THE EFFECTS OF THE WILD AFRICAN POTATO (HYPOXIS HEMEROCALLIDEA) SUPPLEMENTATION ON STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS REPRODUCTIVE FUNCTION

By

AUDREY EMMERENTIA JORDAAN

Thesis submitted in fulfillment of the requirements for the degree Master of Technology: Biomedical Technology

In the

Faculty of Health and Wellness Sciences

At the

CAPE PENINSULA UNIVERSITY OF TECHNOLOGY

Supervisor: Dr YG Aboua

Co-supervisor: Prof SS du Plessis

Bellville

September 2015

DECLARATION

I, Audrey Emmerentia Jordaan, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Date**

ABSTRACT

Diabetes mellitus (DM) has been reported to be one of the greatest global public health threats. Statistics of the fertility status of modern society has linked increased DM to a decrease in fertility rates. Hyperglycaemia is characteristic of DM that results in a disturbance of proteins, lipids and carbohydrate metabolism leading to an increase production of reactive oxygen species (ROS). In the case where ROS overwhelms antioxidant mechanisms, the body goes into state of oxidative stress (OS). OS plays a vital role in the progression of DM which leads to dysfunction and damage of various organs including that of the reproductive system. Os has shown to cause damage to the sperm membraneby oxidation of polyunsaturated fatty acids (PUFA's) as the sperm membrane are rich in PUFA's. This damage contributes to reduced sperm motility, concentration, morphological abnormalities and the sperms ability to fuse with the ZP of the oocyte. DM has been observed to cause testicular degeneration by interrupting sertoli cell production and maintenance thus resulting in a disturbance of the normal functioning of the reproductive system.

Experimental studies have targeted more natural sources for treating DM and its complications of the reproductive system. Plants and natural dietary substances have shown to have high antioxidant contents that combat DM induced oxidative stress. This study explored the effect the *Hypoxis hemerocallidea* (*H. hemerocallidea*) supplementation on testicular and epididymal tissue, sperm motility and reproductive hormones in male wistar rats. The experiment were conducted for 6 weeks and the rats (230-260 grams) were randomly divided into 5 groups (n=12 per group). Diabetes was induced in 3 of the 5 groups. The first group was the normal control group (A), second the diabetic control group (B), third was the diabetic group treated with 800mg/kg *H. hemerocallidea* (group C), fourth the diabetic group treated with 200mg/kg *H. hemerocallidea* (group D) and fifth the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea* (group E).

Blood glucose showed a significant increase in the diabetic group when compared to the normal control and treated groups. *H. hemerocallidea* showed improvement in sperm motility and sperm morphology more at 800mg/kg when compared to diabetic group and diabetic group treated with 200mg/kg. Body, testicular and epipidymal weights of diabetic control were significantly lower when compared to the other groups. Testicular and epididymal Malondialdehyde levels were decreased in normal control, diabetic groups treated with different doses of *H. hemerocallidea* and the non-diabetic group supplemented with *H. hemerocallidea*on comparing with the diabetic control group. Antioxidants such as Superoxide dismutase, Catalase and total Glutathione activity was observed to be dosage dependent in certin groups but most showed a significant increase when compared to the

diabetic control group. The total antioxidant capacity was measured using Oxygen radical absorbance capacity (ORAC) and Ferric ion reducing antioxidant power (FRAP); increase was observed when normal control group and treated groups were compared to the diabetic group. Testosterone and estradiol levels were also increased when the normal control group and treated groups were compared to the diabetic control group.

Based on our findings it can be concluded that *H. hemerocallidea* supplementation can potentially be used to counteract deleterious effects of DM on the male reproductive system.

Key words: Oxidative stress, Diabetes mellitus, male reproduction system, *H. hemerocallidea*, Testicular, epididymal, antioxidants

ACKNOWLEDGEMENTS

I wish to thank:

- First and foremost, I would like to thank the Lord God Almighty without nothing would be made possible. Thank You for Your strength, love, protection, provision, and also for the gifts of knowledge, wisdom, patience and favour.

- My parents and my sisters; for the motivation, encouragement, care, love and prayer.

- My supervisor Dr YG Aboua for his dedication and devotion in making my research a complete success. Thank you for the encouragement and support.

- My co-supervisor Prof SS du Plessis for his engagement to make my work an outstanding.

- Special thanks to Mr Fanie Rautenbach from the Oxidative Stress Laboratory for making me technically fit as a scientist, imparting your knowledge, experience, for the patience, guidance and availability.

- CPUT University Research Funding (URF) for the financial support for the realisation of this project. CPUT Centre for Postgraduate Studies (CPGS) for the provision of full-time CPUT postgraduate bursary.

- My friends, family, and colleagues: William Jordaan, Emily Jordaan, Michelle Andrews, Marissa Dixon, Boitumelo Mosito, Keamogetswe Ntamu, Mediline Goboza, Mandisa Sobantu for their willingness to help and support me.

- CPUT postgraduate residence and residence co-ordinator Mrs Sharon du Plessis and her family for making my stay on campus feel like home.

- To Stellenbosch University that housed our rats and allowed us to use their Andrology laboratory facilities. A special thanks to Mr. Noel and Mr. Williams for helping us with sacrificing the rats.

- To the Life Science Faculty at the University of the Western Cape for allowing us to use their laboratory for analysis of sperm morphology.

DEDICATION

This thesis is dedicated to the glory of the **Almighty God**

&

my beloved belated father **William Aubrey Moses Jordaan**

LIST OF OPERATIONAL TERMS AND CONCEPTS

Antioxidants	Compounds that act as scavengers of free radicals. Molecules that prevent oxidation of other molecules
Chain breaking molecules	Molecule that inserts itself in the cell membranes of cells and ceases LPO
Endogenous	Originating from within an organism
Exogenous	Taken up from ext ernal environment
Free radical	Reactive oxidizing molecules that has atleast one or more unpaired electrons and in excess can induce LPO and DNA damage
In vivo	A measurement or a process taking place in a live body
Lipid peroxidation	Oxidative degradation of lipids and propagating lipid chain breaking reaction initiated by the attack of free radicals
Oxidation	The combination of a substance with oxygen with subsequent loss of electrons
Oxidative Stress	Metabolic imbalance between the production of free radicals and their scavenging counteracting antioxidants in favour of free radical overload and subsequent cellular damages
Reactive oxygen species	Any compound derived from oxygen which contains one or more unpaired electron(s)

LIST OF ABBREVIATIONS

A

AC	Adenyl cyclases
AR	Acrosome reaction
ATP	Adenosine Triphosphate

B

BCA	Bicinchoninic acid
BSA	Bovine Serum Albumin

C

Ca^{2+}	Calcium
cAMP	Cyclic Adenosine Monophosphate
CAT	Catalase

D

DM	Diabetes mellitus

F

FSH	Follicle stimulating hormone
FRAP	Ferric ion reducing antioxidant power

G

GIT	Gastrointestinal tract
GnRH	Gonadotropin- releasing hormone
GSHt	Total Glutathione

GPx	Glutathion peroxidase

H

H_2O_2	Hydrogen peroxide
HCO_3^-	Bicarbonate
Hypoxis	*H. hemerocallidea*

I

IDDM	Insulin- dependent diabetes mellitus

L

LH	Luteinizing hormone
LPO	Lipid peroxidation

M

MDA	Malondialdehyde

N

NADPH	Nicotine adenine dinucleotide phosphate
NIDDM	Non-Insulin dependent diabetes mellitus
NO	Nitric oxide

O

O_2	Oxygen
ORAC	Oxygen radical absorbance capacity
OS	Oxidative stress

P

PHPGx	Phospholipid hydroperoxide glutathione peroxide
PKA	Protein kinase A

R

ROS	Reactive Oxygen species

S

SOD	Superoxide dismutase
STZ	Streptozotocin

T

TK’s	Tyrosine kinases

Z

ZP	Zona pellucida

CONTENT

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1. Statement of research problem

Male reproductive problems have been confirmed, by clinical and epidemiological studies, to be growing increasingly. Statistics have shown that infertility affects 13-18% of couple's worldwide. On reviewing the fertility status of modern society, it was revealed that decreased fertility rates are closely related to increased prevalence of DM (Hamilton and Ventura, 2006; Alves *et al*., 2013). Diabetes mellitus (DM) has been reported as one of the greatest global public health threats and its prevalence is rapidly increasing (WHO, 2002; Agbaje *et al*., 2007). Diabetic induced infertility has been linked to the overproduction of reactive oxygen species (ROS) consequently leading to oxidative stress status in the male reproductive system. Oxidative stress (OS) decrease sperm quality, sperm count as well as male reproductive function (Bacetti *et al*., 2002; Mallidis *et al*., 2009; Navarro-Casado *et al*., 2010). The World Health Organisation (WHO), (1994) has emphasised the use of phytomedicine as it is rich in antioxidants and has been used to treat many diseases including DM. Plants like *H. hemerocallidea* has been used traditionally to relief disease states for centuries. Therefore, it is important to investigate the benefits of plants such as *H. hemerocallidea* and its remedial effects on diabetes induced male infertility.

1.2. Research problem

It has been indicated that 15% of couples struggle to achieve pregnancy within one year and of those, 5% are unable to conceive (Te Velde and Pearson, 2002). Male factor infertility has been found in 45-50% of these cases (WHO, 2000). Many factors have been associated with male infertility but DM is one of the most rapidly increasing concerns (WHO, 2002). Obesity, lifestyle, population growth and ageing have contributed to DM. DM causes an increase of glucose and the body goes into hyperglycaemic state. Hyperglycaemia generates excess ROS and attenuates the body's natural defence mechanism, inactivating antioxidant activity (Vincent *et al*., 2004; Pop-Busui *et al*., 2006). When ROS overwhelms endogenous antioxidants it is vital to supplement the body with exogenous antioxidants as it assist endogenous antioxidants with its function in maintaining homeostasis.

Reports indicate that *H. hemerocallidea* is a rich source of antioxidants. However, there is lack of pertinent information in scientific literature on the effect of *H. hemerocallidea* on oxidative status, reproductive functions and signalling mechanisms related to diabetes. This study proposes the use of *H. hemerocallidea* as treatment for DM and its effects on male reproduction.

1.3. Aim of research study

The present study aims to observe whether the *H. hemerocallidea* could inhibit the destructive effects of oxidation and prevent damage to the reproductive system of diabetic male wistar rats.

1.4. Research aim and objectives

The present study is designed to assess the effect of *H. hemerocallidea* supplementation on oxidative stress status of testicular and epididymal tissue in diabetic and non-diabetic wistar rats. The tendency of oxidative stress results in the production of abnormal sperm therefore sperm parameters will also be analysed to observe if *H. hemerocallidea* has any positive effects on spermatogenesis of STZ-induced diabetic wistar rats. The following procedures will be done to achieve the objectives.

Investigate sperm motility and morphology of cauda epididymal sperm.

Measured antioxidant enzymes such as catalase, superoxide dismutase activity and total glutathione levels in both testicular and epididymal tissues.

Measured oxidative stress biomarkers such as thiobarbituric acid (TBA) and total antioxidant capacity (TAC) of testicular and epidymal tissue.

Estimation of serum levels of reproductive hormones such as testosterone and estradiol.

CHAPTER 2

LITERATURE REVIEW

The male reproductive system consists of various organs that help in the production of functional spermatozoa and assists in aiding the release of the spermatozoa into the female reproductive tract for conception (Treuting and Dintzis, 2011; Mawhinney and Mariotti, 2013). Any dysfunction in this can lead to male infertility. Infertility is the term doctor's use when acouple is unable to conceive after one year of unprotected intercourse (WHO, 2000). About 15% of couples do not achieve pregnancy in one year thus they seek medical treatment for infertility. According to the Male infertility best practice policy committee of the American Urological association and Practice committee of the American Society of reproductive medicine (2006), 50% of infertility is contributory of male factors together with abnormal semen parameters (Dohle *et al.,* 2010). When semen analysis was done on infertile men, it was observed that there were a decreased number of spermatozoa, also known as oligozoospermia, decreased sperm motility (asthenozoospermia) and many morphological changes (teratozoospermia). These sperm abnormalities usually occur together and is called oligo-astheno-tetatozoospermia (OAT) syndrome (Arikawe *et al*., 2006; Jungwirth *et al.,* 2012). Male infertility has been characterised by the WHO (2000) as the presence of more than one reproductive anomalies, these include obstruction in the reproductive tract, abnormal spermatogenesis and inadequate sexual or ejaculatory functions (Isidori *et al*., 2005; Patki *et al*., 2004). Reproduction can be affected by environmental, physiological, lifestyle, genetic factors and physical stress (Gaur *et al*., 2007; Desai *et al.*, 2010). Studies indicated that certain disorders or conditions can lead to the manifestation of infertility, due to oxidative damage in the body, these include cancer, cardiac failure and DM (Hamilton and Ventura, 2006; Lutz, 2006).

A basic and thorough understanding of the male reproductive system's physiology and anatomy is of utmost importance when having to work on effective treatment strategies for male infertility (Roberts, 2010).

2.1. Organs of the male reproductive system and their functions

The male reproductive system is composed of the testes, epididymis and accessory organs (vas deferens, seminal vesicle, prostate and penis) (Hermo *et al*., 1994; Sullivan, 2004; Dacheux *et al*., 2005; Roberts, 2010). The whole reproductive system is mainly dependant on sufficient production of hormones. The most important hormone of these being, testosterone. Testosterone is produced in the testes and the regulation centres includes the hypothalamus and anterior pituitary. Other hormones that are vital for healthy sperm production and normal functioning of the male reproductive system includes follicle-

stimulating hormone (FSH), luteinizing hormone (LH) and estradiol. Hormones such as thyroid hormone and inhibin as well as proteins such as androgen-binding protein (ABP) play a huge role in regulation of the male reproductive system (Roberts, 2010; Mawhinney and Mariotti, 2013).

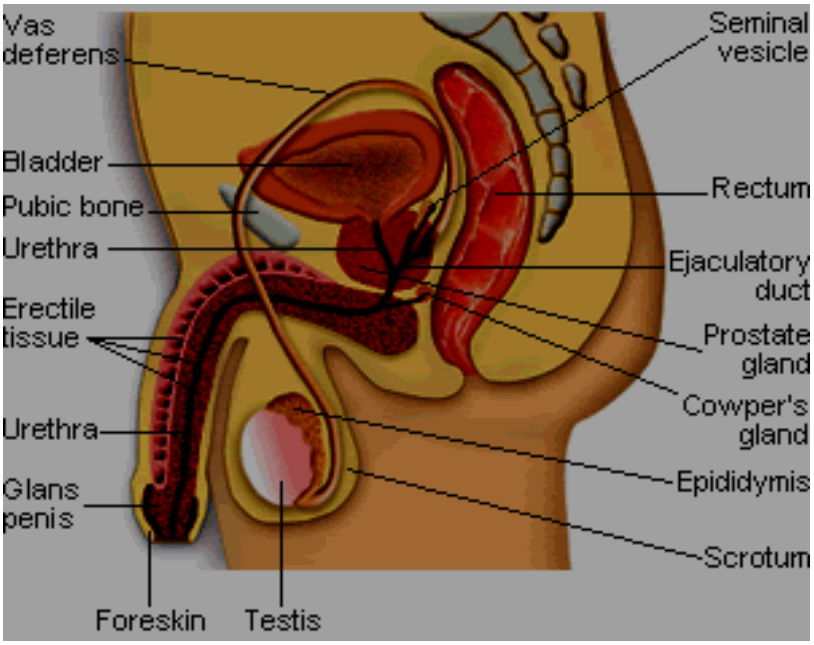

Figure 2.1: Diagramatic overview of the male reproductive tract (Adapted from Torta and Derrickson, 2012)

2.1.1. Testes

The testes are the primary organs of the reproductive system. It has an ellipsoid shape with a diameter of about 2.5-4 cm and engulfed by a capsule of strong connective tissue, the tunica albuginea (Middendorff *et al.,* 2002; Shokri *et al*., 2010). The tunica albuginea has an external covering known as the serosa. A conical mass of tissue called the mediastinum exuding to the tunica albuginea causing a division of the parenchyma of the testes into ± 300 lobuli. The lobuli contains 1-4 convoluted seminiferous tubules each of 150-300µm in diameter and 30-80cm long. Interstitial tissue is found between the convoluted tubules known as the seminiferous tubules which are the main site of testicular function (Shokri *et al*., 2010; Naraghi *et al*., 2010). The seminiferous tubules are convoluted structures consisting of two types of cells; the leydig cells and the sertoli cells which are responsible for spermatogenesis. The testes are protected and housed by the scrotum, which is responsible for maintenance of optimal temperature of the testes for spermatogenesis to take place and production of healthy sperm. The two main functions of the testes include 1) the production of the male gametes (spermatozoa) and 2) it is also responsible for the synthesis and secretion of androgens, specifically the male hormone testosterone and estradiol (De Kretser *et al*., 1982; Roberts, 2010).

2.1.2. Epididymis

The epididymis is a convoluted structure with an estimated length of 5-7cm and attached to the testes by efferent ducts at its lower pole (Sallivan 2004; O'Hara *et al.,* 2011). The epididymis is divided in to three segments the caput, corpus and the cauda (Turner, 2006; Robaire *et al.,* 2006). The segments are mainly composed of a lumen and polarized epithelium that consists of principal cells, apical cells, narrow cells, clear cells, basal cells and halo cells (Lasserre *et al*., 2001; Dacheux *et al*., 2005; Robaire *et al.,* 2006). Principal cell constitutes about 80% of the epididymal epithelium. They are columnar cells with stereocilia that functions as secretory and endocytotic cells (Robaire *et al*., 2000; Dacheux *et al*., 2005; Robaire *et al*., 2006; Cornwall, 2009). Basal cells are the second most predominant cells type that constitutes about 10-15% of the epididymal epithelium. Basal cells are flat, triangular cells that are located in the basement membrane of the epididymal epithelium (Robaire *et al*., 2000; Cornwall, 2009). The functions of basal cells are not yet understood, but it has been suggested to aid principle cells in its secretory function. Basal cells have also been associated with the immune system as it has shown to respond to the presence of sperm auto-antibodies in the lumen of the epididymis (Olson *et al*., 1995; Seiler *et al*., 2000). The epididymis also contains apical cells that have a characteristic apically located nucleus. They function to endocytose substances from the lumen and it has also been observed that apical cells contain many proteolytic enzymes (Adamali and Hermo, 1996; Adamali *et al*., 1999). Narrow cells are cup-shaped vesicles that constitute about 10% of the epididymal epithelium. Narrow cells are involved in endocytosis and the aids in the intracellular transport between the epithelium cells and the lumen (Adamali and Hermo, 1996; Robaire *et al*., 2006). Halo cells and clear cells both constitutes for about 5% of the epididymal epithelium population. Clear cells are also endocytotic cells and are responsible for clearing proteins from the lumen (Hermo *et al*., 2005; Robaire *et al*., 2006). As for halo cells, they contain dense granules in their cytoplasm and has been known to be the primary immune cell that initiates immune response in the epididymis (Robaire *et al*., 2006). These cells all have a contributory function for the epididymis to accomplish its function. The structure of the cells in the different parts of the epididymis is indicated in more detail in figure 2.2.

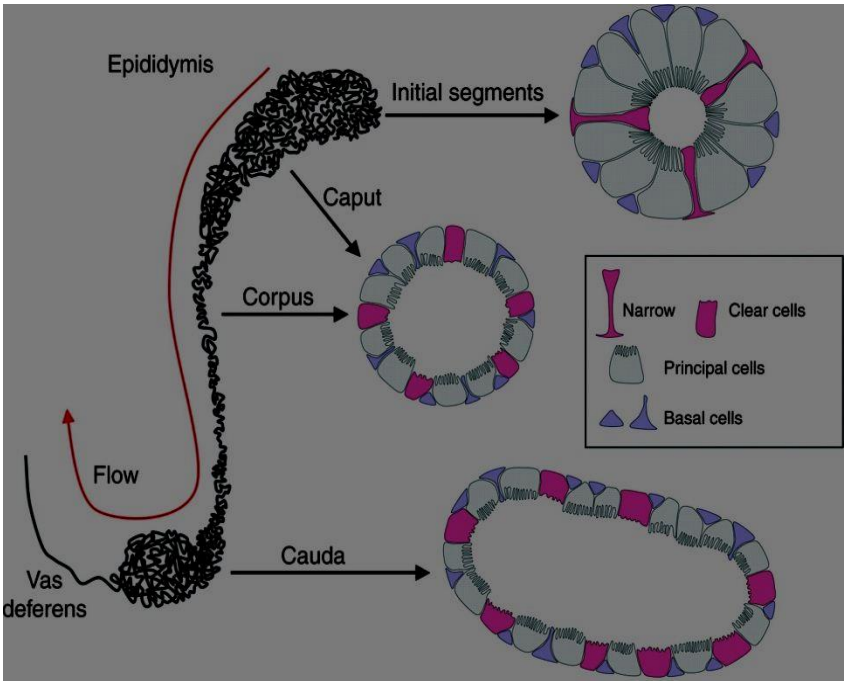

Figure 2.2: Cell composition of the epididymis (Adapted from Shum *et al*., 2009)

The main function of the epididymis is to create a favourable environment for immature spermatozoa to undergo successful morphological, physiological and biochemical processes to develop into a functional mature sperm cell that is capable of fertilizing an oocyte (Yanagimachi 1994, Flesch and Gadella 2000; Toshimori, 2003, Gatti *et al.,* 2004). The epididymis contributes to this by secreting proteins, glycoproteins and enzymes that will help this process along (Orgebin-Crist, 1967; Robaire *et al*., 2000, Robaire *et al*., 2006). The process start when the sperm enters the epididymis and the testicular fluid gets replaced with epididymal fluid secreted by the epididymal epithelium (Da Silva, 2006; Gadea *et al*., 2013). The fluid contains certain proteins, molecules and nutrients that allow for the first phase of modifications such as migration of cytoplasmic droplet, beating of the sperm flagellae and later assistance in binding to the zona pellucida (ZP) of the female oocyte (Ellerman *et al*., 1998; Flesch and Gadella, 2006; Von Horsten *et al*., 2007). The replacement of fluid also functions in increasing the concentration of spermatozoa from 10 fold to 100 fold (Toshimori, 2003). The next steps as the sperm passes from the caput epididymis to the cauda epididymis is change in net surface charge, adenylate cyclase activity, composition of membrane proteins, immunoreactivity, and phospholipid and fatty acid content (Fouchecourt *et al*., 2000; Da Silva *et al*., 2006; Belleannee *et al*., 2011; Dacheux *et al*., 2012). These changes contribute to the spermatozoa to acquire motility, maturation and capacitation (Sallivan *et al*., 2007). These processes are androgen dependant (Hermo *et al.,* 1994; Sullivan, 2004). Factors such as optimal temperature, oxygen, tension, pH and available energy source are essential for immature spermatozoa to develop into mature spermatozoa (Dacheux *et al.,* 2005).

To assure these processes occur uninterrupted, the epididymis contains an antioxidant defence mechanism consisting of enzymatic and non-enzymatic strategies. The antioxidant defence mechanism is attributed to the secretory function of the epididymal epithelium aiding in the protection of the spermatozoa (Robaire *et al*., 2000; Gatti *et al*., 2004; Robaire *et al*., 2006; Gadea *et al*., 2013). These changes improve the structural integrity of the membrane of the sperm and contribute to fertilisation ability of spermatozoa. Rendering the sperm fully matured and allowed to be stored until ejaculation, ready to fertilize an oocyte (Yanagimashi, 1994; Gadea, 2013).

Other accessory organs that play a role in the male reproductive system include;

2.1.3. Vas deferens

The vas deferens (or ductus deferens or spermatic duct) is a tubular structure made up of a smooth muscle coat. The muscle coat consists of an outer adventitia and inner mucosa. The vas deferes is 1-1.5mm thick, its cells 30-40µm in length and 2-5µm in diameter (Koslov and Andersson, 2013). Overall the vas deferens is 30-40cm from the cauda portion of the epididymis to where it fuses with the seminal vesicle to create the ejaculatory duct (Ernest *et al*., 2011; Costabile, 2013). The primary function of the vas deferens functions to transport seminal secretions from the epididymis that contains matured spermatozoa to the prostatic urethra (Koslov and Andersson, 2013).

2.1.4. Seminal vesicle

Seminal vesicles resides above the prostate gland and measures about 3cm, in length and 1.5cm in diameter (Kim *et al*., 2002). It contains tubular alveoli that are lined with very active secretory epithelial that contributes to at least 70% of the fluid volume of the ejaculate. The secretions produced by the epithelium are rich in fructose and prostaglandins, as well as proteins. Seminal vesicles function is androgen dependent and testosterone levels have been associated with corrected fructose levels. Prostaglandins, potassium bicarbonate and prolactin that are also secreted by the seminal vesicles, has been directly associated with stimulating motility of spermatozoa as well as preventing the ejaculate from clotting (Gonzales, 1989; Bromfield *et al*., 2014).

2.1.5. Prostate

The prostate is found inferior to the bladder and anterior to the rectum. The prostate originates from several distinct tubules that form from the primitive urethra and each develops into a separate lobe. These lobes are made up of alveoli and lined with secretory epithelium that drains into the prostatic urethra (Roberts, 2010; Sooriakumaran *et al*., 2012).

The secretion of the prostate makes up ±25 % of the ejaculate and is high in citric acid, zinc and choline. A number of secretory proteins are also found in the secretion as well as acid phosphatise, seminin, plasminogen activator and prostate specific antigen (PSA). It is presumed that the prostatic secretions are important for the function of spermatozoa during and after ejaculation (Owen and Katz, 2005; Alshahrani *et al*., 2012).

2.2. The process of Spermatogenesis

Spermatogenesis can be defined as the process where immature germ cells undergo three distinct phases namely proliferation of diploid spermatogonia, meiosis of spermatocyte and differentiation of haploid spermatids (O'Donnell *et al*., 2001; Sharma, 2007; Chung *et al.,* 2009). This process normally takes 60 days in rats and 72 days in humans (Hess *et al*., 1999) and produces ±1000 spermatozoa for every heartbeat (Griswold and Oatley, 2013). Spermatogenesis occurs mainly in the seminiferous tubules of the testes. However, full maturation takes place in the epididymis where spermatids undergo a number of biochemical, physiological and morphological changes which results in motile spermatozoa that is proficient for fertilisation (O'Donnell *et al*., 2001). During spermiogenesis spermatids differentiate into full elongated spermatozoa with a head, neck and tail (O'Donnell *et al*., 2001; Chung *et al*., 2009).

Spermatogonia undergo a number of mitotic divisions. Mitosis allows generating and maintaining of the population of primary spermatocytes. Primary spermatocytes moves from the base membrane, passes through a tight junction between tightly packed sertoli cells and reaches the luminal compartment where it will undergo the prophase stage of meiosis (Hess, 1999). During prophase, spermatogonia divide into two daughter cells. This process involves the replication of genetic material, cleavage of nuclear envelope and equal division of homologous chromosomes and cells cytoplasm (Paulson, 1977; Handel, 2010). After division, one of the daughter cells become a precursor cell (remains a stem cell) which remains at the base of the sertoli cells and the other cell will carry on in the process of spermatogenesis. The type B cell is known as the preleptotene primary spermatocyte. The aim of this phase is for secondary spermatocytes to give rise to four identical spermatids (Hermo *et al.,* 2010a).

The last changes that occur are more morphological, including spermatids being transformed into specialised spermatozoa (Hermo *et al*., 2010 a, b). This phase is known as spermiogenesis. Spermiogenesis has at least 8 steps in humans, 19 steps have been identified in rats and 16 in mice. Changes include that of transformation of the golgi apparatus and mitochondria as well as differentiation thereof. For the spermatozoa to be functional in fertilisation it also needs to complete the acrosome reaction, formation of the

radial body of the endoplasmic reticulum and annulate lamellae, development of chromatoid body, restructuring and remodelling of the spermatids head. Other changes include the formation of the neck of sperm and observation of tail developing (Breucker *et al*., 1985; Kwon and Hecht, 1991; Fouquet *et al*., 2000; Hermo *et al.,* 2010 a, b). These are the last changes that spermatozoa undergo before they leave the testes and move on to the epididymis. The epididymis with further allow maturation of sperm as well as storing it until ejaculation (Breucker *et al*., 1985; Russell *et al*., 1989; Lie *et al*., 2010). The process of spermatogenesis is depicted in figure 2.3.

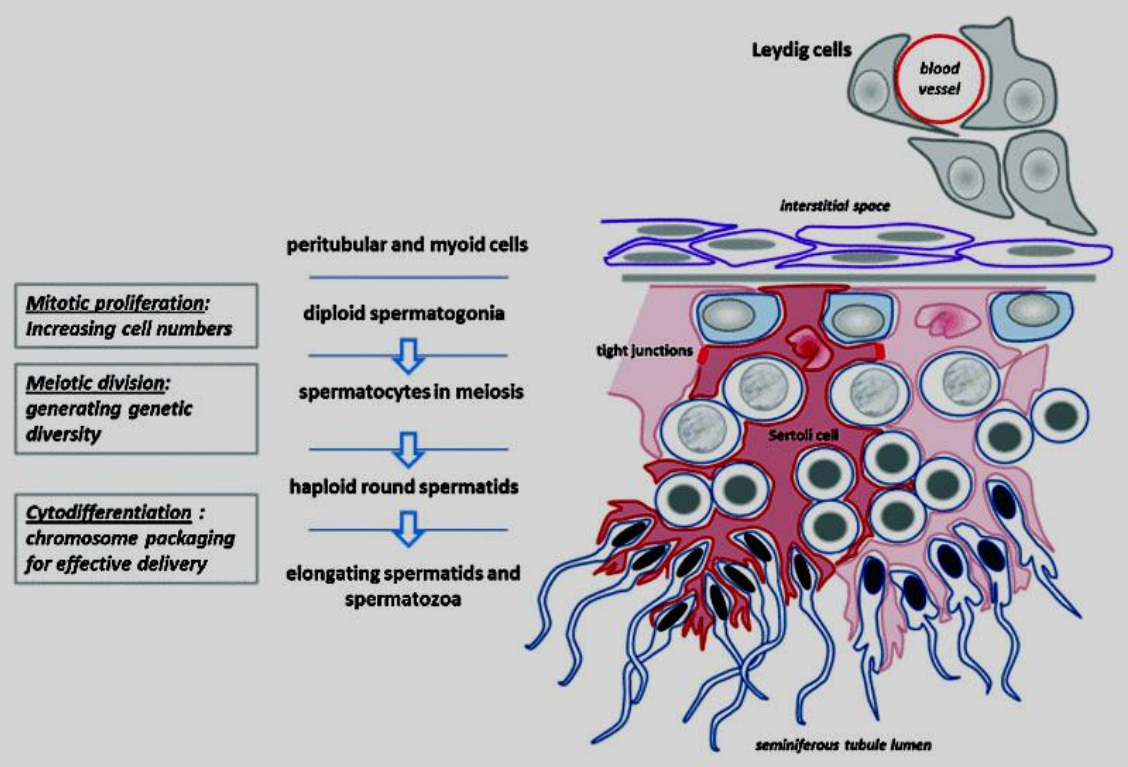

Figure 2.3: Cell composition of the epididymisphases of spermatogenesis (Hunter *et al*., 2012).

