause a rise in intracellular Ca^{2+} which then attaches to calmodulin and then activate myosin light chain kinase. Myosin light chain kinase (MLCK) then causes smooth muscle contraction by phosphorylation of myosin light chains (Nunes and Webb, 2012).

Binding of the vasodilator substances with G-coupled receptors also cause smooth muscle contraction through the RhoA/Rho-kinase pathway. The RhoA/Rho-kinase pathway largely serves to sustain smooth muscle contraction after the transient increase in intracellular Ca^{2+} caused by IP_3 and DAG. RhoA/Rho-kinase pathway acts by increasing Ca^{2+} sensitization i.e. it sustains contraction without an increase in the concentration of intracellular Ca^{2+}. The membrane G-proteins activates RhoA. The RhoA then stimulates Rho-kinase which impedes the activity of Myosin light chain phosphatase (MLCP), thereby inhibiting the desphosphorylation of the myosin light chains, thus delaying the termination of contraction (Hirano, 2007).

Locally, erection is brought about by biomolecules released by the parasympathetic nerves, non-adrenergic non-cholinergic nerves (NANC) and the endothelium. The parasympathetic nerves in the penis release acetylcholine, which acts on the muscarinic receptors present in the endothelium. This leads to the stimulation of endothelial nitric oxide synthase (eNOS) and invariably nitric oxide (NO) synthesis. The NO is synthesized in the NANC by the action of neuronal nitric oxide synthase (nNOS). This NO is released directly by NANC to the smooth muscle of the penis (Nunes and Webb, 2012).

The NO is the principal vasodilator involved in the process of erection. In other words, the whole process of erectile mainly depends on the release of NO (El-Wakeel *et al*., 2020). The NO activates soluble guanylyl-cyclase in the cavernosal smooth muscle cells translating guanosine triphosphate (GTP) to cGMP (Linder *et al*., 2006). The cGMP acts on protein kinase G (PKG), which acts through cascade of mechanisms to block cell membrane calcium channels so as to prevent the entry of Ca^{2+} into the cell, open up potassium channels and promote uptake of cystolic Ca^{2+} into the intracellular Ca^{2+} stores. Thus, there is decreased intracellular Ca^{2+} thereby inhibiting the contraction of the smooth muscles due to disabling of the calcium-calmodulin complex. The opening and closing of potassium and calcium channels, respectively, also leads to hyperpolarization resulting in relaxation of the penile smooth muscles (Christ and Lue, 2004).

Another vasodilator of importance is prostaglandin E1 (PGE1). It also serves to decrease intracellular Ca^{2+}, however, unlike NO its action is mediated by protein kinase A (Alexandre *et al.*, 2007).

For detumescence to occur, cGMP and cAMP, which are largely produced during the process of erection is hydrolyzed by phosphodiesterase (PDE). The specific type of PDE largely found in the corpus cavernosum is PDE5. Reduction in the bioavailability of cGMP by PDE5 in the corpus cavernosum thus, reverses the process of erection by causing a rise cystolic Ca^{2+} and invariably leads to contraction of the smooth muscles of the penis and surrounding blood vessels. This returns the penis to the state of flaccidity (Ignarro *et al*., 1990).

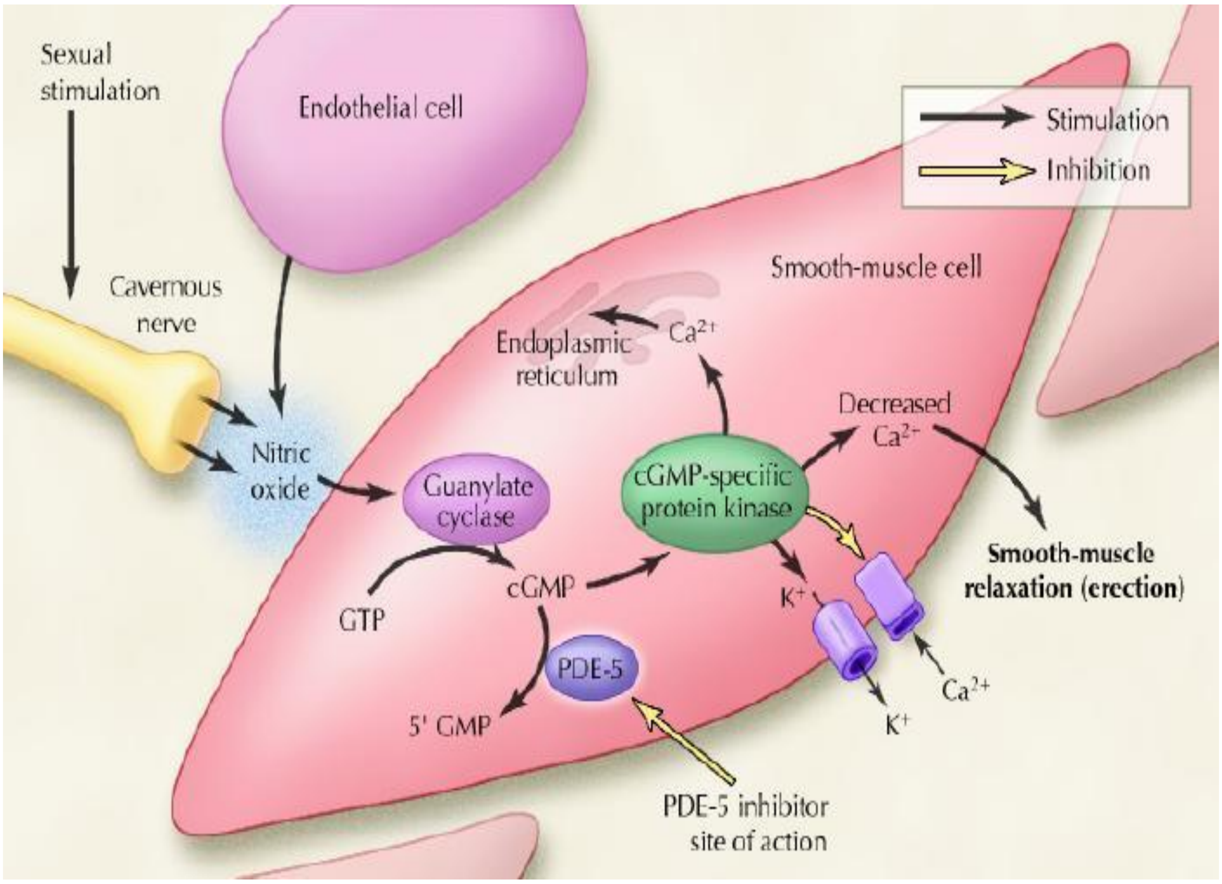

Figure 2.3: Cellular mechanism of erection (Fazio & Brock, 2004)

2.1.3 Erectile dysfunction

Erectile dysfunction (ED) is the failure to get or sustain an erection needed for a pleasing sexual intercourse. Naturally it becomes more severe with age (El-Wakeel, *et al.,* 2020). Because of the cultural stigma, ego and also the ignorance that ED is not just a consequence of aging but it can be an associated disorder of cardiovascular and metabolic diseases, most cases of ED are not reported to the physicians for appropriate treatment (Oyelade *et al.*, 2016)

Erectile dysfunction is a disease of multifaceted and complex aetiology. Considering erectile function is hinged on neurovascular events with the modulatory inputs of psychological and endocrine system, medical conditions that impair these systems or processes are said to be risk factors for erectile dysfunction. Therefore, it is not uncommon for erectile dysfunction to be recorded among the large population of aged men due to the general decline in body function associated with aging (El-Wakeel *et al*., 2020).

2.1.3.1 *Classification of the Causes of Erectile Dysfunction*

Earlier, the aetiology of erectile dysfunction was believed to be solely psychological (psychogenic), but overtime physiological causes have been shown to be responsible for 80% of the cases of erectile dysfunction. Physiological causes, also referred to as organic can be classified as vasculogenic, neurogenic, anatomical, hormonal, and iatrogenic (drug induced and surgery). Interestingly, there is a mixed aetiology for a number of cases of erectile dysfunction in that they fall both into the organic and the psychogenic category (Gratzke *et al*., 2010)

Table 2.1: Classification of the Causes of Erectile Dysfunction (Yafi *et al*., 2016)

Class	Causal factors
Psychogenic	Performance anxiety, depression, fatigue and stress
Neurogenic	Spinal cord injury, multiple sclerosis, Parkison's disease, diabetic neuropathy.
Endocrinologic	Hypogonadism (low testosterone level), hyperprolactinoma
Vasculogenic	Diabetes, hypertension, atherosclerosis, hyperlipidaemia
Iatrogenic	Radical pelvic surgery, antihypertensives (thiazide diuretics and β-blockers), antidepressants, anti-ulcer drugs, opiates and digoxin
Other causes	Alcohol, tobacco

2.1.3.2 *Diagnosis and assessment of erectile dysfunction*

Erectile dysfunction is indicated in a patient by a persistent difficulty in erection for at least a period of 3 months (Hatzimouratidis, 2007). Erectile function can be evaluated experimentally by the use of kits that monitor the turgidity of the penis following sexual stimulation or administered substance. However, this method presents with some difficulties and limitations (Snow, 2006)

The perception and experience of erectile dysfunction is relative and subjective from patient to patient, thus getting the standard measurement or evaluation of erectile dysfunction can be challenging. Clinically, the use of questionnaires has been adopted in the evaluation of erectile function. Although there are many questionnaires that can be used, that proposed by the International Index of Erectile Function (IIEF) is mostly validated and globally accepted in the evaluation of erectile dysfunction. The IIEF scores determine the severity of erectile dysfunction. A score of 1–7 indicates severe, 8–11 moderate, 12–16 mild–moderate, 17–21 mild and 22–25 no erectile dysfunction (Yafi *et al*., 2016)

2.1.3.2 *Available treatments for erectile dysfunction*

Oral drugs are the first-line of treatment for erectile dysfunction and the most commonly used drugs are Phosphodiesterase 5 (PDE5) inhibitors (Lue, 2000). This is because they are easy to use and patients generally report a favourable response (Snow, 2006). The PDE5 breaks down cGMP, which is involved in vasodilation, and relaxation of the smooth muscles of the corpus cavernosum, thus inhibition of PDE5 favours erection (Lue, 2000). Thus, PDE5 inhibitors are specific for vasculogenic erectile dysfunction (VED) (Jeremy *et al*., 2000). A classic example of PDE5 inhibitor is Sildenafil, which was the first prescribed PDE5 inhibitor for erectile dysfunction (Goldstein *et al*., 1998), and it has been proven an effective oral pharmacotherapy cut across patients suffering from erectile dysfunction. The onset of action of Sildenafil is about 30-60 minutes, thus it is recommended to be taken one hour before sexual activity. Due to its half-life it has a long duration of action of 4-5 hours after administration and can be up to 12 hour in some patients (Hatzimouratidis, 2006). For the sake of patients who have difficulty taking solid drugs, sildenafil was manufactured in the form of sildenafil citrate. Sildenafil is an orally disintegrating tablet (ODT) (Bokarica, 2015).

Other treatments available include:

1) Vacuum erection devices (less satisfaction rates)
2) Intra-urethral suppositories (less effective)
3) Intra-cavernosal injection therapy (Painful)
4) Penile implant surgery (last resort) (Penson & Wessells, 2004)

2.1.3.2 *Limitations of Phosphodiesterase 5 (PDE5) inhibitors*

Even though PDE5 inhibitors like sildenafil have been proven to improve erectile function, they do not particularly cure erectile dysfunction but only provide a temporary relief to the symptoms. PDE5 inhibitors fail to deal with the root cause of the disorder as seen for example in diabetes induced erectile dysfunction. Thus, the long term efficacy of PDE5 inhibitors cannot be guaranteed (Jeremy *et al*., 2000). The use of PDE5 inhibitors is also associated with some common side effects like: headache, flushing, impaired vision, indigestion, nasal congestion, light-headedness and palpitation. Thus, there may be some long term risks linked with the frequent usage of PDE5 inhibitors (Hatzimouratidis, 2006)

2.2 Testicular Function

The testes play a huge role in male reproductive and sexual function. The testes are the site of the production of sperm and androgens. The production of sperm is referred to as spermatogenesis, while the production of androgens is known as steroidogenesis (Weinbauer *et al*., 2010).

2.2.1 Structure and function of the testis

The testis is oval in shape, about 5 cm in longitudinal length and has a volume of about 20 ml in the average young male. Three layers cover the testis namely: the tunica vaginalis, tunica albuginea, and the tunica vasculosa (Costabile, 2013). An offshoot of the tunica albuginea referred to as septa divides the testes into about 300 lobules. Each lobule contains about 1-3 coiled tubules referred to as seminiferous tubules which makes up about 70% of the entire volume of the testis. The seminiferous tubules contain germ cells and other supporting cells called like Sertoli cells and peritubular cells (Weinbauer *et al.*, 2010).

Spermatogenesis occurs in the seminiferous tubules and it is regulated by follicle stimulating hormone (FSH) and testosterone. Interestingly, the receptors for FSH and testosterone are not found on the male germ cells but on the sertoli cells that are proposed to mediate the actions of these hormones in stimulating the process of spermatogenesis (Costabile, 2013).

Apart from the tubular component of the testes which is made up of the seminiferous tubules, the testes also has an interstitial component which is the interstitium surrounding and between the seminiferous tubules. The interstitium represents about 14% of the entire volume of the testes. The most important cells in the interstitium are the Leydig cells. The testis contains about 200 million Leydig cells that accounts for about 15% of the content of the interstitial compartment. The leydig cells occupy a small portion of the testes yet produces testosterone, which is the major androgenic hormone. The function of the Leydig cell is regulated by luteinizing hormone (LH) produced by the anterior pituitary gland (Weinbauer *et al.*, 2010).

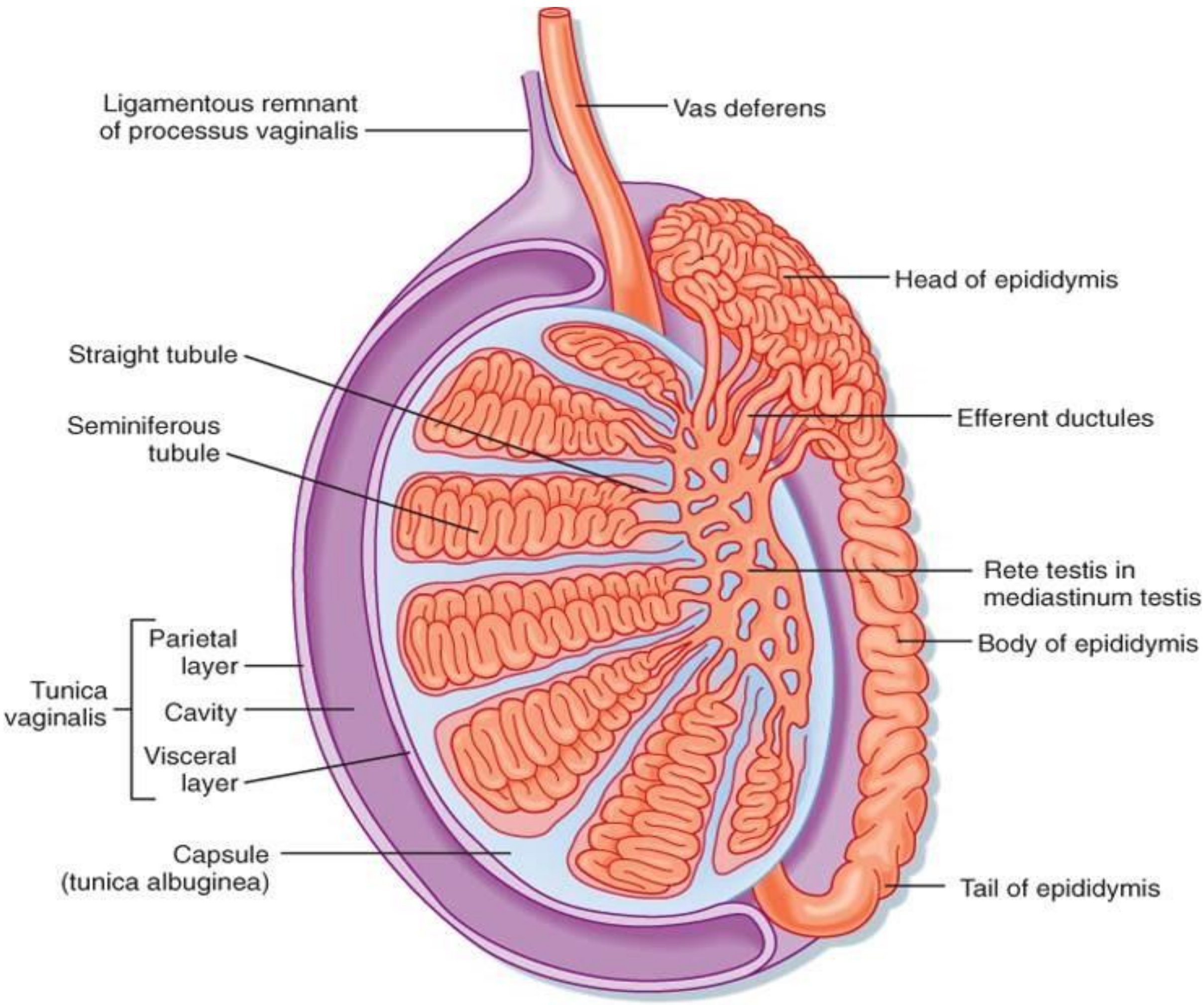

Figure 2.4: A cross section of the Testis (Koeppen and Stanton, 2008)

2.3 Testosterone

Testosterone was discovered by Arnold Berthold in 1849. He found out that by the removal of the testes in the experimental animals, male secondary sexual characteristics and aggressive behaviour were stifled. Replacing the testes in the male experimental animals restored the above-mentioned characteristics. The androgenic substance being produced by the testes thus became the centre of attention and was found out to be testosterone (Bird & Zilioli, 2018)

2.3.1 Synthesis and secretion of testosterone

In intra-uterine life, testosterone is produced in the seventh week by the foetal Leydig cells. The secretion of testosterone peaks in the second trimester. The testosterone level remains low during childhood until puberty when it significantly increases, thus causing the various male sexual characteristics associated with the beginning of puberty (Douglas College Human Anatomy & Physiology II, 2017).

Unlike the female steroid hormones, testosterone is produced throughout the lifetime in men. However, after reaching its peak before the age of 40, the testosterone level in the circulation begin to wane (Nassar *et al*., 2020)

Production of testosterone takes place continuously in the Leydig cells located in the interstitium of the testes. There is a de-novo synthesis by the Leydig cells, as they cannot store testosterone. Each day, about 6-7 mg of testosterone is synthesized. Extra-testicular production of testosterone takes place in the adrenal glands, although in trace amounts. In females, testosterone is also produced in the ovaries (Weinbauer *et al.*, 2010).

Testosterone, being a steroid hormone, is produced from cholesterol. The source of the cholesterol is either from low-density lipoproteins (LDL) which moves into the Leydig cells or synthesized in the Leydig cells. The production of testosterone is an enzymatic process starting from the conversion of cholesterol to pregnenolone in the mitochondria. In the endoplasmic reticulum, the proceeding stages from pregnenolone production can continue in two pathways. Along the Δ4 pathway, pregnenolone is converted to progesterone while along the Δ5 pathway it is converted to 17-hydroxypregnenolone (Weinbauer *et al.*, 2010). From these intermediate substances, testosterone is formed by stages highlighted in figure 2.5.

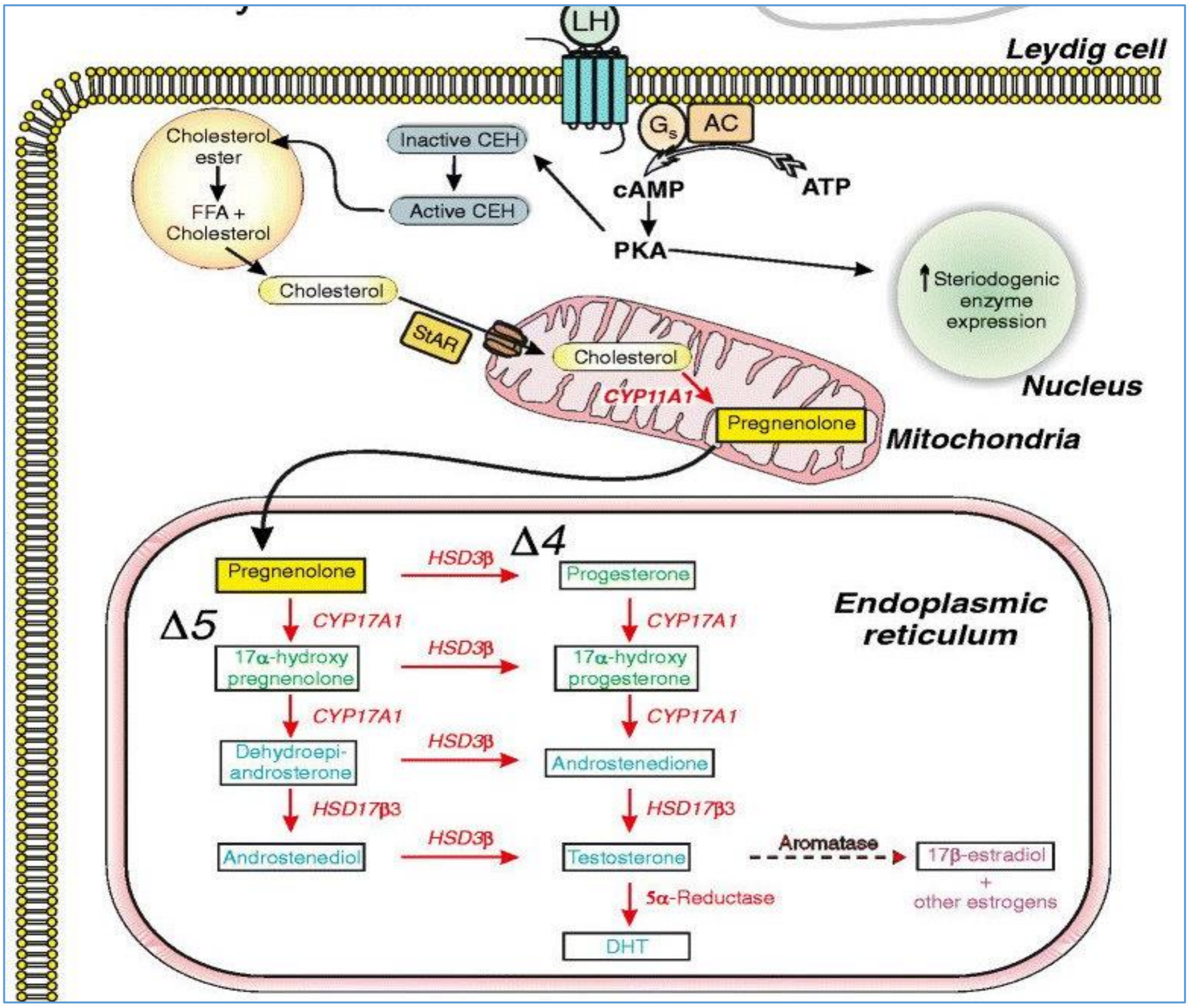

Figure 2.5: Pathway for the synthesis of Testosterone (Ayaz & Howlett, 2015)

2.3.2 Function of testosterone

In the intra-uterine life, testosterone causes sex differentiation in the foetus. This initiates the development of male sexual characteristics thus causing a distinction between the male and female foetus (Douglas College, 2017). In addition, at about the

7th month of gestation testosterone causes the descent of the testes as seen by the outward appearance of the scrotum. Non-descent of the testes after about 6 months of extra-uterine life can amended by the administration of testosterone (Nassar *et al*., 2020).

Testosterone plays a great role in male sexual and reproductive function. Testosterone stimulates penile and testicular development, spermatogenesis and increase in libido. The development of secondary male sexual characteristics like facial and pubic hair, receding hairline, deepening of the voice, broadening of the chest, etc. are also stimulated by testosterone. Testosterone is referred to as an anabolic hormone as it promotes protein synthesis. It is responsible for skeletal muscle growth and promotes erythropoiesis (Nassar et al., 2020).

When there is a reduction in the level of testosterone levels as seen in aged men (above 40 years), there is a decrease in the production of sperm cells. This is thus responsible for the higher rates of infertility in aged men (Douglas College, 2017). The clinical manifestations of the decline in testosterone level include reduced libido and general sexual function, reduced vigour, decreased bone and muscle thickness, depression and decline in cognition (Costabile, 2013).