2.3. Endocrinological control of Spermatogenesis

The endocrine system has a vital role in normal functioning of the both male and female reproductive systems. In males, it assists with spermatogenesis as well as producing hormones responsible for masculine characteristics and libido (Nieschlag and Behre, 2004).

2.3.1. Hypothalamus

The hypothalamus is the main centre of control of the reproductive axis. The hypothalamus releases GnRH, which travels to the anterior pituitary via blood vessels located in interstitial spaces. GnRH stimulates the anterior pituitary gland to secrete both LH and FSH (Winters and Moore, 2004; Smith *et al*., 2006; Matsumoto and Bremner, 2012).

2.3.1.1. Gonadotropin-releasing hormone (GnRH)

GnRH is the key regulator in the reproductive hormonal cascade that triggers a whole cascade of hormones (Schally *et al*., 1971; Baba *et al*., 1971; Matsumato, 2012) through the hypothalamic-pituitary-gonadotropic axis that control the reproductive system. The peptide is released from the nerve endings (about 1000 neurons) into the hypophyseal portal circulation in synchronized pulses (1 pulse every 70-90 minutes). GnRH does not stay in the bloodstream for too long. It has a half-life of 2-3 minutes. The pulsating release is important for the stimulatory effects on LH and FSH (Veldhuis, 1997; Griffin and Wilson, 1998). GnRH stimulates the production of gonadotropic hormones, LH and FSH by binding to receptors on the anterior pituitary. This allow GnRH to have indirect control of gametogenesis and the production of sex steroids (White and Fernald, 1998; Carolsfeld *et al*., 2000; Okubo *et al*., 2000; Montaner *et al*., 2001; Adams *et al*., 2002).

Continuous exposure of GnRH to the receptors on the pituitary may lead to inhibitory effects of LH and FSH due to desensitisation of the receptors on pituitary gland. The secretion of GnRH can be influenced by number of factors such as age, diet, stress and harsh exercise (Veldhuis, 1997).

2.3.2. Anterior pituitary gland

LH and FSH have separate functions and respectively bind to different cells of the testes. FSH binds to sertoli cells triggering spermatogenesis in the presence of ABP. LH binds to the Leydig cells that will trigger the production of testosterone (Nieschlag *et al*., 1999; Luetjens *et al*., 2007; Grigorva *et al*., 2011; Matsumoto and Bremner, 2012; Alves *et al*., 2013).

2.3.2.1. Gonadotropins: Luteinizing hormone (LH) and follicle-stimulating hormone (FSH)

Both LH and FSH are dimeric glycoproteins secreted by the anterior pituitary that play an important role in testicular function (Bardin and Paulsen, 1981; Huhtaniemi, 2010). Due to their protein structure these hormones cannot enter the sertoli or leydig cells of the testes; binding to receptors found on the cell membranes of these cells to exert their actions in facilitating spermatogenesis. Gonadotropins are normally secreted an episodic manner but it has been observed that circulatory FSH has a longer half-life and is constant in serum level when compared to LH that gets metabolised more rapidly (Santen and Bardin 1973; Costabile, 2013).

The secretion of LH and FSH are essential for gametogenesis as well as production and maintenance of testicular sex hormones. FSH stimulates the proliferation and diffrentiation of prepuberal sertoli cells during neonatal stage. During puberty FSH mediate the maturation of

sertoli cells into functional cells. In adult males, FSH indirectly sustains spermatogenesis by controlling an array of metabolic functions. As for LH, its action is on the leydig cells and allows for production of testosterone (O'Donnel *et al*., 1994; Sharpe, 2001; Huhtaniemi, 2010).

2.3.3. Hormones produced by the testes

2.3.3.1. Testosterone

Testosterone forms part of a class of hormones known as androgens. Testosterone is a steroid hormone derived from cholesterol and synthesised by the leydig cell in large amounts in male testes, and in lesser amounts by adrenal glands and female ovaries (Mazur and Booth, 1998; Eisenegger *et al*., 2011). It is responsible for the maintenance of libido and masculine characteristics, stimulates muscle and bone growth and plays a vital role in the development of male reproductive organs (penis, prostate and scrotum) (Mooradian *et al*., 1987; Serra *et al*., 2013). Testosterone is considered to be the main androgen responsible for male fertility and spermatogenesis (Wang *et al*., 2009; Smith and Walker, 2014).

Hormone-withdrawal experiments on rats indicated that testosterone is essential for completing meiosis and differentiating between round spermatids to elongated spermatozoa. The androgen receptor (AR) has shown to have an important part in this observation (Ghosh *et al*., 1991; Yeh *et al*., 2002; Chang *et al*., 2004). Testosterone is responsible for the function of sertoli cells in maintaining and supporting the seminiferous tubular fluid (Al-Attar *et al*., 1997; Wang *et al*., 2006). It has also been observed that testosterone plays an important role in the secretion of functional proteins and peptides from the sertoli cells. These proteins and peptides aid in nourishing germ cell development (Wang *et al*., 2006; Wistabu *et al*., 2007). Testosterone is also linked to numerous social behaviours including aggression, power, sexual behaviour as well as social dominance (Eisenegger *et al*., 2011).

2.3.3.2. Estradiol

Estradiol is an important sex hormone in both males and females as it plays a vital role in development and maintenance of the fertility and the reproductive system (Nilsson *et al*., 2001; Carreau *et al*., 2008). In females, estradiol is mainly produced by the ovary and placenta and in males, by the testes and in both sexes by the adrenal cortex. Estradiol normally circulated bound to sex hormone binding globulin (SHBG) (Kahn *et al*., 2002; Wildman *et al*., 2013). Estradiol forms part of a hormone estrogen and like other steroid hormones it is derived from cholesterol. During reproductive years in both sexes, most estradiol is produced by aromatisation of testosterone from adrostenedione then coverted to estrone, estrone in turn gets converted to estradiol. The enzyme responsible for this cascade

of conversions is called aromatase (Careau *et al*., 2002; Peters *et al.,* 2003; Salway, 2004; Lehninger, 2005; Antal *et al*., 2008).

Studies done by Nilsson *et al*. (2001) have demonstrated that estradiol yield an array of biological effects in the musculoskeletal, cardiovascular, immune and central nervous systems. Estradiol has also been observed to have an important role in preventing pathological processes of various tissues of the reproductive system (Prins and Korach, 2008; Ellem and Risbridger, 2009; Lazari *et al*., 2009).

2.2.3. Feedback mechanism

To maintain normal function the body has developed a feedback mechanism, ensuring that secretions of these hormones are monitored and controlled. Testosterone allows for a negative feedback inhibition on GnRH release by binding to androgen receptors of the pituitary and hypothalamic neurons. Testosterone as well as estrogen has a vital role in maintaining and regulating the male reproductive system on both tissue and cellular level (Shupnik and Schreihofer, 1997; Costabile, 2013). Testosterone is metabolised either to a potent metabolite dihydrotestosterone or potent estrogen, in the form of estradiol, which both has an inhibitory effect on LH. The seminiferous tubules secrete another hormone, inhibin. Inhibin mainly inhibits FSH, which aid in the regulation of sperm production (Simoni *et al*., 1997; Matsumoto and Bremner, 2012). The feedback mechanism is depicted in figure 2.4.

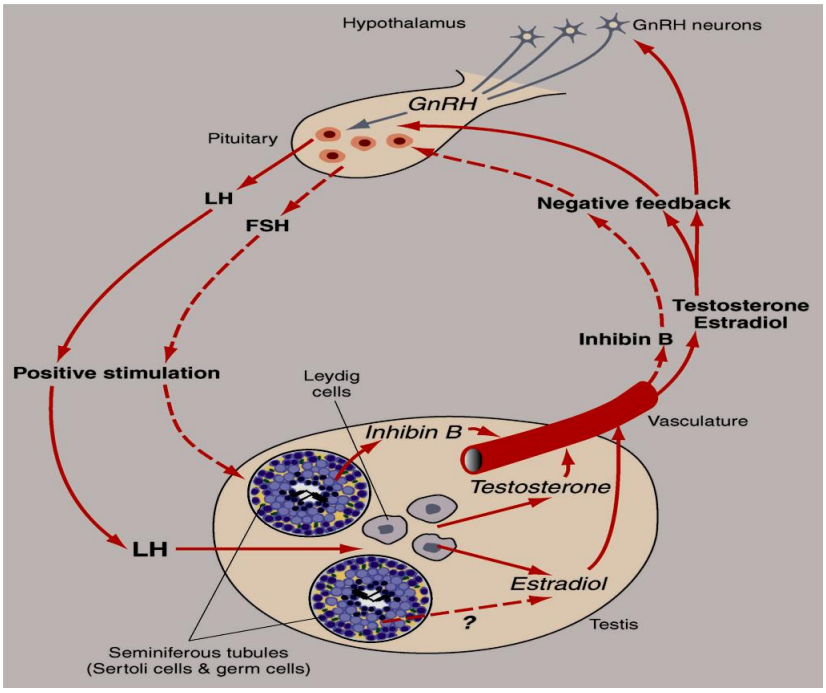

Figure 2.4: Depiction of negative feedback mechanism and the hormones involed (Adapted from WHO, 2002)

2.4. Reactive oxygen species (ROS) and Oxidative stress (OS)

ROS belongs to the class of free radicals, are chemically active agents that includes a number of molecules like superoxide (O_2^-), nitric oxide (NO), hydroxyl (OH^-), peroxyl (RO_2), lipid peroxidise (LOO^-), hydrogen peroxide (H_2O_2) and ozone (O_3) (Koppenol *et al*., 1992; Sikka, 2001; Bansal and Bilaspuri, 2011). When ROS overwhelms the body's defence mechanism, OS occur (Sharma and Agarwal, 1996; Kemal Duru *et al*., 2000; Saalu, 2010; Hampl *et al*., 2012; Walczak-Jedrzejowska *et al*., 2013) resulting in detrimental effects leading to cellular and tissue damage that may cause various pathological effects (Agarwal *et al*., 2008; Galley, 2011; Pirinccioglu *et al*., 2012; Holmström *et al*., 2014). However, low levels of ROS play an important role in signal transduction that contributes to maintaining homeostasis of the male reproductive system (de Lamirande *et al*., 1997).

2.5. Physiological role of ROS in sperm function

ROS is predominantly associated with negative effects on the human body specifically the male reproductive system. It has been proven that in physiological amounts of ROS it has a beneficial role in a number physiological processes of normal sperm function (Agarwal and Saleh, 2002; Choudhary *et al*., 2010). For sperm to be functional is has to undergo a number of morphological and physiological changes such as sperm maturation, capacitation, hyperactivation motility, acrosome reaction and oocyte fusion (Agarwal *et al*., 2004; Choudhary *et al*., 2010)

2.5.1. Role of ROS in sperm maturation

When spermatozoa are released from the testes they are still immature as they do not display progressive motility and have not undergone capacitation. These abilities are acquired as they pass through the epididymis (Gadella *et al.,* 2001). A number of morphological and metabolic changes need to occur for sperm to be rendered matured and capable of fertilizing an ovum (Aitken, 1999). One of these changes includes a tightly packed chromatin and changes of molecules being expressed and distributed on the sperm membrane (Moore, 1996; Ford, 2004). These processes are largely conducted by ROS. In the presence of low amounts of ROS allows the substitute of histone beads by proteins known as protamines. The cysteine residues found in the protamines forms disulfide bonds and results in a compacted stabilised nuclear DNA (Soawaros and Panyim, 1975; Aitken *et al*., 1999). The enhanced stabilised sperm chromatin maintains genetic integrity (Twigg *et al*., 1998). ROS is also required to in force changes in mitochondrial membrane forming a mitochondrial capsule that serve as protection (Roveri *et al.*, 2001). The formation of the mitochondrial capsule is induced by H_2O_2 which is responsible for the oxidation of phospholipid hydroperoxide glutathione peroxidise (PHPGx). In its oxidised form, PHPGx allows a reaction that forms an intermediate with a reduced protein thiol groups and results in

the formation of a mitochondrial capsule. The capsule is tightly held together with a selenadisulphide bond (Baker and Aitken, 2004). It has also been suspected that ROS might be involved in initiation of motility by enhancing cyclic adenosine monophosphate (cAMP) synthesis and protein phosphorylation during ejaculation (Aitken *et al*., 2004).

2.5.2. Role of ROS in sperm capacitation

Capacitation is the physiological and cellular changes spermatozoa undergo to render it suitable to reach and penetrate the zona pellucida of the female ovum (Yanagimachi, 1981; Yanagamachi, 1994; Aitken and Nixon, 2013; Fierro *et al*., 2013). The changes include the spermatozoons ability to undergo acrosome reaction and obtain hypermotility (Kovalski *et al*., 1992; Jannsen *et al*., 1993; Yanagimachi, 1994; de Lamirande 1997; Gaboriau *et al*., 2007; Chiu *et al*., 2014). During capacitation a number of conditions are required. These include the increase of intracellular cAMP and pH level; calcium and bicarbonate influx, efflux of cholesterol, protein phosphorylation, and changes in the membrane fluidity (Schoeller *et al*., 2007; Chung *et al*., 2014).

Hyperactivation and capacitation of sperm goes hand-in-hand and both of these processes take place only when ROS is in moderate physiological amounts (de Lamirande and Gagnon, 1993; Tsai *et al*., 2013). The biochemical process of capacitation is responsible for activation of protein kinase A (PKA). This process is conducted by an influx of Ca^{2+} and HCO_3^-, that causes stimulation of adenyl cyclases (AC). AC in turn converts ATP to cAMP and cAMP is responsible for the activation of PKA. Activated PKA eventually leads to the phosporylation of tyrosine through inhibition of phosphotyrosine phosphatases (PTPases) and increasing activation of tyrosine kinases (TK's). ROS is also responsible for the amount of tyrosine phosphorylation being produced. O_2^- has been observed to be responsible for AC activation by oxidizing an important thiol group. Studies also speculate that O_2^- is essential for hyperactivation of sperm (Zini *et al*., 1995; Aitken *et al*., 2004). As for H_2O_2, it has been associated with tyrosine phosphorylation by inhibiting PTPases and stimulating activation of TK's (Zini *et al*., 1995). H_2O_2 has been proposed to be a vital element in capacitation (de Lamirande and Gagnon, 1993).

2.5.3. Acrosome reaction (AR)

The AR is the exocytotic release of proteolytic enzymes that assists in spermatozoon penetration of the ZP and allowing it to fuse with the female oocute (Yanagimachi, 1981; Yanagimachi, 1994; Fierro *et al*., 2013). Capacitation and the acrosome reaction share many biochemical processes, similarities including phosphorylation of tyrosine kinases and tyrosine phosphatases, the influx of Ca^+ and activation of cAMP, PKA and AC (de Lamirande and O'Flaherty, 2008). All these events are necessary as it allows the spermatozoa to penetrate

the ZP. All these events are necessary as upon penetration of spermatozoon to ZP it initiates the AR that is important for fusion of the spermatozoon to ZP.

In vivo studies has revealed that initiation of the AR occur when the spermatozoa binds to the ZP. The sperm affinity is attributed to the stimulation of ROS. ROS production is stimulated through the phosphorylation of three plasma membrane proteins that is found at the apex portion of the spermatozoon's head. Plasma proteins being phosphorylated include fertin beta (ADAM2), P47 (the porcine homologue of SEDI) and spemadhesin family (AZN-3 and other proteins classed under this family) (Breitbart and Noar, 1999). Studies have demonstrated that the addition of low concentrations of O_2^-, H_2O_2 and NO has a favourable effect on AR (Leclerc *et al*., 1998; O'Flaherty *et al*., 1999).

For both capacitation and AR to occur, activation of AC is of importance. The activation of AC triggers the cascade of molecule activation (including cAMP and PKA) that will result in the initiation of exocytotic events of the AR (Zini *et al*., 1995; de Lamirande and O'Flaherty, 2008).

2.6. Pathological role of ROS in the male reproductive system

ROS are responsible for a number of cell signalling transductions in the male reproductive system. However, when high levels of ROS overwhelms antioxidant levels results in pathological effects on spermatozoa. Increased amounts of ROS result in significant amounts of damage to proteins, lipids and nucleic acids (Riffo and Parrago, 1996; Agarwal and Prabakaran, 2005). The extent of the damage to the male reproductive system depends on duration of exposure to ROS, the type of ROS and the amount of ROS exposed to. Factors such as temperature, oxygen tension and levels of antioxidants present are contributes to the level of damage induced to sperm (Agarwal and Prabakaran, 2005; Agarwal, 2006).

2.6.1. Causes of increased ROS that results in OS and its effects on the male reproductive system

Among the many causes of infertility, overexposure to certain environmental factors seems to be one that impairs sperm production and lower sperm count (Gaur *et al*., 2007; Desai *et al*, 2010; Aleksander *et al*., 2011). Therefore, male infertility cannot be mentioned without taking into consideration the effects of OS (Agarwal *et al*., 2005).

Tobacco smoking plays a contributing role in infertility. Tobacco smoking has been observed to cause damage to sperm DNA (Gaur *et al*., 2007), some damage is irreversible but ceasation of smoking reduces any further damage (Olooto, 2012).

Aging has played a big role in male infertility (de la Rochebrochard *et al*., 2006; Wiener-Megnazi *et al*., 2012). It has been observed that in older males there is decreased androgen levels, decreased sexual activity, alterations in sperm motility as well as morphology and the deterioration of sperm quality and DNA integrity (de La Rochebrochard *et al*., 2006; Sartorius and Nieschlag, 2010; Wiener-Megnazi *et al*., 2012).

Environmental factors including toxins such as glues, volatile, organic solvent, silicones physical agents, chemical dust, pesticides and pollutants have been notarised to cause infertility (Hruska *et al*., 2000; Mendiola *et al*., 2008). It has also been proven that intensive heat and radiation to the genitalia can cause damage to the testicles that can lead to defects in sperm function and quality. Phthalates, an estrogen-like compound has been observed to impair spermatogenesis and increase sperm DNA damage (Irvine *et al*., 1996; Bujan *et al*., 1996; Hauser, 2006; Ozmen *et al*., 2007). These finding can easily be linked to the increase in ROS in the body (Lee *et al*., 2007).

A number of andrological fertility disorders that has been classified as idiopathic has a genetic origin. Many can only be detected by karyotyping and looking into family history (Dohle *et al*., 2005). Chromosomal abnormalities are commonly found and are in higher incidence in infertile men and are seen to be inversely related to the sperm count. The chromosomal linked disorders can either be found to be numerical (trisomy) or structural (inversion or translocations) (Johnson, 1998; Jungwirth *et al*., 2012). These changes can be observed in disorders such as Klinefelter's disease, which is one of the common sex hormone abnormalities (Jungwirth *et al*., 2012). Other disorders like Kallmann syndrome and mild androgen insensitivity syndrome, which are X-linked genetic disorders also been seen to have and antifertility effect in males. There are also a number of Y-chromosome abnormalities that contribute to male infertility causing azoospermia.

Numerous hormonal problems have also been associated with infertility of males. Conditions that may affect the hypothalamic-gonado-pituitary axis has a great influence on GnRH and in turn affects follicle stimulating, luiteinizing hormone and testosterone production. Disorders that may influence these hormones are Kallmann syndrome, hyperprolactinemia (that can lead to hypogonadism and hypopituitarism). These conditions can cause erectile dysfunction, decreased libido, gynecomastia and infertility in males (Madhukar and Rajender, 2009; Oloota *et al*., 2012).

A number of physiological changes in the body also contributes to male infertility, these include hormonal problems, variocoele (Costabile and Spevak, 2001), infections (Ochsendorf *et al*., 1999; Momoh *et al*., 2011), damage to sperm ducts and obstruction in the vas deferens (Olooto *et al*., 2012). One physiological factor that has been commonly associated with male infertility is hyperglycemia or DM. About 90% of diabetics have fertility associated

disturbances causing a decrease in libido, impotence and infertility (Cameron *et al*., 1990; Glenn *et al*., 2003; Aboua *et al*., 2013). Figure 2.5 illistrates possible causes that leads to male infertility.

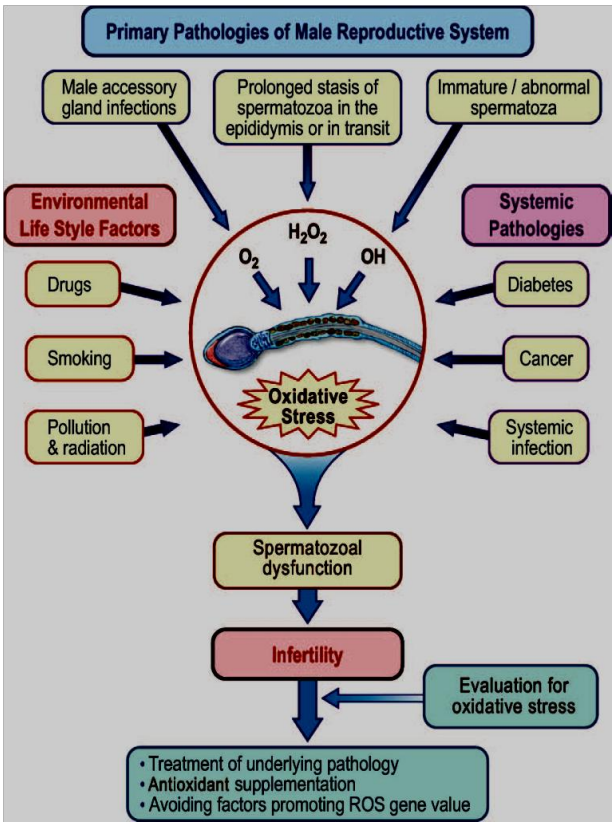

Figure 2.5: Environmental and pathological conditions that cause ROS that may result in male infertility (Adapted from Agarwal and Sekhon, 2010)

2.6.2. Diabetes mellitus (DM) and oxidative stress

DM is a metabolic disorder of multiple aetiologies and is usually characterised by chronic hyperglycaemia which disturbs the metabolism of carbohydrates, fat and proteins (WHO, 1999; Kumar, 2005; American diabetes association, 2012). This is a result of a defect in insulin secretion, insulin action or both. The growth of the population has also lead to an increase number of people with DM. It has been estimated that the prevalence of diabetes will reach about 370-439 million people by the year 2030 (Shaw *et al*., 2010; Rowley and Bezold, 2012).

DM and its effects on the body may lead to damage as well as dysfunction of many organs. DM may be in the form of 4 different types of which type 1 DM orInsulin dependent DM (IDDM) and type 2 DM also known as Non-insulin dependent DM (NIDDM) are the more common forms. Type 1 DM refers to the processes of beta cell destruction and characterized with the development of ketoacidosis (Brownlee and Cerami, 1981; Resnick and Howard,

2002; Rother, 2007; Singh *et al*., 2011). Type 2 DM is the most common form and is characterised by disorders of insulin action and insulin secretion (Kumar, 2005; Singh *et al*., 2011). Other forms of DM also exist, these include gestational diabetes and an unclassified form of DM. Gestational DM usually occurs during pregnancy. Unclassified DM also known as other type DM under is a less common form and is mainly a result of underlying defect or disease process (WHO, 1999). Diabetics are prone to severe metabolic complications, these including increased ketone production, hypoglycaemia and hyperglycaemic induced coma due to hyperosmolality (Umpierrez *et al.,* 2002; English and Williams, 2004; Tiwari *et al*., 2013). Studies has also documented that in addition to these, diabetics have an increased risk of acquiring coronary heart diseases, neuropathy, foot ulcers, nephropathy and retinopathy (American diabetes association, 1998; Tiwari *et al*, 2013). Studies also revealed that DM is commonly associated with oxidative damage induced infertility (Hamilton and Ventura, 2006; Lutz, 2006).

OS is an important element in diabetes as it contributes to its progression and development of the disorder (Ceriello, 2000). DM leads to an increase of ROS and depletion of antioxidants causing OS that will alter the body's normal redox state (Giron *et al*., 1999; Wiernsperger, 2003; Bloch-Damti and Bashan, 2005).

2.6.3. Hyperglycaemia and oxidative stress

A relationship has been established between hyperglycaemia and OS. Numerous pathways can lead to the development of diabetic complications that may affect the male reproductive system, specifically spermatogenesis. Four of these pathways are very important and includes the activation of protein kinase C (PKC) isoforms, disruption of mitochondrial signalling by increased production of superoxide, increased advanced glycation end-product (AGE) formation (Viassara and Palace, 2002; Peppa *et al*., 2004), and increased polyol (sorbitol) pathway flux (Rolo and Palmeira, 2006). OS has been implicated to play a central role in these pathways. OS occurs as a result of excessive formation of ROS and reactive nitrogen species (RNS) collectively described as free radicals. Free radicals are highly unstable and have the ability to abstract electrons from macromolecules such as carbohydrates, protein, lipid and DNA (WHO, 1999; Valko *et al.,* 2007). Excessive ROS can cause structural deterioration and instability of the macromolecules, consequently affecting proper cellular signalling pathways, gene regulation and function (Pop-Busui *et al.,* 2006). Although, the human system has check-in mechanisms to deal with oxidative damage and free radical formation through endogenous and exogenous antioxidants. However, when the rate of formation of ROS overwhelms the detoxifying ability of the antioxidants OS can occur (Ridnour *et al*., 2004; Valko *et al*., 2007, Halliwell, 2011).

2.6.4. Four pathways affected by hyperglycaemia causing ROS in the body

2.6.4.1. Polyol (sorbitol) pathway

This pathway involves glucose being cleaved to sorbitol. The action is mainly dependent on Nicotine adenine dinucleotide phosphate (NADPH). The enzyme responsible for cleavage of glucose to sorbitol is known as aldose-reductase (Oates and Mylari, 1999; Rain and Jain, 2011). Under normal conditions aldose-reductase has a low affinity to glucose but in diabetic patients, where there is an increase in glucose, it has been observed to have an increase production of sorbitol due to enhanced affinity to glucose. As mentioned this process is NADPH-dependent and it will cause a decrease in NADPH. NADPH also acts as co-factor for glutathione (GSH), which is known as the most vital antioxidant present in the cell and key factor in antioxidant defence mechanism (Rains and Jain, 2011). This pathway is not directly responsible for ROS production but it does cause a decrease in GSH levels in the cell resulting in a redox impairement (Srivastava *et al*., 2005). A summary of the polyol (sorbitol) pathway is observed in figure 2.6.

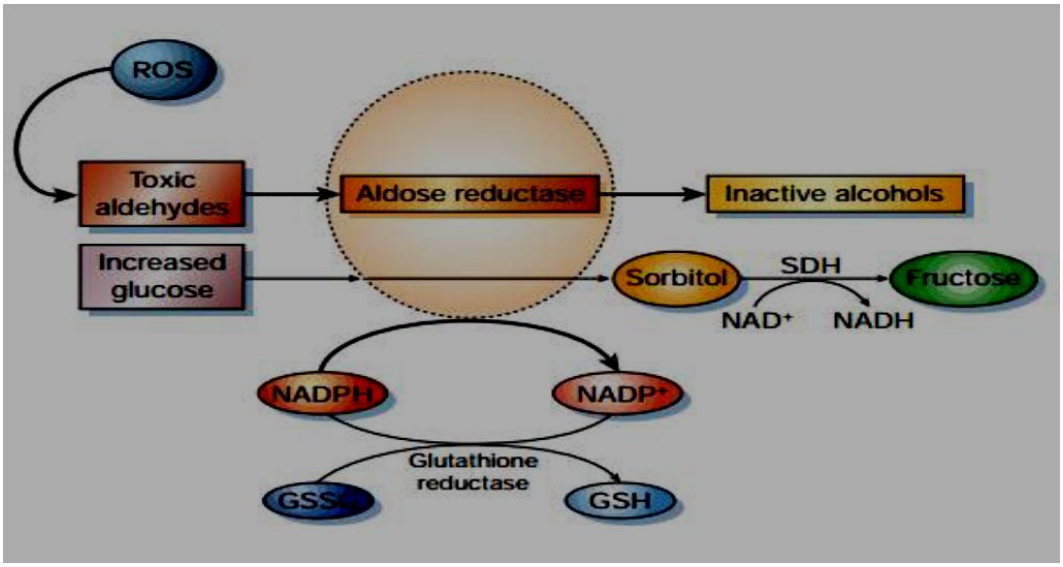

Figure 2.6: Polyol (sorbitol) pathway: Schematic diagram illustrating the increased affinity of aldose reductase during hyperglycaemic conditions, leading to GSH levels decreasing due to NADPH depletion (Adapted from Brownlee, 2001)

2.6.4.2. Increased formation of advanced glycation end- (AGE's) products

This is a pathway that is characteristically found to occur in diabetic patients which involves ROS (Brownlee, 2001). The pathway include AGE's that are formed when groups from reducing sugars, aldehydes or ketones, binds covalently to protein that contains free amino acids that creates a chemical structure known as a Schiff's base. This structure will later lead to formation of carbonyl intermediates. Carbonyl intermediates has also been associated with glucose auto-oxidation. A number of chemical mutations of carbonyl intermediates make AGEs irrevocable (Sato *et al*., 2006). Initiation of NADPH oxidation is started when receptors (RAGE's) on the cell surface is activated which will lead to ROS activation. ROS then

activates the Ras-Mapk pathway, which is responsible for the activation of nuclear factor kappa- light chain- enhancer activation (NF-kB) by activating B-cells. Pathway of increased formation of AGE's is summarised in figure 2.7.

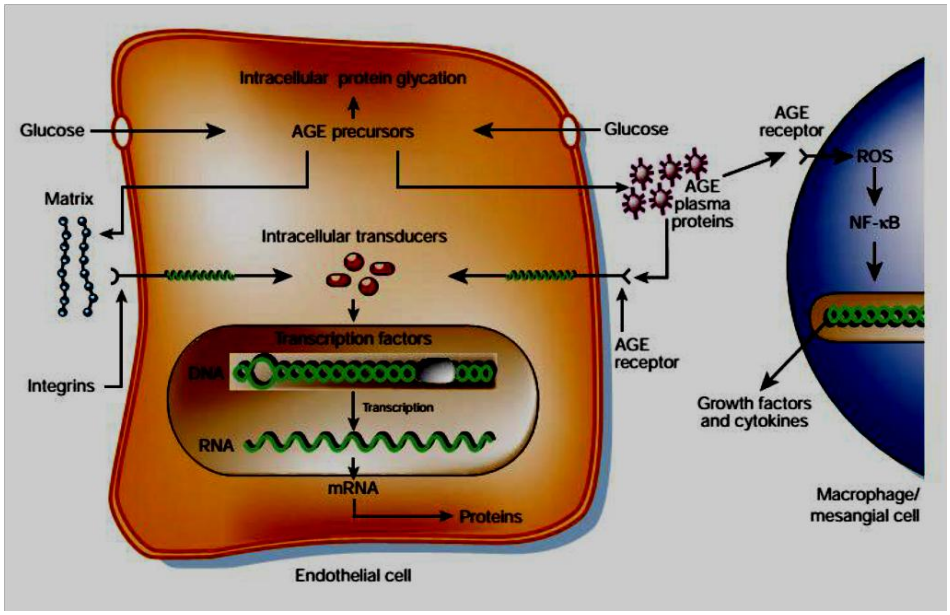

Figure 2.7: Diagram depicts the formation of AGEs during hyperglycaemic state showing impairment of expression of genes inside the cell that leads to deregulating numerous cellular processes (Adapted from Brownlee, 2001)

2.6.4.3. Activation of PKC pathway

The activation of PKC affects diabetic patients. Diacylglycerol (DAG) is responsible for activation of a variation of protein kinase C (PKC) isoforms. In hypoglycaemic conditions, dihydroxyacetone phosphate (a glycolytic compound) is found in increase and gets reduced to glycerol 3-phosphate. The reduction action allows for an increase synthesis of DAG that leads to PKC activation (Rains and Jain, 2011). Studies have affirmed that AGEs and the polyol pathway can also indirectly lead to PKC activation (Ahmed *et al*., 2005). PKC activation has contributed to impairments of numerous pathways that lead to complications such as DM associated circulation problems (Rains and Jain, 2011). The effects of PKC activation has on the human body is summarised in figure 2.8.

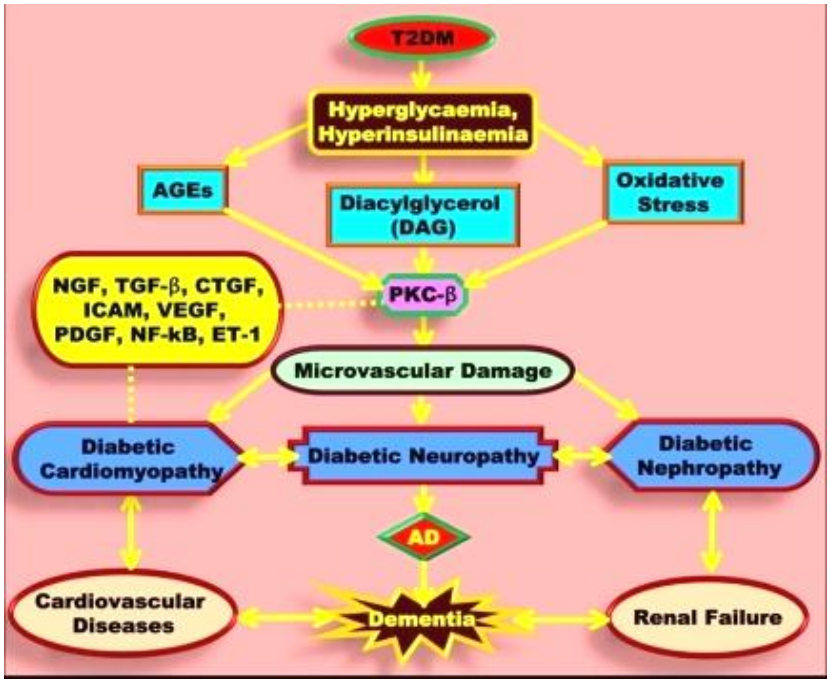

Figure 2.8: Diagrammatic expression of PKC activation in hypoglycaemic conditions and its harmful consequences associated with diabetes (Adapted from Kumari and Heere, 2010)

2.6.4.4. Disruption of mitochondrial signalling due to increase superoxide production (Increase of hexosamine pathway flux)

This pathway induces OS by increasing superoxide in the mitochondria. In this pathway there is a production of NADPH and pyruvate which will enters the tricarboxylic acid (TCA) cycle and result in the production of NADH and $FADH_2$ (Rains and Jain, 2011). NADH and $FADH_2$ allows for electron transport and ATP production (Green *et al*., 2004). In hyperglycaemic conditions, there will be an increased production of NADPH and pyruvate that will enter the TCA cycle. This will consequently lead to an increase of electron donors and overproduction of superoxide in the mitochondria (Du *et al*., 2001).

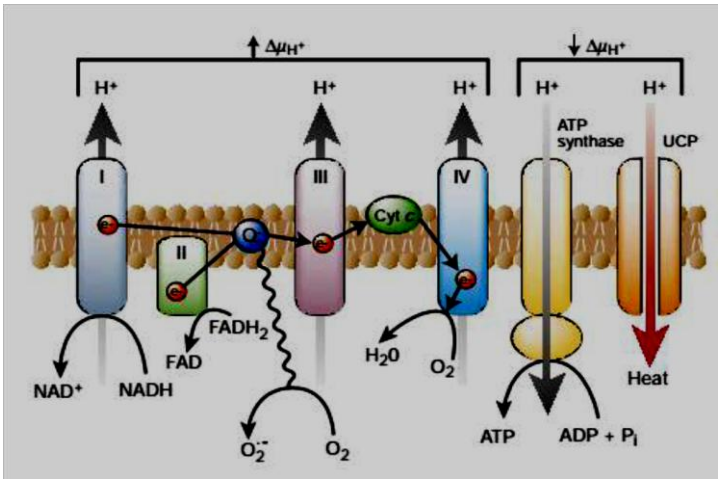

Figure 2.9: Hyperglycaemia causing disruption in mitochondrial signalling leading to inhibition of electron transfer, resulting in increased reduction of oxygen to superoxide (Adapted from Brownlee, 2001)

2.6.5. Hyperglycemia, oxidative stress and its effects on male reproductive function

There have been various studies done concerning the role of ROS in causing disease in the male reproduction. These studies have shown that ROS may affect various physiological functions in the reproductive tract and increased or excessive levels can result in a number of pathologies that may affect male reproductive tract and sperm parameters (Dennery, 2004). Hyperglycemia has been associated with male sexual dysfunctions in experimental rats (Altay *et al*., 2003) and in humans (Baccetti *et al.,* 2002). Several reproductive changes have been observed in streptozotocin (STZ)-induced hyperglycemic male rats. These includes reduction of organ and body weights (Scarano *et al.,* 2006; Suthagar *et al.,* 2009); oligospermia (Scarano *et al.,* 2006; Olivares *et al.,* 2009); decreased testosterone and gonadotropin levels (Sudha *et al*., 2000; Olivares *et al.,* 2009) and reduced spermatogenesis and testicular damage (Cai *et al.,* 2000; Guneli *et al.,* 2008). A decrease in sperm count has been attributed to the influence of hyperglycemia on late stages of spermatogenesis, possibly through an increase of ROS (Sikka, 2001). The high content of polyunsaturated fatty acids (PUFA's) within the plasma membrane of spermatozoa increases susceptibility to oxidative damage. Increased lipid peroxidation (LPO) and altered plasma membrane can affect the sperm function by impairing sperm metabolism, motility, acrosome reaction as well as sperm DNA damage. The changes may lead to increase of morphological changes in spermatozoa and decrease in caudal sperm count (Tramer *et al.,* 1998; Kumar *et al.,* 2002; Sanchez *et al.,* 2006; Rabbani *et al.,* 2009). Hemachand and Shaba (2003) have shown that the increase of hydroperoxide (H_2O_2) levels can affect spermatogenesis, since germ cells are more susceptible to peroxidative damage.

Studies done on rat models have documented that diabetes induced oxidative damage diminishes reproductive organ and body weights as well as sperm content and cause sperm

defects (Saudamani *et al*., 2005; Sacarano *et al.,* 2006; Amaral *et al*., 2008). It has also been observed in STZ-induced diabetic rats that testosterone levels are decreased due to the changes in Leydig cells (Cameron *et al.,* 1990; Sacarano *et al*., 2006). It has also been observed that few diabetic rats complete ejaculation when compared to normal control rats (Hassan *et al*., 1993; Sacarano *et al*., 2006). Histological studies revealed that STZ-induced diabetic rats when compared to normal control group showed a noticeable reduction in seminiferous tubules and epididymal lumen (Saudamani *et al*., 2005; Tolba, 2014). Observations also revealed an increase in ROS in diabetic rats, influences the hypothalmic-pituitary-gonadal axis causing decreased in reproductive hormones. A decrease was observed in LH and FSH influencing spermatogenesis (Cameron *et al*., 1990).

2.6.5.1. Lipid peroxidase (LPO) in sperm

In sperm cells, lipids have a fundamental role in the fluidity of the cell membrane layers as well as changes that take place in the female reproductive tract during capacitation of spermatozoa (Sanocka and Kurpisz, 2005). The plasma membranes of mammalian spermatozoa are largely composed of PUFA's, therefore spermatozoa has a higher susceptibility to oxidative damage (Agarwal and Saleh, 2002). PUFA's contains unconjugated double bonds (Halliwell, 1984) that are separated by a methylene group. This arrangement of a double bond joined to a methylene group causes a weakened methyl-carbon-hydrogen bond, exposing thus the hydrogen to oxidative damage. When ROS in the cell increases beyond physiological levels, it will start attacking the PUFA's at the weak bond; this cascade triggered is then known as LPO (Makker *et al*., 2009).

Mammalian spermatozoa consist of approximately 50% of fatty acids which are mostly composed of decosahexanoic (DHA). During the process of LPO, a sperm cell can loose up to 60% of the fatty acids in the membrane leading to a decrease of membrane fluidity, ion gradients, and process of transportation and inactivation of enzymes and enzyme receptors (Sikka *et al*., 1995; Tremellen, 2008; Aitken *et al*., 2010). Consequently, such sperm are impaired and may not fertilise the oocyte (Riffo and Parrago, 1996; Tremellen, 2008).

LPO is an autocatalytic self proliferating reaction that result is impaired fertilisation. This process has three main phases namely initiation, propagation and termination (Sanocka and Kurpisz, 2004; Tremellen, 2008). The initiation phase involves the abstraction of hydrogen atoms, from unsaturated lipids, associated with carbon-carbon double bonds found next to methylene groups. The abstraction of hydrogen atoms results in the production of free radicals. The lipid radicals react with oxygen to form peroxyl radicals (HO^{-}_{2}) which will inturn result in lipid peroxides with the aid of antioxidant defence mechanism (Saalu, 2010). The production of lipid peroxides actually help stabilise the sperm plasma membrane which

stimulates the propagation phase. During propagation, lipid peroxides are degraded and result in alkoxyl and peroxyl radicals that acts on additional lipids until lipid damage is widespread and irreversible (Riffo and Parrago, 1996; Sikka, 2001; Sanocka and Kurpisz, 2004; Agarwal *et al.,* 2014). The last phase is the termination phase where free radicals reacts with another molecule and results in the formation of an end-product, MDA (Sikka, 2001; Sanock and Kurpisz, 2004; Kothari *et al*., 2010). MDA is used in biochemistry to quantify the peroxidative damage to spermatozoa (Sikka, 2001; Sanocka and Kurpisz, 2004; Agarwal and Prabakaran, 2005).

2.6.5.2. Sperm DNA damage

Parameters such a sperm concentration, motility and morphology are normally measured to confirm the sperm's ability to fertilise an oocyte. These parameters however, only give an overview of the quality of the sperm and do not look at the most important functional unit of the spermatozoa which is sperm DNA (Zribi *et al*., 2011). DNA damage is a result of OS inducing base modifications, specifically guanine, by exposing it to either lipid peroxyl or alkoxyl radicals. This action results in the breaking of DNA strands, cross-linking DNA by binding covalently with MDA, deletions, frameshifts, rearrangements of chromosomes and exposed free base sites that lead to DNA damage (Twigg *et al*., 1998; Sikka *et al*., 1999; Irvine *et al*., 2000; Duru *et al*., 2000; Aitken and Krausz, 2001; Aitken and Deluliis, 2010).

Spermatozoa, like any other aerobic cells are rich in mitochondria. Oxidative damage isprone to occur mostly to mitochondrial DNA of spermatozoa thus leading to impaired sperm motility (Grivaeu *et al*., 1995). Studies have proved that DNA damage may consequently lead to infertility (Sawyer *et al*., 2003). DNA damage can be caused in a number of ways of including apoptosis, error in spermatogenesis and OS (Sawyer *et al*., 2003; Aitken *et al*., 2007). Amri *et al*. (2007) showed that free radicals such as NO have caused DNA damage. Irvine *et al*. (2000) has observed that DNA fragmentation, poor semen parameters and high levels of ROS is common in infertile men. Other findings associated with DNA damage are those of early embryo death (Duru *et al*., 2000; Aitken *et al*., 2004; de Lamirande and O'Flaherty, 2008), reduced fertilisation capacity (Sun *et al*., 1997; Aitken, 1999) and apoptosis of cells (Zini and Sigman, 2009; Aitken and De Iuliis, 2010; Avendaño and Oehninger, 2011; Chen *et al*., 2013).

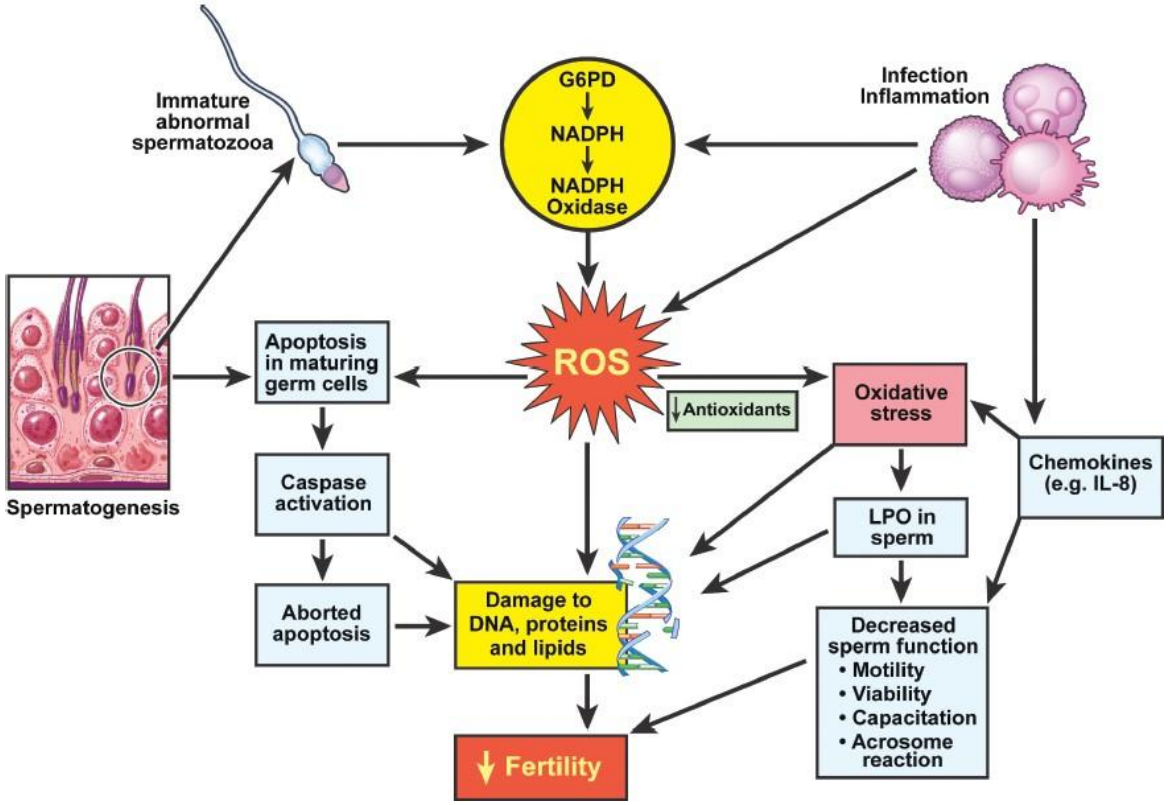

Figure 2.10: Demonstrates the influence increased ROS has on DNA of spermatozoa (Adapted from Agarwal and Sekhon, 2011)

2.6.5.3. Apoptosis

Apoptosis can be defined as a physiological process that involves morphological and biochemical changes that encourage cell death (Makker *et al*., 2009). In the early development of the male testes, apoptosis serve as a mechanism to regulate the ratio of sertoli cells and germ line cells in the seminiferous tubules by using both intrinsic and extrinsic pathways in the presence of different stimuli (Agarwal, 2005). The extrinsic pathway uses the binding of a death receptor on Type I membrane protein (Fas) and allows it to bind to its ExoS ligand (FasL) (Danial and Korsmeyer, 2004; Czabotar *et al*., 2014). The membrane death receptors form a complex known as death inducing signalling and recruits procaspase-8 to activated caspase 8. Caspase 8 will initiate a cascade with other downstream caspases that will result in apoptosis (Bejarano *et al*., 2011). The second is the intrinsic pathway; where the mitochondria undergo alterations. The mitochondria have a vital role in the process of apoptosis. When apoptosis is stimulated the mitochondrial alteration initiates the production of ROS. Increase of ROS stimulates the inner and outer membrane of the mitochondria to activate a signalling molecule cytochrome C (Agarwal *et al*., 2004; Agarwal *et al*., 2008; Bejarano *et al*., 2008). Cytochrome C will stimulate a cascade of caspases including caspases 3 and 9 that will eventually lead to apoptosis (Agarwal *et al*., 2008; Aitken and Baker, 2013). These processes are all ROS dependent (Sentman *et al*., 1991).

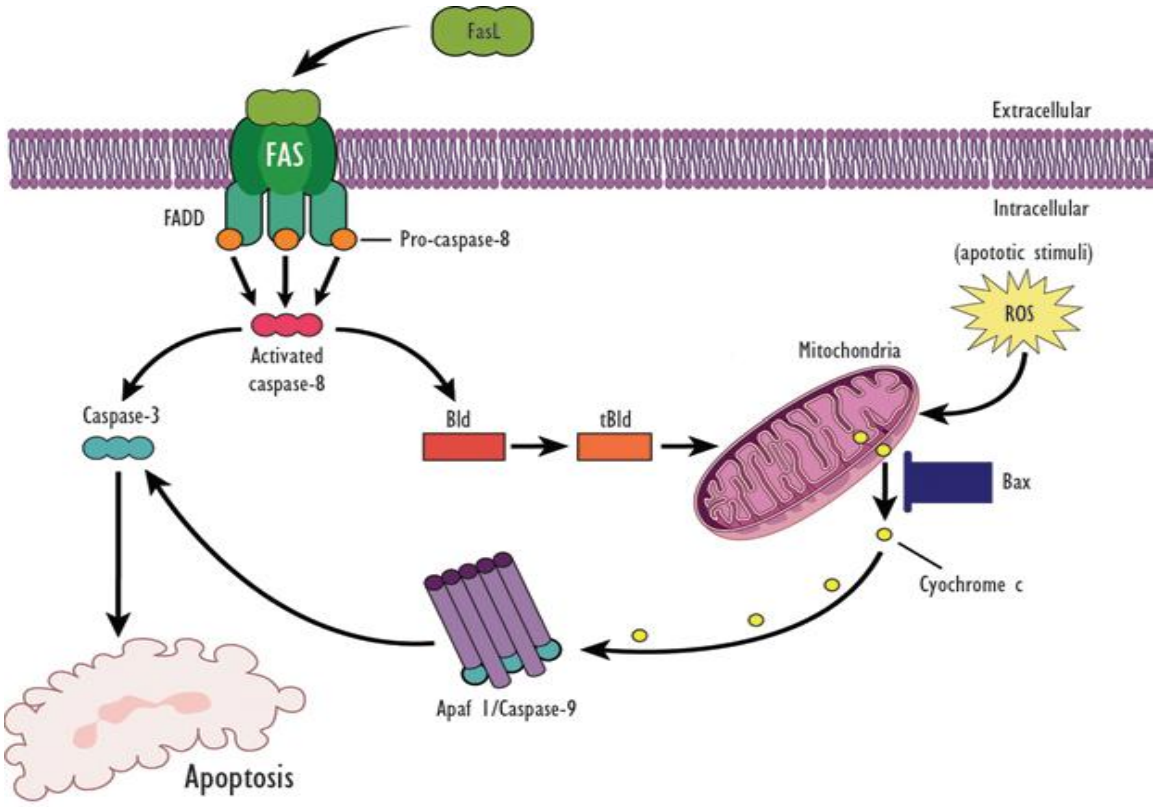

Figure 2.11: Demonstrated the activation of caspases and its involvement in apoptosis (Adapted from Agarwal, 2005)

When apoptosis occur independently of ROS, apoptotic markers like Fas, phosphatidylserine (PS) Bcl-XI and P53 are seen to be involved in initiation of apoptosis (Lee *et al*., 1997; Agarwal *et al*., 2003). To prevent this degree of damage the body has put into place defence mechanisms that help fight against OS in the seminal plasma.

2.7. Management of ROS in the male reproductive function

Antioxidants can be divided into two groups, enzymatic and non-enzymatic. These two groups work in close connection with each other to ensure maximum protection from free radicals (Walczak–Jedrzejowska *et al.,* 2012; Akbari and PJelodar, 2013). The antioxidant defence mechanism use three ways of protection against free radicals which includes stopping oxidation by eliminating ROS, taking up, or minimising ROS production (Bansal and Bilaspuri, 2011; Lampiao, 2012). In the seminal plasma three main enzymatic antioxidants exist, these include SOD, CAT and glutathione peroxidase (GPx) (Tunco *et al*., 2011; Walczak–Jedrzejowska *et al*., 2012). Non-enzymatic antioxidants are mainly obtained from dietary products such as fruits and vegetables. Non-enzymatic antioxidant includes vitamin C, E and B, carotenoids, carnitines, metals, GSH and polyphenols (Agarwal and Sekhon, 2010).

It has been observed that ROS has both physiological and pathological roles in the male reproductive system it is important that an array of antioxidants help maintain homeostasis of ROS (Agarwal and Saleh, 2002). Antioxidants are molecules that functions as free radical scavengers. Their function is to protect essential molecules of biological systems against oxidation by regulating ROS activity (Matés, 1999; Agarwal and Saleh, 2002; Choudhari *et al*., 2008). In the male reproductive system, antioxidants are mainly responsible for protecting spermatozoa and precursor germ cells from free radical activity (Sikka, 2001; Agarwal and Saleh, 2002; Gharagozloo and Aitken, 2011).

2.7.1. Endogenous antioxidants

Endogenous antioxidants are characterised as enzymatic antioxidants and are molecules generated by the seminal plasma (Agarwal *et al*., 2005; Mancuso *et al*., 2012; Murphy, 2014). Three main enzymatic antioxidants are SOD, CAT and GPx (Willcox *et al*., 2004; Halliwell, 2007). The primary responsible of these antioxidants is the ability to neutralise and prevent the oxidation of biological molecules by ROS (Agarwal *et al*., 2005). GPx work with other molecules like glutathione reductase (GR) and GSH to achieve its action on ROS (Choudary *et al*., 2010). When ROS are produced in excess SOD is the first antioxidant that acts to catalyse O_2^- into H_2O_2 and oxygen. CAT or GPx then comes and transforms H_2O_2 to H_2O and O_2 (Droge, 2002; Bahorun *et al*., 2006).

2.7.1.1. Superoxide dismutase (SOD)

SOD is a metalloenzyme (Johnson and Giulivi, 2005) that has both extracellular and intracellular scavenging abilities for superoxide radicals and prevents lipid peroxidation of spermatozoa plasma membrane. Superoxide anion radicals are produced *in vivo* by aerobic mechanisms and SOD allows for dismutation of O_2^- into H_2O_2 and O_2 as final products (Johnson and Giuvili, 2005; Zhang *et al*., 2011; Akbari and PJelodar, 2013).

SOD can be divided into three different forms and its classification is dependent on either the ion found bound to the active site or the number of subunits present (Miller, 1996). SOD-1 consists of two intracellular forms; copper-zinc SOD whichare found in the cytoplasma and is expressed as Cu-ZnSOD, SOD-1. SOD found in the mitochondrial axis has a manganese attached to its active site and is expressed as MnSOD, SOD-2. The third form of SOD is found extracellularly, EC-SOD, SOD-3, has a similar structure as SOD-2, but instead of a manganese at its active site it has Cu and Zn at its active site and is mostly attached topolysaccharides but can also be found in free form (Walczak–Jedrzejowska *et al*., 2012). All three SOD isoenzymes have been found to have different susceptibility in the way they uniquely bind to single charged anions (Su *et al*., 2010). Studies have reported that SOD-1 accounts for atleast 75% of antioxidant activity in the seminal plasma and the remaining 25%

are related to SOD-3 and all three these isoforms may be derived from the prostate (Peeker *et al*., 1997; Walczak–Jedrzejowska *et al*., 2012). Studies also reported that SOD prevents premature activation of hyperactivation and capacitation caused by superoxide radicals prior to ejaculation (de Lamirande*et al*., 1997; Aitken and Roman, 2008). However, if SOD produced abnormally may cause damage to spermatozoa as well as testicular tissue (Maiorino and Ursini, 2002).

2.7.1.2. Catalase (CAT)

CAT is an intracellular enzyme with a characteristic heme structure with an iron atom attached to its centre. CAT activity has been reported in the peroxisomes, endoplasmic reticulum, cytosol and the mitochondria of many mammalian cells. CAT activity has been known to conduct the decomposition of H_2O_2 into H_2O and O_2 and allows oxidation of hydrogen donors. Therefore, CAT has both catalytic and peroxidic activity (Devasagayam *et al*., 2004; Agarwal *et al*., 2008). CAT was found in human and rat sperm as well as the seminal plasma sourced from the prostate (Fraczekand Kurpisz, 2005). CAT can activate capacitation using NO which utilizes H_2O_2. This mechanism however is a very intricate processand is not really understood (de Lamirande *et al*., 1997; Walczak–Jedrzejowska *et al*., 2012).

2.7.1.3. Glutathione peroxidase (GPx)

GPx is a selenoprotein (Bahorun *et al*., 2006) which contains selenium as a co-factor and a selenocysteine residue at it active site (Margis *et al*., 2008). The enzyme is mainly responsible for detoxifying peroxides to prevent lipid peroxidation in cell membranes of spermatozoa (Szasz *et al*., 2007; Saraswathi and Devaraj, 2013). GPx is categorised into four isoforms, GPx1 help prevent oxidative stress induced apoptosis and is found in tissues of the kidney, liver and also in erythrocytes. GPx2 is found in the gastrointestinal tract and GPx3 is found in plasma. GPx4 is found in high levels in the testes and is responsible for detoxification actions in the cell membrane of spermatozoa (Ashok and Sushil, 2005; Margis *et al*., 2008). GPx has been associated with the protection of sperm DNA from ROS and aids in chromatin condensation (Yeung *et al*., 1998). Its detoxifying abilities contribute to improve sperm motility (Pfeifer *et al*., 2001; Moreno *et al*., 2003).

2.7.2. Exogenous antioxidants therapy

Exogenous antioxidants mostly found in dietary products like fruits, vegetables, plant foods like whole grain, nuts, legumes, meat, fish and dairy products (Moure *et al*., 2001; Van Langendonckt *et al*., 2002). Exogenous antioxidants can also be found in synthetic food additive (Bouayed and Bohn, 2010). Exogenous antioxidants have an important role in assisting endogenous antioxidants to neutralise OS. Deficiency of nutrients is one of the

main causes of serious pathologies. Each nutrient has its own unique structure and is specific for a particular function (Willcox *et al*., 2004; Donaldson, 2004; Carlsen, 2010). When infertility is due to blockage of the reproductive tract, it can be rectified by surgery. Infertility which involves the hypothalamic-pituitary-gonadal axis is normally treated with hormone therapy (Siemons and Mahler, 1987; Jungwirth *et al*., 2012). As for other treatment of disorders such as diabetes mellitus, that has been shown to have an effect on reproduction, there are many hypoglycaemic agents available.

2.7.2.1. Vitamin E (α-tocopherol)

Vitamin E is a fat soluble molecule that is high in antioxidant activity. It is a chiral compound and consists of eight stereoisomers of which only α-tocopherol is active in mammals (Nguyen *et al*., 2006; Pham-Huy *et al*., 2008). Due to vitamin E being fat soluble it acts to protect against LPO and oxidative damage induced by free radicals (Bansal and Bilaspuri, 2011; Lampiao, 2012). Vitamin E also contributes to improve the scavenging abilities of other antioxidants (Mora-Esteves and Shin, 2013). It is a vital chain breaking (inserts itself in the cell membranes of cells and ceases LPO) antioxidant found in cell membranes of spermatozoa and its effects are dose dependent (Hansen and Deguchi, 1996). Vitamin E scavenges O_2^-, H_2O_2 and hydroxyl radicals (OH^-) (Wang *et al*., 2003).

Studies have proven that vitamin E can prevent ischemia, cardiovascular disorders, breasts, colon and prostate cancers, cataracts, arthritis and some neurological disorders (Myo Clinic Medical Information, 2005). Administration of 100mg of vitamin E in a group of males with decreased sperm motility and normal female partners three times a day for six months showed significant decrease in LPO, increased sperm motility and increased pregnancy rates (Suleiman *et al*., 1996; Choudhary *et al*., 2010). Another study done by Comhaire *et al*. (2000) demonstrated that administration of vitamin E in combination with vitamin A and essential fatty acid led to improved sperm concentration of oligozoospermic men. It was also observed in the study that there was a reduction in free radicals in the seminal plasma, but no changes in sperm morphology and motility were observed (Comhaire *et al*., 2000).

2.7.2.2. Vitamin C (ascorbate acid)

Vitamin C or ascorbic acid is a water soluble compound and is important in the biosynthesis of collagen, carnitine and neurotransmitters (Li and Schellhorn, 2007). It has been found intracellular and extracellular fluid of cells and interacts directly with free radicals (Agarwal *et al*., 2004; Pugliese *et al*., 2013). Vitamin C is also a chain-breaking antioxidant and is responsible for 65% of antioxidant activity in the seminal plasma (Saleh *et al*., 2002; Agarwal *et al*., 2014). Its function is to neutralise O_2^-, OH^- and H_2O_2 radicals and plays an important role in preventing sperm agglutination (Sharma and Argawal., 1996; Agarwal *et al*., 2004).

Vitamin C also prevents LPO, recycles vitamin E and aid in the protection against DNA damage induced by H_2O_2 (Ashok and Sushil, 2005; Ghareeb and Sarhan, 2014).

Kodama *et al*. (1986) reported that oral administration of 200mg of vitamin C, vitamin E and glutathione for a period of two months resulted in significantly reduced 8-OH-dG levels in spermatozoa and increased sperm count. Another study showed that administration of vitamin C, vitamin E and glutathione in combination also improve sperm morphology and motility (Akmal *et al*., 2006; Agarwal *et al*., 2014).

2.7.2.3. Vitamin B12

Vitamin B12 is also a water soluble enzyme that functions as a coenzyme in methionine synthesis and metabolism of branched amino acids (Juanchi *et al*., 2000). This enzyme is essential for cellular replication, especially of RNA and DNA synthesis. Vitamin B12 deficiency has been associated with decreased sperm motility and sperm count (Ghareen and Sarhan, 2014). Busetto *et al*. (2012) conducted a study where 1500mg of methylcobalamin was given to a group of men daily for a period of 8-60 weeks. Periodic semen analysis revealed that 60% of semen parameters were increased.

2.7.2.4. Coenzyme Q10 (CoQ10)

CoQ10 non-enzymatic antioxidant and related to low-density lipoproteins and functions to protecting against lipid peroxidative damage (Frei *et al*., 1990; Lee *et al*., 2012). CoQ10 reacts directly with oxygen and results in decreased superoxide production. It also reacts with peroxide radicals thus reducing OS. CoQ10 is an energy-promoting agent and is found in the mitochondria of the sperm mid-piece. As a result of this characteristic it enhances sperm motility (Lewin and Levon, 1997). CoQ10 has been associated with recycling of vitamin E and prevent its pro-oxidant activity (Lewin and Levon, 1997; Karbownik *et al*., 2001; Tremellen, 2012).

In *vitro* studies have shown that when semen samples of infertile men was incubated with 50mM of CoQ10 there was a significant increase in sperm motility (Thomas *et al*., 1997; Safarinejad, 2009). Safarinejad *et al*. (2012) reported that there was in improvement in semen parameters after treating with 600mg CoQ10 daily for 12 months. There was also an increase in spontaneous pregnancy rates. Another study demonstrated that oral supplementation of 60mg CoQ10 daily improved fertilisation of normospermic infertile males using intracytoplasmic sperm injections (ICSI) (Lewin and Lavon, 1997).