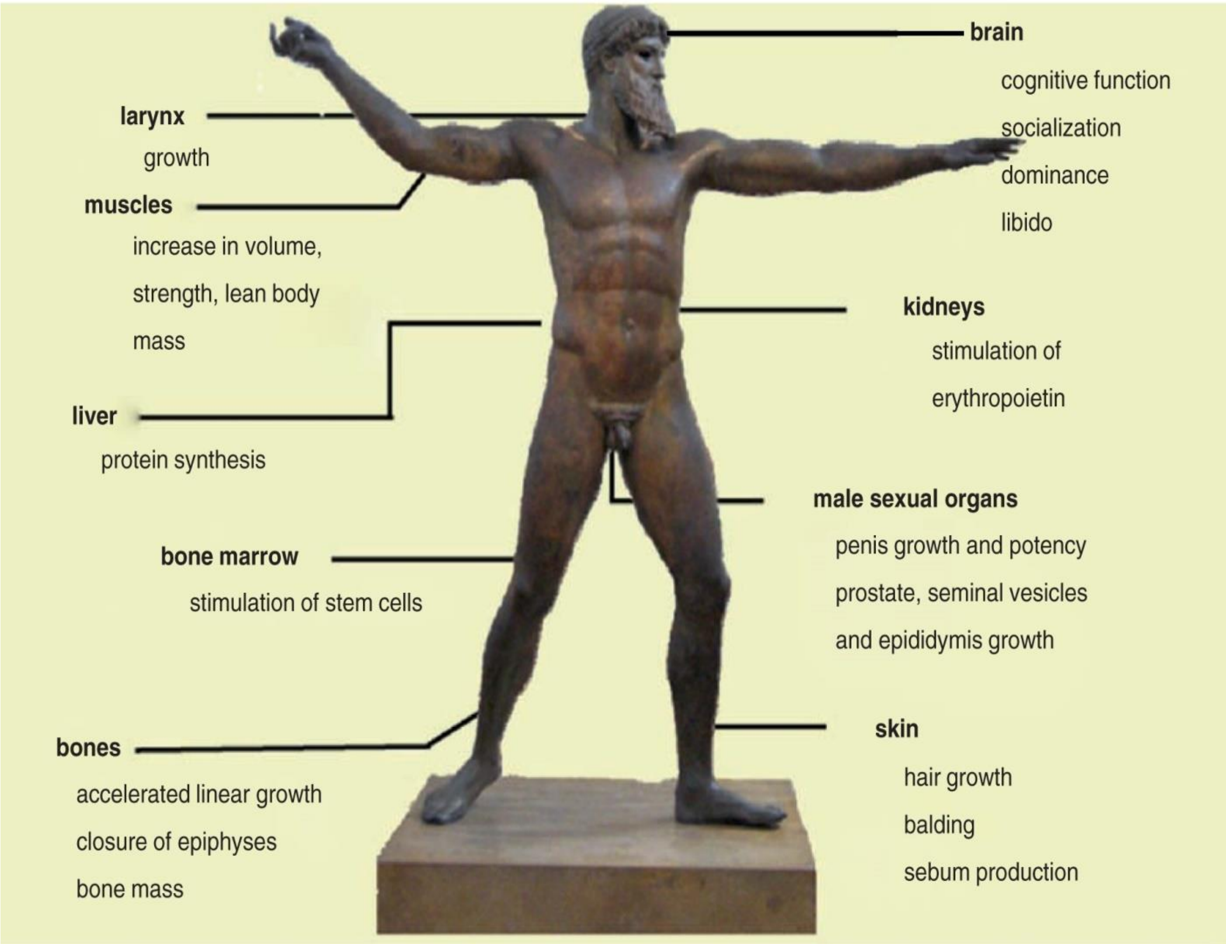

Figure 2.6: Actions of testosterone (Weinbauer *et al.,* 2010)

2.3.3 Mechanism of action of testosterone

Depending on the mechanism, the actions of testosterone on the target cells is classified as either genomic or non-genomic. Genomic actions of testosterone refer to its modulatory action on the transcription of genes. Testosterone being a steroid hormone passes freely across the cell membrane and first binds with androgenic receptors in the cytoplasm. The steroid-receptor complex now migrates to the nucleus and binds to DNA to either stimulate or inhibit gene transcription (Bird and Zilolli, 2018).

Non-genomic actions of testosterone on the other hand, only involves the binding of testosterone to receptors on the cell membrane. This of course takes a shorter durationcompared to the genomic mechanism of testosterone action. The effect of the genomic actions of testosterone occurs after 30 minutes and sometimes takes days, while it takes less than 30 minutes for the non-genomic mechanism.

In addition, within the target cell, testosterone can be acted upon by aromatase or 5-α-reductase and is then converted to oestradiol or dihydrotestosterone (DHT), respectively (Bird and Zilolli, 2018).

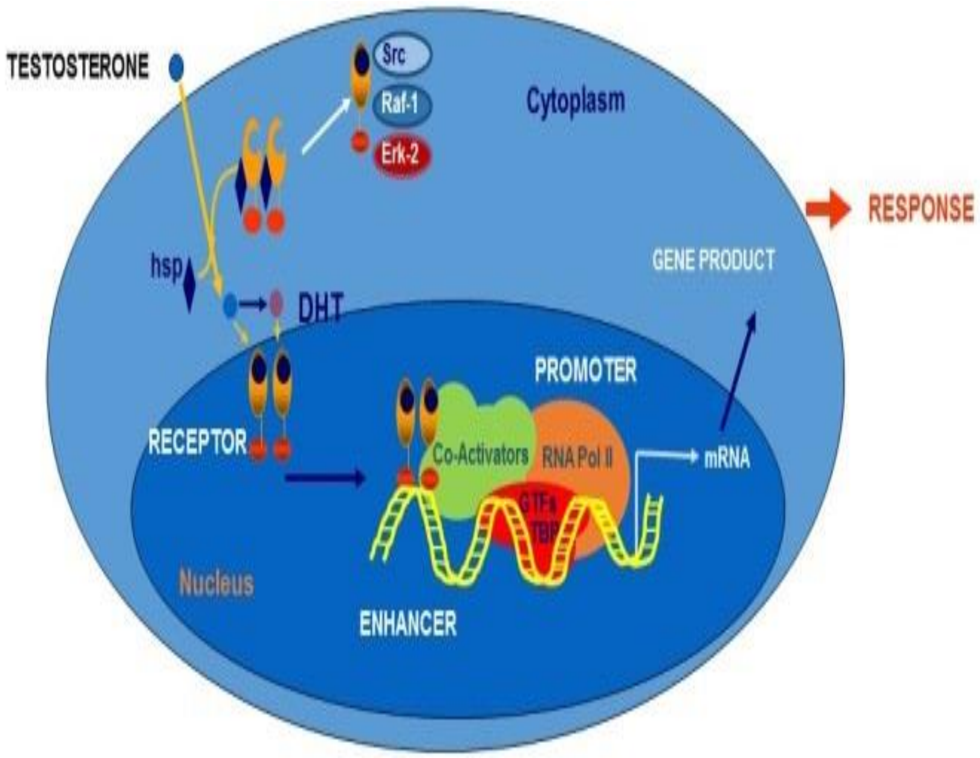

Figure 2.7: Mechanism of testosterone action in target cells (Mcewan & Brinkmann, 2020)

2.4 Sperm

Anton van Leeuwenhoek is credited with the first scientific information about the structure of the mature sperm. His finding was submitted in 1677 to the Royal Society of London. He described the spermatozoa as several *animacula* in the semen of animals. Follow up studies in the eighteenth and nineteenth centuries proved that the sperm cells were produced in the testes and are important to the process of fertilization (Chemes & Rawe, 2003).

A spermatozoon is about 45-50 μm long and it is made up of a head and tail. The head is egg-shaped with a length and width of about five micrometres and three micrometres, respectively. The head contains a nucleus, which occupies 65% of its content. The nucleus houses DNA, which contains 23 chromosomes with the Y chromosome inclusive. The head is thus, the most significant part of the male gamete in that it contains the male parent genetic material that binds with the female genetic material in the oocyte during fertilization to produce a diploid cell zygote (Esteves and Miyaoska, 2015).

The head also cell also contains acrosome which covers the anterior two-third. It is made up of the Golgi complex and contains hydrolytic enzymes. These hydrolytic enzymes are released during fertilization. The fusion of the membrane of the acrosome with the oocyte membrane causes the hydrolysing enzymes to be discharged from the acrosome. This process is referred to as acrosome reaction and it aids the breakdown of the oocyte membrane thereby facilitating the process of fertilization (Durairajanayagam *et al*., 2015).

The tail of the sperm cell is about ten times longer than the head and about 0.5μm in diameter. The tail is responsible for the motility of the sperm. It consists of three parts namely: mid piece, principal portion, and the end piece. The mid piece is the thickest part of the tail. It has a length of about eight micrometres and it supports the sperm head. It is bounded by a sheath and it contains mitochondria which generates ATP, the energy needed for the movement of the tail. The principal piece is the longest portion of the tail and it contains the majority of the structures needed for tail movement. The end piece is distinct and not convoluted (Esteves and Miyaoska, 2015). Proper tail movement is important for sperm motility, which is vital to the movement of sperm cells within the fluid of the female reproductive tract, as it moves through the cervix into the fallopian tube where it fertilizes the oocyte. The fluid above contains phagocytes that can destroy sperm cells; hence, fast movement of sperm cells within the female tract is necessary to prevent damage (Durairajanayagam *et al*., 2015).

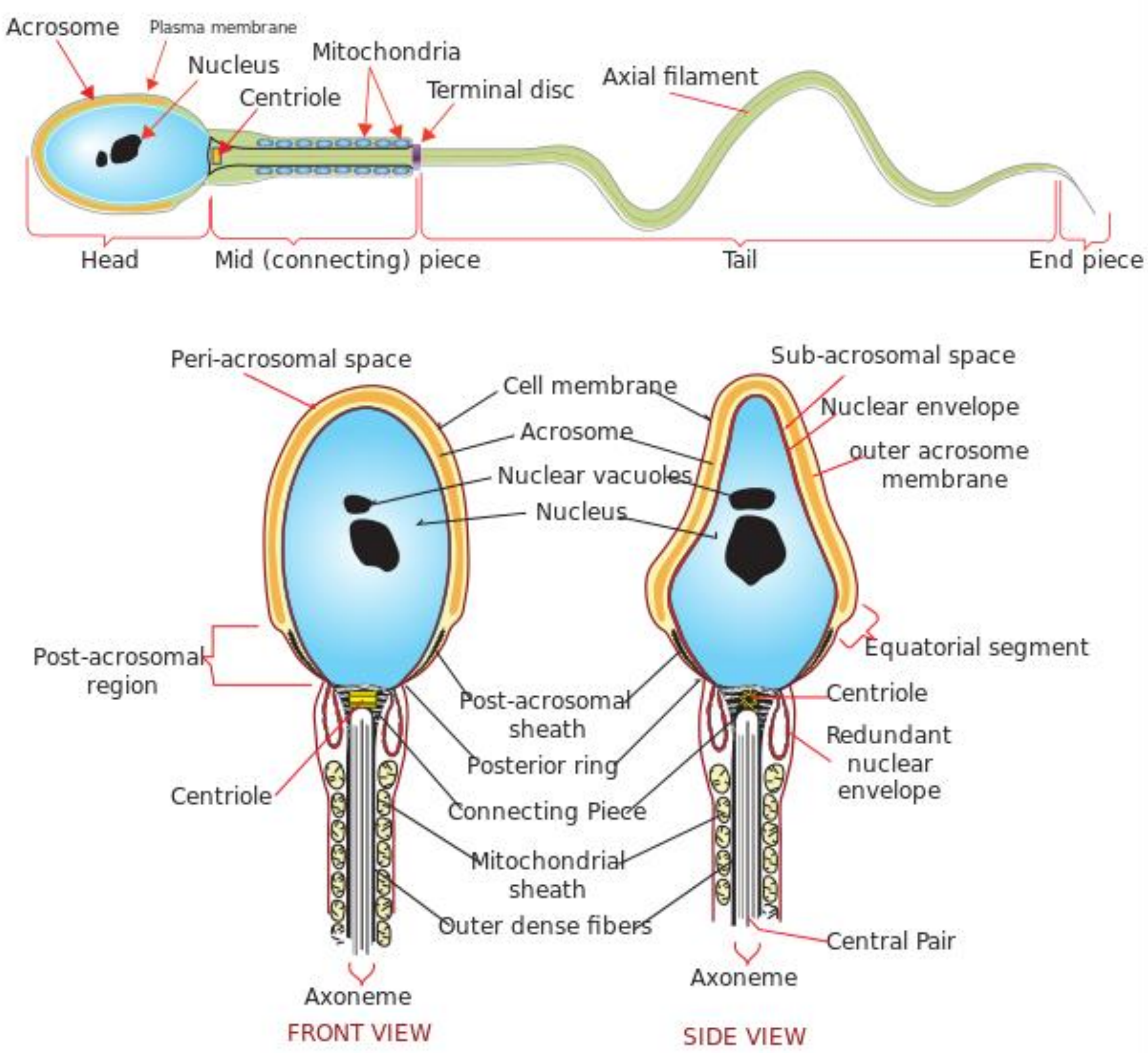

Figure 2.8: The human mature sperm cell (Villarreal, 2006)

2.4.1 Pathology of the sperm

Identification of sperm defects is important in the evaluation of fertility. This knowledge is also important in assisted reproductive techniques. Semen analysis is a commonly used clinical procedure, which detects sperm disorder or dysfunction, and it aids the diagnosis of infertility in males. Teratospermia or teratozoospermia refers to the presence of a large number of sperm cells with abnormal morphology. This abnormality can be due to head or tail aberrations (Franken and Henkel, 2012).

Asthenospermia or asthenozoospermia refers to a reduced percentage of motile sperm cells in the semen sample. This reduction in motility hinders the ability of the sperm cell to travel to the fallopian tube for fertilization to occur**.** Necrospermia or necrozoospermia is a reduction in the sperm vitality i.e. a reduction in the percentage of live sperms. Azoospermia refers to absence of sperm cells (Franken and Henkel, 2012).

2.5 Semen Analysis

Semen consists of sperm cells and seminal fluid. Sperm is produced by the testes and stored in the epididymis until the process of ejaculation. During the process of ejaculation, as sperm is being transported to the penis through the ejaculatory duct secretions from accessory glands (seminal vesicle, bulbourethral gland and the prostate) form the seminal fluid and mix with the sperm. This mixture i.e. the semen is now expelled through the urethra in the process called emission (Lucio *et al*., 2011).

Semen analysis is a common clinical diagnostic procedure for evaluating fertility in males. The quality of semen is a pointer to the fertility of the male. In a given semen sample, the following analyses are usually carried out among others: sperm count, semen volume, sperm motility, sperm viability and sperm morphology. Sperm count refers to the number of sperm cells in a semen sample, while the semen volume refers to the quantity of the seminal fluid produced. Sperm motility refers to the percentage of sperm cells exhibiting any form of motion. Sperm vitality or viability refers to the live sperm cells. Sperm morphology refers to the percentage of sperm cells with a normal or abnormal anatomical structure (Patel *et al*., 2018)

According to the 2010 WHO study carried out on men from different countries, the table below shows the lower limit reference value for normal semen parameters

Table 2.2 : Lower reference limit for semen analyses as defined by WHO (Cooper *et al*., 2010)

Semen parameters	**Lower reference limit**
Semen volume	1.5 mL
Sperm concentration	39×10^6/mL
Sperm count	15×10^6
Total motility	40%
Progressive motility	32%
Live sperm cells	58%
Sperm morphology (normal cells)	4%
Seminal fructose	At least 13 µmol/ejaculate
pH	7.2 and above

2.6 Diabetes Mellitus

Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood'. The persistent hyperglycaemia seen in diabetes is involved in nephropathy, retinopathy, neuropathy and cardiovascular dysfunction and other associated malfunction across body systems observed in diabetics (WHO, 2021).

2.6.1 Prevalence and burden of diabetes mellitus

Between the years 1980 and 2014, the prevalence of diabetes worldwide rose from about 108 million to 422 million. Among the adult population (above 18 years), the prevalence rate was shown to have increased from 4.7% to 8.5% within those 34 years. The prevalence rate of diabetes is more in the developing and developed nations than the first world countries.

In 2016, diabetes was ranked seventh among the causes of death worldwide with about 1.6 million deaths. About 50% of deaths caused by diabetes occur before the age of 70

Diabetes ranks top four among the non-communicable diseases worldwide and its persistent rise in prevalence over the years has continued over the years and is still estimated to further rise in coming years despite innovations in medical research (WHO, 2016)

2.6.2 Classification of diabetes mellitus

The two major classes of diabetes are Type 1 and Type 2 diabetes.

2.6.2.1 *Type 1 diabetes*

Type 1 diabetes was formerly referred to as insulin-dependent, juvenile or childhood-onset diabetes (WHO, 2019). It is common in children and young adults below the age of 25 years. However, it is now being found in adults. It occurs because of damage to beta cells of the pancreas, thus leading to a deficiency in the production of insulin by the pancreas. The cause of type 1 diabetes is unknown but it is largely attributed to the autoimmune destruction of the beta cells. The insulin deficiency results in the reduced peripheral utilization of blood glucose by the body cells thus, resulting in retention of glucose in the blood and consequently chronic hyperglycemia (Punthakee *et al*., 2018).

Symptoms of type 1 diabetes include polyuria, polydipsia, polyphagia, weight loss, vision impairment, and body weakness.

2.6.2.2 *Type 2 diabetes*

It was earlier referred to as non-insulin-dependent or adult-onset diabetes. It affects mostly adults; however, it is now common to find it among young adults. About 90% of diabetic cases globally are type 2 diabetes (WHO, 2021)

Type 2 diabetes results from the body's ineffective use of insulin; a condition termed as insulin resistance. It is linked with obesity and lack of proper physical activity (WHO, 2021).The recent increase in the rate of obesity among children and adolescents is largely responsible for the increasing rate of type 2 diabetes among the young population (Punthakee *et al*., 2018).

2.6.2.3 *Other classes of diabetes mellitus*

Other classes of diabetes mellitus include:

i) Gestational diabetes: It refers to impaired glucose tolerance associated with pregnancy. The blood glucose level is above normal but not high enough to be diagnosed as diabetes. Gestational diabetes is a risk factor for pregnancy and delivery complications, and the children stands a high chance of being developing type 2 diabetes later in life. A proper dietary adjustment can mitigate the glucose impairment in pregnancy and its associated complications

ii) Other types of diabetes with regards to the causes include: genetic defects of the beta cells or insulin secretion and action, disorder of the exocrine pancreas, medication induced e.g. HIV drugs, glucorcorticoids, etc (WHO, 2021)

The symptoms of type 2 diabetes are more or less the same as that of type 1 although they are often less noticeable initially. The symptoms become obvious several years after the inception of the disease when it has reached a complicated state (WHO, 2021).

2.6.3 Diagnosis of diabetes

Diabetes can be detected by assessing fasting or random blood glucose level, or by oral glucose tolerance test (OGTT) which is evaluated within 2 hours after a 1g/kg oral dose of glucose.

Another diagnostic test is done by estimating glycated haemoglobin (HbA1c).Although this reveals the mean blood glucose level over a longer period it is however a more expensive test.

The diagnostic values for diabetes in humans are shown below:

1) A fasting blood glucose level $\geq$ 126 mg/dl
2) A random blood glucose level $\geq$ 200 mg/dl
3) A 2 hour blood glucose level $\geq$ 200 mg/dl in the course of OGTT
4) HbA1c $\geq$ 6.5

It is important to note that some diagnostic values, even though not categorically termed as diabetic show a high risk for the development of diabetes. These are referred to as prediabetes values. The values are shown below:

i) Fasting blood glucose level of between 100mg/dl and 125mg/dl. This is termed as impaired fasting glucose (IFG)
ii) 2- hour blood glucose of between 140 mg/dl and 199 mg/dl as observed for the period of OGTT. This is termed as impaired glucose tolerance (IGT)
iii) HbA1c of between 5.7 and 6.4% % (American Diabetes Association, 2018)

2.7 Diabetes and Erectile Dysfunction

Diabetes mellitus induced erectile dysfunction has multiple aetiologies. This poses a challenge in the treatment of erectile dysfunction in diabetics. An understanding of the different mechanisms involved can aid an effective approach to the treatment. The associated complications of diabetes, which include vascular dysfunction, neural damage and hypogonadism, are proposed to be responsible for erectile dysfunction in diabetics (Maiorino, *et al*., 2014).

Chronic hyperglycaemia, which is a major characteristic of diabetes mellitus has been proven to trigger a pathologic process which eventually leads to the damage of neural and cardiovascular tissues and then invariably negatively impacts erectile function. (Moore & Wang, 2006). Chronic hyperglycaemia causes a pathologic reaction between glucose and proteins, lipids or nucleic acids resulting in the production of advanced glycation products (AGEs). This increased levels of AGEs cause vascular damage by causing thickening of the walls of the blood vessels, reducing their compliance. They also cause impairment in endothelial function (Cartledge *et al.*, 2001).

The endothelium and non-adrenergic non-cholinergic nerves produce nitric oxide (NO), which plays an important role in erection. Neural damage and endothelial dysfunction, which are associated with diabetes, thus causes a reduction in the level of neuronal and endothelial nitric oxide synthase (nNOS and eNOS). This results in reduced production and supply of NO necessary for relaxation of the smooth muscles of the corpus cavernosum thus resulting in erectile dysfunction (Vernet *et al*., 1995). In addition, there is a proposition that cGMP, which mediates the action of NO is reduced in diabetes due to the inhibition of guanylyl cyclase (Moore & Wang, 2006).

In addition, there are reports of enhanced responses to vaso-constrictors in diabetes. This has been attributed to increased expression of Rho-kinase in diabetic conditions. RhoA-Rho kinase is involved in increased Ca^{2+} sensitization. This leads to increased contraction of the penile smooth muscles thus, causing flaccidity and impairment in erectile function (Penson & Wessells, 2004).

Diabetes is associated with a reduction in the level of the testosterone and gonadotropic hormones (luteinizing and follicle stimulating hormone). This testosterone deficiency has been highlighted as one of the causes of erectile dysfunction in diabetics; considering the role testosterone plays in general male reproductive function including the growth and maintenance of the penile structure (Maiorino *et al*., 2014). Testosterone has also been reported to be vital to the integrity of the endothelium. Testosterone deficiency has been associated with endothelial dysfunction which impairs erectile function. Also, studies have shown that testosterone plays a role in the production of nitric oxide (NO) by increasing the activity of nitric oxide synthase (NOS). This results in increased relaxation of the penile erectile tissues thus, promoting erection (Traish & Galoosian, 2013).

2.8 Diabetes and Testicular Function

Diabetes causes damage of the testes. The chronic hyperglycemia in diabetes causes testicular DNA damage. This then leads to a decline in the overall function of the testes as seen in impairment in sperm parameters and testosterone deficiency (Atta *et al.*, 2017).

Degeneration in Leydig cell structure and function has been reported in diabetic conditions. In addition, there is decreased production of luteinizing hormone (LH) which invariably causes a reduction in testosterone level. In addition, there is a decline in the level of follicle stimulating hormone (FSH). The resultant effect of this hormonal disorder is an inhibition or interruption of the process of spermatogenesis. This causes reduced sperm quantity and quality. Furthermore, the elevated oxidative stress in diabetes due to increased AGE production causes damage of sperm DNA which is partly responsible for the infertility associated with diabetes (La Vignera *et al*., 2012).

A number of studies have been carried out on the relationship between testosterone levels and diabetes. Some reports have shown impairment in glucose homeostasis in men with testosterone deficiency. Others have shown that diabetes causes hypogonadism.

Insulin has been suggested to be involved in the regulation of testosterone production by stimulating the Leydig cells in concert with LH. The activity of insulin has also been shown to regulate LH and FSH secretions. Thus, the decline in testosterone level in diabetes especially type I is proposed to be due to the insulin deficiency (La Vignera *et al*., 2012).

2.9 Lauric acid

Lauric acid also known as Dodecanoic acid is a saturated fatty acid. It contains 12 carbon atoms and thus, considered as a medium chain fatty acid with the molecular formula $C_{12}H_{24}O_2$ (Ghosh, *et al*., 2014).

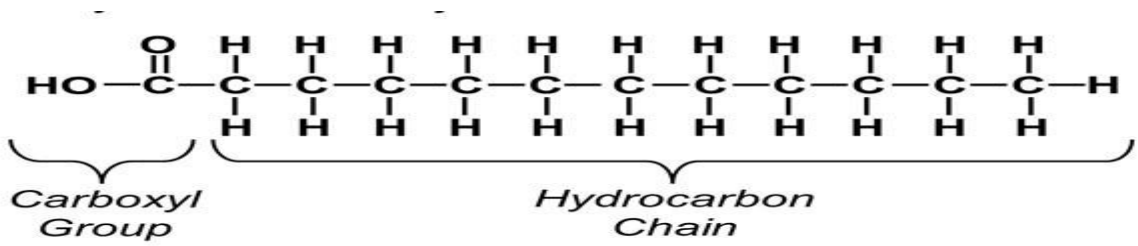

Figure 2.9: Chemical structure of Lauric acid (Dallas learning cloud, 2020)

As typical of saturated fatty acids, lauric acid is solid at room temperature. It is in form of small white granules with a faint soapy odour. The natural sources of lauric acid include coconut oil, Laurel Oil, Palm Kernel Oil, Human Breast Milk, Cow's Milk And Goat's Milk (Beare-Rogers *et al.*, 2001). *Coconut oil has been the centre of a number of scientific research due to its ameliorative properties in a number of disease conditions.* Lauric acid constitutes about 50% of the fatty acids in coconut oil and has been reported in a number of studies to be responsible the actions of coconut oil (Dayrit, 2015).

Consumption of saturated fatty acids have been widely reported to be unhealthy because it causes dyslipidaemia, which eventually leads to cardiovascular damage. However, recent studies on coconut oil which is mostly made up of medium chain saturated fatty acids has shown that medium chain saturated fatty acids are safe for consumption as they increase the level of high density lipoprotein (HDL) consequently reducing the total HDL cholesterol ratio. This has a positive effect on the cardiovascular system. Attention is thus being drawn to lauric acid, which is the most abundant fatty acid in coconut oil and is proposed to be responsible for the physiological roles of coconut oil (Mensink, *et al*., 2003; Dayrit, 2015).