2.7.2.5. Other non enzymatic compounds

Carotenoids are tetraterpenoid compounds that are natural antioxidants produced by coloured fruits and vegetables, also eggs, algae, certain seafood, produced by plants, bacteria and fungi (Bohn, 2008). Carotenoids are pigments that have several conjugated double bonds, called polyene chains. Their main mode of action is to scavenge and quench excited free radical activity. Carotenoids are water-soluble compounds that act as lipophilic antioxidants thus prevent PUFA's from oxidative damage. They are also chain-breaking antioxidants as they can incorporate themselves into lipid membranes and cease free radical chain reactions (Mortensen *et al*., 2001; Krinsky and Yeum, 2003). Two important carotenoids are β-carotene, which prevents peroxidation of lipids in the cell membrane and lycopene, which is the most readily and most potent carotenoid found in the cell. Lycopene functions to prevent peroxidation in the seminal plasma and testes (Kao *et al*., 2008; Agarwal and Sekhon, 2010). Gupta and Kumar (2002) reported that carotenoids at a dose of 200mg/day for 3 months significantly increased sperm concentration in 66% of patients and sperm motility of 53%.

Polyphenols have shown to possess antioxidant activity. They are synthesised by plants as a defence mechanism and has been found in food sources like potatoes, plums, leafy vegetables, coffee and whole grain products (Souci *et al*., 2000). Polyphenol structures consist of an aromatic benzene ring and two hydroxyl groups (Bouayed, 2010; Romero *et al*., 2013). Flavonoids are the most common polyphenol and can be divided into 6 subgroups namely flavonols, flavones, isoflavones, flavanones, anthocyanidins and flavanols. Non-flavonoid compounds include phenolic acids, lignans and stilbenes (Miller, 1996; Manach *et al*., 2004). Flavonoids work synergistically with other vitamins to reduce oxidation (Romero *et al*., 2013) and also help endogenous antioxidants to prevent oxidative damage by neutralising ROS (Schroeter *et al*., 2002). Several animal studies confirmed that flavonoids have antihyperglycaemic effects on diabetic induced OS (Rahman, 2003; Rahimi *et al*., 2005). It was confirmed that fruits and vegetables are major contributors of polyphenols (Bouayed, 2010).

Other non-enzymatic exogenous antioxidant include selenium, omega 3 and 6 fatty acids (Pham-Huy *et al*., 2008), cysteine, pentoxifylline, glutathione (GSH) and trace metals like zinc and copper (Agarwal *et al*., 2008; Agarwal and Sekhon, 2010).

2.8. Management of DM for improvement of male reproduction

The aim of treating DM is primarily to ensure longer life expectancy, relieving symptoms and preventing long-term complications (Watkins *et al*., 1990; Alwan, 1994; Bastaki, 2005). Uncontrolled DM can result in major complications of the reproductive system, pregnancy

and morbidity and mortality of the offspring (Kitzmiller *et al*., 1996). To restore fertility status it is essential to treat the diabetes and control the blood glucose levels. This is normally achieved by lifestyle and dietary modification, insulin therapy and oral hypoglycaemic agents (Kumar and Clark, 2002; Bastaki, 2005).

2.8.1. Pharmaceutical management of DM

Pharmaceutical management refers to the supplementation of synthetic hypoglycaemic agents in order to control blood glucose levels. Type 1 DM is normally treated with insulin replacement therapy, whereas management of type 2 DM is more dependent on lifestyle and diet adjustments. If type 2 DM cannot be controlled by diet, weight loss, exercise or oral medications, insulin can be used in combination with these. Hypoglycaemic agents that are normally used in the management of type 2 DM include sulphonylureas, biguanides (metformin), alpha glucosidase inhibitors, thiazolidenedione and insulin secretagogues (Kumar and Clark, 2002; Loghmani, 2005). The main objectives of these drugs are to correct underlying metabolic insulin disorders and hopefully keep the body in homeostasis (National Institutes of Health, 1995; Bastaki, 2005).

However, reduced response as well as toxic side effects has been observed after prolonged use (Chattopadhyay, 1999; Kavishankar *et al*., 2012). Due to limitation, unavailability and side effects associated with existing synthetic oral hypoglycaemic agents as well as treatment of reproductive disorders has lead to search for chemicals of a more natural source. Plants have shown to have antioxidant and antidiabetic properties. The polyphenolic compounds such as vitamins, flavonoids, phenols, flavonols, phytosterols and proanthocyanidins in plants contributes to this (Mariod *et al*., 2008).

2.8.2. Phytochemical treatment

2.8.2.1. Hypoxis hemerocallidae (H. hemerocallidea)

Several species of *hypoxis* plants have been used for medicinal purposes in various parts of Africa. Species that have been identified under the Hypoxidaceae family that has been used for medicinal purposes include *H.interjecta, H.nyasica, H.rooperi (*also known as *H.hemerocallidea), H.multicips, H.sololifera* and *H.obtuse* (Abegaz *et al*., 1999; Leistner, 2000; Ojewole, 2002). Amongst all these species, *H. hemerocallidea* is the most researched and used in traditional medicine. Scientific research has discovered that *H. hemerocallidea* possess therapeutic properties that allows it to be used as treatment for numerous diseases (Abegaz *et al*., 1999; Nair and Kanfer, 2006; Drewes *et al*., 2008; Katerere and Eloff, 2008).

H. hemercallidae is commercially known as the African potato (AP) (Boukes *et al*., 2008). In different parts of Southern Africa the plant has a number of vernacular names that includes

yellow-star or star lily in English, while it is known as sterblom in Afrikaans, in Swati it is referred to aslilabatseka or zifozonke, isiZulu it is called inkomfe or igudu, in isiXhosa inongwe, tshuka in seTwana and in seSotho as lotsane or molikharatsa (Singh, 1999; Erasto *et al*., 2005; Mills *et al*., 2005a; Drewes *et al*., 2008; Katerere and Eloff, 2008).

H. hemerocallidea is classified under the *Hypoxidaceae* family (Drewes *et al*., 1984; Singh, 1999; Fasinu *et al*., 2013) or lily family that has about 8 genera and consists of 130 species and of those 90 species are found in South Africa (Drewes *et al*., 2008). The AP is normally located in savanna regions of South Africa, Lesotho, Mosambique, Swaziland, Zimbabwe, Botswanna and North- Eastern Africa. In South Africa the AP is mainly found in Limpopo, Kwazulu-Natal, Gauteng and Eastern Cape. It has also seen to be located on the mountainous regions of Australia, South-America and on the coast of Asia (William *et al*., 2000; Singh, 2007; Katerere and Eloff, 2008; Drewes *et al*, 2008).

It is a geophytic, tuberous, perennial herb with long strap-like leaves (about 30 cm long and 3.2 cm wide) with large dark brown to black corms or also known as rhizomes tubers (about 10 cm in diameter and a half a kilogram in weight). The leaves of *H. hemerocallidea* are broad with a slight hairy appearance and arranged in an orderly way that they lay one above another forming three separate groups of leaves. These leaves spread outwards from the centre of the plant giving rise to a bright yellow star-shaped flower embedded on long slender stalks. The part of the plant mostly used for medicinal purposes are the corms or rhizomes tubers (Albrecht *et al*., 1995 a, b; Van Wyk *et al*., 2002; Ndong *et al*., 2006; Ojewole *et al*., 2006; Drewes *et al*., 2008; Boukes, 2010).

Figure 2.12: *H. hemerocallidea* plant (left) (Adapted from McMaster, 2007) and corm (right) (Adapted from Drewes *et al*., 2008)

2.8.3. Traditional medicinal uses of *H. hemerocallidea*

Traditionally, *H. hemerocallidea* has always been used as medicinal agent to treat many ailments (Hutchings *et al*., 1996; Van Wyk *et al*., 2009). A corm infusion is normally used as

emetic for dizziness and mental disorders. Hot aqueous extracts has been used to treat the symptoms of benign prostate hypertrophy (BPH) (Berges *et al*., 1995; Bouic and Vanderhaeghe, 1999). Other disorders also treated with *H. hemerocallidea* include colds, hypertention, psoriasis, gastrointestinal complaints, urinary tract infections (Brandt and Muller, 1995; Mills *et al*., 2005a; Laporta *et al*., 2007a, b), cardiovascular diseases, impotency, depression, bad dreams, barrenness and infertility, headaches, burns, polyarthritis, apprehension, intestinal parasites (Drewes *et al*., 2008; Katerere and Eloff., 2008), diabetes (Erasto *et al*., 2005; Drewes *et al*., 2008), sore throat, bronchitis, wasting disease, wounds, blemishes on skin, rush, lice, anxiety, allergies, herpes simplex virus infections, cancer, tuberculosis (TB), haemorrhoids, asthma, chronic viral diseases, ulcers, and vomiting and nausea (Hutchings *et al*., 1996; Van Wyk *et al*., 2002; Steenkamp, 2003; Steenkamp *et al*., 2006; Van Wyk, 2008; Ojewale *et al*., 2006; Brown *et al*., 2008; Van Wyk, 2009; Ojewole *et al*., 2009; Aremu *et al*., 2010). *H. hemerocallidea* has also been used in the treatment of Human Immunodeficiency Virus (HIV), AIDS (Hutchings *et al*., 1996; Van Wyk *et al*., 2002; South African Development Community, 2000; Giraldo, 2003; Verschaeve and Van Staden, 2003; Mills *et al*., 2005a, b; Street *et al*., 2008), epilepsy and other central nervous system disorders (Hutchings *et al*., 1996; Van Wyk *et al*., 2002). *H. hemerocallidea* is normally prepared by chopping the corms into cubes and boiled for approximately 20 minutes, after boiling, the concoction is administered orally (Nair and Kanfer, 2006).

2.8.4. Biochemical compounds found in *H. hemerocallidea*

Several compounds have been extracted from *H. hemerocallidea* which may contribute to its therapeutic abilities. The primary compounds isolated were hypoxide, rooperol, sterols, monoterpene glucosides and lectins. Apart from these, compounds such as polyphenols, steroids, glycosides, tannins and saponins have also been extracted from the corms (Oluwule *et al*., 2007; Street and Prinsloo, 2013). Three cytokinins that have also been yield are named zeatin, zeatin riboside and zeatin glucoside (Hutchings *et al*., 1996; Street and Prinsloo, 2013).

2.8.4.1. Hypoxoside

African potato extracts have shown to contain mainly hypoxoside which is later converted in the gastrointestinal tract to a compound called rooperol (Mills *et al*., 2005a, Owira and Ojewole, 2009). Albrecht *et al*. (1995) extracted from the corms of the plant a yellow crystalline water-soluble substance which was named hypoxoside. The hypoxoside molecular structure consists of two benzene rings linked with a pent-4en-1-yne and central methyl group (Drewes *et al*., 1984; Albrecht *et al*., 1995 a, b), therefore, it is also known as (E)-1,5-bis-(4'-β-D-glucopyranosyloxy-3'-hydroxyphenyl)pent-4-en-1-yne. This compound is categorised as a norlignan diglucoside (Drewes *et al*., 1984; Albrecht *et al*., 1995 a, b; Nair

and Kanfer, 2006). Albrecht *et al*. (1995b) also looked at the pharmacokinetics of both hypoxide and rooperol. They demonstrated that when *H. hemerocallidea* was administered orally, β-glucosidase produced by the bacteria in the gastrointestinal tract hydrolysed hypoxoside into a lipophilic dicatechol aglucone, called rooperol (Drewes *et al*., 1984; Smith *et al*., 1995; Drewes *et al*., 2008). Beta-glucosidase, apart from it being present in the human gastrointestinal tract, has also been observed to be released when cells are rapidly dividing especially that of cancer and bacterial cells (Boukes *et al*., 2008).

Rooperol has been characterised as a cytotoxic compound due to its ability to inhibit the growth of 60 cancer cell lines in humans (Nair and Kanfer, 2006; Boukes *et al*., 2008). These cell lines include those of the breasts, uterus, colon, melanomas and non-small cell lung cancer cell lines (Albrecht *et al*., 1995b; Smith *et al*., 1995; Boukes *et al*., 2008). Albrecht *et al.* (1995b) observed that rooperol had a role in maintaining the integrity of the chromosomal structure as well as the dividing process during mitosis. Research studies has confirmed rooperol to posses antioxidant (Laporta *et al*., 2007a, b), bactericidal, bacteriostatic (Drewes and Liebenberg 1982; Guzdek *et al*., 1996; Laporta *et al*., 2007b; Drewes and Khan, 2004; Owira and Ojewole, 2009), anti-inflammatory (Jager *et al*., 1996; Ojewale, 2002; Steenkamp *et al*., 2006; Gaidamashivilli and Van Staden, 2006; Owira and Ojewole, 2009), analgesic and antinociceptive (Ojewale, 2006), anticonvulsant (Ojewale, 2008), hyperglycaemic and antidiabetic (Zibula and Ojewale, 2002; Ojewale, 2002b; Musabayane *et al*., 2005; Drewes *et al*., 2008; Owira and Ojewale, 2009) capabilities. A study done by Ojewale *et al*. (2006), proved that rooperol have cardio-depressant and hypotensive effects and also displayed properties of bronchorelaxant (Ojewale *et al*., 2009). Rooperol has been associated with cytochrome P-450 (CYP) enzyme, P- glucoprotein and the receptor of pregnane X (Albrecht *et al*., 1995a; Mills *et al* 2005b; Chinsembu and Hendimbi, 2010). Rooperol has demonstrated to have greater affinity for biological actions associated with cell membranes (Laporta *et al*., 2007a).

2.8.4.2. Phytosterols

Sterols have been observed to be one of the main compounds of *H. hemerocallidea*, which contribute to the therapeutic and medicinal role of the plant (Bouic *et al*., 2001; Van Wyk *et al*., 2002; Boukes *et al*., 2008). Phytosterols are commonly found in cell walls of plants and belongs to the terpene family that umbrella more than a 100 different phytosterols. Phytosterols molecular structure is almost similar to cholesterol but phytosterols contains methyl or ethyl groups, with side chains and double bonds. The main function of phytosterols is to stabilise and maintain the integrity of the cell membranes of plants (Awad and Fink, 2000; Heldt, 2005; Boukes *et al*., 2008; Du Plessis-Stoman *et al*., 2009; Nair and Kanfer,

2006b). Phytosterols has hydrophilic heads mapped out on a sterane skeleton containing side chains that forms hydrophobic tails, making the molecule amphiphilic (Heldt, 2005).

H. hemerocallidea contains a sterol known as β-sitosterol (BSS) and its metabolite β-sitosterol glucoside (BSSG) (Hostetmann *et al*., 2000). BSS, stigmasterols and campesterols are the main sterols normally produced by plants, humans thus has to obtain these phytosterols through their diet. Sources of sterols include unrefined plant oils, nuts, seeds, cereals, fruit, vegetables and legumes (Mosby, 1996; Moghadasian, 2000; De-Eknamkul and Potduang, 2003; Boukes *et al*., 2010). Studies have demonstrated that BSS possess anticancer properties in cells of the colon, prostate and breasts (Boukes *et al*., 2008; Careri *et al*., 2001). They obtain this protection against cancer by altering the concentration of phospholipids interfering with the fluidity and integrity of the cell membrane structure (Awad and Fink, 2000). BSS has observed to have stimulatory effects on proliferation on lymphocytes, especially T-cells (Bouic *et al*., 1996) and helps immunodeficient patients boost their immune systems (Mosby, 1996; Bouic *et al*., 1996; Bouic *et al*., 2001; Van Wyk *et al*., 2002). Numerous studies have connected BSS to assist in the management and treatment of testicular tumours and benign prostate hyperplasia (BPH) (Rhodes *et al*., 1993; Pegel, 1997; Berges *et al*., 2000; Lowe and Fagelman, 2008; Steenkamp, 2003; Kanterere and Eloff, 2008; Wilt *et al*., 2011; Street and Prinsloo, 2012). Management of BPH lay in the ability of BSS to inhibit both 5α-reductase and aromatase enzymes, in doing so it prevents the enzymes from binding to dihydrotestosterone found in the prostate (Berges *et al*., 1995; Bouic *et al*., 1999; Kanterere and Eloff, 2008; Van Wyk, 2008). Weber *et al.* (2002) used a research study to demonstrate that BSS aid in the decreasing of cholesterol and LDL in both humans and animals. Phytosterols, specifically β-sitosterols, has been successful in the treatment of cardiovascular diseases, pulmonary tuberculosis and HIV (Pegel, 1997; Moghadasian, 2000; Nair *et al*., 2006; Boukes *et al*., 2008). Phytosterols has also exhibited anti-ulcer, anti-inflammatory and antidiabetic properties (Pegel, 1997; Careri *et al*., 2001; Boukes *et al*., 2008; Katerere and Eloff, 2008).

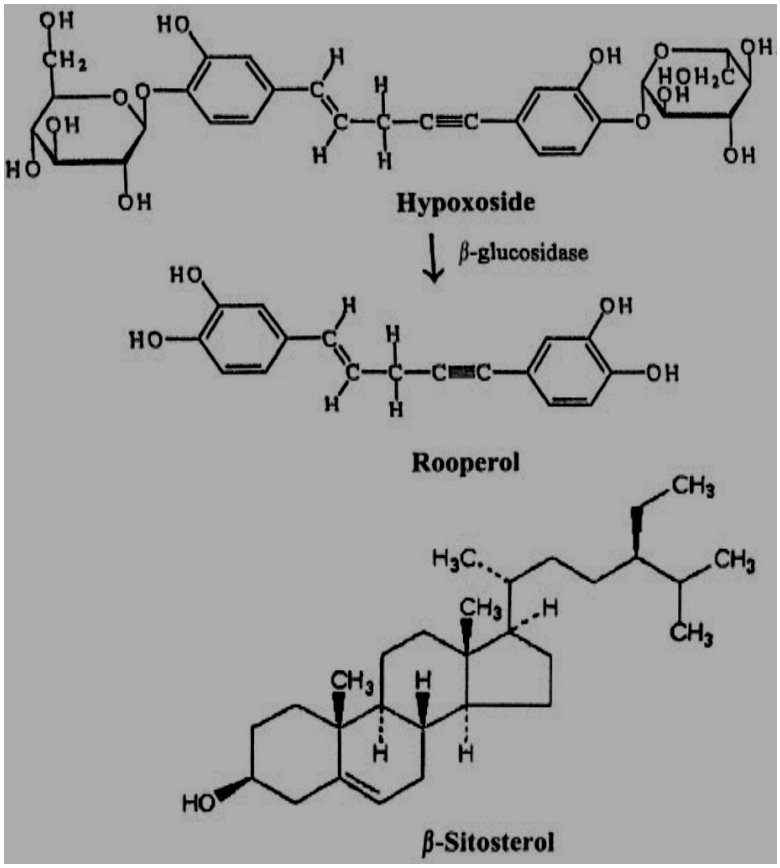

Figure 2.13: Biochemical structures of norlignan Hypoxiside and it being converted by β-glucosidase to its diglucoside, Rooperol. Biochemical structure of β-sitosterol (bottom) (Adapted from Owira and Ojewale, 2009)

2.8.4.3. Lectins

Lectin-like protein has also been extracted from *H. hemerocallidea* (Gaidamashvilli and Van Staden, 2002; Erlwanger and Cooper, 2008). Lectins also known as agglutinins are non-enzymatic glycoproteins with a main function to bind to specific carbohydrates found on cell membranes of pathogenic bacteria that results in agglutination, this process however is reversible (Gaidamashvilli and Van Staden, 2002; Garib-Fakim, 2006). It has been demonstrated that lectins have the ability to inhibit multiplication; growth and motility of certain bacteria found on plants and play an important role in the protection mechanism of the plant. Clinically, lectins can be used in analysis to identify bacteria and recognise as well as differentiate between tumours that are malignant (Hutchings *et al*., 1996; Gaidamashvilli and Van staden, 2002; De Hoff *et al*., 2009; Ghazarian *et al*., 2011).

Lectins have the ability to prevent the interaction and adhesion of pathogenic bacteria to the cells of the gastrointestinal tract. When harmful pathogens have been detected, lectins interact with lymphocytes and erythrocytes to activate an immune response in the human

body. It is believed that lectins may also possess anticancer properties and is capable of inducing apoptosis of cancer cells (Fu *et al*., 2011; Ghazarian *et al*., 2011).

In the current study we will investigate the effect *H. hemerocallidea* have on the reproductive system on STZ-induced diabetic wistar rats by observing a number of analytic methods that will allow us to reach our objectives for this study.

CHAPTER 3

RESEARCH DESIGN AND METHODOLOGY

3.1. Ethical consideration

All the experimental protocols were conducted according to the guidelines for the care and use of experimental animals. All animals received humane care according to the principles of Laboratory Animal Care of the National Society of Medical Research and the National Institutes of Health Guide for the Care and Use of Laboratory Animals of the National Academy of Sciences (National Institutes of Health publication no. 80-23, revised 1978). Approval from the appropriate Institutional Faculty Ethics Committee was sought. An ethics clearance was obtained from the Cape Peninsula University of Technology Faculty of Health and Wellness Ethics Committee (Cape Town, South Africa) (NHREC: 230408-014). Specific training for handling of laboratory wistar rats was undertaken.

3.2. Place of Study

This study was conducted on the Cape Peninsula University of Technology, Bellville Campus, South Africa. Rats were housed and sacrificed at Stellenbosch Animal facility. Sperm parameters was analysed at Stellenbosch University Physiology Department, Tygerberg campus and the University of the Western Cape. The Oxidative Stress Research Centre (OSRC) was used for antioxidant analysis and blood sample analysis under supervision of Mr F Rautenbach (OSRC Laboratory Manager).

3.3. Experimental study

The study assessed the baseline OS in the male wistar rats. Male wistar rats were randomly allocated to 5 groups according to the dietary supplementation they will receive. The rats was individually housed according to the groups they were divided in to ensure that each animal receive equal amounts of supplements, which was prepared fresh on a daily basis. The number of rats was 60 and they were divided into 5 groups, with 12 rats per group. Each rat was weighed and the weight was recorded before the study was started. All rats were fed standard rat chowand have free access to water daily.

3.3.1. Induction of diabetes

Diabetes was induced after overnight fasting with a single intraperitoneal injection of a freshly prepared STZ solution (Sigma, USA) dissolved in 0.1M cold citrate buffer pH 4.5 (Atila *et al*., 2012). Twenty four hours after diabetes was induced with STZ solution, blood glucose levels were measured using a portable Accucheck Glucometer (Roche, Germany). Blood was

obtained with tailprick and animals diabetic status was confirmed when blood glucose levels were above 18mmol/l (Ravi *et al.,* 2005).

3.3.2. Experimental groups in summary:

Group I (Normal control): Control, normoglycemic rats were fed a standard rat chow and water daily for 6 weeks.

Group II (Diabetic control): STZ-induced diabetic rats, fed standard rat chow and water daily for 6 weeks

Group III: STZ- induced diabetic rats and treated with 800mg/*kg H. hemerocallidea* extract by daily gastric intubation for 6 weeks.

Group IV: STZ- induced diabetic rats and treated with 200 mg/kg *H. hemerocallidea* extract daily by gastric intubation for 6 weeks.

Group V: Non-diabetic rats supplemented with 800 mg/kg *H. hemerocallidea* by gastric intubation daily for a period of 6 weeks.

All groups received fresh water and were fed a standard protein rat chow daily for a period of 6 weeks.

3.4. Sample collection and organ harvesting

At completion of the 6 weeks of treatment period, the rats were weighed and fasting blood glucose levels was taken using tail prick method. The rats were then anaesthetized with an intraperitoneal injection of 1ml (± 60mg/kg) sodium pentabarbitone. Once anaesthetized, animals were sacrificed and blood was collected from the abdominal aorta into serum separator clot activator tubes (BD Vacutainers, Plymouth, UK) and placed on ice. Tubes were centrifuged at 4000rpm at 4°C for 10 minutes to obtain serum and stored at -80°C pending further analysis. The epididymis and testes were excised, weighed and washed in phosphate buffer, before it was snap frozen in liquid nitrogen and stored at -80°.

3.5. Sperm Motility analysis

Upon excision, the epididymis was immediately placed in a petri dish containing 5ml of a-HAMS F10 supplemented with 3% BSA (Roche Diagnostics GmbH Mannheim, Germany) heated at 37°C. The cauda epididymis was cut into several fragments to allow the spermatozoa to swim out from the epididymis. The quality of sperm was accessed by its concentration, motility and morphology. Sperm motility was performed with the computer-aided sperm analysis (CASA) using the Sperm Class Analyser (SCA®; Microptic, Barcelona, Spain) which was able to do motion analysis of the sperm.

A 5µl sample was loaded into a pre-warmed eight-chamber standard count analysis slide (Leja products, GN Nieuw-Vennep, The Netherlands) at 37^{o}C. The following CASA settings for rat spermatozoa analyses were used: *Pseudo Negative phase, Ph2/3 condenser, 4x objective lens, no filter, Brightness ± 450, Contrast ±100.* The system analysed the total motility, progressive motility (percentage of A+B level of spermatozoa) and kinematic and velocity parameters such as curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), amplitude of lateral head displacement (ALH), linearity (LIN), straightness (STR) and beat cross-frequency (BCF).

3.6. Sperm morphology analysis

Sperm morphology was performed using SpermBlue (SB) fixative and stain supplied by Microptic SL, Barcelona, Spain. A 10µl of the sperm suspension was placed on the slide and a thin smear was made. The smear was air-dried for 24 hours after which it was carefully placed vertically into a staining tray containing SB fixative for 20 minutes after which it was removed from the staining tray and placed at a 60° to 80° angle to drain off excess fixative. Fixed smears were then placed vertically into a staining tray containing SB stain for 20 minutes after which the slides were then slowly dipped and fully immersed for 3 seconds in distilled water to remove excess stain. The slides there after left in an upright position (at about 70° angle), allowing excess fluid to run off and left to air-dry. On completion of the staining procedures, the slides were mounted using DPX mounting glue and clover-slipped. For morphometric evaluation, the Sperm Class Analyzer® (SCA®; Microptic, Barcelona, Spain) was used, using a blue filter, a 60x magnification. All spermatozoa which did not overlap with each other or with background staining or debris were considered for analysis. The SCA system automatically detects the acrosome, head and midpiece of spermatozoa and made rapid and accurate measurements of various variables and subsequently classify as either normal or abnormal.

3.7. Tissue homogenate preparation

Prior to analysis, tissues were homogenised to 200mg of tissue by adding 2ml of phosphate buffer (59mM $NaH_2PO_4.H_2O$, o.5% (v/v) TritonX-100, pH 7.5) in a glass homogeniser. The tissue was homogenised using a Potter Elvehjam homogeniser stroking five times. The whole process was performed on ice. The homogenate was then transferred to new eppendorf tubes and sonicated for 15 seconds on ice. The tubes containing the homogenate were then centrifuged at 15000rpm for 10 minutes using a microcentrifuge at a temperature of 4°C. The supernatant was transferred into new marked eppendorfs and protein determination was done using Bicinchoninic acid (BCA) method before freezing at -80°C.

3.8. Experimental Assays

3.8.1. Protein determination

Protein analysis was done using the BCA method quantitating the concentration of protein in the tissues before enzyme assays were performed. The BCA method relies on the formation of Cu^{2+} under alkaline conditions that leads to the reduction of Cu^{1+} as end-product, this reaction is known as the biuret reaction (Smith *et al*., 1985). Bicinchoninic acid forms a blue-purple complex with Cu^{1+} in alkaline environment and also indicates that reduction has taken place. The amount of reduction is directly proportional with the amount of protein present. This complex is water-soluble and has strong absorbance at 562nm.

The protein contents are normally reported with reference to a standard protein such as bovine serum albumin (BSA). A series of dilutions were prepared with known concentrations from BSA as well as dilutions of unknown(s) of testicular and epididymal tissues were assayed, concentration of dilution was chosen for the unknown(s) based on the standard curve. BCA working reagent, five albumin standards and samples were prepared for protein determination. In the microplate wells, 25µl of each standard and sample were put up in triplicates as per manufacturer's instruction assay kit supplied by Sigma Aldrich. The next step is the addition of 200µl of the working reagent to the each well containing the standards and samples and thoroughly mixed for 30 seconds. The microplate was then covered and incubated at 37°C for 30 minutes. After incubation, plate was left at room temperature to cool down and absorbance was measured at 562nm on a plate reader. Protein determination was quantified using the standard curve, expressed in ug/ml and used in measuring CAT concentrations.

3.8.2. Lipid peroxidation (TBA)

Lipid peroxidation or Thiobarbituric acid (TBA) assay is high specific that quantifies the amount of MDA production. The principle of the method is based on the reaction of MDA and TBA in acidic conditions and increased temperature that will result in a pink colour complex of MDA-$(TBA)_2$ which will then be quantified using a microplate reader (Thermo Electron Corporation, multiscan spectrum, USA) at a reading between A_{532}- A_{572}.

A neat sample volume of 100 µl of tissue homogenate was added to a tube with 12.5µl of 4mM of cold BHT/ethanol and 100 µl of 0.2M ortho-phosphoric acid in eppindorf tubes. The tubes were vortexed for 10 seconds to thoroughly mix the content. After mixing, 12.5µl of TBA (0.11M in 0.1M NaOH) was added to the tubes and vortexed for 10 seconds. Tubes with content were then placed in a water-bath that was heated to 100°C for one hour to allow reaction to take place (little punctures were made in the lids of the tubes to prevent it from

popping open while in the water-bath). Exactly after the one hour the tubes were directly placed on ice for 2 minutes allowing for rapid cooling and then left at room temperature for 5 minutes. On completion of the 5 minutes, 1000µl of n-butanol and 100µl of saturated NaCl were added to aid in better separation of phases followed with 10 seconds of vortexing for thorough mixing. The tubes and its contents were then placed in a microcentrifuge and centrifuged for 2 minutes at 12000rpm at 4°C. 200µl of the butanol layer was then added to a 96-well microplate in triplicates and read at A_{532}- A_{572}.

3.9. Antioxidant assessment

3.9.1. Superoxide dismutase (SOD)

SOD activity is determined from the conversion of xanthine and oxygen to uric acid and hydrogen peroxide by xanthine oxidase to form O_2^-. SOD activity was determined using a modified method from Ellerby and Bredesen (2000). The assay uses 6-Hydroxydopamine (6-HD) and diethylenetriaminepentaacetic acid (DTAPAS) that aids in generating superoxide anions. Samples were run in triplicates using a 96-well plate, 170µl DETAPAC solution (0.1mM in SOD buffer, 0.4mg in 10ml) was added to 6µl of lysate and 24µl of superoxide dismutase buffer was added to each well. A blank of distilled water was used in the first three wells. Fifteen microliters of stock 6-HD was added to each well and read immediately at 490nm at 1 minute intervals. Each sample was run in triplicates. The activity of SOD was calculated from a linear calibration curve, over a range of 2-20U/mg.

3.9.2. Catalase (CAT)

CAT activity was determined by measuring the change in absorbance of H_2O_2 and sample mixture. The rate of H_2O_2 decomposition into water and oxygen was proportional to the concentration of CAT activity. This assay was carried out at room temperature in UV microplate. The first duplicates were distilled water that served as a blank; the wells that followedwere 10µl of sample homogenate. All samples were run in duplicates 170µl phosphate buffer was added to each well followed by 75µl of H_2O_2 stock solution. The plate contents were mixed well and read at a wavelength of 240nm using a spectrophotometer (Thermo Electron Corporation, Multiscan spectrum, USA). The CAT activity was expressed as µmol/mg of protein.