Contrary to much earlier reports, lauric acid, like all other medium chain saturated fatty acids has been proven not to cause obesity. After absorption they are transported directly to the liver through the portal vein as opposed to being packaged as chylomicrons and released into the circulation through the lymphatic system. Also, in the liver they are more rapidly metabolized by β-oxidation to produce ketone bodies which are converted to acetyl-CoA in extra-hepatic tissues like the brain, heart and msucles and used as a quick source of energy. This implies that lauric acid does not stay in the body for long and thus, does not get stored in adipose tissues thus preventing obesity (Mccarty & Dinicolantonio, 2016; Dayrit, 2015).

Lauric acid has been reported to have a number of therapeutic actions including; antimicrobial and anti-inflammatory properties (Huang *et al*., 2014), reduction in blood pressure and oxidative stress (Alves *et al*., 2017), increase in high density lipoproteins , inhibition of testosterone induced prostatic hyperplasia (Patil *et al.*, 2016), cure of androgenetic alopecia (Mccoy and Ziering, 2012), etc.

2.9.1 Lauric acid as a potential alternative therapy

Research has recently being channelled in the direction of natural substances which have been used traditionally over the years to improve sexual function. This has also led to the concept of 'nutraceuticals' which serve as a dietary therapy for various diseases (Chang *et al*., 2013). Compared to standarad medication, these natural products have the advantage of being affordable, accessible, palatable and sometimes even offer health benefits beyond the intended treatment. They also do not have the side effects associated with the use of standard drugs. However, little is known about the active components of these nutraceuticals. Such knowledge will aid the development of a standard drug formulation and dosage for this nutraceuticals (Modak *et al*., 2007).

Lauric acid is the most abundant constituent coconut oil. A number of scientific research has been focused on coconut oil because of its therapeutic functions (Dayrit, 2013). For example, coconut oil is shown to have anti-hyperglycaemic and anti-oxidant properties, which contributes to ameliorative function in diabetic conditions (Iranloye *et al*., 2013). Also coconut oil has been shown to have therapeutic function in reproductive disorders (Dosumu *et al*., 2010; Ogedengbe *et al*., 2018). Lauric acid is proposed and in some cases, scientifically proven to be responsible for the actions of coconut oil (Dayrit, 2015). The benefits of lauric acid in diabetes induced reproductive dysfunction is thus worth investigating**.**

2.9.2 Anti-diabetic properties of lauric acid

Studies have shown the anti-diabetic function of medium chain fatty acids (MCFAs) in coconut oil, of which lauric acid is the most abundant constituent. The MCFAs cause weight loss and combat obesity. This prevents insulin resistance as seen in type 2 diabetes and thus, enhances insulin action. In type 1 diabetes, MCFAs have been shown to promote insulin production and release into the circulation (Dayrit, 2015). In addition, ketone bodies produced by the metabolism of lauric acid in the liver, stimulates insulin secretion leading to a decrease in blood glucose. Thus the possibility of lauric acid, like other MCFAs being used as nutritional supplements in the treatment of diabetes should be explored (Dayrit, 2015)

2.9.3 Lauric acid and reproductive function

Although there is a dearth of information on the direct physiological roles of lauric acid on sexual function, it is however hypothesized to be largely responsible for the actions of coconut oil in particular on reproductive function. Coconut oil has been reported to ameliorate testicular damage and thus, improve testicular function (Ogedengbe *et al*., 2018 and Dosumu *et al*., 2010).

Metabolic syndrome, which include obesity, dyslipidaemia, hypertension, and insulin resistance, has been reported to have a negative impact on reproductive function. Drugs targeted at ameliorating these metabolic diseases have the potential of improving reproductive function (Martins *et al*., 2019)(Martins 2019). Lauric acid directly or in coconut oil has been reported to have hypocholesterolaemia (Dayrit, 2015), anti-diabetic (Iranloye *et al*., 2013), antioxidant, smooth muscle relaxant effects (Alves *et al*., 2017); and thus has the potential of having a positive effect on erectile dysfunction.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Chemicals and Drugs

Lauric acid, N-nitro-L-arginine methyl ester (L-Name), Sodium nitroprusside (SNP), Acetylcholine (Ach), Phenylephrine (PE), Ethylene glycol tetraacetic acid (EGTA) were obtained from Ak Scientific Inc. (U.S.A) Streptozotocin was obtained from Santa Cruz Biotechnology (USA). Sildenafil was obtained from Pfizer Inc. (USA). Coconut oil was prepared by wet extraction method as described by Nevin & Rajamohan (2009).

3.2 Management of Experimental Animals

Seventy (70) male Wistar rats weighing 150-200 g were used. The animals were kept in the Animal House of the Department of Human Physiology, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria. The animals were maintained under standard conditions of temperature, humidity and light and given standard chow diet and water *ad libitum* (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). Ethical approval was obtained from the Ahmadu Bello University Animal Care and Use Committee with the approval number: ABUCAUC/2017/019

3.3 Grouping of Experimental Animals

The animals (N=70) were divided into 7 groups of 10 rats each (n=10) and treated as follows:

Group 1 (NC): Distilled water 1 ml/kg (control)

Group 2 (DM-UT): Diabetic untreated

Group 3 (DM + LA 90): Diabetic treated with lauric acid at 90 mg/Kg orally

Group 4 (DM-UT + LA 180): Diabetic treated with lauric acid at 180 mg/Kg orally (Patil *et al.*, 2016)

Group 5 (DM + LA 360): Diabetic treated with lauric acid at 360 mg/Kg orally (Patil *et al.*, 2016)

Group 6: (DM + CO): Diabetic treated with coconut oil at 1.42 ml/Kg (Shariq *et al.*, 2015).

Group 7 (DM + Sild): Diabetic treated with Sildenafil at 20 mg/Kg (Ahn *et al.*, 2011).

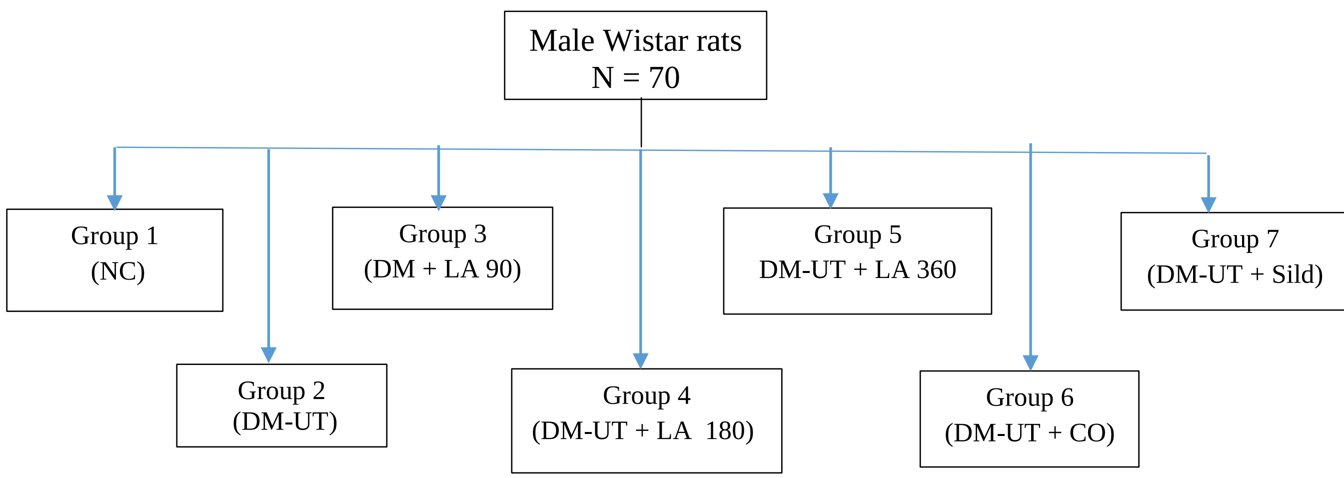

Figure 3.1: Animal grouping

3.4 Induction of Diabetes and Treatment

Type 1 diabetes was induced by the administration of a single intraperitoneal injection of streptozotocin (65 mg/kg) in 0.1 M citric acid buffer, pH 4.5 (Simas *et al*., 2017). The blood glucose level of the rats was checked three days after. Animals with fasting blood glucose level greater than 200 mg/dl were marked as diabetic and placed in the diabetic groups (groups 2, 3, 4, 5, 6 and 7). Lauric acid was suspended in distilled water using Tween 80. Sildenafil was dissolved in distilled water. Coconut oil, prepared by wet extraction method according to Nevin and Rajamohan (2006) was used. All treatments were administered through oral gavage (Ahn *et al.,* 2011 and Patil *et al.,* 2016) for 4 weeks. Following an overnight fast, animals were euthanized by chloroform inhalation and cervical dislocation, after which the blood and relevant tissue samples were obtained for further analysis.

3.5 Measurement of Blood Glucose Level

Blood glucose level was measured using a glucometer with Accu-Chek glucose test strips (Roche, USA). The glucometer works based on glucose-oxidase principle (Mcmillin, 1990). After the test strips have been inserted into the glucometer, a drop of blood from the pricked tail is dropped on the test strip. The glucometer then displays the blood glucose levels.

3.6 Organ and Body Weight

The body weight of the animals was taken before the beginning of treatment and at week 4. At the end of the experiment, the testes were harvested and weighed. The gonadosomatic index was then evaluated by the formula below (Oliveira *et al*., 2015)

$$\frac{\text{Testes weight (g)}}{\text{Body weight (g)}} \times 100$$

3.7 Testosterone Assay

Blood sample collected by cardiac puncture with the use of a 5 ml syringe was emptied into plain tubes. It was then spun in a centrifuge for 10 minutes at 3000 ×g to separate out the serum. Estimation of serum testosterone was done by Enzyme-linked immunosorbent assay (ELISA) (McCann and Kirkish, 1985) using rat testosterone ELISA kits (Wuhan Fine Biotech Co., Ltd). Briefly, 50 µl of the standard or sample was added into each well. Then, 50 ul of Biotin-labelled antibody was added and then incubated for 45 minutes at 37^{o}C after which it was washed and aspirated. The 100 µl of SABC working solution was then added, incubated for 30 minutes at 37^{o}C, washed and aspirated. Thereafter, 90 µl TMB substrate was then added to the solution, incubated for 15-20 minutes at 37^{o}C. Then 50 µl stop solution was added after which absorbance at 450 nm was recorded.

3.8 Semen Analysis

Sperm fluid was obtained from the caudal epididymis of the left testes by macerating the epididymis in a petri dish containing warm normal saline (Yokoi *et al*., 2003) Thereafter the following parameters were determined:

3.8.1 Sperm count

After the epididymis has been macerated, the supernatant was diluted in the ratio 1 to 100 with a solution containing 5 g $NaHCO_3$, and 1 ml formalin (35%) per 100 ml distilled water. About 10 µl was dropped in the improved Neubaur haemocytometer and then placed under a microscope (x 200). The number of sperm cells seen in five 16-celled squares in focus was then counted. The sperm concentration was then calculated by multiplying the total counted sperm cells by 5 and expressed as S x 10^6/ml; where S is the total sperm count (Yokoi *et al*., 2003).

3.8.2 Sperm motility

The supernatant described in section 3.8.1 was diluted with tris buffer solution to 0.5 mL and dropped on a slide, which is placed on a light microscope with magnification of x 400. The sperm cells were observed under the microscope. Sperm cells showing movement were classified as motile, while those who were stationary were classified as non-motile (Sönmez, *et al*., 2005)

3.8.3 Sperm morphology

From the original dilution for sperm motility, the sperm fluid was diluted with 10% neutral buffered formalin in the ratio 1:20 and smeared on histological slides. Staining of the slides was with Giemsa's dye after which they examined under a microscope. For each rat, 500 sperm cells were evaluated for structural abnormalities. The percentages sperm cells with normal and abnormal structures were then evaluated (Atessahin *et al*., 2006).

3.8.4 Sperm viability

Live and dead sperms were distinguished by the method of Siegel (1993). A drop of Eosin-Nigrosin was added to a drop of semen and allowed to settle for a 1-2 minutes after which this combination was smeared on a microscopic slide and viewed under a microscope. The dead sperm cells stained red, while the live sperm cells were unstained.

3.9 Histology of the Testes

The right testes were extracted from the animals and then a section of the medial portion was fixed in a phosphate buffered formalin solution (10%), after which it was stained with hematoxylin and eosin (H and E) to highlight the general histological features as described by Traish *et al*, (2005).

3.10 Isolated Tissue Study (Model of Erectile Function)

After the rats were sacrificed, the penis was surgically removed as a whole and placed straightaway in a Petri dish containing Krebs solution in the following composition: NaCl (118 mM), KCl (5 mM), $NaHCO_3$ (25 mM), KH 2 PO_4 (1.0 mM), $MgSO_4$ (1.2 mM), $CaCl_2$ (2.5 mM), and glucose (11.2 mM). The glans penis, urethra was resected and the corpus cavernosum tissue was then isolated from the surrounding tissues. Each cavernosal strip with both ends tied with a silk thread was placed in an organ chamber, one end fixed to a tissue holder and the other secured to a force transducer. Cavernosal strips were maintained in the organ baths filled with Krebs solution, maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. Isometric tension was continuously measured using a 17400 data capsule connected to a computer that recorded contraction and relaxation responses and analysis of the data. A 2 g tension was applied to each strip, then the strips were allowed to equilibrate for 60-90 minutes During the equilibration periods, the tissue was pre-contracted with phenylephrine every 30 minutes and the organ bath solution was washed off and replaced five minutes after injection of phenylephrine. Thereafter the contractile or relaxant responses of the tissue to the test drugs were evaluated (Salahdeen *et al.*, 2012).

3.10.1 Dose concentration response to phenylephrine

After the equilibration period, the degree of adrenergic mediated contraction was evaluated by injecting a cumulative dose of phenyleprine (10^{-9} to 10^{-5} M) (Salahdeen *et al*., 2014).

3.10.2 Dose response to potassium chloride

After the equilibration period, the degree of depolarization -induced contraction was evaluated by injecting a cumulative dose of KCl (10 to 60 mM) (Salahdeen *et al.*, 2014).

3.10.3 Dose response to acetylcholine

For cavernosal strips of all groups, acetylcholine (Ach) was used to evaluate the endothelium-dependent relaxation. After pre-contraction with phenylephrine (10^{-7} M) and KCl (60 mM) in separate experiments, cumulative doses of Ach (10^{-9} – 10^{-3} M) were added to the organ bath. The concentration-relaxation response of the tissue strip was then obtained (Salahdeen *et al*., 2012).

3.10.4 Dose response to sodium nitroprusside

Sodium nitroprusside (SNP) was used to evaluate the endothelium independent relaxation in the cavernosal tissue strips for all the groups. Cumulative doses of sodium nitroprusside (SNP) (10^{-9} – 10^{-4} M) were added to the organ bath after pre-contracting the tissues with phenylephrine (10^{-7} M) and KCl (60 mM) in separate experiments (Salahdeen *et al.*, 2012).

3.10.5 Influence of nitric oxide synthase on the effect of lauric acid

Cavernosal strips from Group 1 animals only were used. The corpus cavernosum strips were incubated with N-nitro-L-arginine methyl ester (L-NAME) (10^{-4} M) for 30 minutes before the study (Salahdeen *et al.*, 2012). Following pre-contraction with KCl and phenylephrine in separate experiments, a dose response to cumulative doses of Lauric acid (10^{-9} – 10^{-5} M) was then evaluated (Salahdeen *et al*., 2015).

3.10.6 Influence of lauric acid on the dose response to calcium chloride (Ca^{2+} sensitivity)

Cavernosal strips from only group 1 animals were used. A calcium free physiological solution was used. The tissue was incubated in 2 mM of N,N-ethylene glycol tetraacetic acid (EGTA) for 30 minutes and then incubated with Lauric acid for 15 minutes. Thereafter, a cumulative dose of $CaCl_2$ (10 to 60 mM was then injected) and the contractile responses were evaluated (Salahdeen *et al*., 2014)

3.11 Statistical Analysis

Results were presented as mean ± S.E.M. Data was analyzed using one way and two way ANOVA and Tukey post-hoc test with the aid of the *Graph pad 6*. Values with $p < 0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Gonadosomatic Index in Diabetic Male Wistar Rats Treated with Lauric Acid

Figure 4.1 shows the gonadosomatic index (GSI) across the groups of male Wistar rats. GSI in NC (0.79 ± 0.04) and DM + CO (0.74 ± 0.03) rats, respectively were significantly higher ($p < 0.05$) compared to the other groups: DM-UT (0.27 ± 0.07); DM + LA 90 (0.34 ± 0.04); DM + LA 180(0.27 ± 0.04); DM + LA 360 (0.44 ± 0.04); DM + Sild (0.48 ± 0.05), respectively.

4.2 Serum Testosterone Level in Diabetic Male Wistar Rats Treated with Lauric Acid

Figure 4.2 shows the serum testosterone across the groups of male Wistar rats. Serum testosterone in all treatment groups were significantly lower ($p < 0.05$) compared to the control rats: NC (0.92 ± 0.10 ng/ml) vs DM-UT (0.31 ± 0.03 ng/ml); DM + LA 90 (0.52 ± 0.08 ng/ml); DM + LA 180 (0.40 ± 0.09 ng/ml); DM + LA 360 (0.24 ± 0.04 ng/ml); DM + CO (0.29 ± 0.02 ng/ml); DM + Sild (0.57 ± 0.09 ng/ml), respectively.

4.3 Sperm Count in Diabetic Male Wistar Rats Treated with Lauric Acid

Figure 4.3 shows sperm count across the groups of male Wistar rats. Sperm count in NC (51.93 ± 1.85 × 10^6/ml) and DM + CO (40.17 ± 1.69 × 10^6/ml) rats respectively, were significantly higher ($p < 0.05$) compared to other groups: DM-UT (6.20 ± 1.15 × 10^6/ml); DM + LA 90 (8.88 ± 1.91 × 10^6/ml); DM + LA 180(4.30 ± 1.15 × 10^6/ml); DM + LA 360 (6.80 ± 0.30 × 10^6/ml); DM + Sild(4.40 ± 0.86 × 10^6/ml), respectively.

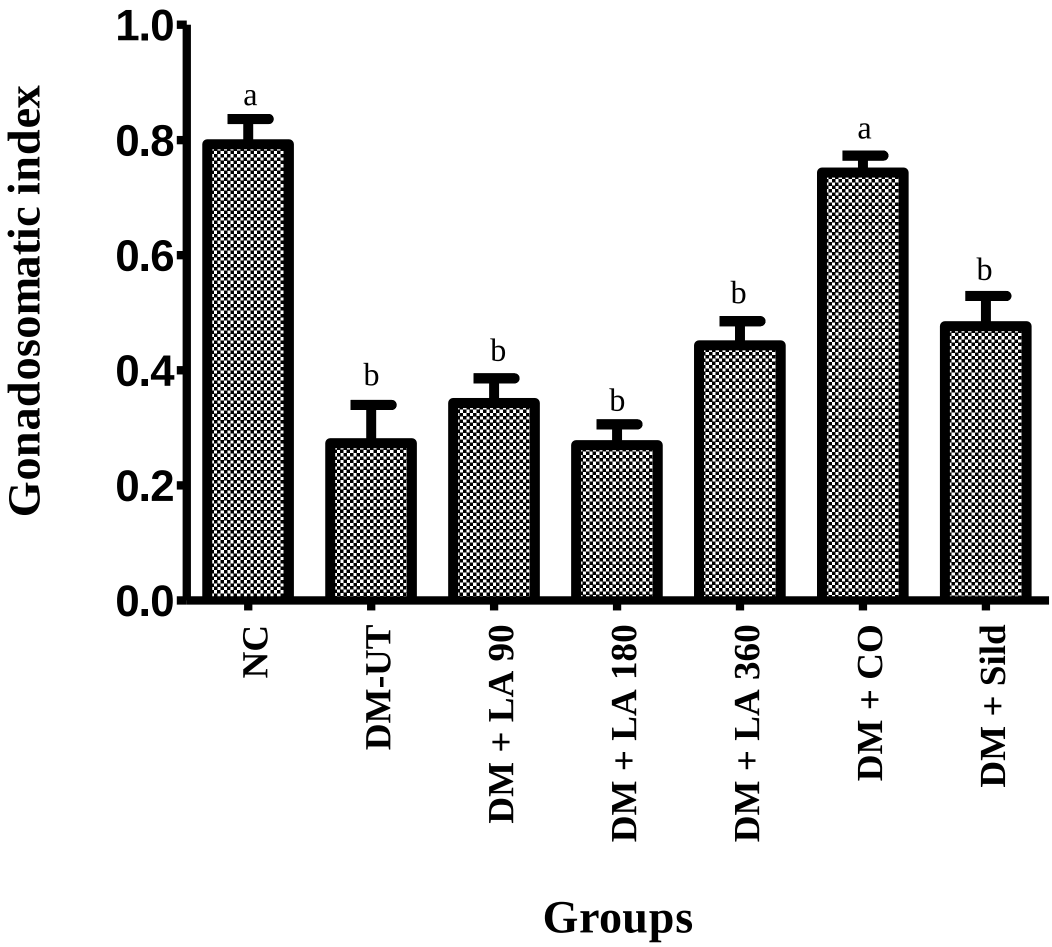

Figure 4.1: Effect of lauric acid on gonadosomatic index in diabetic male Wistar rats

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180- Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; DM + Sild - Diabetic treated with Sildenafil; Different superscripts represent significant difference ($p < 0.05$)

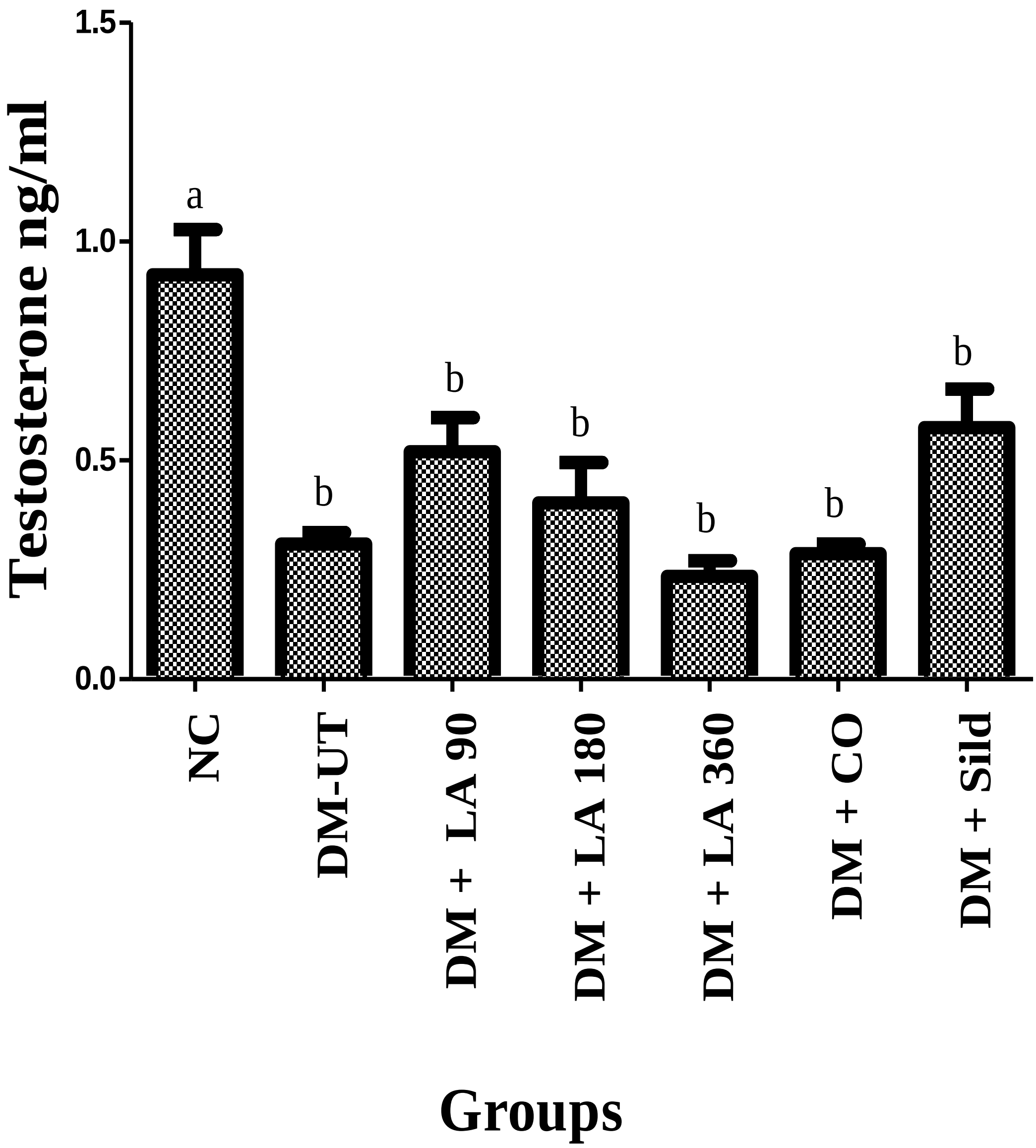

Figure 4.2: Effect of lauric acid on serum testosterone level in diabetic male Wistar rats

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180- Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; DM + SILD- Diabetic treated with Sildenafil; Different superscripts represent significant difference ($p < 0.05$)

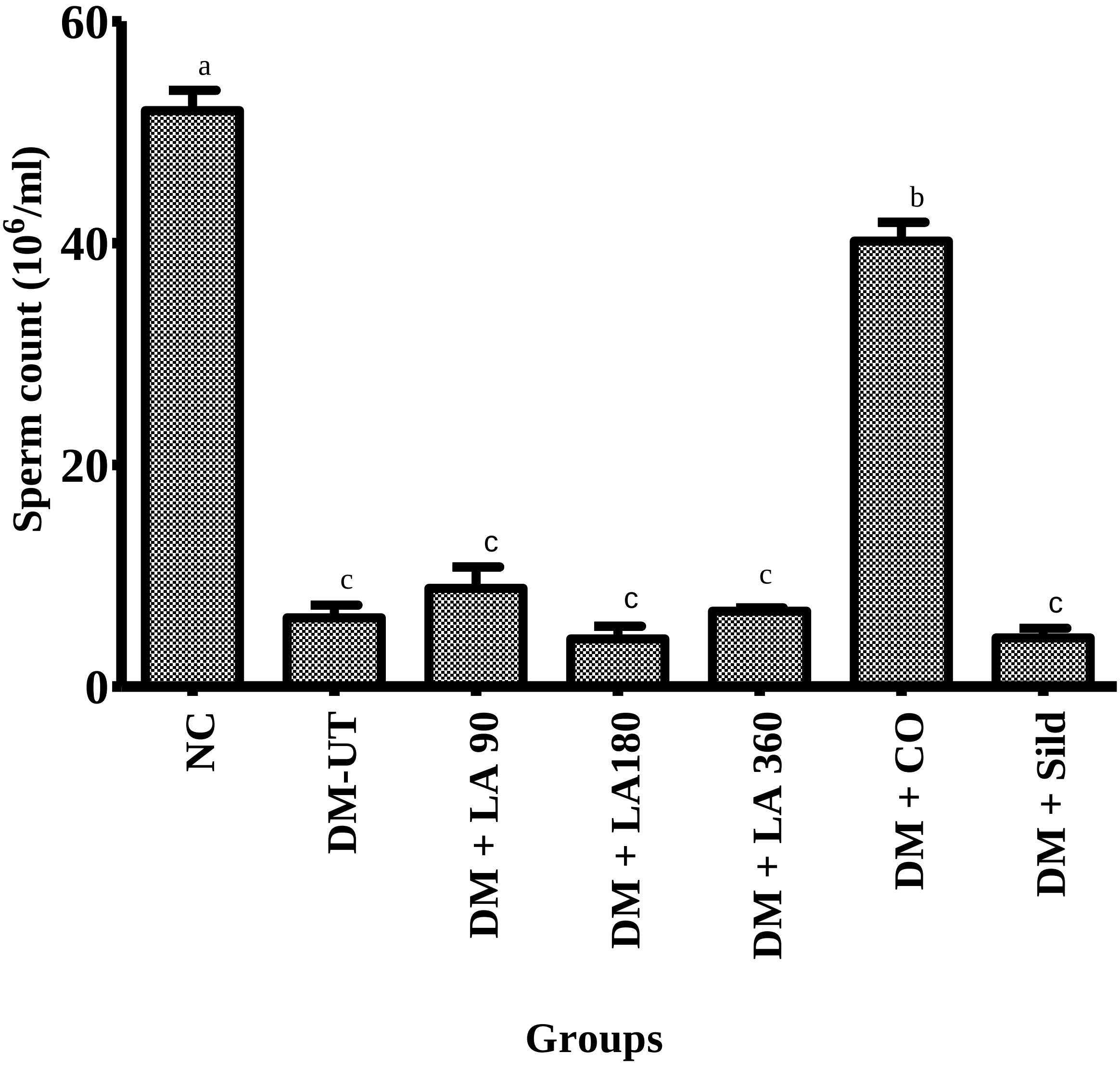

Figure 4.3: Effect of Lauric acid on sperm count in diabetic male Wistar rats

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180- Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; DM + Sild- Diabetic treated with Sildenafil; Different superscripts represent significant difference ($p < 0.05$)

4.4 Sperm motility in diabetic male Wistar rats treated with lauric acid

4.4.1 Motile sperm cells (%)

Figure 4.4 shows that the percentage of motile sperm cells across the groups of male Wistar rats. Motile sperm cells in NC (71%) and DM + CO (79%) rats respectively, were significantly increased (p < 0.05) compared to the other treatment groups: DM-UT (26%); DM + LA 90 (25%); DM + LA 180(8%); DM + LA 360 (12%); DM + Sild (3.00%), respectively

The percentage of motile sperm cells in: DM + LA 180 (8.33%); DM + LA 360 (12.33%); DM + Sild (3%) rats respectively, were significantly lower (p < 0.05) compared to DM-UT (26%); and DM + LA 90 (25%), respectively

Also, the percentage of motile sperm cells in DM + Sild (3%) was significantly lower (p < 0.05) compared to DM + LA 360 (12%) rats

4.4.2 Progressive motile sperm cells (%)

Figure 4.4 shows the percentage of progressive motile sperm cells across the groups of male Wistar rats. In all the treatment groups, progressive motile sperm cells were significantly lower (p < 0.05) compared to the control: NC (43%) vs DM-UT (0%); DM + LA 90 (9%); DM + LA 180(0%); DM + LA 360 (0%); DM + CO (34%); DM + Sild (0%), respectively

The percentage of progressive motile sperm cells in: DM-UT (0%); DM + LA 180 (0%); DM + LA 360 (0%); DM + Sild (0%) rats respectively, were significantly lower (p < 0.05) compared to DM + LA 90 (9%) and DM + CO (34%) rats respectively.

Also, the percentage of progressively motile sperm cells in DM + CO (34%) rats was significantly higher ($p < 0.05$) compared to DM + LA 90 (9%).

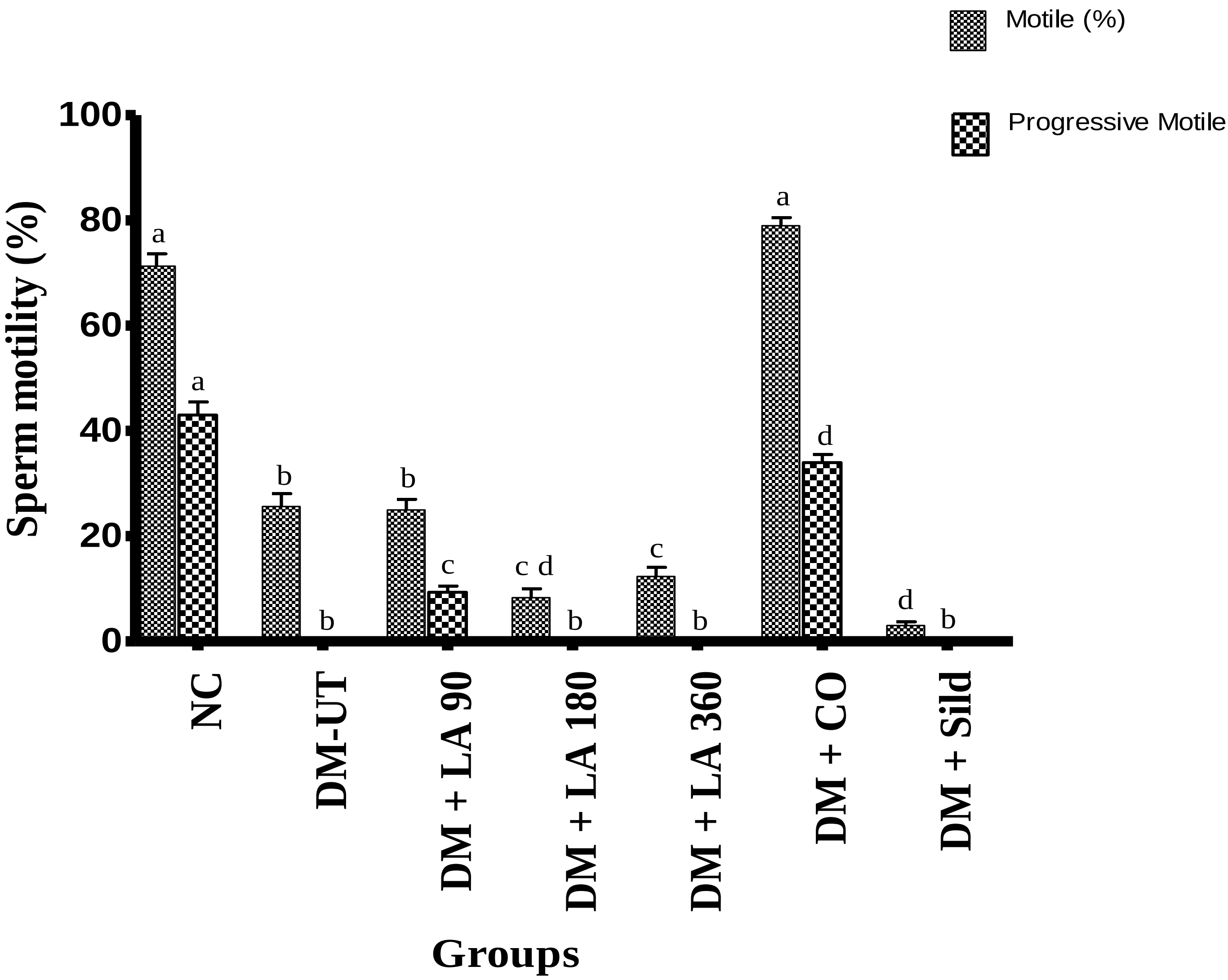

Figure 4.4: Effect of Lauric acid on sperm motility in diabetic male Wistar rats

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180- Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; DM + Sild - Diabetic treated with Silldenafil; Different superscripts represent significant difference ($p < 0.05$)

4.5 Sperm morphology in diabetic male Wistar rats treated with lauric acid

4.5.1 Normal sperm cells

Figure 4.5 shows the percentage of normal sperm cells in all the groups was significantly lower ($p < 0.05$) compared to the control: NC (80%) vs DM-UT (43%); DM + LA 90 (58%); DM + LA 180 (32%); DM + LA 360 (62%); DM + CO (61%); DM + Sild (66%), respectively

The percentage of normal sperm cells in: DM-UT (43%); and DM + LA 180 (32%) rats respectively, were significantly lower ($p < 0.05$) than DM + LA 90 (58%); DM + LA 360 (62%); DM + CO (61%); and DM + Sild (66 %) rats, respectively

4.5.2: Sperm cells with tail defects

Figure 4.6 shows that percentage of sperm cells with tail defects was significantly higher ($p < 0.05$) in: DM + LA 90 (22%); and DM + LA 180 (39%) rats respectively compared to the other groups: NC (7%); DM-UT (19%); DM + LA 360 (14%); DM + CO (18%); and DM + Sild(16%) rats, respectively.

The percentage of sperm cells with tail defects was significantly lower ($p < 0.05$) in: DM + LA 90 (22%) rats compared to DM + LA 180 (39%) rats

4.5.3 Sperm cells with head defects

Figure 4.7 shows that percentage of sperm cells with head defects in: NC (10%) and DM + Sild rats (18%) rats, respectively, were significantly lower ($p < 0.05$) compared to the other groups: DM-UT (33%); DM + LA 90 (28%); DM + LA 180(27%); DM + LA 360 (24%); DM + CO (21%), respectively.

The percentage of sperm cells with head defects in: DM + CO (21%); and DM + Sild rats (18%) rats respectively were significantly lower ($p < 0.05$) compared to: DM-UT

(33%); DM + LA 90 (28%); DM + LA 180 (27%); and DM + LA 360 (24%) rats, respectively.

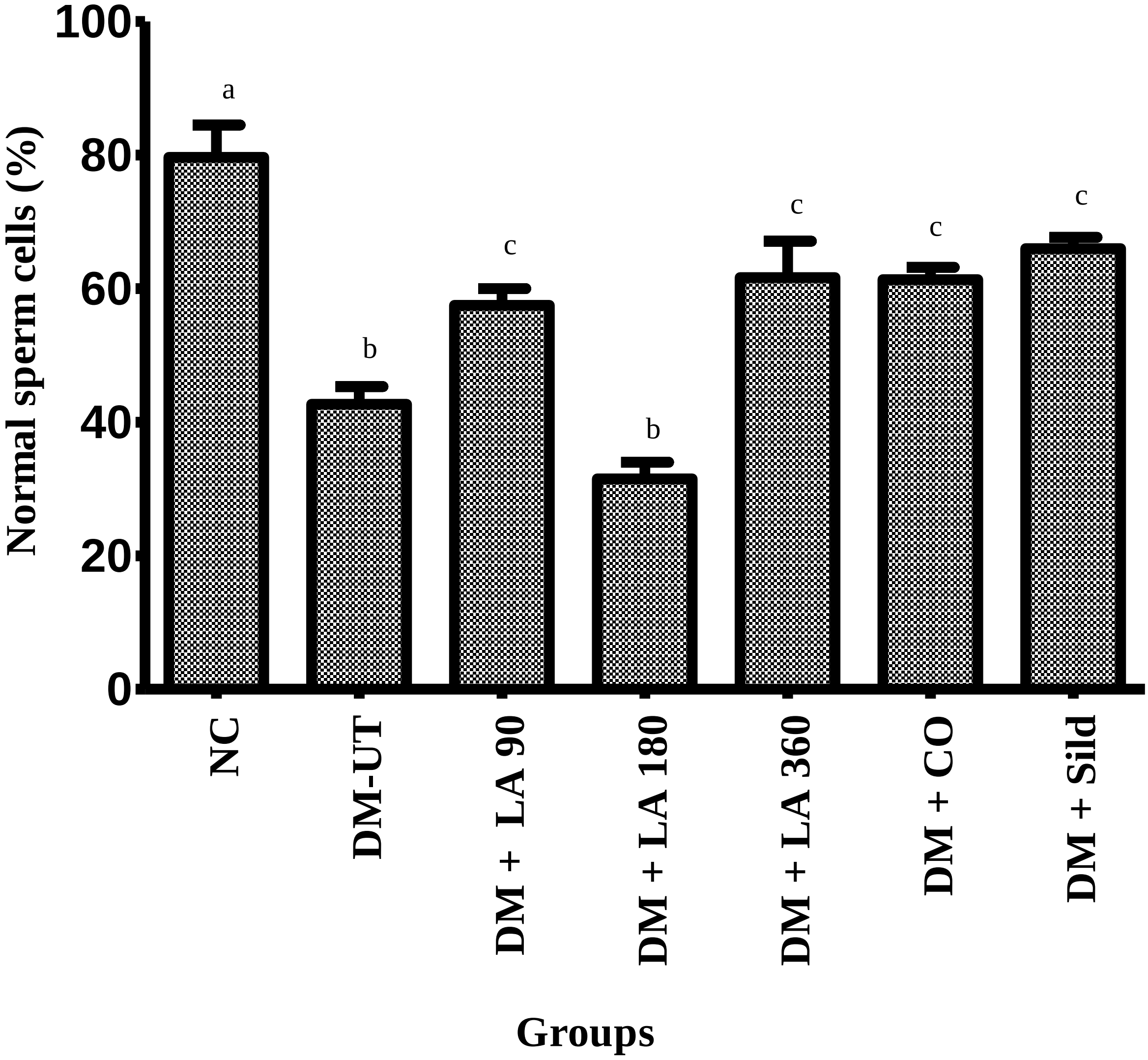

Figure 4.5: Effect of lauric acid on the percentage of sperm cells with normal morphology in diabetic male Wistar rats

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180- Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; DM + Sild- Diabetic treated with Silldenafil; Different superscripts represent significant difference ($p < 0.05$)

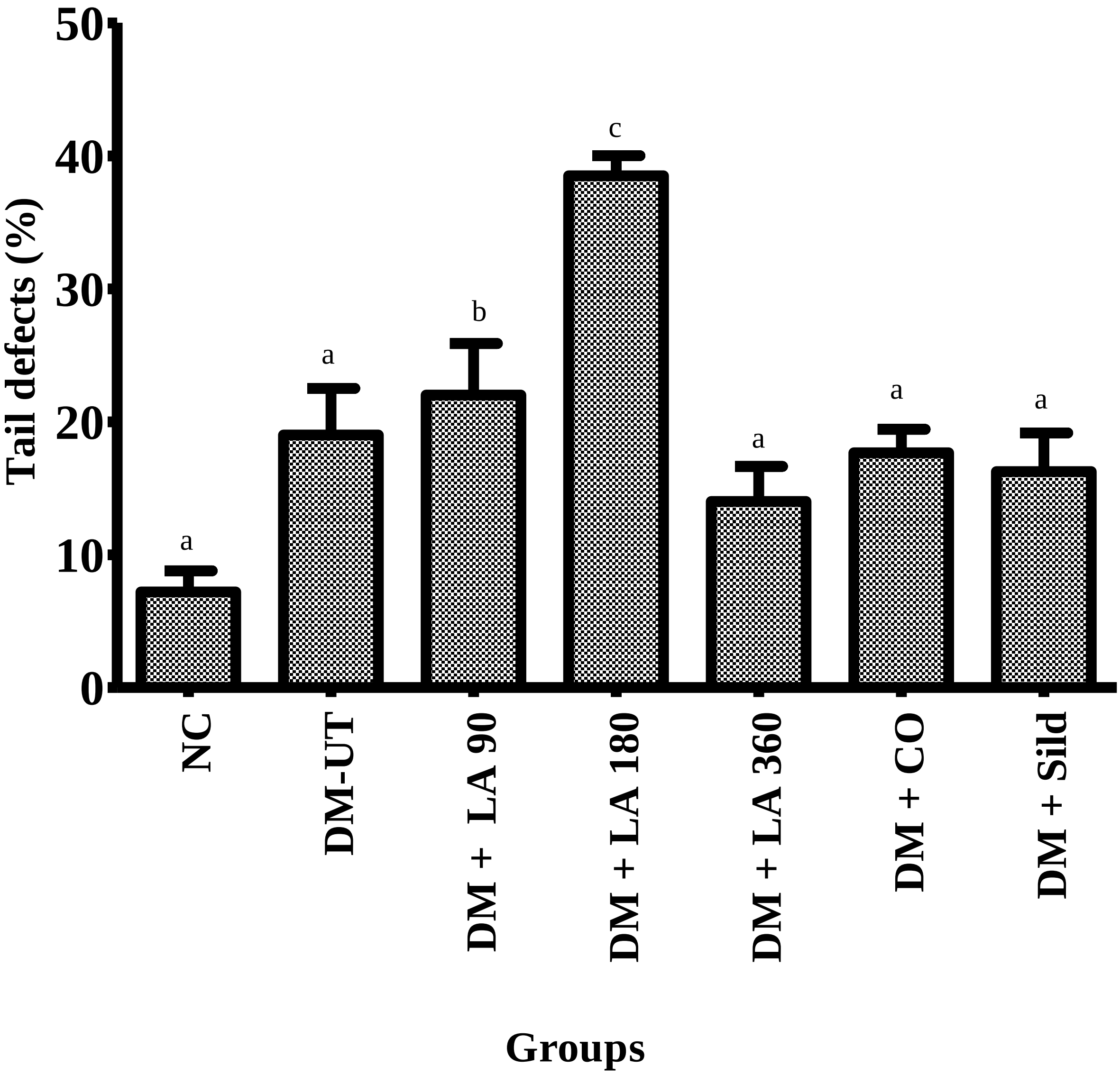

Figure 4.6: The percentage of sperm cells with tail defects in diabetic male Wistar rats treated with lauric acid

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180- Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; DM + Sild- Diabetic treated with Silldenafil; Different superscripts represent significant difference ($p < 0.05$)

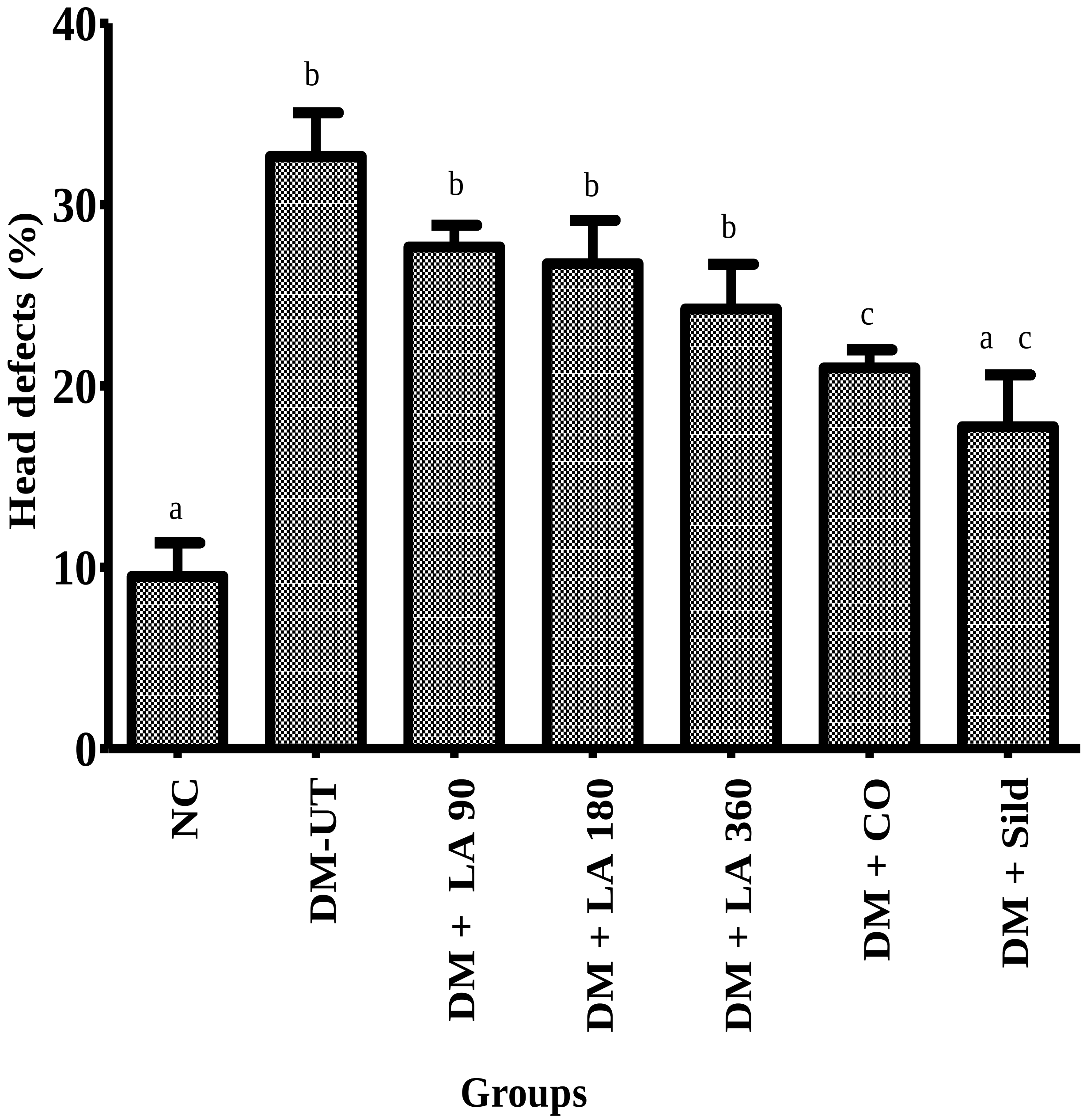

Figure 4.7: The percentage of sperm cells with head defects in diabetic male Wistar rats treated with lauric acid

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180- Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; DM + Sild - Diabetic treated with Silldenafil; Different superscripts represent significant difference ($p < 0.05$)

4.6 Sperm viability in diabetic male Wistar rats treated with lauric acid

Figure 4.8 shows the percentage of viable sperm cells in NC (68%) and DM + CO (58%) rats respectively, were significantly higher ($p < 0.05$) compared to the other treatment groups: DM-UT (22%); DM + LA 90 (25%); DM + LA 180(12%); DM + LA 360 (20%); DM + Sild (18%), respectively.