3.9.3. Spectrophotometric determination of total Gluthatione (GSHt) levels

GSH concentration was determined according to the method of Boyne and Ellman (1972). Tissue homogenates were diluted 20 fold. All reagents were prepared according to the method described by Asensi *et al*. (1999). Sample homogenates were thawed and centrifuged at 15000rpm for 5minutes at 4°C. The Supernatant was used for analysis.

Standards (% standards in total) and supernatant of homogenate were pipetted in triplicates into a 96-well microtiter plate. 50 µl of freshly prepared DTNB (5,5'-Dithio-bis-(2-nitrobenzoic acid)) reagent (40mg DTNB in 100 ml of aqueous 1% trisodium citrate) was added. Next 50µl of enzyme solution (glutathione reductase was enzyme used) was added to each well. The plate was mixed and incubated for 5 minutes at 25°C. For reaction to be initiated 50µl of NADPH was added to each well. The total content of GSHt was quantified using a spectrophotometer which monitored the reduction of DTNB at 412nm within 5 minutes. Final results were obtained by comparison to the calibration curve standard. GSHt concentration was expressed as mmol/ml.

3.10. Evalution of Total Antioxidant capacity

3.10.1. FRAP spectrophotometric determination

FRAP is a colomestric spectrophotometric assay used to measure the ferric reducing ability of biological samples or ferric ion reducing antioxidant power (FRAP). The principle of this assay is based upon the reduction ability of antioxidants to convert the ferric ion (Fe3+) into its oxidised counterpart (Fe2+) in an acidic media. The oxidation/reduction electron transfer redox a reaction occurring in this assay is signalled by the development of a characteristic blue coloration. At low pH, a ferric salt, ferric chloride hexahydrate Fe3(TPTZ)2Cl3 (TPTZ= 2,4,6, Tripyridyl-s-triazine) used as an oxidant it is reduced by biological antioxidants in a sample to give the blue-coloured ferrous tripyridyltriazine complex. The colour development occurs only in the presence of electron donating antioxidants in the sample and is monitored by a spectrophotometer that measures the change in absorbance, maximum at 593nm (Phipps *et al.,* 2007; Gupta *et al.,* 2009).

Ferric ion reducing antioxidant power reaction mix was prepared by mixing 30ml acetate buffer pH 3.6 (300mM), 3ml TPTZ (10mM), 3ml FeCl3 (20mM), and 6.6ml distilled water (dH2O). TPTZ was prepared in 40mM hydrochloric acid (HCl). All other reagents were prepared in water. L-(+) ascorbic acid was used to prepare aqueous antioxidant standard solutions in a range of 0-1000µM. In the 96-well plate, 10µl sample, standard and 300µl FRAP reaction mixture were mixed and incubated 30 minutes at 37ºC before readings. Each sample was run in triplicate and final results were obtained by comparison to the calibration curve standard.

3.10.2. Oxygen radical absorbance capacity (ORAC) spectrofluorometric determination

ORAC is a method used to measure lipophilic, hydrophilic and the antioxidant capacity of a substance. This assay is based upon the measurement of the inhibition of free radical damages to a fluorescent probe by antioxidants. The loss of the fluorescence intensity reflects the intensity of the damage caused by free radicals as well as their concentrations

present. A delay in the degradation of the fluorescent probe indicates the ability of the pre-existing antioxidant to scavenge free radical activity. In this study, fluorescein was used as the probe and the assay is based upon the inhibition of the peroxyl-radical-induced oxidation of fluorescein (FL). The reaction was initiated by the thermal decomposition (at 37°C) of the azo-compound 2, 2'-azobis (2-amidino-propane) dihydrochloride (AAPH) which served as the source of peroxyl radicals. The protective effect of antioxidants in a sample was measured by comparing the areas under the fluorescence decay curve (AUC) obtained from samples to the areas obtained from control mixtures which were prepared using Trolox solution. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid) is a synthetic water-soluble vitamin E antioxidant derivative. The ORAC assay combines both inhibition time and degree of inhibition into one quantity and the results were expressed as micromoles of Trolox® equivalents (TE) per millilitre of sample (µmol of TE/ml).

The procedure used was a modified method of Rautenbach and co-workers (2010). All reagents and standards (AAPH, FL and Trolox) were prepared in phosphate buffer (75mM, with pH 7.4, ORAC buffer). Tissue samples were prepared using 50µl of 5% Perchloric acid (PCA) to 50µl of tissue homogenates. Mixture was spun down for 1min at 14000rpm and supernatant was used for assay. Standards were prepared within a range of 0-417µM Trolox. One hundred and thirty eight microliters of fluorescein (final concentration 14µM per well) and 12µL of the sample were mixed in a black Nunclon 96-well plate. Stock solution of AAPH (500µM) was prepared and 50µl was added to the plate before readings. Fluorescence readings were carried out on a fluoroskan ascent plate reader (Thermo Fisher Scientific, Waltham, Mass., U.S.A.) with 485 and 538nm as excitation and emission wavelengths. Each reading was taken after shaking at the end of every 1 minute cycle for two hours. Antioxidant 35 activity was expressed in Trolox equivalents. One ORAC unit was assigned to the net protection area provided by a 1µM solution of Trolox. The final ORAC values were calculated using a regression equation ($y=a+bx+cx^2$) describing the relationship between the Trolox concentration (µM) and the net area under the fluorescence decay curve. Data are expressed as micromoles of Trolox equivalents (TE) per millilitres of plasma µMTE/ml. The area under the curve (AUC) is calculated as AUC = [(0.5 + f2/f1 + f3/f1 + f4/f1 +...+ fi/f1) x CT] where f1 is the initial fluorescence reading at cycle 1, +…+ is the interval/ratio from f4/f1 to infinity), fi is the fluorescence reading at cycle i and the cycle time in minutes. The net AUC was obtained by subtracting the AUC of the blank from that of the sample. The side wells (columns 1, 2, and 12) of the 96-well plate were not used and the cycle time was reduced to a minute to improve the accuracy of the results. Each sample was repeated in triplicate.

3.11. Hormonal assays

3.11.1. Testosterone

The DEMEDITEC Testosterone rat/mouse ELISA Kit (DRG, Germany) is a solid phase enzyme-linked immunosorbent assay (ELISA). The principle is based on competitive binding where an unknown amount of testosterone present in the sample and a known amount of testosterone conjugated to horseradish peroxide compete for binding sites of testosterone antiserum coated on the wells of the microplate. The addition of substrate solution allow for the concentration of testosterone to be inversely proportional to the optical density measured.

The wash was made up by adding 50ml of 10X concentrated wash solution to 450 ml of deionised water, making it up to a final volume of 500ml which is stable at room temperature for 3 months. The rest of the reagents and calibrators were ready to be used. Before starting procedure, it is important to bring all reagents of kit to room temperature. At room temperature, 10µl of 6 calibrators, controls and blood serum samples was dispensed into the 96-wells microtitreplate coated with anti-testosterone antibody. The next step was dispensing 100µl of incubation buffer into each well and followed with addition of 50µl of enzyme conjugate to each well. The plate was left at room temperature for 60 minutes on a microplate mixer which allowed for optimal reaction to take place. After 60 minutes incubation the content was discarded from the wells and it was rinsed four times with diluted wash of 300µl per well each wash. The last wash was discarded from wells by banging of microplate on absorbent paper till wells were completely dry. 200µl of substrate solution was added to each well and incubated for 30 minutes in the dark without mixing. After incubation, the reaction was stop by adding 50µl of stop solution to each well. The absorbance of each well was then determined by using a microplate reader at a wavelength of 450nm. Absorbances of all wells were read within 15 minutes.

3.11.2. Estradiol

The Estradiol ELISA kit (DRG, Germany) is classified as a solid phase enzyme-linked immunosorbent assay (ELISA). The principle is based on competitive binding where the wells of the microtitre plate are coated with a polyclonal rabbit antibody that is directed to bind an antigenic site on the estradiol molecule. The unknown endogenous estradiol of a patient sample competes with an estradiol-horseradish peroxidase conjugate for binding site to the coated antibody in the wells. After washing, the unbound conjugate is washed off and the amount of peroxidise conjugate left bound is inversely proportional to the concentration of the estradiol in the sample.

The kits reagents were brought to room temperature before use. The wash solution was prepared by diluting 30ml of concentrate wash in 1170ml of deionised water making it up to a final volume of 1200ml. The next step was dispensing 25µl of the seven standards, control and blood serum sample into appropriate wells in triplicates. 300µl of enzyme conjugate was dispensed in each well and mixed for 10 seconds. After mixing, the microplate was left at room temperature for 120 minutes. The content of wells was then briskly discarded and the wells were rinsed three times with 300µl of diluted wash concentrate. To ensure complete removal of wash, the plate was beaten sharply on absorbent paper until dry. Next was the adding of 100µl of substrate to each well, followed with an incubation period of 15min. After incubation, 50µl of stop solution was added to each well to stop the reaction. The absorbance was determined using a microplate reader at a wavelength 450nm. The wells were read within 10 minutes after adding the stop solution.

CHAPTER 4

RESULTS

4.1. Blood glucose level before and after 6 weeks of treatment

Figure 4.1 shows the fasting glucose levels in all five groups before and after four of the groups were supplemented with *H. hemerocallidea* (*Hypoxis*) after diabetes were induced. No significant difference ($p>0.05$) was observed in fasting glucose of normal control (group A) and non-diabetic group supplemented with 800mg/kg *H. hemerocallidea* (group E) before and after 6 weeks of treatment. The diabetic control (group B) showed a significant increase ($p<0.05$) in glucose levels 3 days after DM was induced and on completion of the 6 weeks when compared to initial fasting glucose levels. There was also a significant increase ($p<0.05$) when glucose levels after the 6 week period was compared to glucose levels initially taken from the diabetic group treated with 800mg/kg *H. hemerocallidea* (group C). Another significant increase ($p<0.05$) was observed when initial glucose level of diabetic group treated with 200mg/kg *H. hemerocallidea* (group D) was compared to glucose levels after 6 weeks of treatment.

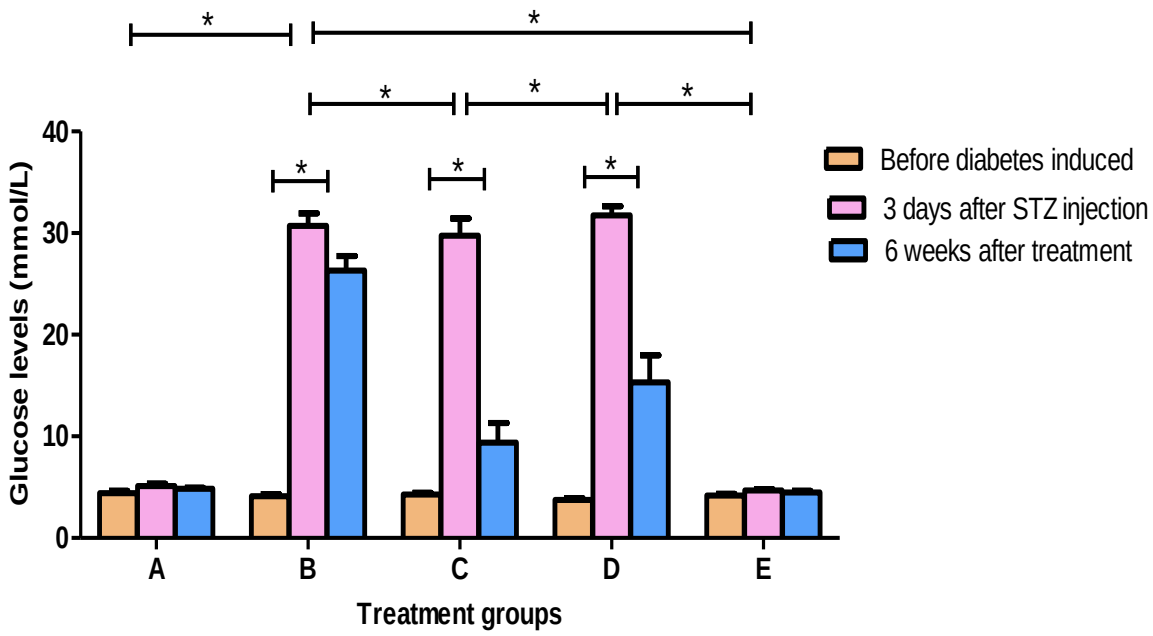

Figure 4.1: Demonstrates the blood glucose levels of the different group before and after diabetes was induced and after treatment

Data presented as mean ± SEM. (*) indicates significance with $p<0.05$, n= 12 per group.

Table 4.1: Blood glucose levels of wistar rats before induction of diabetes, 3 days after diabetes was induced and 6 weeks after treatment

Groups/Treatment	Fasting glucose results (mmol/l)	Blood glucose after 3 days without treatment mmol/l	Blood glucose after 6 weeks after treatment mmol/l
A: Normal control group	4.583 ± 0.1036	5.117 ± 0.2412*#	4.73 ± 0.1034*#
B: Diabetic control group	4.120 ± 0.1812	30.70 ± 1.2630$	26.32 ± 1.4220#$
C:Diabetic+800mg/kg*Hypoxis*	4.280 ± 0.1855	29.76 ± 1.6750$	9.38 ± 1.9410*#$
D:Diabetic+200mg/kg *Hypoxis*	3.767 ± 0.1509	31.75 ± 0.8920$	15.33 ± 2.673*$
E: Non-diabetic+800mg/kg *Hypoxis*	4.175 ± 0.1620	4.692 ± 0.1252*#	4.49 ± 0.1525*#

Data in table is presented as mean ± SEM. (*) represents a significant difference when compared to Diabetic control group, (#) represents a significant difference when compared to Diabetic group+200mg/kg *Hypoxis* and ($) represents significance when compared to Non-diabetic+800mg/kg *Hypoxis*, n= 12 per group.

Abbreviations: mmol/l: milimoles per litre; mg/kg: milligrams per kilogram; *Hypoxis*: *H. hemerocallidea*.

Experimental groups:
A: Normal control group : Only fed with standard rat chow (SRC)
B: Diabetic control group : Diabetes induced using STZ and fed SRC
C: Diabetic+800mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 800mg/kg *H. hemerocallidea*
D: Diabetic+200mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 200mg/kg *H. hemerocallidea*
E: N+ 800mg/kg *Hypoxis* : Non-diabetic group supplemented with 800mg/kg *H. hemerocallidea*

4.2. Body weights

In Figure 4.2, shows the body weights of all five groups before and after 6 week period. The average body weights of the normal control (group A) before and after the 6 weeks showed a significant increase (215.0 ± 3.236mmol/l versus 315.7 ± 9.348mmol/l, $p<0.0001$). The diabetic control (group B) showed no significant difference in body weights at the end of the 6 weeks when compared to initial body weights that was taken (213.3 ± 3.073mmol/l versus 210.2 ± 6.256mmol/l, $p>0.05$). There was a significant increase when body weights after the 6 week period was compared to body weights initially taken from the diabetic group treated with 800mg/kg *H. hemerocallidea* (group C) (213.3 ± 3.945mmol/l versus 239.3 ± 14.47mmol/l, $p<0.05$). No significant difference was observed when initial body weights of the diabetic group treated with 200mg/kg *H. hemerocallidea* (group D) was compared to body weights after 6 weeks of treatment (213.9 ± 2.799mmol/l versus 218.4 ± 4.936mmol/l, $p>0.05$). However, there was a significant increase when body weights of the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea* (group E) was compared to the normal control group before and after the 6 weeks (226.3 ± 2.801mmol/l versus 305.8 ± 7.567mmol/l, $p<0.0001$).

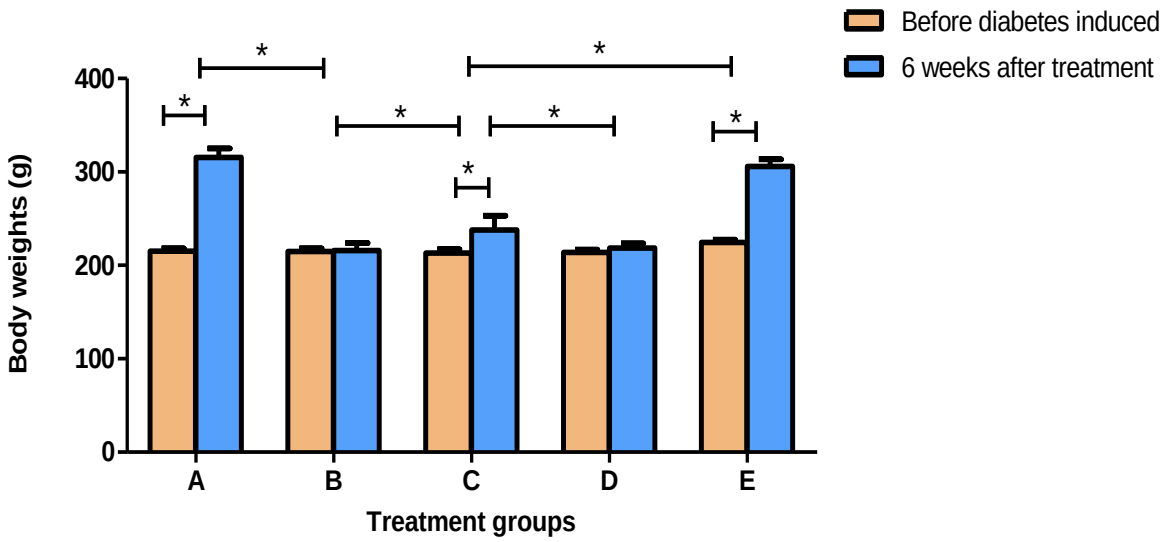

Figure 4.2: Body weight of male wistar rats before and after treatment

Data presented as mean ± SEM. (*) represents a significant difference, n= 12 per group.

Experimental groups:
A: Normal control group : Only fed with standard rat chow (SRC)
B: Diabetic control group : Diabetes induced using STZ and fed SRC
C: Diabetic+ 800mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 800mg/kg *H. hemerocallidea*
D: Diabetic+ 200mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 200mg/kg *H. hemerocallidea*
E: N+ 800mg/kg *Hypoxis* : Non-diabetic group supplemented with 800mg/kg *H. hemerocallidea*

4.3. Evaluation of testicular and epididymal weights of wistar rats after treatment

Weights of testicular and epididymal tissue after treatment with *H. hemerocallidea are* presented in Figure 4.2 and Figure 4.3 and values of weights in Table 4.2.

Epididymal and testicular weights of the normal control, non-diabetic group supplemented with *H. hemerocallidea* and the diabetic groups treated with *H. hemerocallidea* showed no significant difference ($p>0.05$) when compared. However, there was a significant increase ($p<0.05$) in epididymal and testicular weights when the diabetic groups treated with *H. hemerocallidea* were compared to the diabetic control group. No significant difference ($p>0.05$) was observed when epididymal and testicular weights of the diabetic group treated with 800mg/kg *H. hemerocallidea* was compared to the diabetic group treated with 200mg/kg *H. hemerocallidea*.

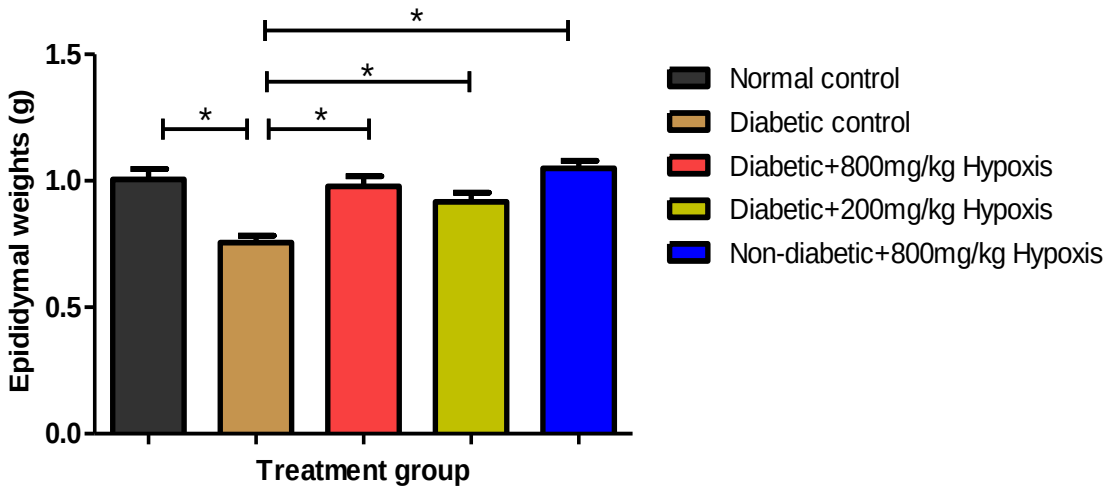

Figure 4.3: Epididymal weight of male Wistar rats after reatment

Data is presented as mean ± SEM. (*) indicates significance with p<0.05, n= 12 per group

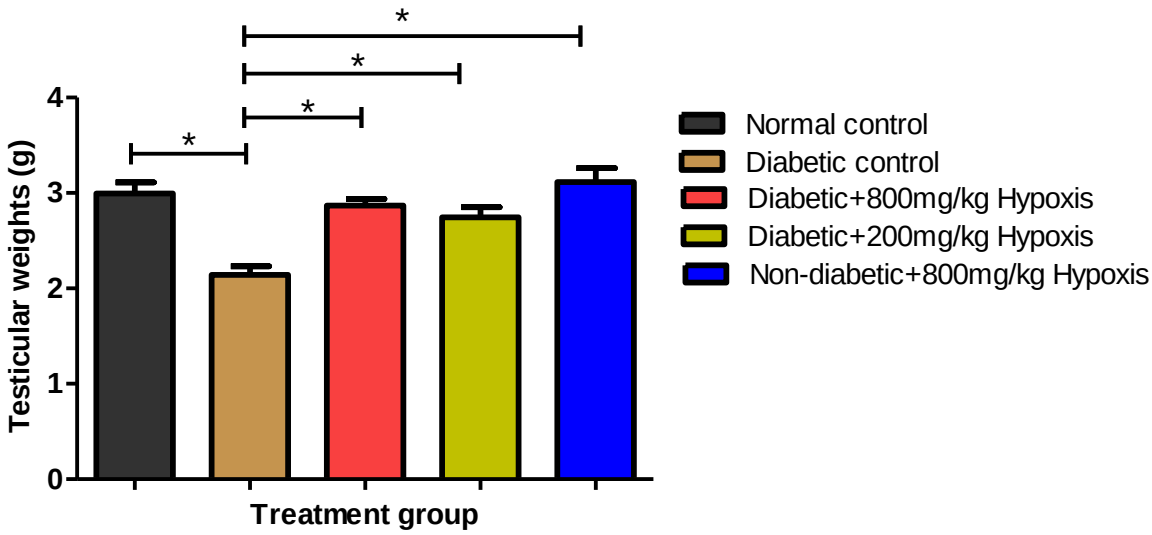

Figure 4.4: Testicular weights of male Wistar rats after treatment

Data is presented as mean ± SEM. (*) indicates significance with p<0.05, n= 12 per group

Table 4.2: Epididymal and testicular tissue weights results upon sacrifice wistar rats after treatment of respective groups at different dosages compared to control groups

Groups/Treatment	Epididymal weights (g)	Testicular weights (g)
A: Normal control	1.005 ± 0.0415*	2.995 ± 0.1179*
B: Diabetic control	0.7557 ± 0.0279$^{\#\$}$	2.140 ± 0.0911$^{\#\$}$
C: Diabetic +800mg/kg *Hypoxis*	0.9777 ± 0.0404*	2.868 ± 0.0718*
D: Diabetic +200mg/kg *Hypoxis*	0.9173 ± 0.0357*	2.744 ± 0.1077*
E: Non-diabetic +800mg/kg *Hypoxis*	1.030 ± 0.0297*	3.116 ± 0.1443*

Data in table is presented as mean ± SEM. (*) represents a significant difference when compared to Diabetic control, ($^{\#}$) represents a significant difference when compared to Diabetic group+200mg/kg *Hypoxis* and ($^{\$}$) represents significance when compared to Non-diabetic+800mg/kg *Hypoxis*, n= 12 per group.

Abbreviations: g: grams; mg/kg: milligrams per kilogram; *Hypoxis*: *H. hemerocallidea*.

Experimental groups:
A: Normal control group : Only fed with standard rat chow (SRC)
B: Diabetic control group : Diabetes induced using STZ and fed SRC
C: Diabetic+ 800mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 800mg/kg *H. hemerocallidea*
D: Diabetic+ 200mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 200mg/kg *H. hemerocallidea*
E: N+ 800mg/kg *Hypoxis* : Non-diabetic group supplemented with 800mg/kg *H. hemerocallidea*

4.3. Assessment of sperm motility and sperm morphology of wistar rats after treatment

This section includes sperm parameters (including motility and morphology) of different groups by observing the fast progression, slow progression, total progression and immotile sperm. Also including velocity parameters such as Curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), linearity (LIN), straightness (STR), wobble (WOB), head displacement (ALH) and beat/cross frequency (BCF). Morpholoy of sperm was classified as normal or abnormal.

Table 4.3: Progression and velocity parameters as well as morphology of spermatozoa of normal control (A), diabetic control (B), diabetic group treated with 800mg/kg *H. hemerocallidea* (C), diabetic group treated with 200mg/kg *H. hemerocallidea* (D) and non-diabetic group supplemented with *H. hemerocallidea* (E)

Sperm motility parameters	Group/ Treatment				
	A: Normal control	B: Diabetic control	C: Diabetic+800mg/kg *Hypoxis*	D: Diabetic+200mg/kg *Hypoxis*	E: Non-diabetic+ 800mg/kg *Hypoxis*
Fast progressive	267.2 ± 38.29*#$	54.86 ± 19.83$	260.0.3 ± 47.48*#$	57.80 ± 14.40#	287.3 ± 34.56*#
Slow progressive	176.0 ± 38.53*$	181.6 ± 46.43#$	171.8 ± 81.75*#	204.8 ± 64.68*$	164.5 ± 27.58*#
Non-progressive	130.9 ± 16.14*#$	154.1 ± 29.76$	133.0 ± 15.96*#$	146.4 ± 26.49$	113.2 ± 7.151*#
Total progressive	190.4 ± 22.79*#$	101.3 ± 15.36#$	175.4 ± 35.08*#$	115.7 ± 19.13*$	204.9 ± 27.84*#
Immotile	46.80 ± 6.35*#	203.00 ± 21.72$	77.00 ± 18.40*#$	208.60 ±28.96$	52.00 ± 4.95*$
VCL	193.1 ± 8.526*#	115.5 ± 8.850#$	188.6 ± 7.218*#	133.9 ± 9.398*$	187.5 ± 12.20*#
VSL	49.65 ± 1.943	36.76 ± 2.140$	48.84 ± 1.613	43.30 ± 2.388	51.64 ± 1.525*
VAP	76.88 ± 5.399*	58.83 ± 3.118#$	83. 42 ± 3.826*	71.12 ± 3.902*	83.78 ±2.583*
LIN	27.79 ± 1.249	29.56 ± 1.353	27.04 ± 1.110	30.30 ± 2.385	27.82 ± 1.179
STR	59.56 ± 1.659	61.18 ± 1.378	58.76 ± 1.852	63.88 ± 2.005	53.88 ± 5.521
Wobble	45.85 ± 0.485	48.73 ± 1.339	45.98 ± 1.210	46.04 ± 3.032	45.98 ± 0.595
ALH	11.99 ± 0.7532	9.488 ± 0.4037	11.38 ± 0.5704	8.675 ± 1.286	11.66 ± 0.7160
BCF	3.350 ± 0.1875	3.543 ± 0.2599	3.640 ± 0.2977	3.125 ± 0.5031	3.400 ± 0.2324
Sperm Morphology					
Normal (%)	92.67 ± 2.917*#	69.33 ± 4.723$	88.67 ± 4.185*#	70.60 ± 8.727$	81.60 ± 3.187*#
Abnormal (%)	7.333 ± 2.917*#$	35.60 ± 4.534	16.50 ± 3.403*	24.50 ± 9.323	18.40 ± 3.187*

Data presented as mean ± SEM, (*) p<0.05 compare to Diabetic control, (#) p<0.05 compared to Diabetic+200mg/kg *Hypoxis*, ($) p<0.05 compared to Non-diabetic supplemented with 800mg/kg *Hypoxis*, n= 12 per group.

4.4. Assessment of lipid peroxidation of epididymal and testicular tissues of wistar rats after treatment

In Figure 4.5, the diabetic control group showed a significant increase in epididymal MDA levels when compared to the normal control group (0.2648 ± 0.0111µmol/g protein versus 0.2116 ± 0.0045µmol/g protein, p<0.05). A significant decrease of MDA was also observed when the diabetic control group was compared to the diabetic group treated with 800mg/kg *H. hemerocallidea* (0.2648 ± 0.0111µmol/g protein versus 0.1782 ± 0.0062µmol/g protein, p<0.05). A significant decrease was also observed when the diabetic control group was compared to the diabetic group treated with 200mg/kg *H. hemerocallidea* (0.2648 ± 0.0111µmol/g protein versus 0.2122 ± 0.0114µmol/g protein, p<0.05).There was a significant increase when diabetic group treated with 800mg/kg *H. hemerocallidea* was compared to the diabetic group treated with 200mg/kg *H. hemerocallidea* (0.1782 ± 0.0062µmol/g protein versus 0.2122 ± 0.0114µmol/g protein, p<0.05). A significant decrease was also observed when the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea* was compared to the normal control (0.2116 ± 0.0045µmol/g protein versus 0.1609 ± 0.0076µmol/g protein, p<0.05).

Figure 4.6 shows a significant increase of testicular MDA levels when the diabetic control group was compared to the normal control group (0.0562 ± 0.023µmol/g protein versus 0.0425 ± 0.0023µmol/g protein, p<0.05). A signicant increase was also observed when diabetic control was compared to diabetic group treated with 800mg/kg *H. hemerocallidea* (0.0425 ± 0.0023µmol/g protein versus 0.0448 ± 0.0015µmol/g protein, p<0.05). A significant decrease was observed when diabetic group treated with 200mg/kg *H. hemerocallidea* was compared with diabetic control (0.0447 ± 0.0031µmol/g versus 0.0425 ± 0.0023µmol/g protein, p<0.05).