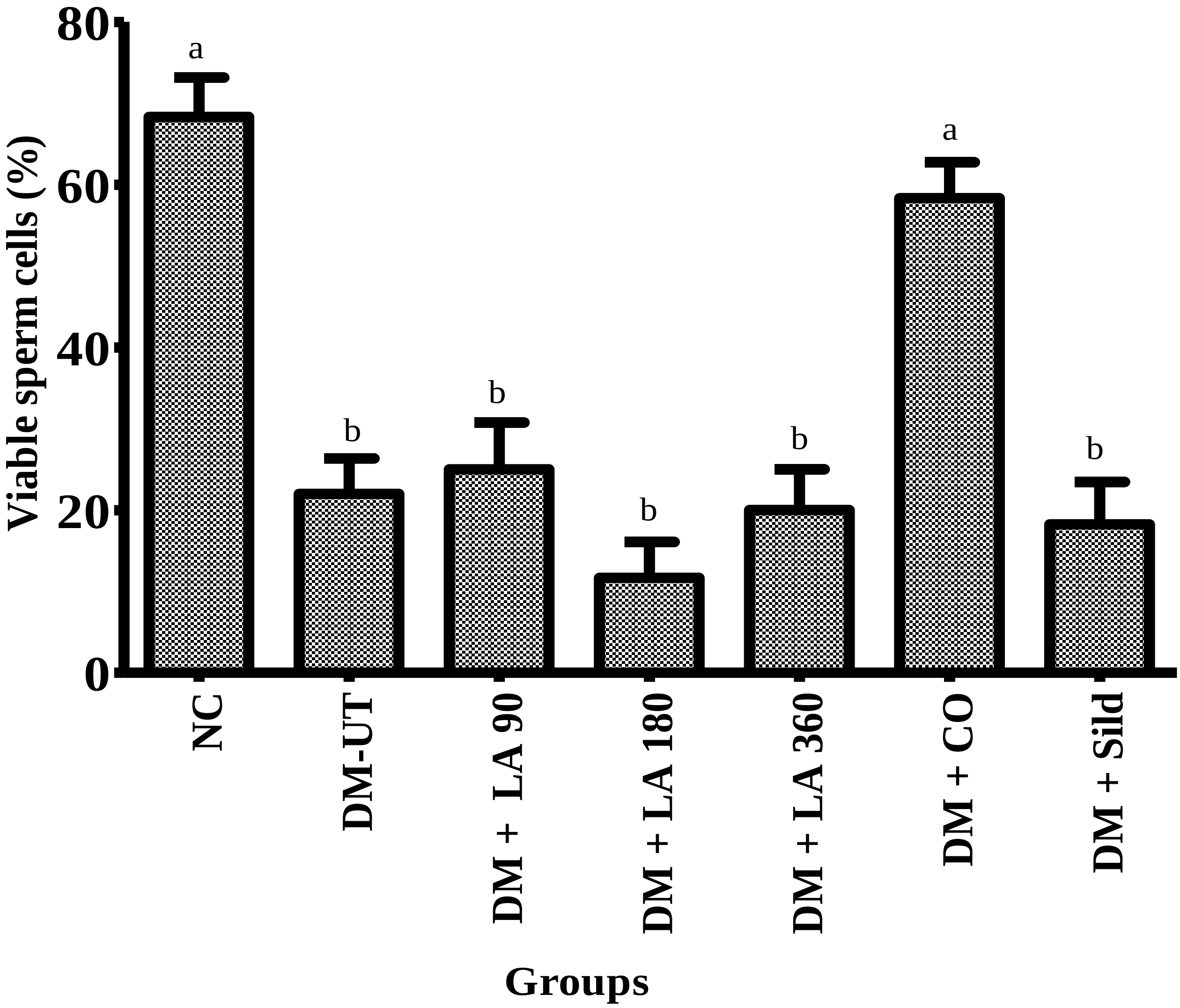

Figure 4.8: Sperm viability in diabetic male Wistar rats treated with lauric acid

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180- Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; DM + Sild - Diabetic treated with Silldenafil; Different superscripts represent significant difference ($p < 0.05$)

4.7 Histology of the Testes

As shown in Plate I, in the NC rats, the Hematoxylin and Eosin staining revealed normal testicular structure with the seminiferous tubules, spermatids and surrounding interstium highlighted. However, in the DM-UT rats as shown in Plate II; the testicular tissue showed degeneration and distortion with a reduction in size of the seminiferous tubules and very sparse spermatids compared to the normal control.

As shown in Plate III, in DM + LA 90 rats, there was testicular degeneration with great reduction in the size of the seminiferous tubules and reduced number of spermatids. Vacuolization was observed within the seminiferous tubules and wide interstitial spaces among the tubules

In DM + LA 180 rats, as shown in Plate IV, the testicular morphology showed normal sized seminiferous tubules with less vacuolization. Spermatids were also seen to be present although sparse.

In DM + LA 360 rats, as shown in Plate V, the testicular morphology showed very few lesions and spermatids were seen to be present

In DM + CO rats, as shown in Plate VI, the testicular tissue showed no lesion. There was abundant presence of spermatids.

In DM + Sild rats, as shown in Plate VII, the testicular tissues showed degeneration with few vacuolizations, although the seminiferous tubules were normal in size. The spermatids in the tubules were also scanty.

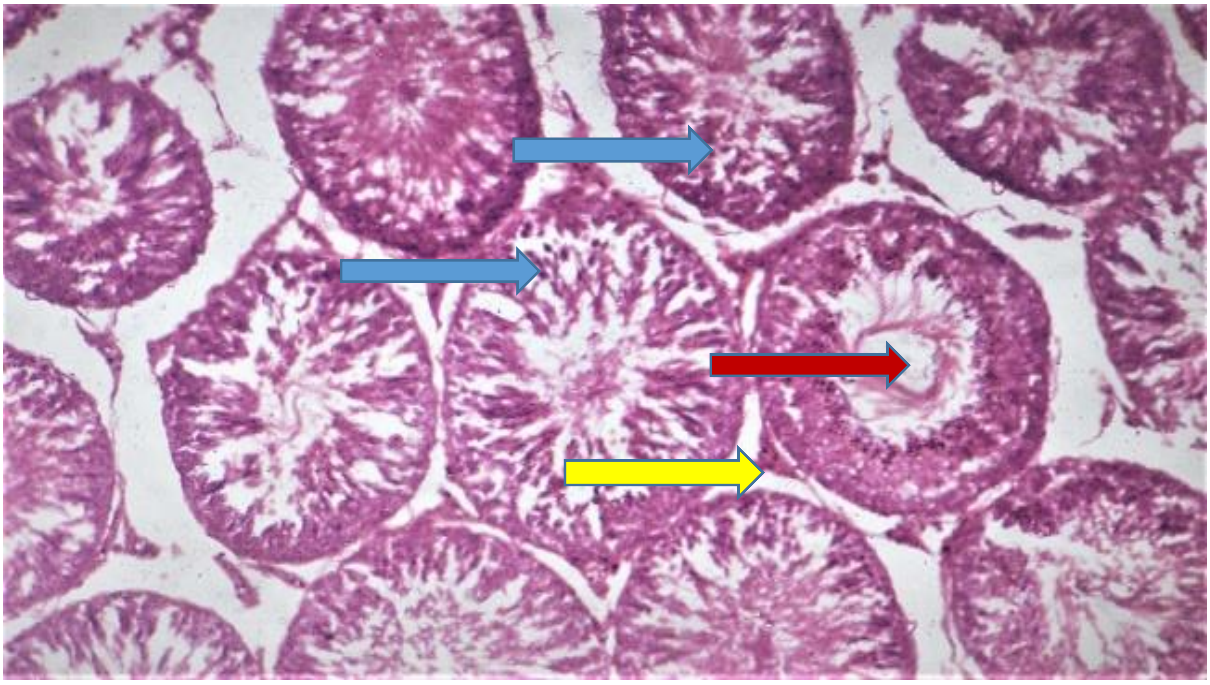

Plate I: Photomicrograph of the the histological section (H & E stain) of a rat testis in
the normal control group (NC) (magnification × 400)

Red arrow represents the lumen. Yellow arrow represents the interstitial space. Blue arrow represents the sperm cells

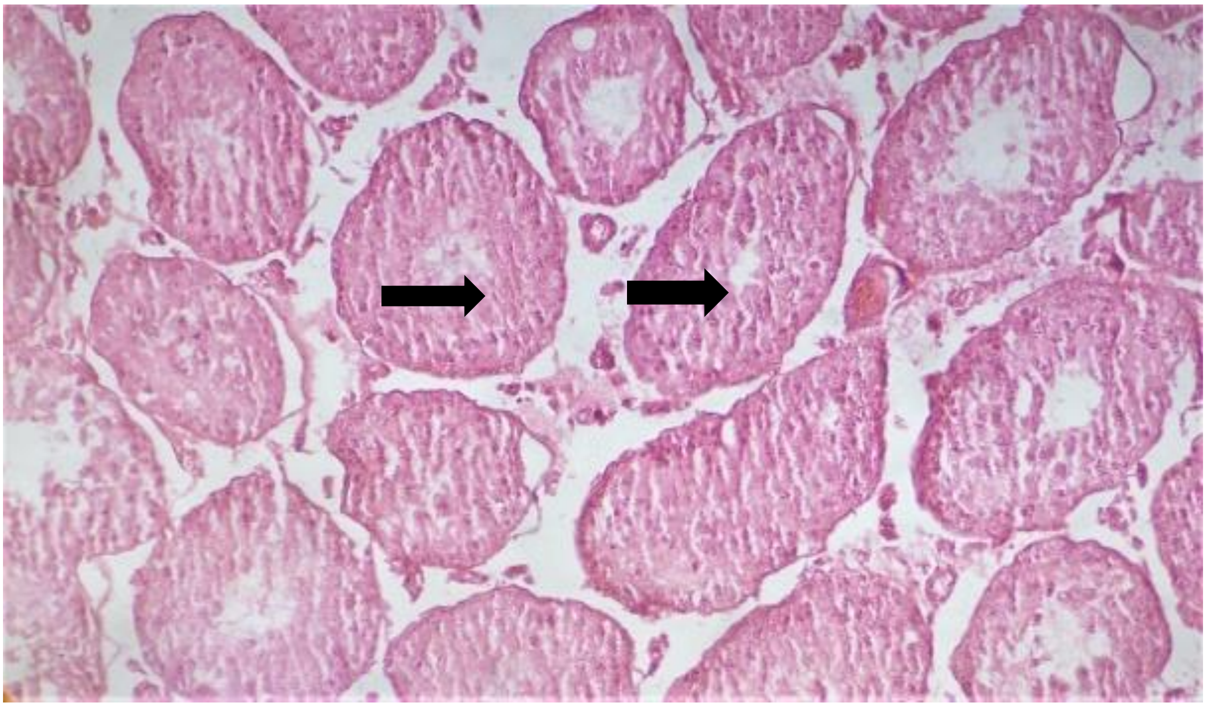

Plate II: Photomicrograph of the the histological section (H & E stain) of a rat testis in
the diabetic untreated group (DM-UT) (magnification × 400)

Black arrows indicate abnormal morphological alterations.

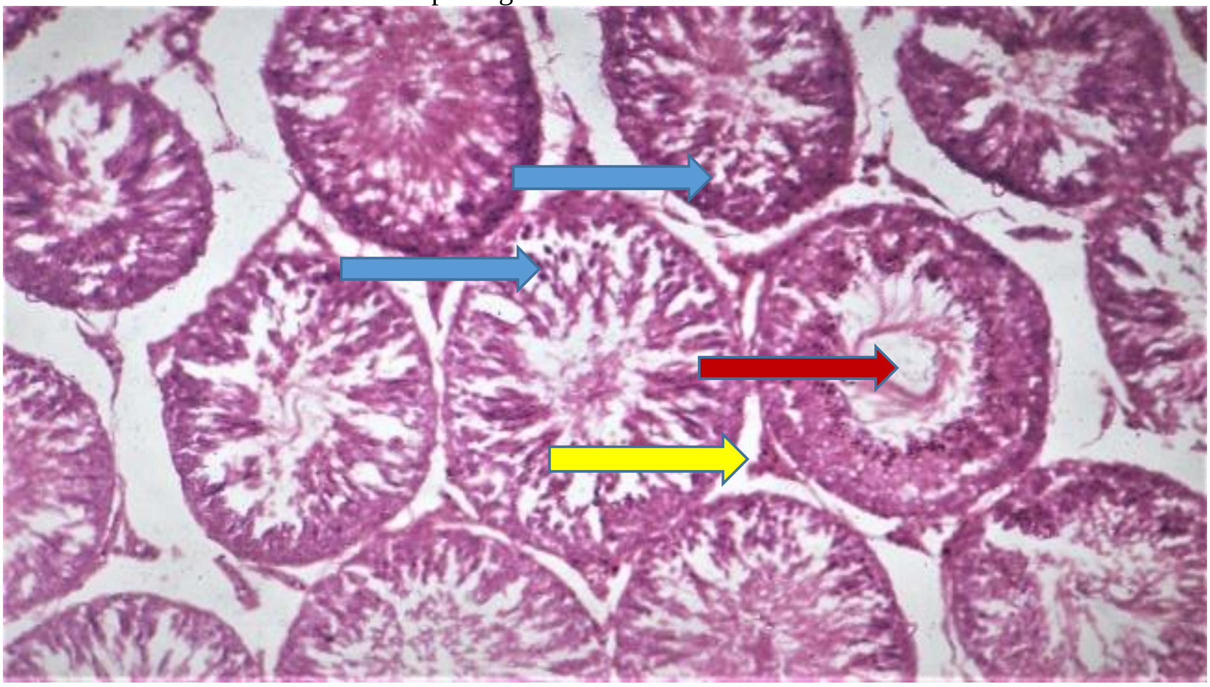

Plate I: Photomicrograph of the the histological section (H & E stain) of a rat testis in
the normal control group (NC) (magnification × 400)

Red arrow represents the lumen. Yellow arrow represents the interstitial space. Blue arrow represents the sperm cells

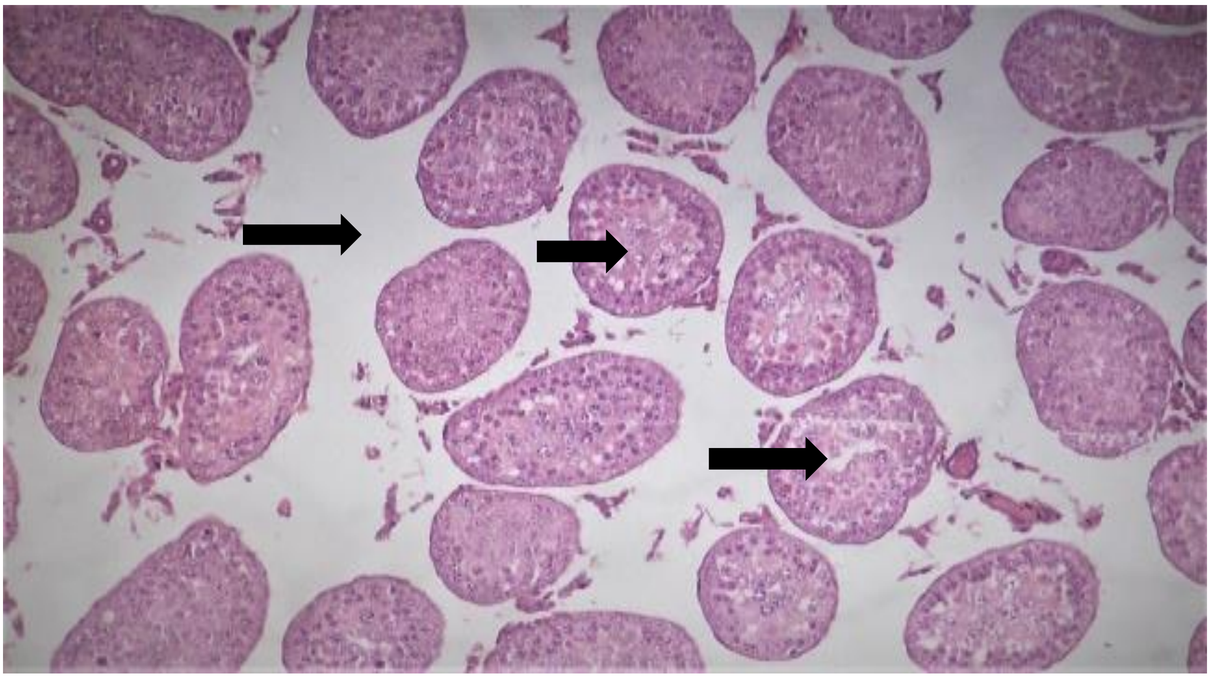

Plate III: Photomicrograph of the the histological section (H & E stain) of a rat testis in

the diabetic group treated with 90 mg/Kg lauric acid (DM + LA 90) (magnification × 400)

Black arrows indicate abnormal morphological alterations.

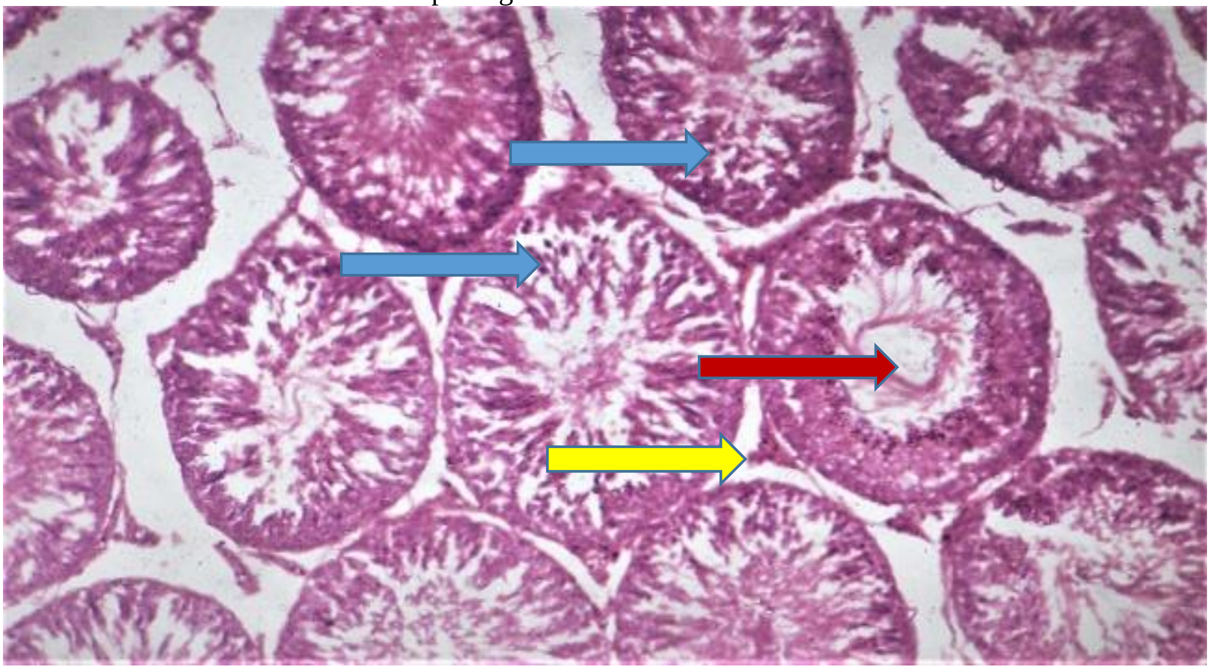

Plate I: Photomicrograph of the the histological section (H & E stain) of a rat testis in
the normal control group (NC) (magnification × 400)

Red arrow represents the lumen. Yellow arrow represents the interstitial space. Blue arrow represents the sperm cells

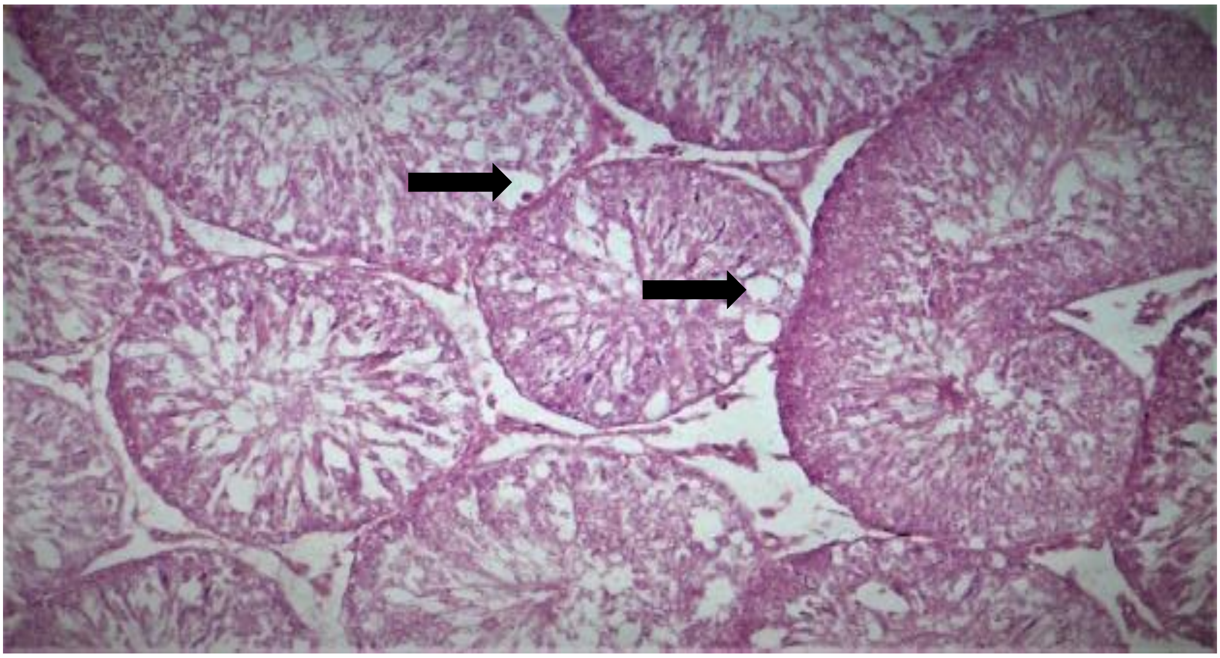

Plate IV: Photomicrograph of the the histological section (H & E stain) of a rat testis in
the diabetic group treated with 180 mg/Kg lauric acid (DM-UT + LA 180)
(magnification × 400)

Black arrows indicate abnormal morphological alterations.

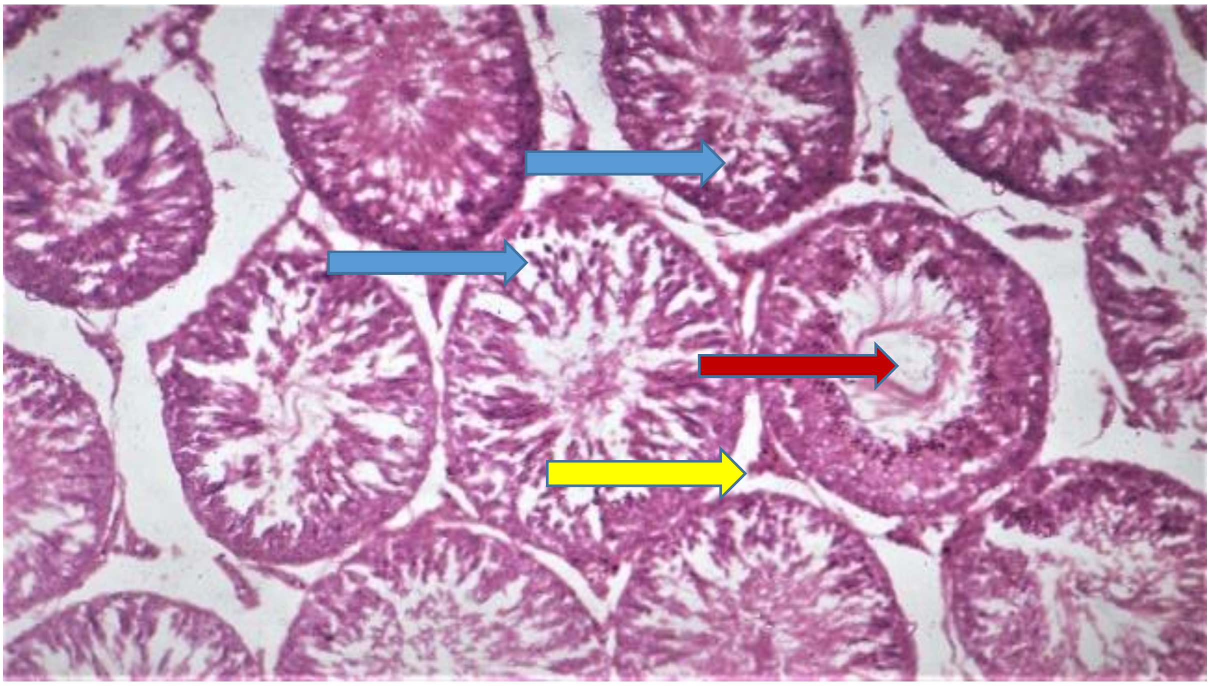

Plate I: Photomicrograph of the the histological section (H & E stain) of a rat testis in
the normal control group (NC) (magnification × 400)

Red arrow represents the lumen. Yellow arrow represents the interstitial space. Blue arrow represents the sperm cells

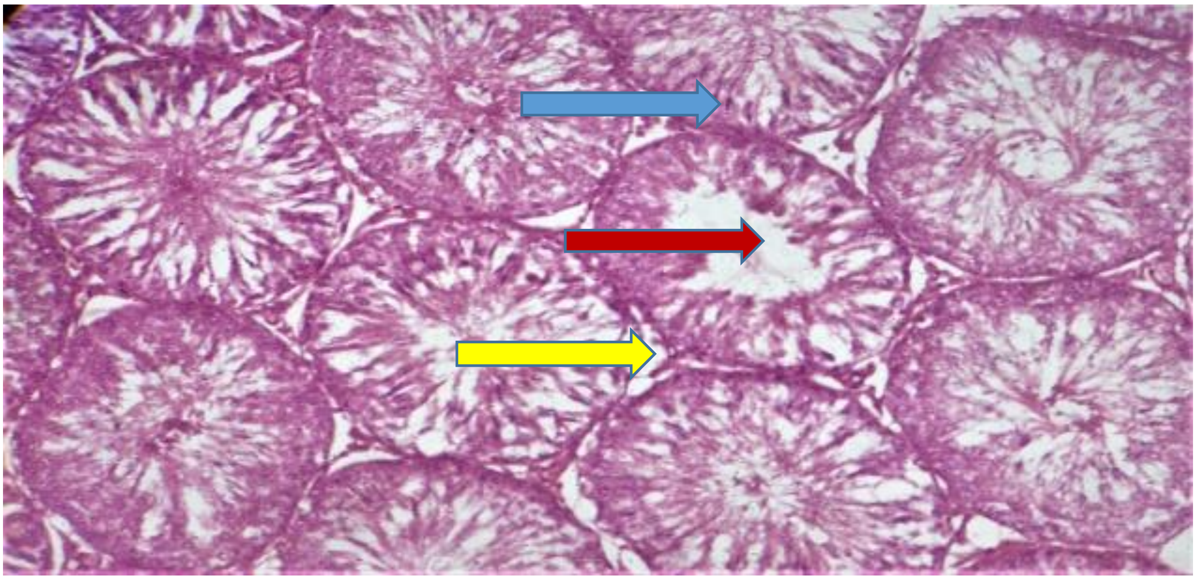

Plate V: Photomicrograph of the the histological section (H & E stain) of a rat testis in
the diabetic group treated with 360 mg/Kg lauric acid (DM + LA 360) (magnification × 400)

Red arrow represents the lumen. Yellow arrow represents the interstitial space. Blue arrow represents the sperm cells

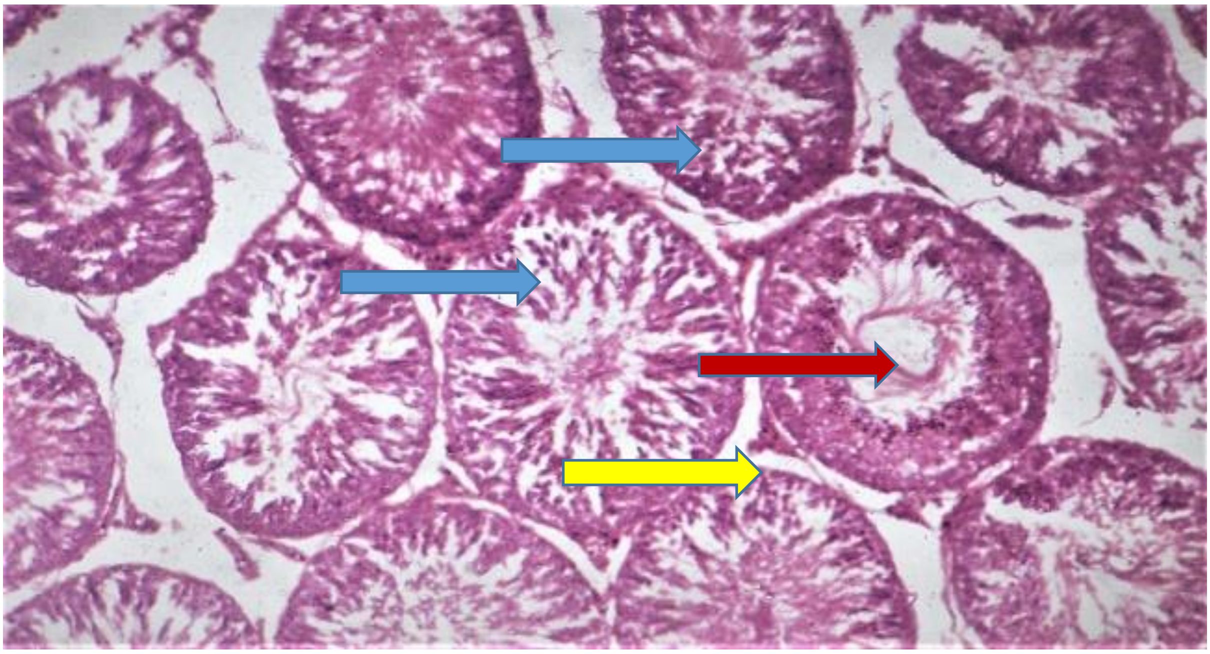

Plate I: Photomicrograph of the the histological section (H & E stain) of a rat testis in
the normal control group (NC) (magnification × 400)

Red arrow represents the lumen. Yellow arrow represents the interstitial space. Blue arrow represents the sperm cells

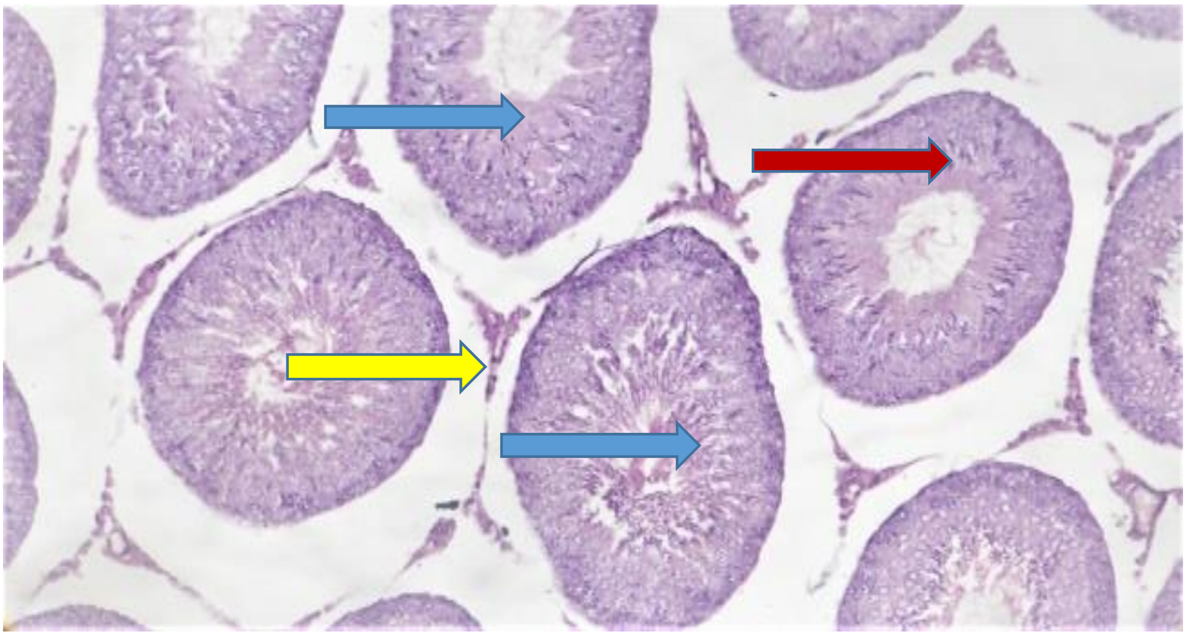

Plate VI: Photomicrograph of the the histological section (H & E stain) of a rat testis in
the diabetic group treated with coconut oil (DM + CO) (magnification × 400)

Red arrow represents the lumen. Yellow arrow represents the interstitial space. Blue arrow represents the sperm cells

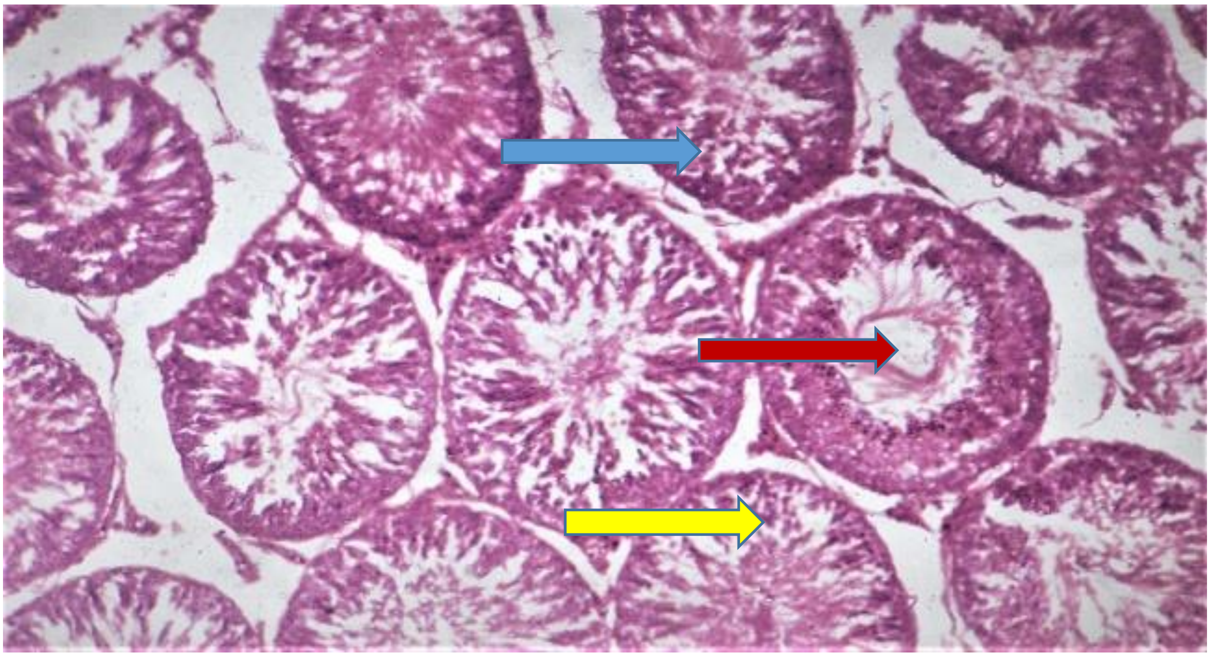

Plate I: Photomicrograph of the the histological section (H & E stain) of a rat testis in
the normal control group (NC) (magnification × 400)

Red arrow represents the lumen. Yellow arrow represents the interstitial space. Blue arrow represents the sperm cells

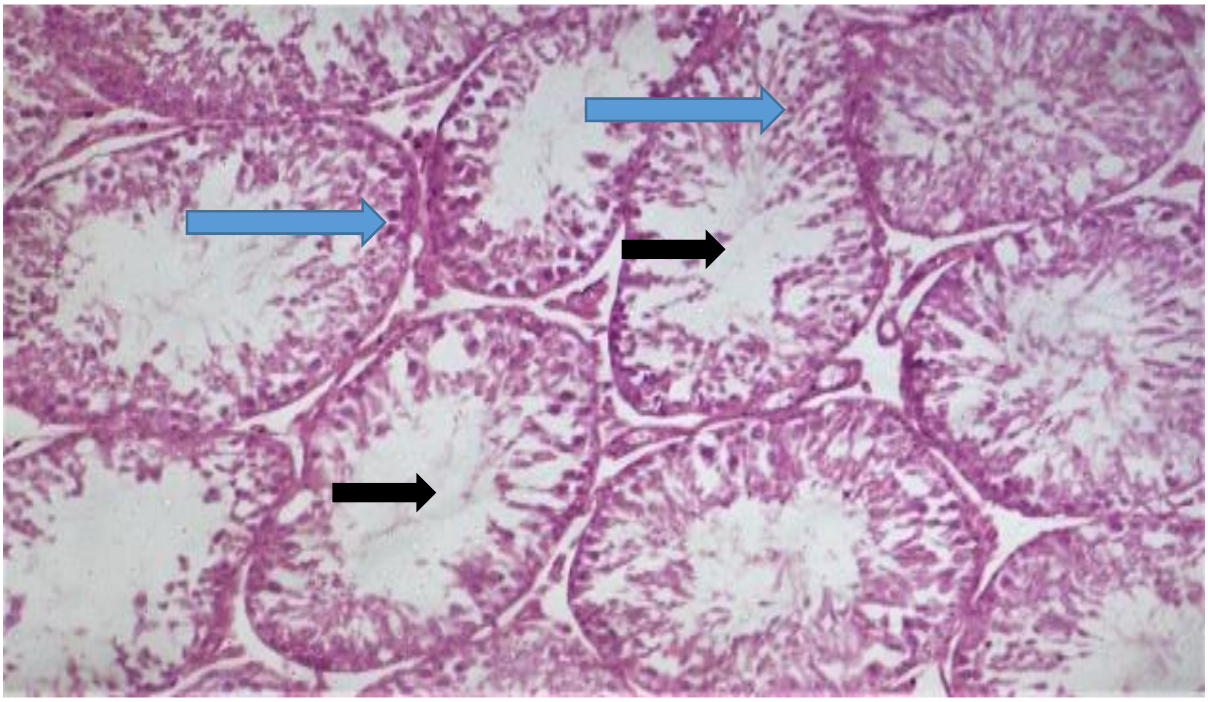

Plate VII: Photomicrograph of the the histological section (H & E stain) of a rat testis in
the diabetic group treated with sildenafil (DM + Sild) (magnification × 400)

Black arrows indicate abnormal morphological alterations. Blue arrow represents the sperm cells

4.8 Dose response to Phenylephrine induced contraction

Figure 4.9 shows percentage contraction of corpus cavernosal tissue strips to cumulative concentrations of phenylephrine in diabetic male Wistar rats. In general there was a concentration-dependent increase in percentage contraction to increasing concentration of phenylephrine (PE).

The percentage contraction was significantly higher ($p < 0.05$) at the concentration of 10^{-8}M in DM-UT rats (38.50 ± 2.25) compared to DM + Sild rats (22.75 ± 3.12).

4.9 Dose response to Potassium chloride induced contraction

Figure 4.10 shows percentage contraction of corpus cavernosal tissue strips to cumulative concentrations of KCl in diabetic male Wistar rats. In general, there was a concentration-dependent increase in percentage contraction to increasing concentration of KCl.

The percentage contraction is significantly higher ($p < 0.05$) in DM-UT rats (51.8 ± 2.4) compared to the NC rats (37.8 ± 2.5) at the concentration of 30 mM. Also, compared to the NC rats, the percentage concentration was significantly higher ($p < 0.05$) in DM + CO rats at concentrations 30 mM (53.8 ± 2.7 vs 37.8 ± 2.5), 40 mM (66.0 ± 4.5 vs 50.3 ± 2.6) and 50mM (77.5 ± 2.5 vs 63.5 ± 2.6), respectively.

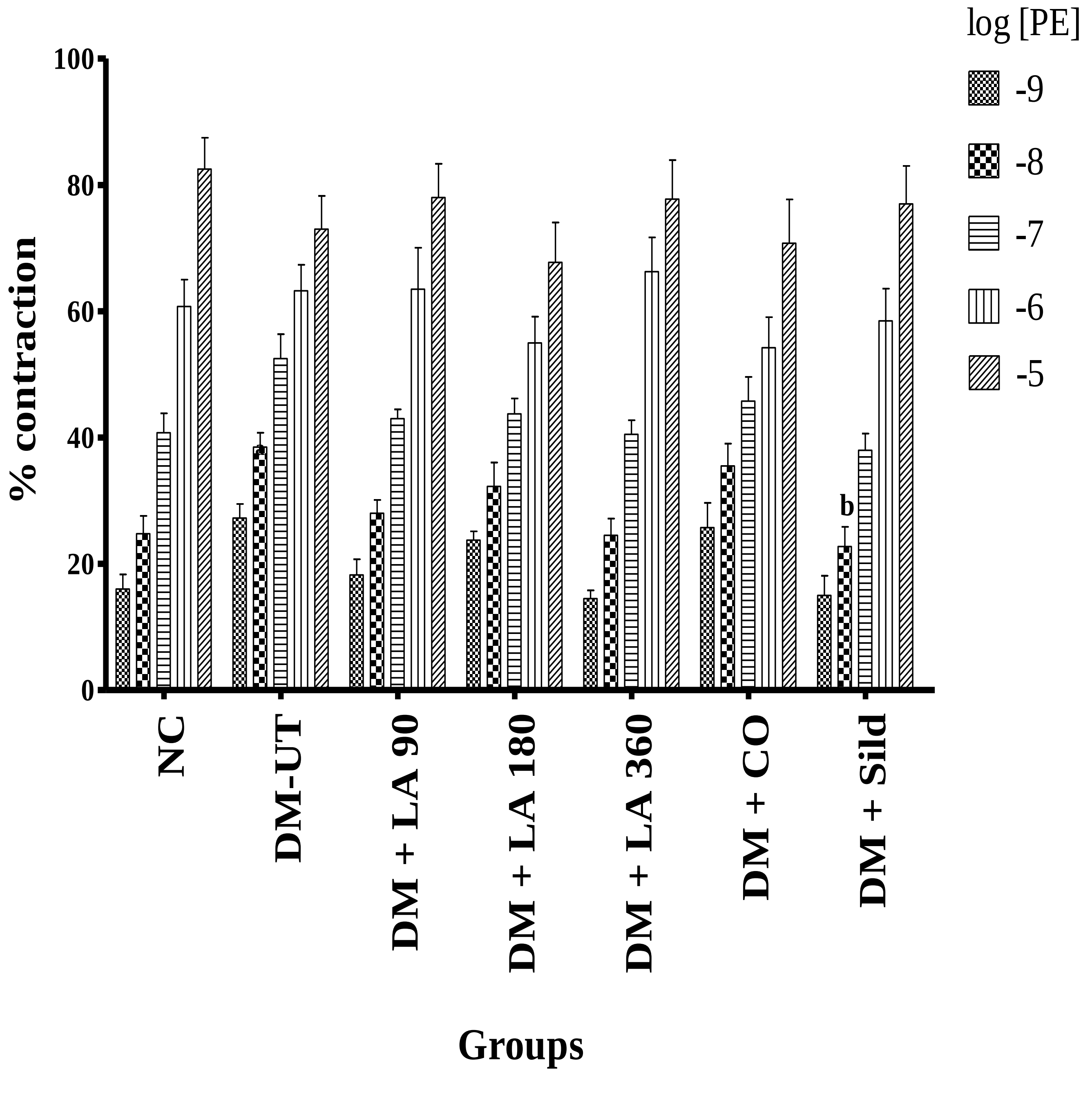

Figure 4.9: Effect of Lauric acid on the percentage contraction to cumulative doses of Phenylephrine (PE) in Diabetic Male Wistar rats

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180 - Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; DM + Sild- Diabetic treated with Silldenafil. **b** - significant compared to DM-UT ($p < 0.05$).

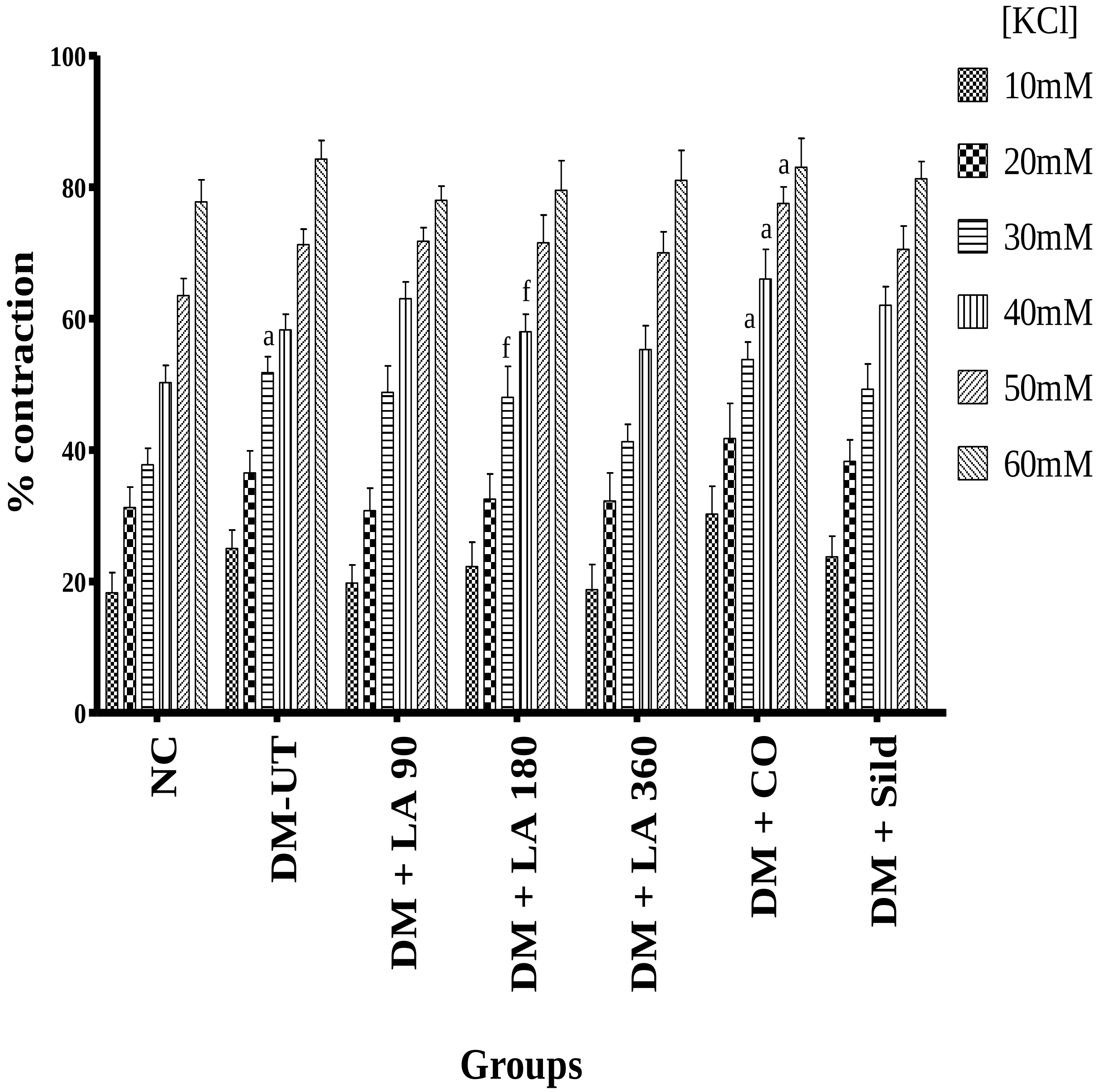

Figure 4.10: Effect of Lauric acid on the percentage contraction to cumulative doses of KCl in diabetic male Wistar rats

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180- Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; DM + Sild- Diabetic treated with Silldenafil. **a -** significant compared to NC ($p < 0.05$)

4.10 Relaxation Response to Acetylcholine in Phenylephrine pre-contracted tissues

Figure 4.11 shows the percentage relaxation of phenylephrine pre-contracted cavernosal tissues to cumulative concentrations of Acetylcholine.

The percentage relaxation was significantly lower ($p < 0.05$) in DM-UT rats compared to NC rats at concentration of 10^{-7} M (35.3 ± 2.8 vs 48.5 ± 4.0), 10^{-6} M (45.5 ± 2.7 vs 59.0 ± 3.8) and 10^{-5} M (51.3 ± 2.3 vs 75.5 ± 2.1), respectively; lower in DM + LA 90 rats compared to NC at the concentration of 10^{-7} M (37.3 ± 3.4 vs 48.5 ± 4.0) and 10^{-5} M (59.0 ± 3.4 vs 75.5 ± 2.1); lower ($p < 0.05$) in DM + LA 180 rats compared to NC rats at concentrations of 10^{-7} M (30.8 ± 2.9 vs 48.5 ± 4.0), 10^{-6} M (41.5 ± 2.6 vs 59.0 ± 3.8) and 10^{-5} M (48.8 ± 2.8 vs 75.5 ± 2.1) respectively; lower ($p < 0.05$) in DM + LA 360 rats compared to NC at concentrations of 10^{-7} M (37.8 ± 3.2 vs 48.5 ± 4.0) and 10^{-5} M: 58.3 ± 3.8 vs 75.5 ± 2.1); lower ($p < 0.05$) in DM + CO rats compared to NC rats at concentrations of 10^{-7} M (27.0 ± 2.5 vs 48.5 ± 4.0), 10^{-6} M (37.8 ± 2.2 vs 59.0 ± 3.8) and 10^{-5} M (41.8 ± 2.6 vs 75.5 ± 2.1), respectively.

The percentage of relaxation was significantly higher ($p < 0.05$) in DM + Sild rats compared to the DM-UT rats at the highest concentration of 10^{-5} M (67.8 ± 2.7 vs 51.3 ± 2.3); higher ($p < 0.05$) in DM + Sild rats compared to DM + LA 180 rats at concentrations of 10^{-6} M (54.5 ± 3.7 vs 41.5 ± 2.6) and 10^{-5} M (67.8 ± 2.7 vs 48.8 ± 2.8); higher ($p < 0.05$) in DM + Sild rats compared to DM + CO rats at concentrations of 10^{-7} M (40.8 ± 3.3 vs 27.0 ± 2.5), 10^{-6} M (54.5 ± 3.7 vs 37.8 ± 2.2) and 10^{-5}M (67.8 ± 2.7 vs 41.8 ± 2.6).

The percentage of relaxation was significantly higher ($p < 0.05$) in DM + LA 90 rats compared to the DM + CO rats at concentrations of 10^{-7} M (37.3 ± 3.4 vs 27.0 ± 2.5),

10^{-6} M (49.0 ± 3.9 vs 37.8 ± 2.2) and of 10^{-5} M (59.0 ± 3.4 vs 41.8 ± 2.6); higher ($p <$ 0.05) in DM + LA 360 rats compared to the DM + CO rats at the concentrations of 10^{-7} M (37.8 ± 3.2 vs 27.0 ± 2.5) 10^{-6} M (51.3 ± 3.7 vs 37.8 ± 2.2) and 10^{-5} M (58.3 ± 3.8 vs 41.8 ± 2.6), respectively.