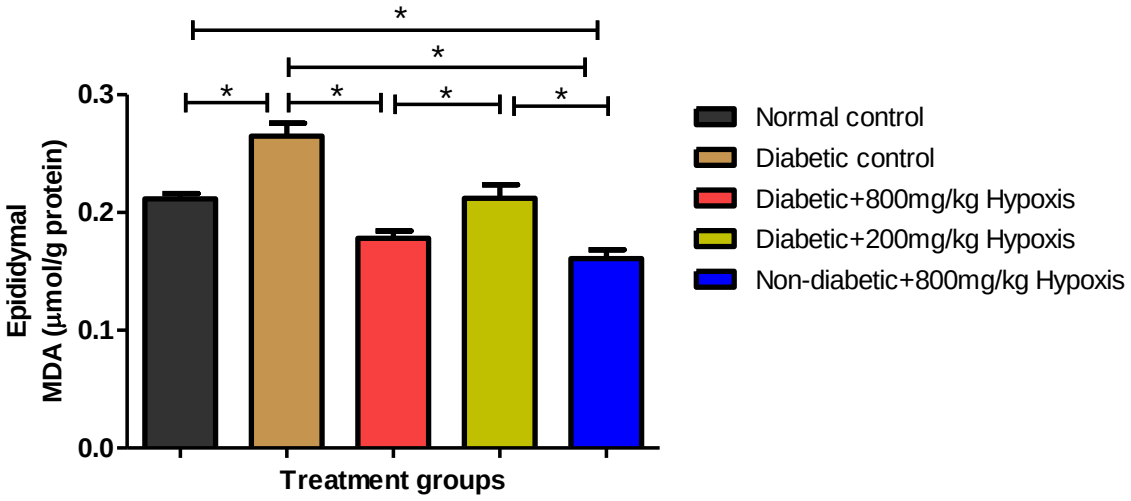

Figure 4.5: LPO activity of epididymal tissue of Wistar rats after treatment

Data presented as mean ± SEM. (*) indicates significant difference with p<0.05, n= 12 per group

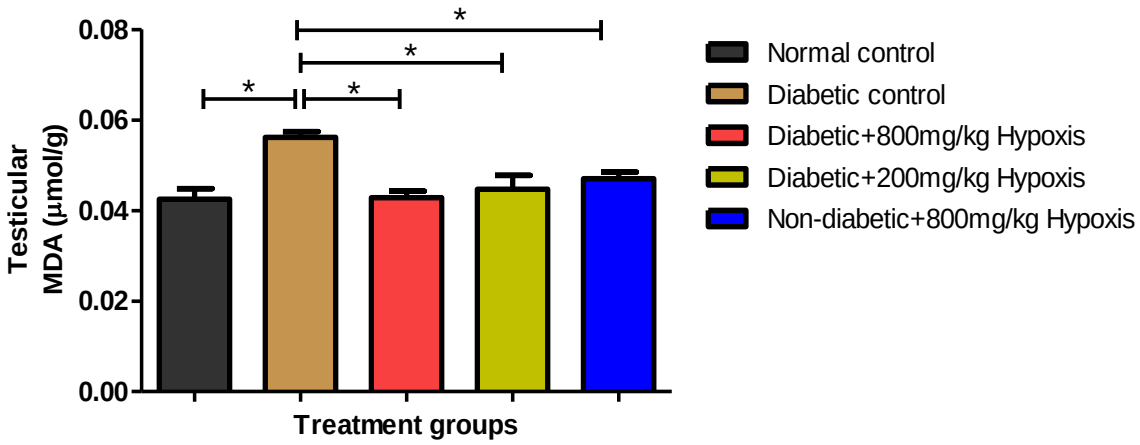

Figure 4.6: LPO activity of epididymal tissue of Wistar rats after treatment

Data presented as mean ± SEM. (*) indicates significant difference with p<0.05, n= 12 per group

4.5. Assessment of antioxidant enzymes in epididymal and testicular tissue

In this section the activity of antioxidant enzymes was investigated after diabetes was induced using STZ and the rats were subjected to different concentrations of *H. hemerocallidea*.

4.5.1. Assessment of SOD in both epididymal and testicular tissue of wistar rats after subjected to treatment

Figure 4.7 and Table 4.4 showed that a significant decrease (p<0.05) was observed when epididymal SOD of the diabetic control group was compared to the normal control group and the non-diabetic group supplemented with *H. hemerocallidea.* Epididymal SOD of diabetic treated groups also showed a significant increase (p<0.05) when compared to the normal control group and the non-diabetic supplemented with *H. hemerocallidea.* Epididymal SOD showed a significant increase (p<0.05) when the diabetic treated groups were compared to the diabetic control group. No significant difference (p>0.05) was observed when the diabetic group treated with 800mg/kg were compared with the diabetic group treated with 200mg/kg *H. hemerocallidea*.

Figure 4.8 and Table 4.4 showed no significance (p>0.05) when testicular SOD activity of the diabetic control group was compared to groups supplemented with *H. hemerocallidea.* However, testicular SOD activity of the diabetic control group was significantly lower (p<0.05) when compared to the normal control group. Testicular SOD activity of the normal group was significantly higher (p<0.05) when compared to groups supplemented with *H. hemerocallidea*.

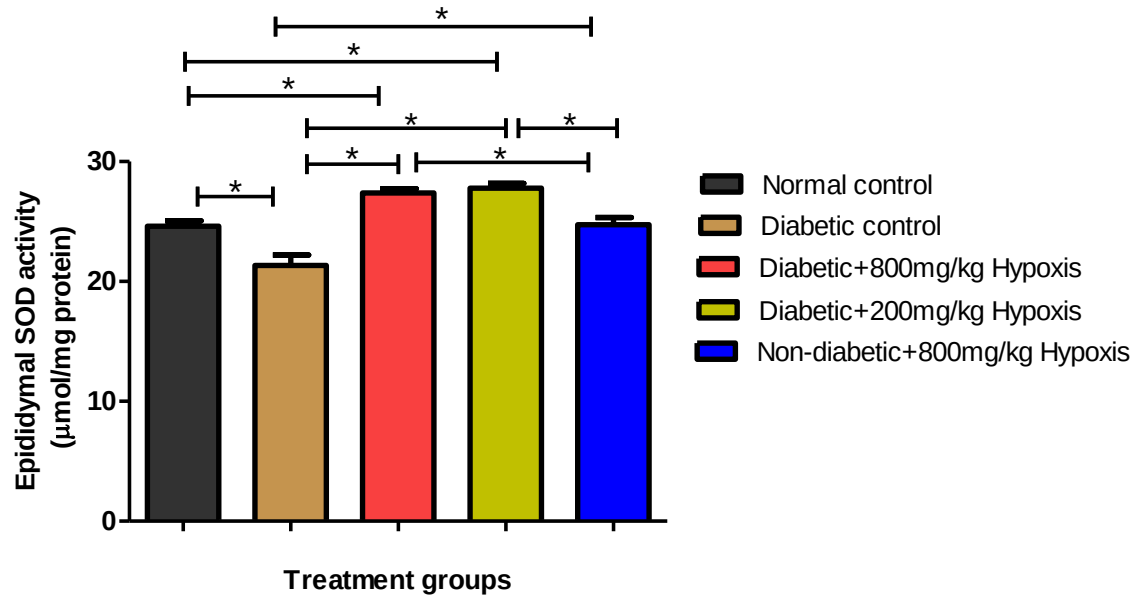

Figure 4.7: Epididymal tissue SOD activity of male wistar rats after treatment

Data is presented as mean ± SEM. (*) indicates significant difference with p<0.05, n= 12 per group.

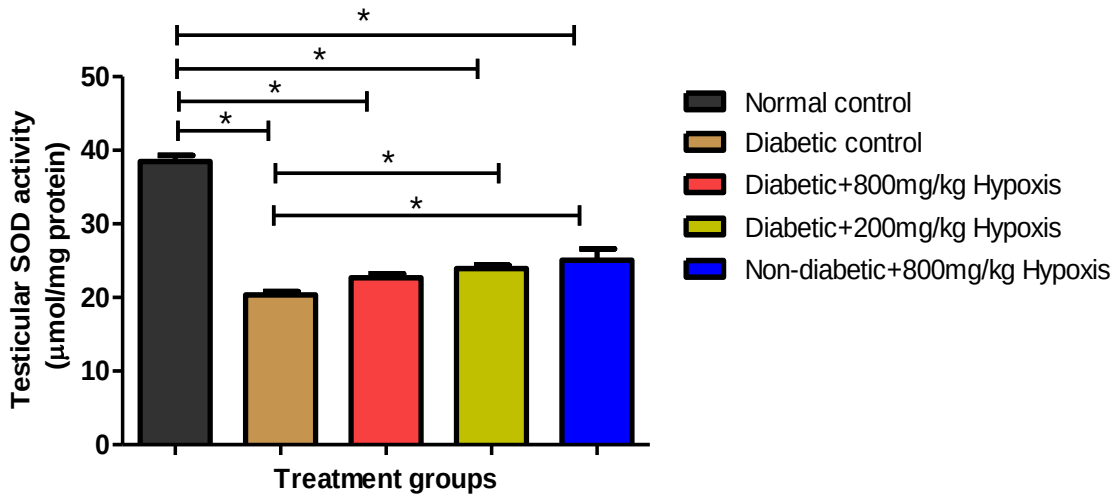

Figure 4.8: Testicular tissue SOD activity of male wistar rats after treatment

Data is presented as mean ± SEM. (*) indicates significant difference with p<0.05, n= 12 per group

Table 4.4: Effects of *H. hemerocallidea* supplementation on both epididymal and testicular SOD activity

Groups/ Treatment	Epididymal SOD (µmol/mg protein)	Testicular SOD (µmol/mg protein)
A: Normal control	24.60 ± 0.7635*#	38.47 ± 0.8523*#$
B: Diabetic control	21.33 ± 0.8898#$	20.35 ± 0.4694#$
C: Diabetic + 800mg/kg *Hypoxis*	27.40 ± 0.3502*$	22.67 ± 0.5434
D: Diabetic+ 200mg/kg *Hypoxis*	27.80 ± 0.3980*$	23.93 ± 0.4491*
E:Non-diabetic+800mg/kg *Hypoxis*	24.73 ± 0.6194*#	25.07 ± 1.5300*

Data in table is presented as mean ± SEM. (*) represents a significant difference when compared to Diabetic control, (#) represents a significant difference when compared to Diabetic group+200mg/kg *Hypoxis* and ($) represents significance when compared to Non-diabetic+800mg/kg *Hypoxis*, n= 12 per group.

Abbreviations: µmol/mg protein: micromole per milligram protein; mg/kg: milligrams per kilogram; *Hypoxis*: *H. hemerocallidea*; SOD: Superoxide dismutase

Experimental groups:
A: Normal control group : Only fed with standard rat chow (SRC)
B: Diabetic control group : Diabetes induced using STZ and fed SRC
C: Diabetic+ 800mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 800mg/kg *H. hemerocallidea*
D: Diabetic+ 200mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 200mg/kg *H. hemerocallidea*
E: N+ 800mg/kg *Hypoxis* : Non-diabetic group supplemented with 800mg/kg *H. hemerocallidea*

4.5.2. Assessment of CAT activity in both epididymal and testicular tissue of wistar rats after subjected to treatment

The CAT activity in epididymal and testicular tissue of wistar rats after treated with *H. hemerocallidea* is represented in Figure 4.9 and Figure 4.10 and values in Table 4.5.

In Figure 4.9 epididymal CAT activity did not show a significant difference ($p>0.05$) when the normal control was compared to the diabetic control group. However, the diabetic group, diabetic group treated with 800mg/kg *H. hemerocallidea* and the non-diabetic group supplemented with 800mg/kg *H. Hemerocallidea* showed a significant increase ($p<0.05$) when compared to the diabetic group treated with 200mg/kg *H. hemerocallidea*.

Figure 4.10 shows a significant difference ($p<0.05$) when testicular CAT activity of treated groups was compared to the normal control groups and the diabetic control group. No significant difference ($p>0.05$) was observed between treated groups.

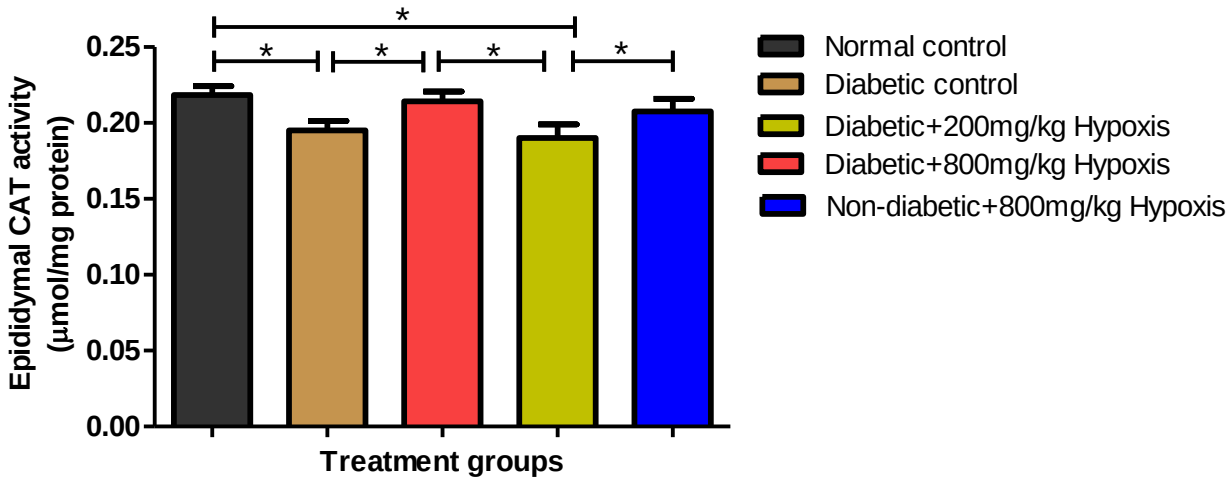

Figure 4.9: CAT activity in Epididymal tissue of Wistar rats after treatment

Data is presented as mean ± SEM. (*) indicates significant difference with p<0.05, n= 12 per group

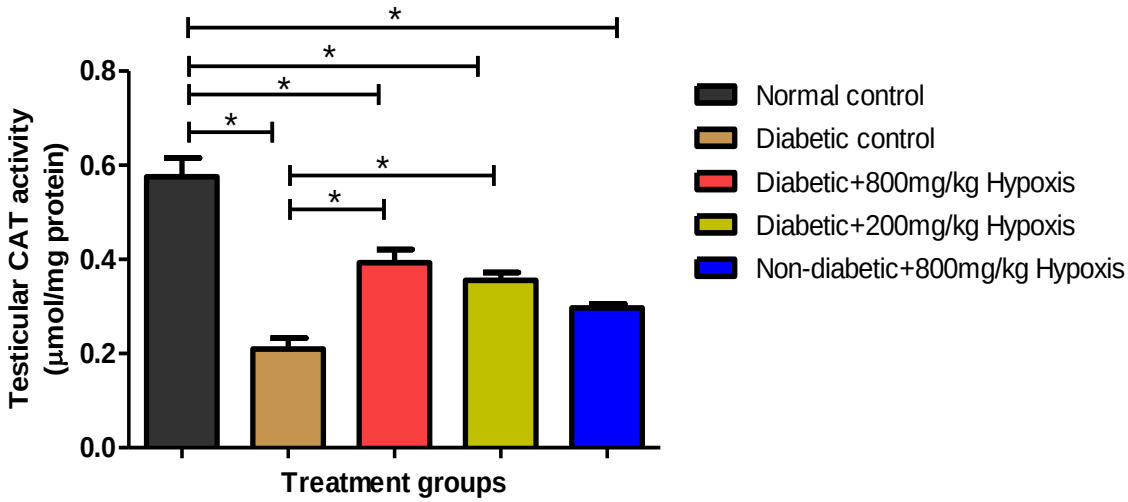

Figure 4.10: CAT activity in Testicular tissue of Wistar rats after treatment

Data is presented as mean ± SEM. (*) indicates significant difference with p<0.05, n= 12 per group

Table 4.5: CAT activity in both epididymal and testicular tissue after treatment of respective groups at different dosages compared to control groups

Group/Treatment	Epididymal CAT (µmol/L)	Testicular CAT (µmol/L)
A: Normal control group	0.2183 ± 0.0060*#	0.5760 ± 0.0397*$#
B: Diabetic control	0.1950 ± 0.0064	0.2100 ± 0.0228#
C: Diabetic + 800mg/kg *Hypoxis*	0.2143 ± 0.0065*#	0.3929 ± 0.0281*
D: Diabetic + 200mg/kg *Hypoxis*	0.1900 ± 0.0091$	0.3557 ± 0.0165*
E: Non-diabetic + 800mg/kg *Hypoxis*	0.2075 ± 0.0085#	0.2967 ± 0.0088

Data in table is presented as mean ± SEM. (*) represents a significant difference when compared to Diabetic control, (#) represents a significant difference when compared to Diabetic group+200mg/kg *Hypoxis* and ($) represents significance when compared to Non-diabetic+800mg/kg *Hypoxis*, n= 12 per group.

Abbreviations: µmol/L: micromole per millilitre; mg/kg: milligrams per kilogram; CAT: Catalase; *Hypoxis*: *H. hemerocallidea*.

Experimental groups:
A: Normal control group : Only fed with standard rat chow (SRC)
B: Diabetic control group : Diabetes induced using STZ and fed SRC
C: Diabetic+ 800mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 800mg/kg *H. hemerocallidea*
D: Diabetic+ 200mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 200mg/kg *H. hemerocallidea*
E: N+ 800mg/kg *Hypoxis* : Non-diabetic group supplemented with 800mg/kg *H. hemerocallidea*

4.5.3. Assessement of GSHt activity in epididymal and testicular tissue after subjected to treatment

Results of testicular and epididymal GSHt activity after treatment with *H. hemerocallidea* presented in Figure 4.11 and Figure 4.12 and mean ± SEM values in Table 4.6.

Figure 4.11 shows no significance ($p>0.05$) in epididymal GSHt levels when *H. hemerocallidea* supplemented groups were compared to each other and the normal control group. *H. hemerocallidea* supplemented groups showed a significant increase ($p<0.05$) in epididymal GSHt levels when compared to the diabetic group. There was also a significantincrease ($p<0.05$) in epididymal GSHt levels of the non-diabetic group supplemented with *H. hemerocallidea* when compared to the normal control group.

Figure 4.12 shows a significance ($p<0.05$) in testicular GSHt levels in most of the groups. However, there was no significant difference ($p>0.05$) when testicular GSHt levels of the diabetic group treated with 800mg/kg *H. hemerocallidea* was compared to the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea*.

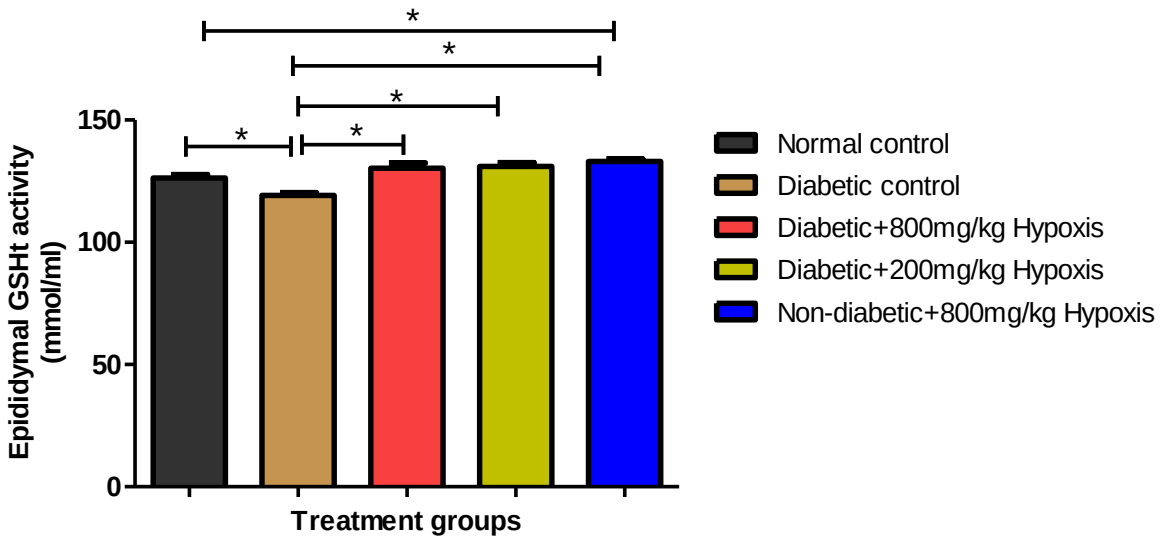

Figure 4.11: Epididymal tissue GSHt activity of male Wistar rats after subjected to treatment

Data is presented as mean ± SEM. (*) indicates significant difference with p<0.05, n= 12 per group

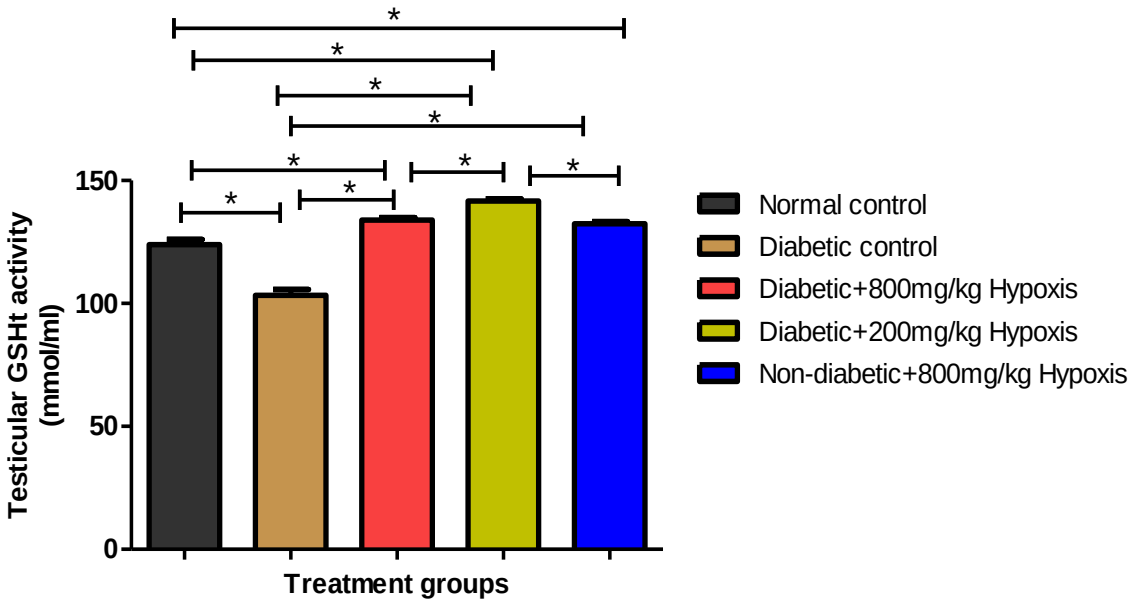

Figure 4.12: Testicular tissue GSHt activity of male Wistar rats after subjected to treatment

Data is presented as mean ± SEM. (*) indicates significant difference with p<0.05, n= 12 per group

Table 4.6: GSHt activity in both epididymal and testicular tissue after treatment of respective groups at different dosages compared to control groups

Group/Treatment	Epididymal GSHt (mmol/ml)	Testicular GSHt (mmol/ml)
A: Normal control	126.4 ± 1.330*$	123.9 ± 2.150*
B: Diabetic control	119.1 ± 1.122#$	103.3 ± 2.407#$
C: Diabetic + 800mg/kg *Hypoxis*	131.0 ± 2.429*	133.9 ± 1.060*#
D: Diabetic + 200mg/kg *Hypoxis*	131.1 ± 1.464*	141.6 ± 0.963*$
E: Non-diabetic + 800mg/kg *Hypoxis*	132.0 ± 1.095*	132.4 ± 0.905*#

Data in table is presented as mean ± SEM. (*) represents a significant difference when compared to Diabetic control, (#) represents a significant difference when compared to Diabetic group+200mg/kg *Hypoxis* and ($) represents significance when compared to Non-diabetic+800mg/kg *Hypoxis*, n= 12 per group.

Abbreviations: µmol/L: millimole per millilitre; mg/kg: milligrams per kilogram; GSHt: Glutathione total; *Hypoxis*: *H. hemerocallidea*.

Experimental groups:
A: Normal control group : Only fed with standard rat chow (SRC)
B: Diabetic control group : Diabetes induced using STZ and fed SRC
C: Diabetic+ 800mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 800mg/kg *H. hemerocallidea*
D: Diabetic+ 200mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 200mg/kg *H. hemerocallidea*
E: N+ 800mg/kg *Hypoxis* : Non-diabetic group supplemented with 800mg/kg *H. hemerocallidea*

4.6. Effects of *H. hemerocallidea* on total antioxidant capacity in epididymal and testicular tissue

This section demonstrates the antioxidant capacity of epididymal and testicular tissue after groups were treated with different concentrations of *H. hemerocallidea*. FRAP and ORAC was used to measure antioxidant capacity was.

4.6.1. Assessement of FRAP on epididymal and testicular tissue after subjected to treatment

FRAP results of both epididymal and testicular tissue of wistar rats after 6 weeks is represented in Figure 4.13 and Figure 4.14 and mean ± SEM values in Table 4.7.

Figure 4.13 shows epididymal FRAP results of the diabetic control group was significantly decreased ($p<0.05$) when compared to the normal control group, diabetic group treated with 800mg/kg and 200mg/kg *H. hemerocallidea* as well as when compared to the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea.* No significant difference ($p>0.05$) was observed when the normal control group was compared to the non-diabetic group supplemented with 800mg/kg and the diabetic groups treated with 800mg/kg and 200mg/kg *H. hemerocallidea*.

Figure 4.14 shows a significant difference ($p<0.05$) when testicular FRAP results of the normal control group was compared to the diabetic control group. There was a significant difference ($p<0.05$) in testicular FRAP results when groups supplemented with different doses of *H. Hemerocallidea* were compared (Diabetic+800mg/kg *Hypoxis*; Diabetic+200mg/kg *Hypoxis*; and Non-diabetic+800mg/kg*Hypoxis*).

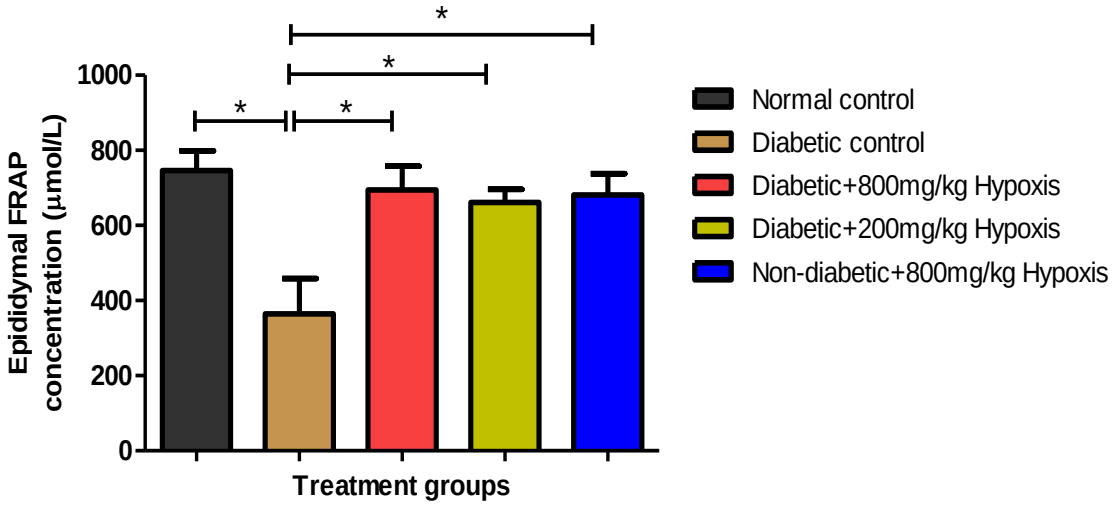

Figure 4.13: FRAP results of epididymal tissue of male Wistar rats after treatment

Data is presented as mean ± SEM. (*) indicates significant difference with $p<0.05$, n= 12 per group

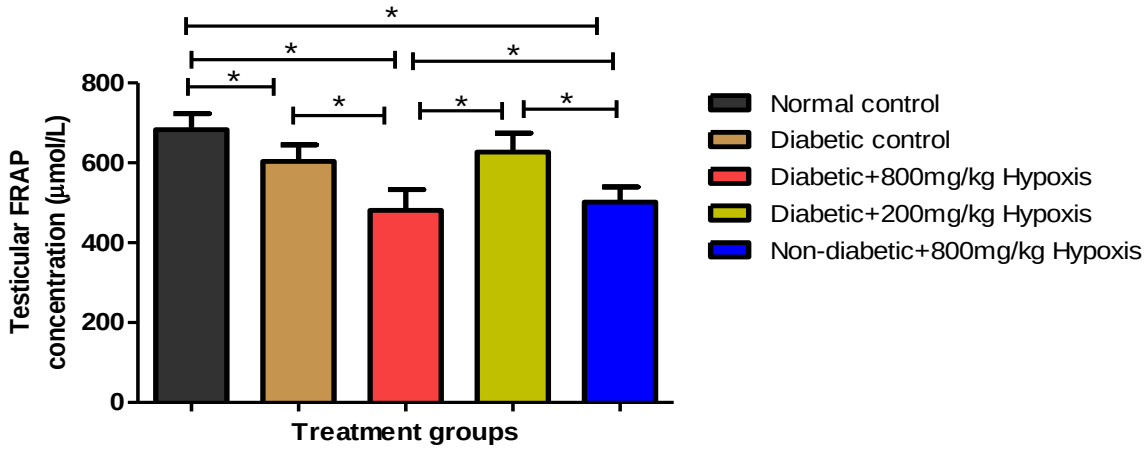

Figure 4.14: FRAP results of testicular tissue after male Wistar rats subjected to *H. hemerocallidea* treatment

Data is presented as mean ± SEM. (*) indicates significant difference with $p<0.05$, n= 12 per group

Table 4.7: FRAP results in both epididymal and testicular tissue after treatment of respective groups at different dosages compared to control groups

Group/Treatment	Epididymal FRAP (µmol/L)	Testicular FRAP (µmol/L)
A: Normal control	746.5 ± 52.67*	682.7 ± 40.55*$
B: Diabetic control	364.6 ± 93.85#$	603.7 ± 41.15$
C: Diabetic + 800mg/kg *Hypoxis*	694.6 ± 63.34*	480.8 ± 52.02*#$
D: Diabetic + 200mg/kg *Hypoxis*	661.1 ± 35.41*	627.0 ± 47.15$
E: Non-diabetic + 800mg/kg *Hypoxis*	681.1 ± 57.05*	501.9 ± 37.65*#

Data in table is presented as mean ± SEM. (*) represents a significant difference when compared to Diabetic control, (#) represents a significant difference when compared to Diabetic group+200mg/kg *Hypoxis* and ($) represents significance when compared to Non-diabetic+800mg/kg *Hypoxis*, n= 12 per group.

Abbreviations: µmol/L: micromole per millilitre; mg/kg: milligrams per kilogram; FRAP: Ferric ion antioxidant power; *Hypoxis*: *H. hemerocallidea*.

Experimental groups:
A: Normal control group : Only fed with standard rat chow (SRC)
B: Diabetic control group : Diabetes induced using STZ and fed SRC
C: Diabetic+ 800mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 800mg/kg *H. hemerocallidea*
D: Diabetic+ 200mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 200mg/kg *H. hemerocallidea*
E: N+ 800mg/kg *Hypoxis* : Non-diabetic group supplemented with 800mg/kg *H. hemerocallidea*

4.6.2. Assessement of ORAC results on epididymal and testicular tissue after subjected to treatment

ORAC results of both epididymal and testicular tissue of STZ induced diabetic wistar rats after treatment with *H. hemerocallidea* is represented in Figure 4.15 and Figure 4.16 and values in Table 4.8.