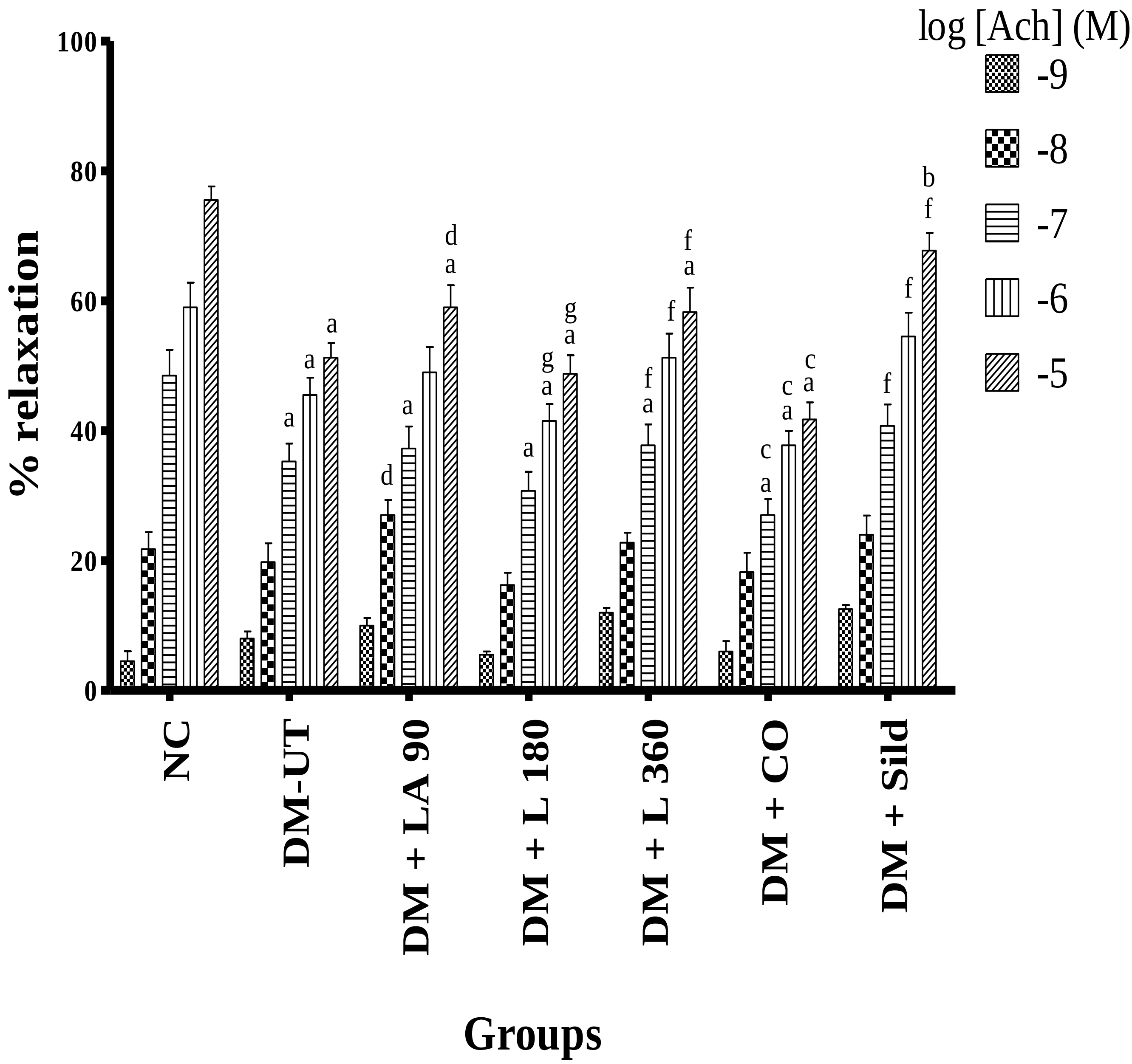

Figure 4.11: Effect of lauric acid on the percentage relaxation of phenylephrine pre-contracted cavernosal tissues to cumulative doses of acetylcholine in diabetic male Wistar rats

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180 - Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; DM + Sild- Diabetic treated with Sildenafil. **a-** significant compared to NC ($p < 0.05$); **b -** significant compared to DM-UT ($p < 0.05$); **c -** significant compared to DM + LA 90; **d** – significant compared to DM + LA 180; **f** – significant compared to DM + CO ($p < 0.05$); **g** – significant compared to DM + Sild

4.11 Relaxation response to Acetylcholine in KCl pre-contracted tissues

Figure 4.12 shows the percentage relaxation to cumulative concentrations of Acetylcholine following a KCl induced contraction.

Compared to the NC rats, the percentage relaxation in DM-UT rats was significantly lower ($p < 0.05$) at concentrations of 10^{-6} M (41.3 ± 3.0 vs 50.0 ± 2.8) and 10^{-5} M (51.3 ± 2.1 vs 61.3 ± 1.3); lower ($p < 0.05$) in DM + LA 180 rats compared to the NC rats at concentrations of 10^{-6} M (38.3 ± 2.0 vs 50.0 ± 2.8) and 10^{-5} M (45.5 ± 1.2 vs 61.3 ± 1.3); lower ($p < 0.05$) in DM + LA 360 rats compared to the NC rats at concentrations of 10^{-6} M (41.5 ± 2.2 vs 50.0 ± 2.8) and 10^{-5} M (53.0 ± 2.0 vs 61.3 ± 1.3); lower ($p < 0.05$) in DM + CO rats compared to the NC rats at concentrations of 10^{-6} M (41.0 ± 2.6 vs 50.0 ± 2.8) and 10^{-5} M (50.8 ± 2.5 vs 61.3 ± 1.3); lower ($p < 0.05$) in DM + Sild rats compared to the NC rats at concentrations of 10^{-7} M (24.3 ± 2.8 vs 35.3 ± 21.6) and 10^{-5} M (50.3 ± 2.1 vs 61.3 ± 1.3).

Compared to the DM + Sild rats, the percentage relaxation in DM-UT rats was significantly higher ($p < 0.05$) at the concentration of 10^{-7} M (35.0 ± 1.9 vs 24.3 ± 2.8); higher ($p < 0.05$) in DM + LA 90 rats compared to DM + Sild rats at the concentrations of 10^{-7} M (32.8 ± 2.5 vs 24.3 ± 2.8); higher ($p < 0.05$) in DM + LA 360 rats compared to DM + Sild rats at the concentrations of 10^{-7} M (36.8 ± 2.8 vs 24.3 ± 2.8); higher (p < 0.05) in DM + CO rats compared to DM + Sild rats at the concentrations of 10^{-7} M (36.8 ± 2.8 vs 24.3 ± 2.8); higher (p < 0.05) in DM + LA 90 rats compared to DM + LA 180 rats at the concentrations of 10^{-6} M (46.8 ± 2.2 vs 38.3 ± 2.0) and 10^{-5} M (56.8 ± 2.1 vs 45.5 ± 1.2).

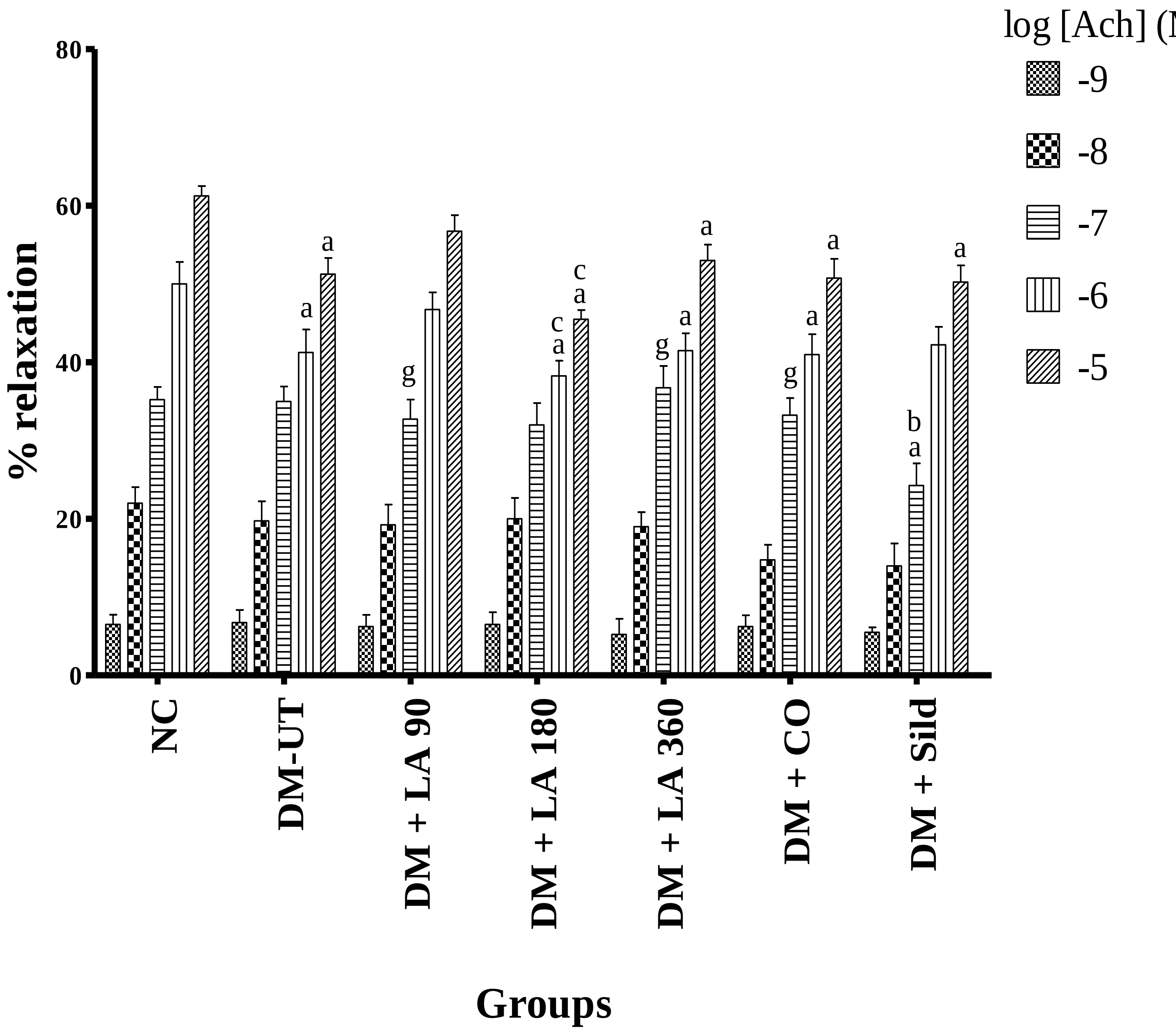

Figure 4.12: Effect of lauric acid on the percentage relaxation in KCl pre-contracted cavernosal tissues to acetylcholine in diabetic male Wistar rats

NC – Normal control. DM-UT – Diabetic untreated. DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg). DM + LA 180- Diabetic treated with Lauric acid (180 mg/kg). DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg). DM + CO – Diabetic treated with coconut oil. DM + Sild- Diabetic treated with Sildenafil. **a -** significant compared to NC ($p < 0.05$); **c** - significant compared to DM + LA 90 ($p < 0.05$). **g** – significant compared to DM + Sild ($p < 0.05$).

4.12 Concentration dependent response to Sodium nitroprusside (SNP) in Phenylephrine pre-contracted tissues

Figure 4.13 shows the percentage relaxation of corpus cavernosal tissues to cumulative concentrations of SNP following phenylephrine-induced contraction.

Compared to NC rats, the percentage relaxation was significantly lower ($p < 0.05$) in DM-UT rats at the concentration of 10^{-5} M (50.3 ± 1.4 vs 60.3 ± 1.7); lower ($p < 0.05$) in DM + LA 360 rats compared to the NC rats at the concentrations of 10^{-7} M (23.5 ± 2.8 vs 36.3 ± 1.7), 10^{-6} M (33.0 ± 4.0 vs 47.8 ± 3.6), and 10^{-5} M (41.5 ± 1.7 vs 60.3 ± 1.7); lower ($p < 0.05$) in DM + CO rats compared to the NC rats at the concentrations of 10^{-7} M (26.5 ± 2.4 vs 36.3 ± 1.7), 10^{-6} M (36.8 ± 3.1 vs 47.8 ± 3.6), and 10^{-5} M (46.3 ± 2.2 vs 60.3 ± 1.7).

Compared to the DM-UT rats: the percentage relaxation was significantly lower ($p < 0.05$) in DM + LA 360 at the concentrations of 10^{-8} M (11.0 ± 2.3 vs 23.0 ± 1.7), 10^{-7} M (23.5 ± 2.8 vs 34.3 ± 2.4), 10^{-6} M (33.0 ± 4.0 vs 44.8 ± 2.4), and 10^{-5} M (41.5 ± 1.7 vs 50.3 ± 1.4); higher ($p < 0.05$) in DM + Sild at the concentrations of 10^{-6} M (53.8 ± 3.8 vs 44.8 ± 2.4) and 10^{-5} M (67.5 ± 2.8 vs 50.3 ± 1.4).

Compared to DM + LA 90 rats, the percentage relaxation was significantly lower ($p < 0.05$) in DM + LA 360 rats at the concentrations of 10^{-8} M (11.0 ± 2.3 vs 21.8 ± 1.8), 10^{-7} M (23.5 ± 2.8 vs 34.0 ± 1.4), 10^{-6} M (33.0 ± 4.0 vs 45.0 ± 2.9), and 10^{-5} M (41.5 ± 1.7 vs 55.5 ± 2.3); lower ($p < 0.05$) in DM + CO rats compared to the DM + LA 90 rats at the highest concentration of 10^{-5} M (46.3 ± 2.2 vs 55.5 ± 2.3). However, the percentage relaxation was significantly higher ($p < 0.05$) in DM + Sild rats compared to the DM + LA 90 rats at the concentrations of 10^{-6} M (53.8 ± 3.8 vs 45.0 ± 2.9) and 10^{-5} M (67.5 ± 2.8 vs 55.5 ± 2.3).

Compared to DM + LA 180 rats, the percentage relaxation was significantly lower ($p < 0.05$) in DM + LA 360 rats at the concentrations of 10^{-8} M (11.0 ± 2.3 vs 23.0 ± 2.6), 10^{-7} M (23.5 ± 2.8 vs 37.8 ± 2.0), 10^{-6} M (33.0 ± 4.0 vs 43.8 ± 1.0), and 10^{-5} M (41.5 ± 1.7 vs 52.0 ± 2.0); and also was also significantly lower ($p < 0.05$) in DM + CO rats compared to the DM + LA 180 rats at the concentration of 10^{-7} M (26.5 ± 2.4 vs 37.8 ± 2.0).

Compared to the DM + Sild rats, the percentage relaxation was significantly lower ($p < 0.05$) in DM + 180 rats compared at the concentrations of 10^{-6} M (43.8 ± 1.0 vs 53.8 ± 3.8) and 10^{-5} M (52.0 ± 2.0 vs 67.5 ± 2.8); lower ($p < 0.05$) in DM + LA 360 rats compared to the DM + Sild rats at the concentrations of 10^{-8} M (11.0 ± 2.3 vs 24.8 ± 2.1), 10^{-7} M (23.5 ± 2.8 vs 42.0 ± 2.2), 10^{-6} M (33.0 ± 4.0 vs 53.8 ± 3.8), and 10^{-5} M (41.5 ± 1.7 vs 67.5 ± 2.8); and also lower ($p < 0.05$) in DM + CO rats compared to the DM + Sild rats at the concentrations of 10^{-7} M (26.5 ± 2.4 vs 42.0 ± 2.2), 10^{-6} M (33.0 ± 4.0 vs 53.8 ± 3.8), and 10^{-5} M (41.5 ± 1.7 vs 67.5 ± 2.8).

4.13 Concentration dependent response to Sodium nitroprusside (SNP) in KCl pre-contracted tissues

Figure 4.14 shows the percentage relaxation of cavernosal tissues to cumulative concentrations of SNP following KCl induced contraction.

Compared to the NC rats, the percentage relaxation was significantly lower ($p < 0.05$) in DM-UT rats at the concentrations of 10^{-7} M (29.0 ± 2.7 vs 41.0 ± 3.8)

Compared to DM-UT rats: the percentage relaxation was significantly higher ($p < 0.05$) in DM + LA 360 rats at the concentration of 10^{-7} M (41.3 ± 3.1 vs 29.0 ± 2.7); and also higher ($p < 0.05$) in DM + Sild rats at the concentration of 10^{-8} M (30.5 ± 2.5 vs 16.0 ± 2.5).

The percentage relaxation was significantly lower ($p < 0.05$) in DM + LA 90 rats compared to DM + Sild rats at the concentration of 10^{-8} M (17.8 ± 2.9 vs 30.5 ± 2.5)

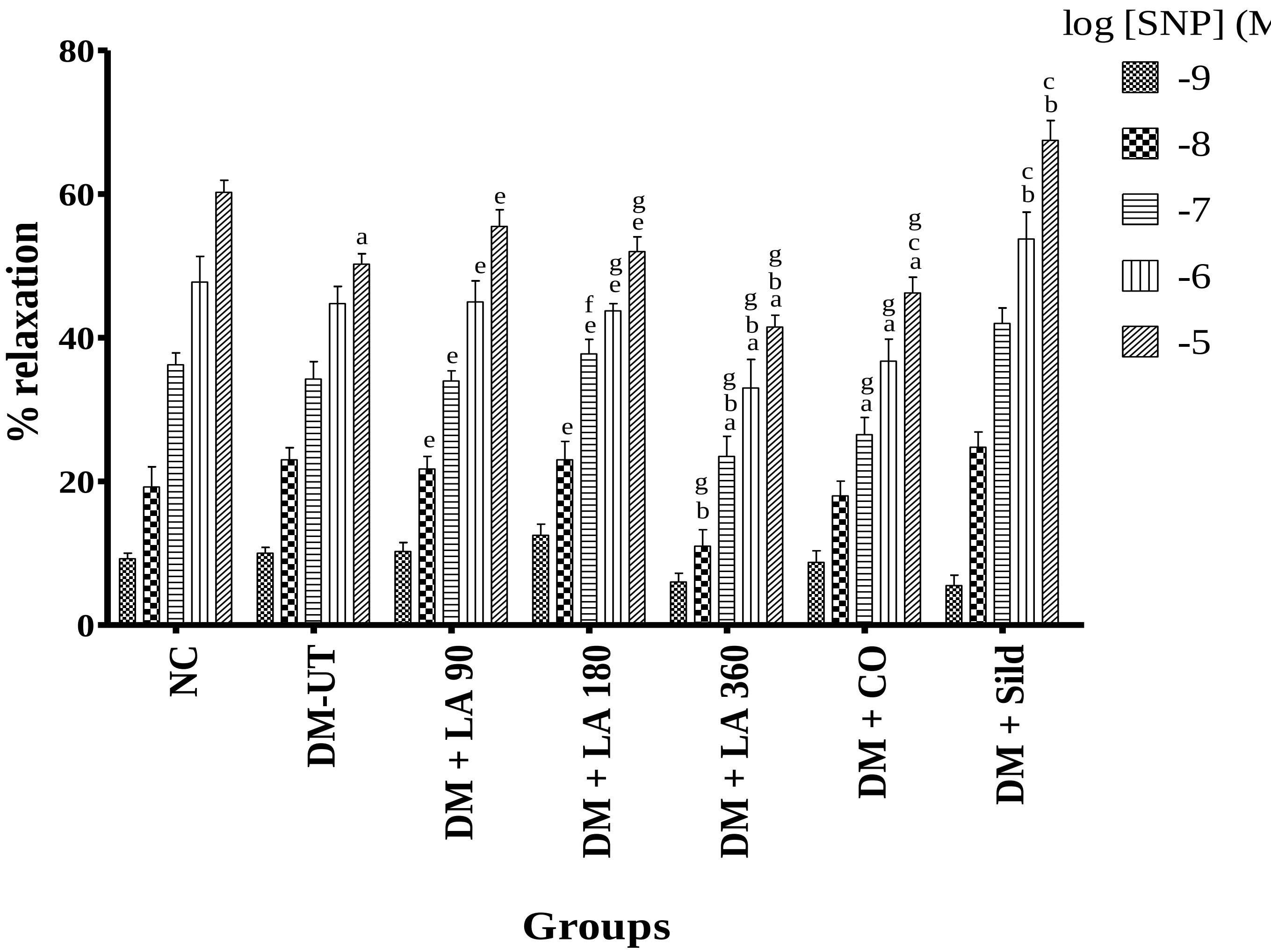

Figure 4.13: Effect of Lauric acid on the percentage relaxation of phenylephrine pre-contracted cavernosal tissues to sodium nitroprusside (SNP) in diabetic male Wistar rats

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180 - Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; DM + Sild- Diabetic treated with Sildenafil. **a-** significant compared to NC ($p < 0.05$); **b** - significant compared to DM-UT ($p < 0.05$); **c** - significant compared to DM + LA 90 ($p < 0.05$); e – significant compared to DM + LA 360; **f** – significant compared to DM + CO ($p < 0.05$); **g** – significant compared to DM + Sild ($p < 0.05$)

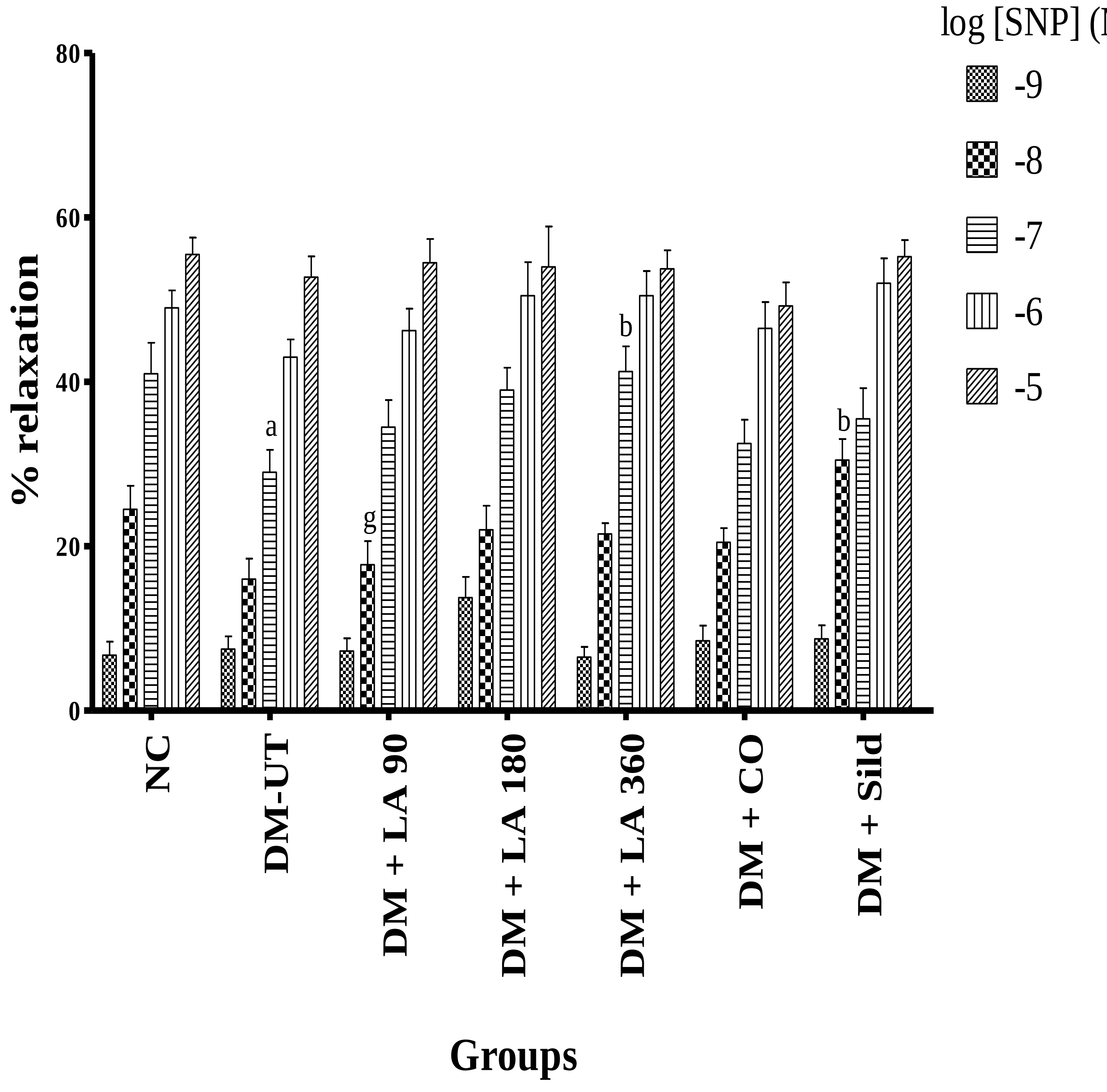

Figure 4.14: Effect of Lauric acid on the percentage relaxation of Sodium nitroprusside in KCl precontracted cavernosal tissues in diabetic male Wistar rats

NC – Normal control; DM-UT – Diabetic untreated; DM + LA 90 – Diabetic treated with Lauric acid (90 mg/kg); DM + LA 180 - Diabetic treated with Lauric acid (180 mg/kg); DM + LA 360 - Diabetic treated with Lauric acid (360 mg/kg); DM + CO – Diabetic treated with coconut oil; DM + Sild- Diabetic treated with Sildenafil. **a-** significant compared to NC ($p < 0.05$); **b** - significant compared to DM-UT ($p < 0.05$); **g** – significant compared to DM + Sild ($p < 0.05$)

4.14: Influence of N-nitro-L-arginine methyl ester (L-NAME) on relaxation response to Lauric acid following Phenylephrine precontraction

Figure 4.15 shows relaxation response of the tissues to cumulative concentrations of lauric acid in the presence of L-NAME following a phenylephrine induced contraction

The percentage relaxation was significantly higher ($p < 0.05$) in the presence of L-NAME compared to the control at the highest concentration of 10^{-5} M (43.3 ± 2.7 vs 33.5 ± 2.6)

4.15 Influence of N-nitro-L-arginine methyl ester (L-NAME) on relaxation response to Lauric acid following KCl pre-contraction

Figure 4.16 shows relaxation response of the tissues to cumulative concentrations of lauric acid in the presence of L-NAME following KCl induced contraction

The percentage relaxation was significantly lower ($p < 0.05$) in the presence of L-NAME compared to the control at the concentrations of 10^{-6} M (21.0 ± 4.7 vs 45.5 ± 5.0) and 10^{-5} M (24.3 ± 5.1 vs 53.0 ± 5.8)

4.16 Effect of Lauric acid on Calcium sensitivity

Figure 4.17 shows the influence of Lauric acid on contraction response to cumulative doses of Ca^{2+}.