In Figure 4.15 epididymal ORAC results were significantly high ($p<0.05$) in the normal control group when compared to the diabetic control and *H. hemerocallidea* supplemented groups. There was significant decrease ($p<0.05$) when the diabetic groups treated with different doses was compared to the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea*. No significant difference ($p>0.05$) was observed when the diabetic control was compared to the diabetic groups supplemented with *H. hemerocallidea*.

Figure 4.16 shows a significant decrease ($p<0.05$) when testicular ORAC results of the normal control group was compared to the diabetic control group. There was a significant difference ($p<0.05$) in testicular ORAC results when diabetic control group was compared to diabetic groups treated with 800mg/kg and 200mg/kg *H. hemerocallidea,* respectively. There was also a significant decrease ($p>0.05$) when the diabetic group was compared to non-

diabetic group supplemented with 800mg/kg *H. hemerocallidea.* However, no significant difference ($p>0.05$) was observed when the normal control was compared to diabetic groups treated with 800mg/kg and 200mg/kg *H. hemerocallidea* as well as the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea*.

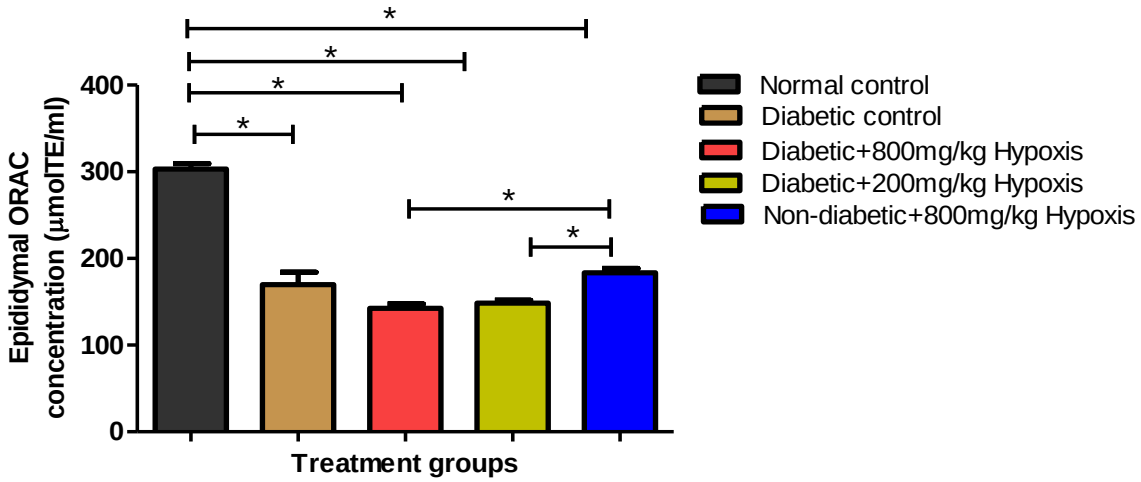

Figure 4.15: ORAC results of epididymal tissue after male Wistar rats subjected to *H. hemerocallidea* treatment

Data is presented as mean ± SEM. (*) indicates significant difference with $p<0.05$, n= 12 per group

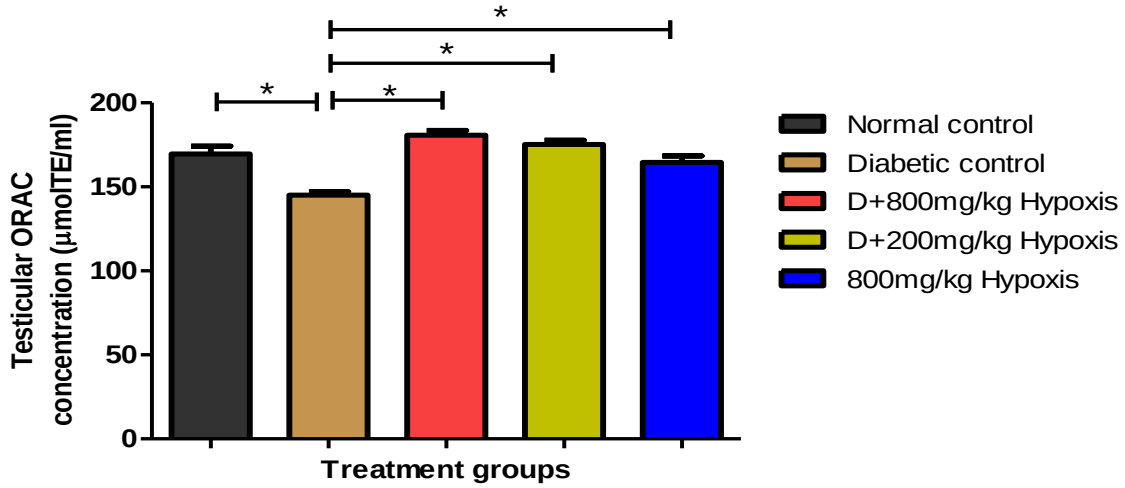

Figure 4.16: ORAC results of testicular tissue after male Wistar rats subjected to *H. hemerocallidea* treatment

Data is presented as mean ± SEM. (*) indicates significant difference with $p<0.05$, n= 12 per group

Table 4.8: ORAC results in both epididymal and testicular tissue after treatment of respective groups at different dosages compared to control groups

Group/Treatment	Epididymal ORAC (µmolTE/ml)	Testicular ORAC (µmolTE/ML)
A: Normal control	303.3 ± 6.321*#$	161.7 ± 4.229*
B: Diabetic control	169.8 ± 14.45	145.0 ± 2.098$#
C: Diabetic + 800mg/kg *Hypoxis*	142.4 ± 5.251$	180.7 ± 2.850*
D: Diabetic + 200mg/kg *Hypoxis*	148.4 ± 3.533$	175.2 ± 2.647*
E: Non-diabetic + 800mg/kg *Hypoxis*	183.6 ± 4.987#	150.5 ± 6.365*

Data in table is presented as mean ± SEM. (*) represents a significant difference when compared to Diabetic control, (#) represents a significant difference when compared to Diabetic group+200mg/kg *Hypoxis* and ($) represents significance when compared to Non-diabetic+800mg/kg *Hypoxis*, n= 12 per group.

Abbreviations: µmolTE/mL: micromole Trolox equivalent per millilitre; mg/kg: milligrams per kilogram; ORAC: Oxygen radical absorbance capacity; *Hypoxis*: *H. hemerocallidea*.

Experimental groups:
A: Normal control group : Only fed with standard rat chow (SRC)
B: Diabetic control group : Diabetes induced using STZ and fed SRC
C: Diabetic+ 800mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 800mg/kg *H. hemerocallidea*
D: Diabetic+ 200mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 200mg/kg *H. hemerocallidea*
E: N+ 800mg/kg *Hypoxis* : Non-diabetic group supplemented with 800mg/kg *H. hemerocallidea*

4.7. Hormonal assessment of epididymal and testicular tissue

This section assesses two hormones, Testosterone and Estradiol in blood serum after diabetes was induced and respective groups were treated with different concentrations of *H. hemerocallidea*. Serum Testosterone concentrations after treatment with *H. hemerocallidea* presented in Figure 4.17 and serum Estradiol concentrations presented in Figure 4.18. Testosterone and estradiol mean ± SEM values in Table 4.9.

4.7.1. Assessment of Testosterone concentration serum of wistar rats after subjected to treatment

Figure 4.17 shows a significant decrease ($p<0.05$) when serum testosterone of the diabetic control group was compared to the normal control group and the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea*. No significant difference ($p>0.05$) was observed when serum testosterone of the diabetic control group was compared to the diabetic group treated with 200mg/kg *H. hemerocallidea.* However, there was a significant increase ($p<0.05$) observed when the diabetic group treated with 800mg/kg *H. hemerocallidea* was compared to the diabetic control group. There was also no significant difference ($p>0.05$) observed in serum testosterone when the diabetic group treated with

800mg/kg *H. hemerocallidea* was compared to the diabetic group treated with 200mg/kg *H. hemerocallidea*. A significant decrease ($p<0.05$) was observed when the diabetic group treated with *H. hemerocallidea* was compared to the non-diabetic group supplemented with *H. hemerocallidea*.

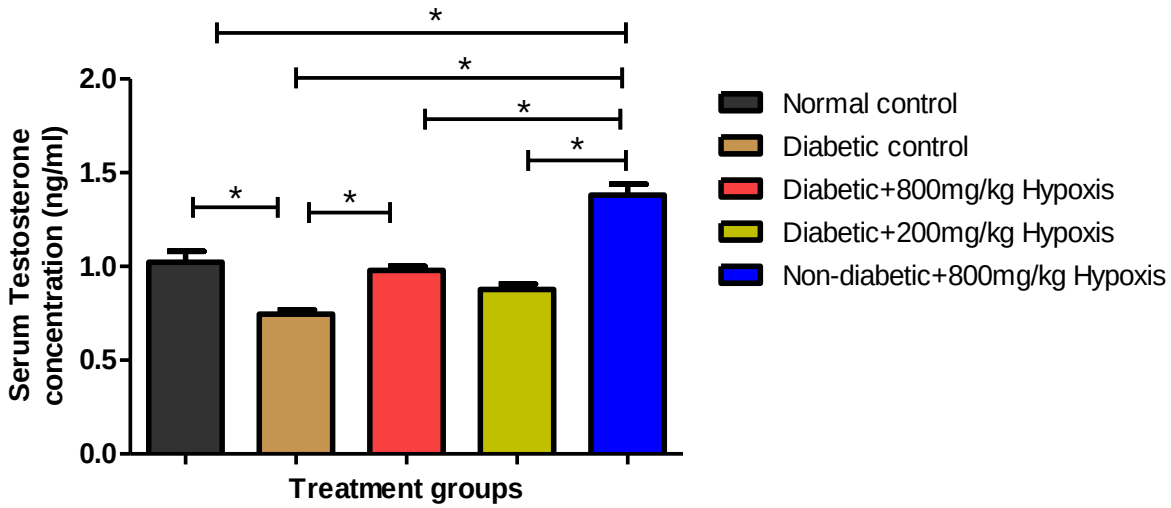

Figure 4.17: Serum testosterone concentrations after male Wistar rats subjected to different doses of *H. hemerocallidea* treatment

Data is presented as mean ± SEM. (*) indicates significant difference with $p<0.05$, n= 12 per group

4.7.2. Assessment of Estradiol concentration serum of wistar rats after subjected to treatment

Figure 4.18 shows serum estradiol of the diabetic control had no significant difference ($p>0.05$) when compared to the normal control group. The diabetic group treated with 800mg/kg *H. hemerocallidea* and the non-diabetic supplemented with 800mg/kg *H. hemerocallidea* shows a significant increase ($p<0.05$) when compared to the diabetic control. There was also a significant increase ($p<0.05$) when the diabetic group treated with 800mg/kg *H. hemerocallidea* and the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea* was compared to the normal control group. No significant difference ($p>0.05$) was observed when the diabetic control group was compared to the diabetic group treated with 200mg/kg *H. hemerocallidea*. However, the diabetic group treated with 800mg/kg *H. hemerocallidea* shows a significant increase ($p<0.05$) when compared to the diabetic group treated with 200mg/kg *H. hemerocallidea*.

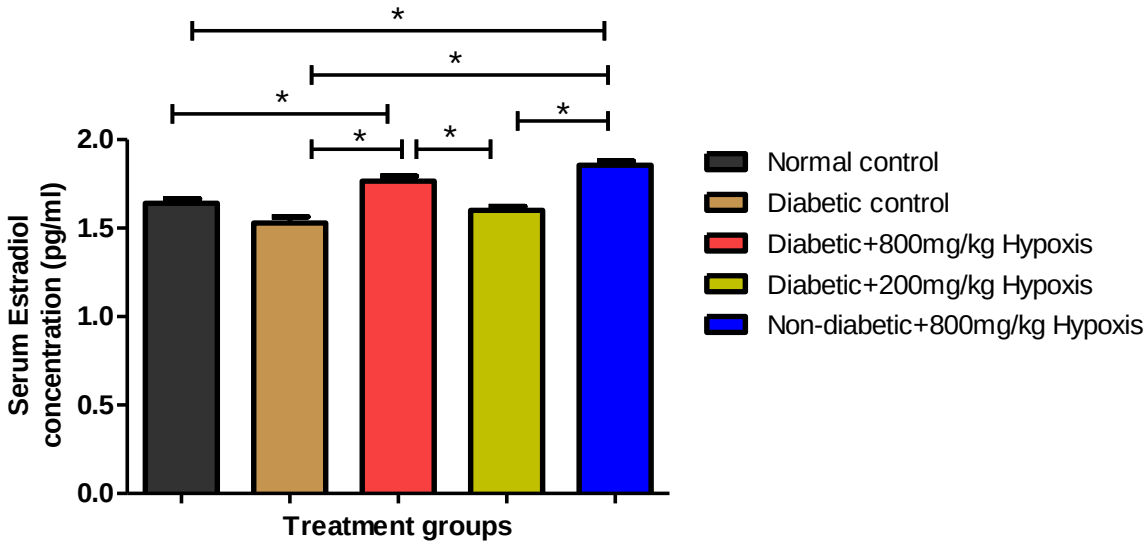

Figure 4.18: Serum estradiol concentrations of male Wistar rats after treatment

Data is presented as mean ± SEM. (*) indicates significant difference with $p<0.05$, n= 12 per group

Table 4.9: Serum testosterone and estradiol concentrations of male Wistar rats after treatment compared to control groups

Groups/ Treatment	Serum Testosterone (ng/ml)	Serum Estradiol (pg/ml)
A: Normal control	1.023 ± 0.0590*$	1.641 ± 0.0242$
B: Diabetic control	0.7466 ± 0.0210$	1.530 ± 0.0354$
C: Diabetic + 800mg/kg *Hypoxis*	0.9795 ± 0.0209*$	1.765 ± 0.0284*#
D: Diabetic + 200mg/kg *Hypoxis*	0.8778 ± 0.0284$	1.601 ± 0.0213$
E: Non-diabetic + 800mg/kg *Hypoxis*	1.381 ± 0.0588*#	1.856 ± 0.0226*#

Data in table is presented as mean ± SEM. (*) represents a significant difference when compared to Diabetic control, (#) represents a significant difference when compared to Diabetic group+200mg/kg *Hypoxis* and ($) represents significance when compared to Non-diabetic+800mg/kg *Hypoxis*, n= 12 per group.

Abbreviations: pg/ml: picograms per millilitre; ng/ml: nanograms per millilitre; mg/kg: milligrams per kilogram; *Hypoxis*: *H. hemerocallidea*.

Experimental groups:
A: Normal control group : Only fed with standard rat chow (SRC)
B: Diabetic control group : Diabetes induced using STZ and fed SRC
C: Diabetic+ 800mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 800mg/kg *H. hemerocallidea*
D: Diabetic+ 200mg/kg *Hypoxis*: Diabetic (STZ) supplemented with 200mg/kg *H. hemerocallidea*
E: N+ 800mg/kg *Hypoxis* : Non-diabetic group supplemented with 800mg/kg *H. hemerocallidea*

CHAPTER 5

DISCUSSION

Many studies have documented that DM is a causatives agent for OS damage that leads to impaired male reproduction (Bacetti *et al*., 2002, Mallidis *et al*., 2009; Navarro-Casado *et al*., 2010), specifically the function of the epididymis and testes (Amaral *et al*., 2008). Numerous international and local initiatives are actively looking into botanical resources of South Africa with great interest in the pharmacologically active compounds these indigenous plants possess (WHO, 2002; Gurib-Fakim *et al*., 2010, Rybicki *et al*., 2012). Some of these compounds isolated from plants have played a contributory role in treating a variety of disorders, DM being one of them (Gericke, 2011).

5.1. Evaluation of induced diabetes using STZ and the effects of *H. hemerocallidea* supplementation on diabetes

Streptozotocin (STZ) is a compound synthesized by *Streptomycetes achromogenes.* It has been mostly used in the treatment of cancers (Weiss, 1982; Dolan, 1997) and also in medical research studies to induce either Type 1 DM or Type 2 DM, depending on the STZ dosage used (Szkudelski, 2001; Islam and Loots du, 2009; Wei *et al*., 2011). STZ is known to exhibit pancreatic cell toxicity and has diabetogenic properties which may manifest through various mechanisms. These mechanisms include, STZ targeting the GLUT-2 receptor on pancreatic β-cells (Raza and John, 2012) or by inducing oxidative damage by releasing NO and increasing free radical production (Hosokawa *et al*., 2001; Friederich *et al*., 2009). At low doses, STZ was observed to cause β-cell dysfunction and at higher doses, β-cell necrosis was observed (Liu *et al*., 2007). Type 2 DM is normally induced by giving multiple low doses of STZ over a period of time which will trigger an immune response that will cause an inflammatory reaction. This reaction is usually linked to the release of glutamic acid and decarboxylase autoantigens (Dufrane *et al*., 2006). Type 1 DM however, is induced with a single large dose of STZ that results in alkalyting the DNA of pancreatic β-cells, this action is normally related to its nitrosurea moiety (Delaney *et al*., 1995; Dufrane *et al*., 2006). STZ alkalyting properties results in DNA fragmentation that leads to the depletion of ATP and stimulation of O_2^- causing OS status in the body (Nukatsuka*et al*., 1990; Szkudelski, 2001).

In the current study hyperglycaemia was observed 3 days after a single intraperitoneaal administration of STZ in adult wistar rats. This was confirmed by significant high blood glucose levels of the diabetic control group (group B) when compared to the initial fasting blood glucose of all five groups (Figure 4.1 and Table 4.1). These findings were typical of that of Type 1 DM, thus we can assume that the diabetic rat model was successfully created.

The results of the current study was supported by previous studies of induction of diabetes in wistar rats by intravenous STZ injection, confirmed hyperglycaemia 24 hours after induction (Ramos-Lobo *et al*., 2015) and another study confirmed hyperglycaemia 7 days after induction (Li *et al*., 2014).

On comparing blood glucose levels of diabetic group that received 800mg/kg (group C) and 200mg/kg (group D) of *H. hemerocallidea* respectively to initial blood glucose taken and normal control group there was a significant increase in blood glucose. However, when diabetic groups treated with 800mg/kg and 200mg/kg *H. hemeroracallidea* was compared to the diabetic control group there was a significant decrease in blood glucose levels. The diabetic group treated with 800mg/kg *H. hemerocallidea* showed a better response in lowering glucose levels than that of the diabetic group treated with 200mg/kg *H. hemerocallidea* (Figure 4.1and Table 4.1). These findings supported a previous study done by Ojewale (2006) that demonstrated that *H. hemerocallidea* has antidiabetic effects, but the results are dosage dependent and it was observed that at 800mg/kg *H. hemerocallidea* there was a significant decrease of blood glucose levels when compared to the 200mg/kg and also when compared to the diabetic control group. There was no significant difference when the normal control group was compared to the non-diabetic group treated with 800mg/kg *H. hemerocallidea*. Therefore, it can be concluded, based on the significance observed in the blood glucose levels that *H. hemerocallidea* has hypoglycaemic effects.

5.2. Evaluation of wistar rat body, epididymal and testicular weights after 6 weeks treatment

In this study it was observed that there was a significant decrease in initial body weight and weights taken after 6 weeks in the diabetic control group when compared to the normal control group and the initial weights taken as well as epididymal and testicular weights (Figures 4.2, 4.3, 4.4 and Table 4.2). These observations of rapid weight loss can be linked to the disturbance of insulin signalling pathways resulting in a glucose disturbance. This causes the body to resort in using other substances such as stored lipids, proteins and carbohydrates as source of energy that results in rapid weight loss and decrease in muscle mass (Sathishsekar and Subramanian, 2005). Decreased size in testicular and epididymal tissue may be attributed to a decrease in testosterone concentrations in the diabetic control group when compared to the normal control group. Testosterone is responsible for regulating and maintaining the growth of these reproductive organs (Klinefelter and Hess, 1998; Nelli *et al*., 2013). Testicular size has been directly linked to sperm quality and spermatogenesis (Sharpe *et al*., 2003; Griffeth *et al*., 2013).

When the diabetic control group was compared to diabetic group treated with 800mg/kg (group C) and diabetic group treated with 200mg/kg (group D) *H. hemerocallidea* respectively, there was high significant difference seen in body, epididymal and testicular weights in these groups. Body, epididymal and testicular weights of diabetic groups treated with 800mg/kg and 200mg/kg *H. hemerocallidea* almost being fully restored to baseline of normal control group and initial body weights taken (Figure 4.2, 4.3, 4.4 and Table 4.2). These findings were similar to those of Ghlissi and co-workers (2013) when checking the antioxidant and androgenic effect of dietary ginger on reproductive function of diabetic rats. It was observed that supplementation of ginger showed a significant increase in body, epididymal and testicular weights. On comparing the body, epididymal and testicular weights with the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea* with the normal control group there was no significant difference observed. These findings demonstrated that *H. hemerocallidea* supplementation had no adverse effects on the rat body and organ weights and that the reduction of weight was due to their diabetic status (Figure 4.2, 4.3, 4.4 and Table 4.2).

Only the diabetic group treated with 800mg/kg *H. hemerocallidea* showed increase in body, epididymal and testicular weights over the 6 week period but was still less than the normal control group and the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea*. These finding may imply that *H. hemerocallidea*'s antioxidant and hypoglycaemic potential may help prevent and improve OS status of individuals with diabetes (Ojewale, 2006). The mechanism on how *H. hemerocallidea* lower glucose levels are not well understood but it has been speculated that protection against weight loss and organ size reduction can be attributed to its hypoglycaemic properties. If there is a regulation of glucose levels as main source of energy, there is no need for the use of alternative sources for energy from body, epididymal and testicular fats, proteins and carbohydrates. Therefore, *H. hemerocallidea* assists in providing a platform for regulating and maintaining the mechanism of glucose usage by the body.

5.3. Assessment of cauda epididymal sperm motility and morphology of wistar rats after treatment

DM has been associated with disturbance in sperm parameters and recent studies have shown that OS attributes to decease in sperm quality parameters of diabetics. In our findings it was observed that epididymal and testicular antioxidant and size improved when rats treated with *H. hemerocallidea* was compared to the diabetic control group. Thus we hypothesised that sperm parameters may also improve specifically sperm motility and morphology for our study. There was a definite improvement observed in sperm motility and morphology when normal control group and diabetic group treated with 800mg/kg *H.*

hemerocallidea were compared to diabetic control group (Table 4.3). *H. hemerocallidea* has shown to decrease both epididymal and testicular ROS when observing antioxidant activity (Figures 4.7, 4.8, 4.9, 4.10, 4.11, 4.12 and Tables 4.4, 4.5, 4.6) and LPO (Figures 4.5, 4.6), therefore resulting in improved sperm motility. Tomás (2013) showed similar results on sperm motility and morphology of diabetic rats after treated with white tea. These results were also in agreement with Zohreh and co-workers (2014) where sperm motility and morphology showed a significant improvement of diabetic rats after ingesting *Aloe vera*. However, there was no significant difference in both sperm motility and morphology observed when diabetic control group was compared to diabetic group treated with 200mg/kg *H. hemerocallidea* (Table 4.3). Oxidative status caused by increased ROS in epididymal and testicular tissue has shown to result in cellular damage resulting in DNA damage and LPO which cause sperm damage. Diabetic patients are known to have reduced sperm concentration, viability, sperm abnormalities and sperm motility. Therefore, we can assume that at 200mg/kg, *H. hemerocallidea* can not correct the damage induced by STZ on inducing DM.

5.4. Assessment of LPO of epididymal and testicular tissue of wistar rats after treatment

LPO is one of the main indicators of the occurrence of oxidative damage (Makker *et al*., 2009). LPO and its action are propagated by free radicals that results in oxidative damage to polyunsaturated fatty acids (PUFA's). LPO causes a disruption in biofunctional membrane structure, membrane fluidity, ion gradients and alterations in transporting enzymes and activation of enzyme receptors. Malondialdehyde (MDA) is a by-product of LPO. It has been observed that an increase in OS is related to increase production of MDA level. In most cases, increased MDA levels are related to pathological condition in both animal and human reproduction.

In the current study, MDA levels of the normal control group showed a significant increased when compared to diabetic control group in both epididymal and testicular tissue (Figures 4.5, 4.6). These findings were in agreement with previous experimental studies done on diabetic animal models (Singab *et al*., 2005; Nelli *et al*., 2013). When the body is in hyperglycaemic state, auto-oxidation of glucose is promoted and results in OS that leads to the damage of biological systems, in our case the reproductive organs of rats. The diabetic control group was also compared to diabetic groups that were treated with 800mg/kg *H. hemerocallidea* and diabetic groups treated with 200mg/kg *H. hemerocallidea* showed a significant increase in testicular MDA levels on comparison with diabetic control group (Figures 4.5, 4.6). Epididymal and testicular MDA also showed a significant decrease in diabetic group treated with 200mg/kg *H. hemerocallidea* when compared to diabetic control

group. This data suggests that *H. hemerocallidea* can prevent cellular damage caused by STZ-induced DM by inhibiting LPO using its antioxidant properties (Oh *et al*., 2002; Nelli *et al*., 2013). On comparing the effect of *H. hemerocallidea* at different doses, there was no significant difference observed between doses in testicular tissue (Figure 4.6). However, in epididymal tissue MDA levels were significantly decreased in the diabetic group treated with 800mg/kg *H. Hemerocallidea* than that of the diabetic group treated with 200mg/kg *H. hemerocallidea* (Figure 4.5). Therefore, it can be assumed that a higher dose of *H. hemerocallidea* reduced LPO in epididymal tissue of diabetic rats. *H. hemerocallidea* not only showed a healthier response in epididymal tissue of diabetic group treated with 800mg/kg *H. hemerocallidea*, but it was also observed that there was significant decrease in epididymal and testicular MDA levels of non-diabetic group supplemented with 800mg/kg *H. hemerocallidea* when compared with normal control group. These findings led to the assumption that *H. hemerocallidea* can also be used as preventative method of LPO ion the absence of DM-induced OS. It can thus be concluded that *H. hemerocallidea* can be therapeutic in restoring LPO induced by disorders such as DM and male infertility.

5.5. Assessment of antioxidants activity in testicular and epididymal tissue of wistar rats after treament

DM is known to be a vehicle of initiating OS. Antioxidant enzymes such as SOD, CAT and GSHt have a vital role in protecting against OS in reproductive organs. However, certain conditions that affect the male reproductive system, such as DM, influence the level of production and activity of these antioxidants (Maritim *et al*., 1999).

In the present study it was observed that epididymal SOD, CAT activity as well as GSHt levels were significantly high when the normal control group was compared to the diabetic control group (Figures 4.7, 4.9, 4.11 and Tables 4.4, 4.5, 4.6). These findings was in agreement with that of Nelli and co-workers (2013) where there was a significant decrease in epididymal SOD, CAT and GSHt in diabetic rats when compared to normal control group. There was a significant increase in epididymal SOD levels when diabetic groups treated with 800mg/kg and 200mg/kg *H. hemerocallidea* respectively, was compared to the diabetic control group. On comparing the epididymal SOD of the two different doses of 800mg/kg and 200mg/kg *H. hemerocallidea* in diabetic rats with each other, there was no significant difference seen between SOD activity as well as GSHt levels (Figures 4.7, 4.11 and Tables 4.4, 4.6). However, there was a significance seen in CAT activity between these two doses (Figure 4.9 and Table 4.5). According to these findings it cannot be accurate to say that *H. hemerocallidea* works optimally in diabetic rats at 800mg/kg and not at 200mg/kg as there were only significance in epididymal CAT activity and no significance seen in epididymal SOD activity and GSHt levels. It was also observed that there was a significant increase in

epididymal SOD activity and GSHt levels when the diabetic control group was compared to the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea* (Figures 4.7, 4.9, 4.11 and Tables 4.4, 4.5, 4.6), confirming that *H. hemerocallidea* thus have some antioxidative properties. When the normal control group was compared to the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea*, no significant difference was observed in epididymal SOD and CAT. This could be due to the body's natural occurring antioxidants interfering with the activity of the antioxidants of *H. hemerocallidea* (Figures 4.7, 4.9, 4.11 and Tables 4.4, 4.5, 4.6).

In this study it was observed that testicular SOD, CAT and GSHt were significantly lower in diabetic control group when compared to normal control group (Figures 4.8, 4.10, 4.12 and Tables 4.4, 4.5, 4.6).These findings were in agreement with the findings of Alkhamees (2013) which demonstrated that STZ-induced DM had a significant decrease in testicular SOD, CAT and GSHt of the diabetic control group when compared to normal control group. These findings were similar to the findings of Ojewale and co-workers (2014) which demonstrated that there was a significantly decrease in testicular SOD, CAT and GSHt alloxan-induced diabetic control group when compared to normal control group.

Previous studies have shown a positive correlation between supplementation of natural antioxidants and increase in testicular SOD and CAT activity as well as GSHt levels. For example, Fatani and co-workers (2015) demonstrated the effects of lutein dietary supplementation attenuating STZ-induced diabetes and resulted in increased SOD and CAT activity and levels of GSHt was also increased. Another study done by Nelli and co-workers (2013) showed that α-mangostin (and extract from a plant *Garcinia mangostana L, Guttiferae*) significantly increased testicular SOD and CAT activity and GSHt levels when STZ-induced diabetic group treated with different doses of α-mangostin was compared to diabetic control group. These studies confirmed that botanical sources help stimulate the activity of SOD and CAT as well as GSHt levels which may reverse diabetes induced male infertility. SOD is a major scavenging enzyme which regulates CAT and GSHt activity. When OS induced (in this case by STZ-induced DM) an over production of free radicals are produced, SOD thus converts superoxide (a free radical) into more stable compounds such as H_2O_2 and water. CAT or GSHt will then come and act upon the H_2O_2 and detoxify it leaving it harmless to the body and OS not being able to occur. Certain plants have shown to cause a favourable response in antioxidant activity, thus it can be beneficial to look at phytochemical treatment as a possible mechanism in reducing diabetic induced or any other forms of disorders that may induce OS damage.

In the current study, a significant increase was observed in testicular SOD, CAT and GSHt activity when diabetic groups treated with 800mg/kg and 200mg/kg *H. hemerocallidea* respectively was compared with the diabetic control group (Figures 4.8, 4.10. 4.12 and Tables 4.4, 4.5, 4.6). There was no significant difference seen in testicular SOD and CAT activity when diabetic group treated with 800mg/kg *H. hemerocallidea* was compared to diabetic group treated with 200mg/kg *H. hemerocallidea*. However, there was a significant increase observed in testicular GSHt levels of diabetic group treated with 200mg/kg *H. hemerocallidea* when compared to diabetic group treated with 800mg/kg *H. hemerocallidea*. The increase of testicular GSHt levels may be due to the *H. hemerocallidea* antioxidant defences stimulating GSH activity in diabetic rats and thus interfering with naturally occurring GSHt levels in the body. However, this finding cannot be used to assume that *H. hemerocallidea* antioxidant activity is more favourable in treating diabetes at 200mg/kg than at 800mg/kg for only GSHt levels was increased not SOD and CAT activity. Another observation that was observed the testicular SOD and CAT activity were significantly lower when non-diabetic group supplemented with 800mg/kg *H. hemerocallidea* was compared to normal control group. But GSHt levels were not significant when non-diabetic group supplemented with *H. hemerocallidea* was compared to normal control group. These finding may be attributed to the fact that there was no oxidative damage induced, therefore not being a need for SOD and CAT to be activated or it can be due to an oversaturation of SOD and CAT supplied by the *H. hemerocallidea* that it over compensating for naturally occurring SOD and CAT activity. These results of a decreased significance in non-diabetic rats supplemented with 800mg/kg *H. hemerocallidea* can also be because of the decreased LPO found thus decreasing CAT and SOD activity. A study done by Nair and co-workers (2007) compared *H. hemerocallidea* and compound Hypoxoside (extracted from *H. hemerocallidea*) and hypoxoside's aglycon, rooperol, to quercetin which is known for its high scavenging enzyme abilities. It was found that rooperol had the same free radical scavenging abilities to that of quercetin, proving that rooperol is a highly potent free radical scavenger. However, hypoxoside did not show any scavenging abilities. Hypoxoside needs to be converted by the GIT to rooperol to be affective in scavenging free radicals.