No significant change was seen in the contraction response in Lauric acid treated tissues compared to the control at all concentrations

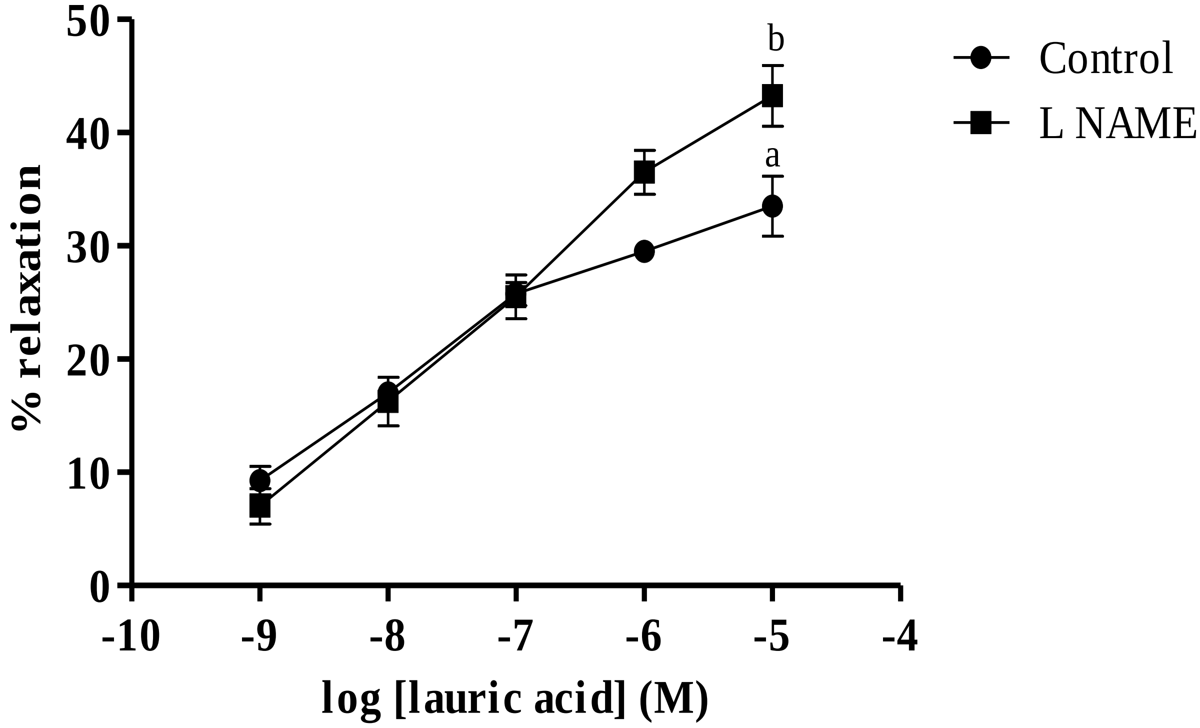

Figure 4.15: Percentage relaxation of phenylephrine pre-contracted cavernosal tissues to cumulative concentrations of lauric acid in the presence of L-NAME

Different superscripts represent significant difference ($p < 0.05$)

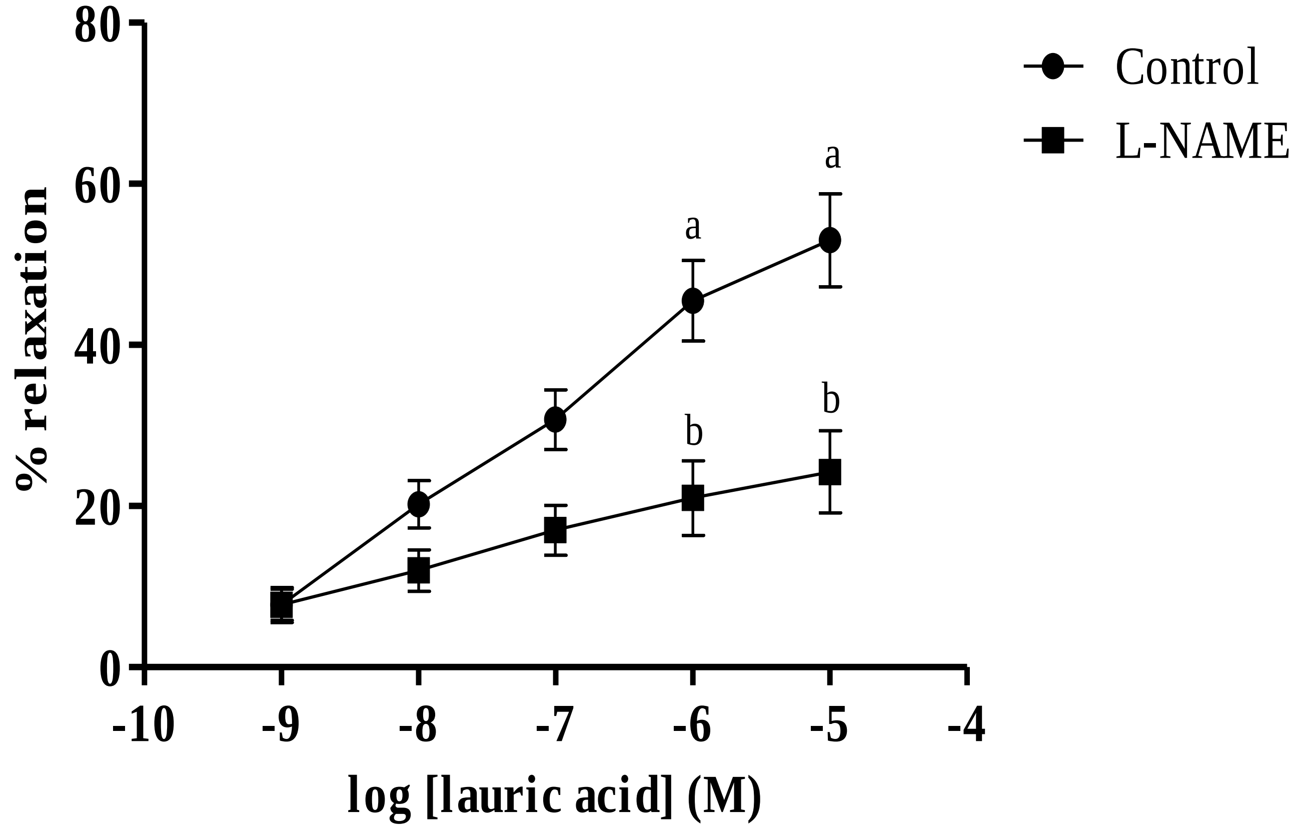

Figure 4.16: Percentage relaxation of KCl pre-contracted cavernosal tissues of male Wistar rats to cumulative concentrations of lauric acid in the presence of L-NAME

Different superscripts represent significant difference ($p < 0.05$)

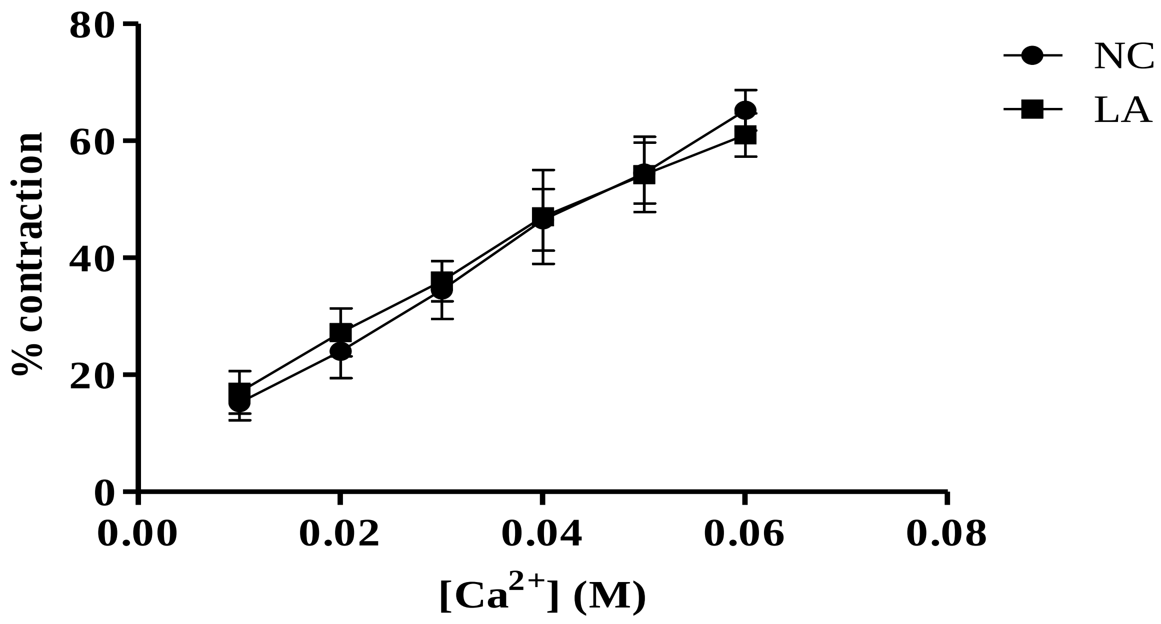

Figure 4.17: Effect of Lauric acid on the percentage contraction of the corpus cavernosum in response to a cumulative concentration of Ca^{2+}

Different superscripts represent significant difference ($p < 0.05$)

CHAPTER FIVE

5.0 DISCUSSION

Attention is being drawn to the physiologic roles of dietary fatty acids on sexual function and there has been a controversy on the health benefits of consuming diet rich in saturated fatty acids (Esmaeili *et al*., 2015). This study showed interesting variations in the effect of lauric acid, a medium chain saturated fatty acid, on the damaging effects of diabetes on some reproductive parameters.

In this study, the decreased gonado-somatic index (GSI) shown in the diabetic untreated rats suggests a reduction in testicular tissue. This usually indicates or leads to compromised testicular structure and function. This was confirmed by the degraded histological structure of the testes observed in the diabetic untreated rats. This agrees with the work of Atta *et al* (2017) who showed that chronic hyperglycaemia observed in diabetes causes oxidative damage to the testes. The failure of lauric acid to ameliorate the decrease in GSI suggests the inability of lauric acid to completely reverse the testicular tissue degradation. In addition, the size of the gonad has been associated with the degree of sperm production in many species (Gomendio *et al*, 2006), thus the lower GSI observed in this study in diabetic untreated rats justifies the observed reduction in sperm count and failure of lauric acid to increase the GSI justifies its inability to boost the sperm count

Reduction in sperm count, motility, viability and percentage of normal sperm cells in diabetic untreated rats observed in this study suggests a decline in or disruption of spermatogenesis. This is in concert with the work of Jangir and Jain (2014), who identified reduced synthesis and secretion of testosterone and testicular degeneration as responsible for the impaired sperm parameters in diabetic conditions. Considering the pivotal role of testosterone in promoting spermatogenesis (Bird & Zilioli, 2018), failure of lauric acid to increase sperm count and some other sperm parameters in diabetic rats in this study could be attributed to its failure to increase serum testosterone level. However, improvement of progressive motility and morphology of the sperm cells by lauric acid points to an improvement in the quality of sperm cells produced despite the reduced quantity.

The reduction in serum testosterone observed in the diabetic untreated rats suggests Leydig cell damage as reported by La Vignera *et al* (2012), and/or disruption in the process of steroidogenesis (Ding *et al.*, 2015). Thus, the failure of lauric acid to reverse the decline in testosterone level could probably be traced to its inability to completely mitigate the leydig cell dysfunction in the diabetic rats. Also, lauric acid has been reported to cause a reduction in low density lipoproteins (LDL) (Dayrit, 2014), which is one of the sources of cholesterol, that serves as a precursor for the production of testosterone (Weinbauer *et al.*, 2010). In addition, Mccoy & Ziering (2012) reported the testosterone reducing property of lauric acid as its mechanism of mitigating androgenetic alopecia. All these may account for the failure of lauric acid to reverse the decline in testosterone level.

Diabetic rats treated with highest dose of lauric acid (360 mg/Kg) showed great improvement in the testicular histology with the presence of a considerable number of spermatids, despite the low gonado-somatic index and impaired sperm parameters. This finding showed that lauric acid prevented some of the diabetes induced testicular damage, portending a potential mitigation of testicular and sperm function as seen by improvement in sperm morphology and motility. This also suggests that with a longer period of treatment, the improved testicular histo-architecture may translate into the complete amelioration of the impaired sperm and testicular parameters.

Interestingly, the potency of lauric acid in mitigating diabetes-induced sperm impairment was not as much as observed with coconut oil treatment even though lauric acid is largely proposed to be responsible for the actions of coconut oil. This could be due to the absence of the polyphenols, which are sources of antioxidants present in coconut oil (Nevin and Rajamohan, 2004), and is proposed to be responsible for the reported antioxidant property of coconut oil (Iranloye et *al*., 2013). Considering oxidative stress is involved in the progression of diabetes and its associated complications, the antioxidant properties of coconut oil could have been responsible for the appreciable mitigation of oxidative damage to the testes, thus improving the testicular and sperm parameters in diabetic rats observed in this study. This is in line with the study carried out by Dosumu *et al*. (2010), showing the ameliorative effect of coconut oil on testicular and sperm dysfunction. This also suggests that for lauric acid to have a higher potency on the sperm and testicular parameters, it requires the synergistic support of other constituents in coconut oil, which are absent when lauric acid is used alone.

Penile erection is favoured by the relaxation of the corpus cavernosum, while it is inhibited by contraction (Carneiro et al., 2009). Phenylephrine and KCl used as contracting agents in this study were used to simulate contraction due to sympathetic stimulation and depolarization respectively in accordance to the report of Karaki & Weiss (1984). This study revealed no impact of diabetes on phenylephrine induced contraction. Considering phenylephrine is an α1-adrenoceptors, this implies that adrenergic receptor mediated contraction of the corpus cavernosum is not modulated in diabetic conditions. There have been controversial reports on vascular reactivity of different smooth muscles to sympathetic stimulation in diabetic conditions. Amplified contraction response was reported by Mohebbati et al (2018), while Owu et al (2013) showed a reduced contraction response. This study however agrees with the work of Sullivan et al (2002) who reported that contraction response of diabetic smooth muscles to sympathetic stimulation is unaltered.

This study revealed slight heightened contraction response of cavernosal tissues to KCl in diabetic untreated rats. A high concentration of K^+ as used in this study has been shown by Kirschstein et al (2009) to increase membrane depolarization leading to the influx of Ca^2 through voltage-gated calcium channels. Thus the result of this study suggests increased depolarization in the cavernosal tissues of diabetic untreated rats and the normal contraction response in lauric acid treated diabetic rats portends a possible reversal of this depolarization induced excess Ca^{2+} influx.

In cavernosal strips pre-contracted with phenylephrine and KCl respectively, there was a decline in the relaxation response of the cavernosal tissues of the diabetic untreated rats to acetylcholine. This suggests the possibility of endothelial dysfunction, an associated complication of diabetes as reported by Sánchez *et al* (2012), thus compromising the potency of acetylcholine in stimulating the production of NO by the endothelium. This could be responsible for the reduced relaxation. The failure of lauric acid to improve the relaxation in phenylephrine contracted tissues reveals the inability to reverse the endothelial damage or impairment in erectile response to parasympathetic stimulation. However, the appreciable improvement in relaxation in KCl pre-contracted tissues of diabetic rats treated with the lowest dose of lauric acid (90 mg/Kg) suggests that lauric acid at a low dose inhibits the voltage-gated calcium channel activated by KCl thus enhancing the relaxant effect of acetylcholine.

The difference in the relaxation response compared to phenylephrine pre-contracted tissues could be due to the differences in their forcez of contraction. Contractile agents like Phenylephrine, which act on G protein coupled receptors have been shown to produce a greater force of contraction compared to KCl. This is because G proteins activates other cellular processes leading to increased calcium sensitivity of the contractile proteins further amplifying contraction (Ratz et al., 2019). This may explain the relatively higher relaxation response to acetylcholine in KCl-contracted cavernosal tissues.

In cavernosal tissues pre-contracted with phenylephrine and KCl, this study showed a slight decline in sodium nitroprusside (SNP) induced relaxation in the diabetic untreated animals. Considering SNP is a nitric oxide donor, this suggests reduced bioavailability of cGMP, which mediates the relaxing action of NO in cavernosal smooth muscles, (Hidalgo-Tamola & Chitaley, 2009). Thus, improvement in relaxation response in tissues of diabetic rats treated with lower doses of lauric acid suggests the prevention of the impairment of cGMP signalling or possible inhibition of PDE5 thus reducing the metabolism of the available cGMP. This thus potentiates the relaxation. The improvement of relaxation in KCl pre-contraced diabetic cavernosal tissues by all the doses of lauric acid as opposed to only lower doses in phenylephrine contracted tissues, further confirms the possibility of lauric acid acting on voltage gated calcium channels to reduce the Ca^{2+} influx through the cell membrane caused by KCl.

Sildenafil, used as the reference drug in this study, is a major standard oral medication for the treatment of erectile dysfunction. Being a phosphodiesterase 5 (PDE5) inhibitor, the actions of sildenafil culminates in sustaining cGMP bioavailability thereby enhancing or prolonging the relaxation of the corpus cavernosum, thus favouring penile erection (Lue, 2000). This is also further supported by the improvement of relaxation to SNP in sildenafil treated diabetic rats. Lauric acid relatively showed to be equally potent in improving relaxation especially to SNP compared to sildenafil and thus can be considered as an alternative therapy.

In this study, lauric acid treatment greatly increased the relaxation of tissues pre-contracted with phenylephrine even in the presence of N-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase (NOS) inhibitor. This indicates that the relaxant effect of lauric acid is not solely dependent on NO production by the endothelium. Considering the production of NO by the endothelium is compromised in diabetic conditions as reported by Dalaklioglu and Ozbey, (2013), lauric acid offers a better alternative therapy for erectile dysfunction in diabetic conditions. However, in tissues pre-contracted with KCl, there was a depressed relaxation response at higher concentrations of lauric acid. This ssuggests that the inhibitory action of lauric acid on the depolarizing action of KCl is dependent on NO bioavailability.

Calcium sensitivity plays a pivotal role in smooth muscle contraction, hence its importance in the process of erection (Jiang & Chitaley, 2012). The contraction response to the $CaCl_2$ in this study was not modulated by lauric acid. This indicates that relaxation mechanism of lauric acid does not involve the reduction of calcium sensitivity

This study aligns with the research conducted by Alves *et al*. (2017) who reported the relaxant action of lauric acid on the superior mesenteric artery of spontaneous hypertensive rats. This was attributed to the antioxidant action of lauric acid. This could also be responsible for the improvement of relaxation observed in the corpus cavernosum of the diabetic rats and thus pointing to its potential in improving erectile function.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Gonadosomatic index was significantly decreased ($p < 0.05$) in diabetic untreated rats compared to the control (0.27 ± 0.07 vs 0.79 ± 0.04) and was not improved by lauric acid treatment.

Sperm count, sperm viability, sperm morphology and sperm motility were all impaired in diabetic untreated rats. Diabetic rats treated with lauric acid had significant increase ($p < 0.05$) in the percentage of normal cells at a dose of 90 mg/Kg (58%) and 360 mg/Kg (62%) compared to the diabetic untreated group (43%). Progressive motile sperm cells were significantly ($p < 0.05$) increased in lauric acid treated diabetic rats at the dose of 90 mg/Kg (9%) compared to the diabetic untreated group (0%).

Serum testosterone level was significantly decreased ($p < 0.05$) in diabetic untreated rats (0.31 ± 0.03 ng/ml) compared to the control rats (0.92 ± 0.10 ng/ml) and lauric acid failed to significantly boost it.

Histological structure showed degradation in the diabetic untreated rats, but was improved in diabetic rats treated with a higher dose of lauric acid

Dose dependent contractile response of the corpus cavernosal tissues to phenylephrine showed no significant change in contraction response of corpus cavernosal smooth muscle in diabetic untreated rats and lauric acid treatment has no modulatory role.

Dose dependent contractile response of the corpus cavernosum smooth muscles to KCl showed significant heightened response ($p < 0.05$) compared to the control in diabetic untreated rats at 30mM (51.8 ± 2.4 vs 37.8 ± 2.5). This was ameliorated in lauric acid treated diabetic rats.

Relaxation response of KCl pre-contracted corpus cavernosal smooth muscle to acetylcholine was significantly improved ($p < 0.05$) in diabetic rats treated with lauric acid at the dose of 90 mg/Kg compared to the diabetic untreated (from 41.25 ± 2.95 to 46.75 ± 2.17; at 10^{-6} M [acetylcholine]) and (from 51.25 ± 2.06 to 56.75 ± 2.06; at 10^{-5} M [acetylcholine]), respectively.

Relaxation to sodium nitroprusside (SNP) in phenylephrine contracted corpus cavernosal smooth muscle was significantly improved ($p < 0.05$) in diabetic male rats treated with lauric acid compared to the diabetic untreated (from 50.25 ± 1.44 to 55.50 ± 2.33; at 10^{-5} M [SNP]) at the dose of 90 mg/Kg and (from 50.25 ± 1.44 to 52.00 ± 2.04; at 10^{-5} M [SNP]) at the dose of 180 mg/kg.

Relaxation to SNP in KCl contracted corpus cavernosal smooth muscle was significantly improved ($p < 0.05$) in diabetic rats treated with lauric acid compared to diabetic untreated rats (from 29.00 ± 2.74 to 34.50 ± 3.28; at 10^{-7} M [SNP]) at the dose of 90 mg/kg, (from 29.00 ± 2.74 to 39.00 ± 2.74; at 10^{-7} M [SNP]) at the dose of 180 mg/kg and (from 29.00 ± 2.74 to 41.25 ± 3.07) at the dose of 360 mg/kg

In the presence of L-NAME, percentage relaxation of phenylephrine contracted corpus cavernosal smooth muscle of male rats to lauric acid was significantly increased ($p < 0.05$) compared to the control (from 33.50 ± 2.63 to 43.25 ± 2.69) at 10^{-5} M). Percentage relaxation of KCl contracted corpus cavernosal tissues was significantly decreased ($p < 0.05$) in the presence of L-NAME compared to the control at the concentrations of 10^{-6} M (21.0 ± 4.7 vs 45.5 ± 5.0) and 10^{-5} M (24.3 ± 5.1 vs 53.0 ± 5.8).

Contraction response of corpus cavernosal smooth muscles in a Ca^{2+} free solution, to cumulative doses of Ca^{2+} following injection with EGTA (a calcium chelating agent) was unchanged in even in the presence of lauric acid.

6.2 Conclusion

Based on the results of this study, it was concluded that lauric acid ameliorated some but not all diabetes mellitus induced testicular dysfunctions. Furthermore, it improved erectile function in diabetic rats by improving the relaxation of corpus cavernosum smooth muscles. The mechanisms proposed include; inhibition of the depolarizing action of KCl, enhancing NO-dependent and independent relaxation. Relaxant role of lauric acid can also be attributed to mechanisms independent of the endothelium, however it cannot be attributed to reduced calcium sensitivity.

6.4 Recommendations

i) Specific calcium channels and potassium channels need to be explored to further investigate the possible therapeutic roles of lauric acid in endothelium independent relaxation in diabetic male rats

ii) A longer period of treatment can be explored to see if lauric acid completely prevent the diabetes induced testicular and sperm dysfunction

iii) More advanced biomedical techniques should be explored in a bid to map the stages of spermatogenesis in the testes and more molecular markers in sperm analysis need to be explored to fully ascertain the role of lauric acid on the disruption of spermatogenesis in diabetics.

iv) The roles of cGMP and cAMP can be further investigated to further confirm the secondary messenger mechanism involved in the relaxant actions of lauric acid

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Appl No.: ABUCAUC/2017/Human Physiology/019
Approval No: ABUCAUC/2017/019

30th April, 2018

Dr. R.A. Magaji
Department of Human Physiology,
College of Health Sciences,
Ahmadu Bello University,
Zaria.

Sir,

APPROVAL OF RESEARCH STUDY 'EFFECT OF LAURIC ACID ON ERECTILE FUNCTION IN DIABETIC MALE RATS'

This is to convey the approval of the ABUCAUC to you for the aforestated study domiciled in the Department of Human Physiology. The approval is predicated on the assumption that you shall maintain and care for the Experimental Animals as approved after the visitation of the Committee.

Monitoring of the Research by spot checks, invitations or any other means the Committee deems fit shall be undertaken at the convenience of the Committee.

This approval can and shall be revoked should a significant breach in the terms and condition of the approval occur. It is hence your responsibility to ensure that the agreed terms are maintained to the end of the Study.

The said approval shall be posted on the ABUCAUC Page on the University's website.
Note upon completion of the research, ethical clearance certificate will be issued.

U.D. Abdullahi
For: Chairman, ABUCAUC.

Cc: Director, DAPM
Director, IC &ICT
Provost, College of Health Sciences,
HOD, Human Physiology,
Prof. Aliyu Mohammed, Dept. of Human Physiology