5.6. Assessment of *H. hemerocallidea* on Total Antioxidant Capacity (TAC) of wistar rats after treatment

A number of assays are available to determine TAC of biological and phytochemical compounds. These assays normally have two main governing principles. Both principles are based on free radical deactivation. TAC determination can either make use of hydrogen transfer (HAT) or electron transfer (ET). The current study used ORAC and FRAP to measure the effect *H. hemerocallidea* has on total antioxidant capacity in both epididymal and testicular tissue of STZ-induced diabetic rats. Numerous research studies has measured

TAC using FRAP and ORAC assays in liver, kidney, blood plasma and serum. However, not much of TAC have been done on epididymal and testicular tissue and therefore it is important to point out that this is the first study measuring TAC of *H. hemerocallidea* in epididymal and testicular tissue of diabetic rats.

ORAC results rely on the amount of damage made to the fluorescent probe by free radicals. Fluoroscein is the most common probe used; the damage caused to the fluorescent probe is caused by an oxidizing agent that will result in loss of fluorescence over a period of time. The inhibition of oxidative damage to the fluorescent probe can be directly related to the antioxidant capacity of the compound measured. FRAP on the other hand measures the ability of antioxidants to reduce Fe^{3+} into Fe^{2+} in acidic medium. It has been observed though that antioxidant activity of what is being measured does not always match its reducing ability when measured against free radicals.

The current study showed that there was a significant difference in epididymal FRAP when all the groups were compared to each other. The diabetic control group was significantly decreased when compared to the normal control group. On comparing the diabetic control group to the diabetic groups treated with *H. hemerocallidea* at respective doses there was a significant increase. These results showed that *H. hemerocallidea* has antioxidants with the ability to reduce Fe^{3+} into Fe^{2+} (Figure 4.13 and Table 4.7). There was no significant difference observed when diabetic group treated with 800mg/kg *H. hemerocallidea* was compared to diabetic group treated with 200mg/kg *H. hemerocallidea* (Figure 4.13 and Table 4.7). A significant decrease was observed in epidiymal ORAC of the diabetic control group when compared to the normal control group (Figure 4.15 and Table 4.8). Therefore, confirming that diabetes does deplete antioxidant capacity. However, epididymal ORAC results also showed no significant difference when the diabetic groups treated with 800mg/kg and 200mg/kg *H. hemerocallidea* respectively was compared to the diabetic control group. These results can be attributed to *H. hemerocallidea* using hypoglycaemic properties to reduce glucose levels rather than its antioxidant properties. A significant decrease was observed when the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea* was compared to the normal control group (Figure 4.15 and Table 4.8). This result confirms that *H. hemerocallidea* antioxidant properties are not the only mechanism that offers protection against oxidative stress.

On comparing testicular FRAP and ORAC, it was observed that there was a significant decrease when the diabetic control group was compared to the normal control group (Figures 4.14, 4.16 and Tables 4.7, 4.8). It was also demonstrated that testicular FRAP of the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea* showed a significant

decrease when compared to the normal control group (Figure 4.14 and Table 4.7). However, testicular FRAP showed a significant increase when the diabetic group treated with 800mg/kg *H. hemerocallidea* was compared to the diabetic control group. No significant difference was observed when testicular FRAP of diabetic control was compared to diabetic group treated with 200mg/kg *H. hemerocallidea*. Thus it may be assumed not many Fe^{3+} reducing antioxidants was present at both doses due to DM-induced OS and *H. hemerocallidea* does also not improve levels of antioxidants that has Fe^{2+} reducing properties. A significant decrease of testicular FRAP was observed when the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea* was compared to the normal control group (Figure 4.16 and Table 4.7). These results are in agreement with that of SOD and CAT results (Figure 4.8, 4.10 and Tables 4.4, 4.5) where there was a significant decrease when the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea* was compared to the normal control group. *H. hemerocallidea* showed that it has the ability to reduce even the normal occurring OS in non-diabetic rats and this may attributed to lower FRAP results. The antioxidants present that reduce Fe^{3+} to Fe^{2+} are less in the non-dabetic group supplemented with 800mg/kg *H. hemerocallidea* than the normal control group. Testicular ORAC results however, showed a significant decrease when diabetic control group was compared to normal control group. There was increase in testicular ORAC when diabetic groups treated with 800mg/kg and 200mg/kg *H. hemerocallidea* was compared to the diabetic control group. The non-diabetic group supplemented with 800mg/kg *H. hemerocallidea* showed a significant increase when compared to normal control group (Figure 4.16 and Table 4.8). This finding shows that the flourescein probe was protected by the antioxidants of *H. hemerocallidea* thus preventing oxidative damage.

5.7. Assessement of different doses of serum testosterone and estradiol wistar rats after treament

Testosterone and estradiol are both steroid hormones that promote male fertility status (Smith and Walker 2014). The enzyme that allows for testosterone conversion to estradiol is called aromatase (Antal *et al*., 2008). Estradiol is important for a number of biological systems, including regulating processes of the male reproductive system (Lazari *et al*., 2009). Testosterone is considered the main androgen and is responsible for male fertility status and spermatogenesis (Wang *et al*., 2009; Smith and Walker, 2014). A number of studies have shown the effects diabetes has on sex hormone levels, specifically testosterone (Khaki *et al*., 2010; Ghorbani *et al*., 2014). However, not many have looked into estradiol of diabetic animal models.

The current study demonstrated that testosterone and estradiol had a significant decrease in the diabetic control group when compared to the normal control group (Figures 4.15, 4.16

and Table 4.9). These findings can be linked to both glucose levels (Figure 4.1 and Table 4.1) and body weights (Figure 4.2). As such is attributed to a disturbance in insulin pathways that allows oxidation of glucose and compels the body to use energy from biological substances such as lipids, proteins and carbohydrates, resulting in increased blood glucose and decrease body weights. Since testosterone is derived from cholesterol, there will be a decrease in testosterone levels due to the decrease in body weights.

A number of studies also looked into the effect certain dietary supplements and phytochemical substances have on testosterone and estradiol concentrations of diabetic animal models. Ghlissi and co-workers (2013) investigated the antioxidant and androgenic effects of dietary ginger on reproductive function of male diabetic rats. Testosterone levels of the diabetic group supplemented with garlic was increased when compared to diabetic control group. Another study done by Kiasalari and co-workers (2009) looked at the effects *Withania somniferia* root has on sex hormones in diabetic male rats. They observed an increase in testosterone levels but no difference in estradiol levels when diabetic group supplemented with *Withania somniferia* was compared to diabetic control group.

In the present study, testosterone and estradiol concentrations of the diabetic group treated with 800mg/kg *H. hemerocallidea* showed a significant increase when compared to the diabetic control group. However, there was no significant difference in testosterone and estradiol observed when the diabetic control group was compared to the diabetic group treated with 200mg/kg *H. hemerocallidea*. The diabetic group treated with 800mg/kg *H. hemerocallidea* showed a significant increase in testosterone and estradiol concentrations when compared to the diabetic group treated with 200mg/kg *H. hemerocallidea.* There was also a significant increase in testosterone and estradiol in the non-diabetic group supplemented with 800mg/kg *H. hemerocallidea* when compared to the normal control group (Figure 4.15, 4.16 and Table 4.9). No studies have been done on *H. hemerocallidea* and its effects on sex hormone levels in diabetic rats. By looking at our results we can say that at a dose of 800mg/kg *H. hemerocallidea*, production of both testosterone and estradiol levels were stimulated and increased in diabetic wistar rats. Testosterone and estradiol concentrations can be linked to improvement to both epididymal and testicular weights as both these hormones play a vital role in the growth and functioning of the reproductive organs (Figure 4.2, 2.3, 4.4 and Table 4.2). These results can also be linked to the improved sperm motility and morphology as both sex hormones play a vital role in spermatogenesis (Table 4.3).

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

In this study we observed how STZ-induced DM can have deleterious effects on epididymal and testicular tissue, sperm motility and morphology as well as disrupting antioxidant enzyme mechanisms and hormone concentrations. We demonstrated how *H. hemerocallidea* can improve OS biomarkers in an *in vivo* experimental model animal using STZ- induced diabetic wistar rats.

Our findings demonstrated that *H. hemerocallidea* can improve antioxidant enzyme activities in normal and OS conditions, serving as protective mechanism against OS in the reproductive system of male wistar rats. This study indeed showed that *H. hemerocallidea* supplementation could have protective effects in lower blood glucose levels and LPO by decreasing MDA levels of the diabetic groups. It was observed that *H. hemerocallidea* has a potential beneficial effect on antioxidant enzyme activities, sperm motility, sperm morphology and serum testosterone and estradiol levels. There was also an improvement in serum testosterone and estradiol levels even when OS was not induced but supplemented with 800mg/kg *H. hemerocallidea*. Most of the results obtained in this study showed that improvement was not dosage dependent. However, sperm motility and morphology showed the greatest improvement in the diabetic group treated with 800mg/kg *H. hemerocallidea*. According to these findings, it was concluded that *H. hemerocallidea* supplementation is an effective approach to ameliorate male infertility in diabetic individuals.

To our knowledge, this is the first study investigating the effect *H. hemerocallidea* has on the reproductive function in the diabetic rat model. The study thus created opportunities for further investigations.

The significance of our results and findings may be influenced by small sample size and materials used contributing to results obtained not being as accurate and distint as intended. Therefore, studies with more advanced techniques of biological investigations and larger sample size are recommended for future research studies.

REFERENCES

Abegaz, B.M., Ngadjui, B.T., Bezabih, M., Mdee, I.K., 1999. Novel natural products from marketed plants of Eastern and Southern Africa. *Pure and Applied Chemistry,* 71:919-926

Aboua, Y.G., Oguntibeju, O.O., & du Plessis, S.S., 2013. Can Lifestyle Factors of Diabetes Mellitus Patients Affect Their Fertility? http://dx.doi.org/10.5772/47838

Adamali, H.I., & Hermo, L., 1996. Apical and narrowcells are distinct cell types differing in their structure, distribution, and functions in the adult rat epididymis. *Journal of Andrology*, 17:208-222.

Adamali, H.I., Somani, I.H., Huang, J.Q., Mahuran, D., Gravel, R.A., Trasler, J.M., Hermo, L., 1999.Abnormalitiesin cells of the testis, efferent ducts, and epididymis in juvenileand adult mice with beta-hexosaminidase A and B deficiency. *Journal of Andrology,* 20:779-802.

Adams, B.A., Vickers, E.D., Warby, C., Park, M., Fischer, W.H., Grey Craig, A., Rivier, J.E., Sherwood, N.M., 2002. Three forms of gonadotropin-releasing hormone, including a novel form, in a basal salmonid, Coregonus clupeaformis. *Biology of Reproduction*, 67:232-239.

Agarwal, A., 2005. Role of oxidative stress in male infertility and oxidant supplementation. *Urology Diseases*, 122-124.

Agarwal, A., 2006. Oxidative stress and human reproduction. *Oxidative stress, Disease and cancer.* Rosewell Park Cancer Institute. New York.

Agarwal, A., Abdelrazik, H., & Sharma, R.K., 2008. Oxidative stress management in patients with male or female infertility. *Handbook of Chemiluminescent Methods in Oxidative Stress Assessment,* 195-218.

Agarwal, A., Durairajanayagam, D., Halabi, J., Peng, J., Vazquez-Levin, M., 2014. Proteomics, oxidative stress and male infertility. *Reproductive Biomedicine online,* 29(1):32-58.

Agarwal, A., Gupta, S., & Sharma, R., 2005. Oxidative stress and its implications in female infertility- a clinician's perspective. *Biomedicine Online,* 11:641-650.

Agarwal, A., Makker, K., & Sharma, R., 2008. Clinical relevance of oxidative stressin male factor infertility: An Update. *American Journal of Reproductive Immunology*, 59:2-11.

Agarwal, A., Nallella, K.P., Allamaneni, S.S., Said, T.M., 2004. Role of antioxidants in treatment of male infertility: An overview of the literature. *Reproductive Biomedicine Online*, 8(6):616-627.

Agarwal, A., & Prabakaran, S.A., 2005. Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology. *Indian Journal of Experimental Biology,* 43:963-974.

Agarwal, A., & Saleh, R.A., 2002. Role of oxidants in male infertility: rationale, significance and treatment. *Urology Clinics of North America,* 29:817-827.

Agarwal, A., & Sekhon, L.H., 2010. The role of antioxidant therapy in the treatment of male infertility: Review. *Human Fertility,* 13(4):217-225.

Agarwal, A., Saleh, R.A., & Bedaiwy, M.A., 2003. Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertility and Sterility*, 79:829-43.

Agarwal, A., Virk, G., Ong, C., du Plessis, S.S., 2008. Effect of Oxidative Stress on Male Reproduction. *World Journal of Men's Health*, 32(1):1-17.

Agbaje, I.M., Rogers, D.A., McVicar, C.M., McClure, N., Atkinson, A.B., Mallidis, C., Lewis, S.E., 2007. Insulin dependent diabetes mellitus: implications for male reproductive function. *Human Reproduction*, 22(7):1871–1877.

Aitken, R.J, & Baker, M.A., 2013. Causes and consequences of apoptosis in spermatozoa; contributions to infertility and impacts on development. *International Journal of Developmental Biology,* 57:265-72.

Aitken, R.J., & Nixon, B., 2013. Sperm capacitation: a distinct landscape glimpse but explored. *Molecular Human Reproduction,* 19(12):785-793.

Aitken, R.J., 1999. The Amoroso lecture: the human spermatozoon—a cell in crisis? *Journal of Reproduction and Fertility*, 115:1-7.

Aitken, R.J., Baker, M.A., De Iuliis, G.N., Nixon, B., 2010. New insights into sperm physiology and pathology. *Handbook of Experimental Pharmacology*, 198:99-115.

Aitken, R.J., & De Iuliis, G.N., 2007. Origins and consequences of DNA damage in male germ cells. *Reproductive Biomedicine Online,* 14:727-733.

Aitken, R.J., & De Iuliis, G.N., 2010. On the possible origins of DNA damage in human spermatozoa. *Molecular Human Reproduction*, 16:3-13.

Aitken, R.J., & Krausz, C., 2001. Oxidative stress, DNA damage and the Y chromosome. *Reproduction*, 122:497-506.

Aitken, R.J., & Roman, S.D., 2008.Antioxidant systems and oxidative stress in the testes. *Oxidative Medicine and Cellular Longevity,* 1(1):15-24.

Aitken, R.J., Ryan, A.L., Baker, M.A., MacLaughlin, E.A., 2004. Redox activity associated with maturation and capacitation of mammalian spermatozoa. *Free Radical Biology and Medicine*, 36:994-1010.

Akbari, A., & PJelodar, G.A., 2013. The effect of oxidative stress and antioxidants on men fertility. *Zahedan Journal of Research in Medical Sciences,* 15(7):1-7.

Akmal, M., Qadri, J.Q., Al-Waili, N.S., Thangal, S., Haq, A., Saloom, K.Y., 2006. Improvement in Human Semen Quality after Oral Supplementation of Vitamin C. *Journal of Medicinal Food*, 9(3):440-442.

Al- Attar, L., Noel, K., Dutertre, M., Belville, C., Forest, M.G., Burgoyne, P.S., Josso, N., Rey, R., 1997. Hormonal and cellular regulation of sertoli cells anti- Mullerian hormone production in the postnatal mouse. *Journal of Clinical Investigation,* 100:1335-1343.

Albrecht, C., Theron, E., & Kruger, P., 1995a. Morphological characterisation of the cell-growthinhibition by Rooperol and pharmacokinetic aspects of hypoxoside as a prodrug of cancer therapy. *Southern African Medical Journal*, 85(9):853-860.

Albrecht, C.F., Kruger, P.B., Smith, B.J., Freestone, M., Gouws, L., Millers, R., Van Jaarsveld, P.P., 1995b. The plant pharmacokinetics behavior of hypoxoside taken orally by patients with lung cancer in phase 1 trial. *Southern African Medical Journal,* 85:861-865.

Alkhamees, O.A., 2013. Quercetin Attenuates Testicular Damage and Oxidative Stress in Streptozotocin-induced Diabetic Rats. *British Journal of Pharmacology and Toxicology*, 5(2): 88-97

Alshahrani, S., McGill, J., & Agarwal, A., 2012. Prostatitis and male infertility. *Journal of Reproductive Immunology,* http://dx.doi.org/10.1016/j.jri.2013.05.004

Altay B, Cetinkalp S, Doganavşargil B, Hekimgil M, Semerci B., 2003. Streptozotocin-induced diabetic effects on spermatogenesis with proliferative cell nuclear antigen imunostaining of adult rat testis. *Fertility and Sterility*, 80:828-831.

Alves, M.G., Martins, A.D., Rato, L., Moreira, P.I., Socorro, S., Oliveira, P.F., 2013. Molecular mechanisms beyond glucose transport in diabetes- realated male infertility: Review. *Biochimica et Biophysica Acta,* 1832:626-635.

Alwan, A.A., 1994. Management of diabetes mellitus standards of care and clinical practice guidelines; noncommunicable diseases, *WHO*, Alexandria.

Amaral, S., Oliveira, P.J., & Ramalho-Santos, J., 2008. Diabetes and the impairement of reproductive function: possible role of mitochondria and reactive oxygen species. *Current Diabetes Revolution,* 4(1):46-54.

American Diabetes Association, 1998. Economic consequences of diabetes mellitus in the U.S. in 1997. *Diabetes Care*, 21(2)296–309.

American Diabetes Association, 2012. Diagnosis and classification of diabetes mellitus. *Diabetes Care, 35(1)*:64-71.

Amri, I., Sheikh, N., & Najafi, R., 2007. Nitric oxide level of seminal plasma and its relation with sperm DNA damage. *Iranian Biomedical Journal*, 11:259-264.

Antal, M.C., Krust, A., Chambon, P., Mark, M., 2008. Sterility and absence of histopathological defects in nonreproductive organs of a mouse ER beta-null mutant. *Proceeding of the National Academy of Sciences of the United States of America*, 105(7):2433-2438.

Aremu, A.O., Ndhlala, A.R., Fawole, O.A., Light, M.E., Finnie, J.L., Van Staden, J., 2010. In vitro pharmacological evaluation and phenolic content of ten South African medicinal plants used as anthelmintics. *South African Journal of Botany*, 76:558-566.

Arikawe, AP., Daramola, A.O., Odofin, A.O., Obika, L.F., 2006. Alloxan-induced and insulin-resistant diabetes mellitus affect semen parameters and impair spermatogenesis in male rats. *African Journal of Reproduction Health*, 10:106-113.

Ashok, A., & Sushil, A.P., 2005. Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology. *Indian Journal of Experimental Biology*, 43(11):963-974.

Atangwho, I. J., Ebong, P. E., Egbung, G. E., Ani, I. F., 2009. Effect of co-adminstration of extracts of *Vernonia amygdalina* and *Azadirachta indica* on serum electrolyte profile of diabetic and nondiabetic rats*. Australian Journal of Basic and Applied Sciences*, 3:2974-2978.

Atila, S., Ayese, S., Yesim, S., & Muhfuz, E., 2012. Evaluation of antidiabetic activity of Cheryl Laurel (*Laurocerasus officinalis*). *The Open Conference Proceedings Journal*, 3:8-12.

Avendaño, C., & Oehninger, S., 2011. DNA fragmentation in morphologically normal spermatozoa: how much should we be concerned in the ICSI era? *Journal of Andrology*, 32:356–363.

Awad, A.B., & Fink, C.S., 2000. Phytosterols as anticancer dietary components: evidence and mechanism of action. *The Journal of Nutrition,* 130:2127-2130.

Baba, Y., Matsuo, H., & Schally, A.V., 1971. Structure of the porcine LH and FSH-releasing hormone. II. Confirmation of the proposed structure by conventional sequential analyses. *Biochemical and Biophysical Research Communications,* 44:459–463

Baccetti, B., La Marca, A., Piomboni, P., Capitani, S., Bruni, E., Petraglia, F., De Leo, V., 2002. Insulin-dependent diabetes in men is associated with hypothalamo-pituitary derangement and with impairment in semen quality. *Human Reproduction*,17:2673-2677.

Bahorun, T., Soobrattee, M.A., Luximon-Ramma, V., Aruoma, O.I., 2006. Free radicals and antioxidants in cardiovascular health and disease. *Internet Journal of Medicine Update*, 1:1-17.

Baker, M.A., & Aitken, R.J., 2004. The importance of redox regulated pathways in sperm cell biology. *Cellular Endocrinology,* 216:47-54.

Bansal, A.K., & Bilaspuri, G.S., 2011.Impacts of oxidative stress and antioxidants on semen functions. *Veterinary Medicine International*, 2011:686137.

Bardin, C.W., & Paulsen, C.A., 1981. The testes. In: Williams, R.H., (ed). *Textbook of Endocrinology*, 6thedn. Saunders, Philadelphia, PA.

Bastaki, S., 2005. Diabetes mellitus and its treatment: Review. *International Journal of Diabetes and Metabolism*, 13:111-134

Bejarano, I., Espino, J., Marchena, A.M., Barriga, C., Paredes, S.D., Rodríguez, A.B., Pariente, J.A., 2011. Melatonin enhances hydrogen peroxide-induced apoptosis inhuman promyelocytic leukaemia HL-60 cells. *Molecular and Cellular Biochemistry,* 353(1-2):167-176.

Bejarano, I., Lozano, G.M., Ortiz, A., Garcia, J.F., Paredes, S.D., Rodriguez, A.B., Pariente, J.A., 2008. Caspase 3 activation in human spermatozoa in response to hydrogenperoxide and progesterone. *Fertility and Sterility,* 90:1340-1347.

Belleannee, C., Labas, V., Teixeira-Gomes, A.P., Gatti, J.L., Dacheux, J.L., Dacheux, F., 2011. Identification of luminal and secreted proteins in bull epididymis. *Journal of Proteomics,* 74:59-78.

Berges, R., Windeler, J., Trampsch, H., Senge, T., 1995. Randomised, placebo controlled double blind clinical trial of Beta-sitosterol in patients with benign prostatic hyperplasia. *Lancet,* 345(8964):1529-1532.

Berges, R.R., Kaasen, A., & Senge, T., 2000. Treatment of symptomatic benign prostatic hyperplasia with beta-sitosterol: an 18-month follow-up. *British Journal of Urology International,* 85:842-846.

Bloch-Damti, A., & Bashan, N., 2005. Proposed mechanisms for the induction of insulin resistance by oxidative stress. *Antioxidants & Redox Signalling*, 7(11-12):1553-1567.

Bohn, T., 2008. Bioavailability of Non-Provitamin A Carotenoids. *Current Nutrition and Food Sciences*, 4:240-258.

Bouayed, J., & Bohn, T., 2010. Exogenous antioxidants: double edged swords in cellular redox state. *Oxidative Medicine and Cellular Longevity,* 3(4):228-237.

Bouic, P., Etsebelt, S., Albrecht, C., Pegel, K., Van Jaarsveld, P., 1996. Beta-sitosterol and betasitosterol glucoside stimulate human peripheral blood lymphocyte proliferation: Implications for their use as an immunomodulatory vitamin combination. *International Journal of Immunopharmacology*, 18(12):693-700.

Bouic, P., & Vanderhaeghe, L., 1999. The immune System Cure, Pyramid Publications.Chapter 1.Out of Africa, plants to the rescue: 3-8.Chapter 2. Understanding immunity, Helper T-cells: 15-22. Chapter 3. Ten nutritional supplements, DHEA-The immune hormone: 38-41. Chapter 8. Protect your prostate, Plant sterols for Benign Prostatic Hypertrophy: 117-122. Chapter 11. Infectious diseases and immunity, HIV and balance between Th1-and Th2 cells: 182-185. Sterols and sterolin: TB research: 190-191.

Bouic, P.J., Clark, A., Brittle, W., Lamprecht, J.H., Freestone, M., Lieberberg, R.W., 2001. Plant sterol/sterolin supplement use in a cohort of South African HIV-infected patients: effects on immunological and virological surrogate markers. *South African Medicinal Journal,* 91:848-890.

Boukes, G.J., 2010*. The in vitro biological activities of three Hypoxis species and their active compounds*. Doctoral thesis. Nelson Mandela Metropolitan University.

Boukes, G.J., Van der Venter, M., & Oosthuizen, V., 2008. Quantitative and qualitative analysis of sterols/ steroins and hypoxide contents of three *Hypoxis* (African Potato) spp. *African Journal of Biotechnology,* 7:1624-1629.

Boyne, A., & Ellman, G., 1972. Assay for glutathione. *Analytical Biochemistry*, 46:639-653.

Brandt, H., & Muller, G., 1995. Traditional medicines and acute poisoning. *Continuing Medical Education*, 13(9):1053-1060.

Breitbart, H., & Noar, Z., 1999. Protein kinases in mammalian sperm capacitation and acrosome reaction. *Review on Reproduction,* 4:151-159.

Breucker, H., Schafer, E., & Holstein, A.F., 1985. Morphogenesis and fate of the residual body in human spermiogenesis. *Cell and Tissue Research,* 240:303-309.

Bromfield, J.J., Schjenken, J.E., Chin, P.Y., Care, A.S., Jasper, M.J., Robertson, S.A., 2014. Maternal tract factors contribute to paternal seminal fluid impact on metabolic phenotype in offspring. *Proceedings of the National Academic of Sciences of the United States of America*, 111(6):2200–2205.

Brown, L., Heyneke, O., Brown, D., Van Wyk, J.P., Hamman, J.H., 2008. Impact of traditional medicinal plant extracts on antiretroviral drug absorption. *Journal of Ethnopharmacology*, 119:588-592.

Brownlee, M., 2001. Biochemistry and molecular cell biology of diabetic complications. *Nature*, 414(6865):813-820.

Brownlee, M., & Cerami, A., 1981. The biochemistry of the complications of diabetes mellitus. *Annual Review of Biochemistry,* 50:385-432.

Bujan, L., Mansat, A., & Pontonnier, F., 1996. Time series analysis of sperm concentration in fertile men in Tolouse, France between 1977 and 1992. *British Medical Journal*, 312:471-72.

Busetto, G.M., Koverech, A., Messano, M., Antonini, G., De Berardinis, E., Gentile, V., 2012. Prospective open-label study on the efficacy and tolerability of a combination of nutritional supplements in primary infertile patients with idiopathic astenoteratozoospermia. *Archivio Italiano di Urologia, Nefrologia, Andrologia*, 84:137-140.

Cai, L., Chen, S., Evans, T., Deng, D.X., Mukherjee, K., Chakrabarti, S., 2000. Apoptotic germ-cell death and testicular damage in experimental diabetes: prevention by endothelin antagonism. *Urology Research*,28:342-347.

Cameron, D.F., Rountree, J., Schultz, R.E., Repetta, D., Murray, F.T., 1990. Sustained hyperglycemia results in testicular dysfunction and reduced fertility potential in BBWOR diabetic rats. *American Journal of Physiology*, 259:881-889.

Careri, M., Elviri, l., & Mangia, A., 2001. Liquid- chromatography- UV determination and liquid chromatography atmospheric pressure chemical ionization mass spectrometric characterization of sitoterol and stimasterol in soy bean oil. *Journal of Chromatography*, 935:249-257.

Carlsen, M., Halvorsen, B., Holte, K., Bohn, S., Dragland, S., Sampson, L., Willey, C., Senoo, H., Umezono, Y., Sanada, C., Barikmo, I., Berhe, N., Willett, W., Phillips, K., Jacobs, D., Blomhoff, R., 2010. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. *Nutrition Journal*, 9:3.

Carolsfeld, J., Powell, J.F., Park, M., Fischer, W.H., Craig, A.G., Chang, J.P., Rivier, J.E., Sherwood, N.M., 2000. Primary structure and function of three gonadotropin-releasing hormones, including a novel form, from an ancient teleost, herring. *Endocrinology,* 141:505-512.

Carreau, S., De Vienne, C., & Galeraud-Denis, I., 2008. Aromatase and estrogens in man reproduction: a review and latest advances. *Advanced Medical Sciences*, 53(5):139-144.

Ceriello, A., 2000. Oxidative stress and glycemic regulation. *Metabolism*, 49(2):27-29.

Chattopadhyay, R.R., 1999. A comparative evaluation of some blood sugar lowering agents of plant origin. *Journal of Ethnopharmacology,* 67:367–372.

Chang, C., Chen, Y.T., Yeh, S.D., Xu, Q., Wang, R.S., Guillou, F., Lardy, H., Yeh, S., 2004. Infertility with defective spermatogenesis and hypotestosteronemia in male mice lacking androgen receptor in sertoli cells. *Proceeding of the National Academy of Sciences of the United States of America,* 101:6876-6881.

Chen, S.J., Allam, J.P., Duan, Y.G., Haidl, G., 2013. Influence of reactive oxygen species on human sperm functions and fertilizing capacity including therapeutical approaches. *Archives of Gynecology Obstetrics*, 288:191-9.

Chinsembu, K.C., & Hedimbi, M., 2010. Ethnomedicinal plants and the natural products with anti-HIV active compounds and their putative modes of actions. *International Journal for Biotechnology and Molecular Biology Research*, 1:74-91.

Chiu, P.C., Lam, K.K., Wong, R.C., Yeung, W.S., 2014. The identity of zona pellucid receptor on spermatozoa: An unresolved issue in developmental biology. *In Seminars in Cell & Developmental Biology*, 30:86-95.

Choudhary, R., Chawala, V.K., Soni, N.D., Kumar, J., Vyas, R.K., 2010. Oxidative stress and role of antioxidants in male infertility. *Pakistan Journal of Physiology*, 6(2):54-59.

Chung, J.J., Shim, S.H., Everley, R.A., Gygi, S.P., Zhuang, X., Clapham, D.E., 2014. Structurally distinct Ca^{2+} signalling domains of sperm flagella orchestrate tyrosine phosphorylation and motility. *Cell*, 157(4):808-822.

Chung, S.S.W., Wang, X., & Wolgemuth, D.J., 2009. Expression of retinoic acid receptor alpha in the germline is essential for proper cellular association and spermiogenesis during spermatogenesis. *Development*, 136:2091-2100.

Comhaire, F.H., Christopher, A.B., Zalata, A.A., Dhooge, W.S., Mahmoud, A.M., Depuydt, C.E., 2000. The effects of combined conventional treatment, oral antioxidants and essential fatty acids on sperm biology in subfertile men. *Prostaglandins Leukotriene Essential Fatty Acids*, 63:159-165.

Cornwall, G.A., 2009. New insights into epididymal biology and function. *Human Reproduction Update***,** 15(2):213-227.

Costabile, R.A., & Spevak, M., 2001. Characterization of patients presenting with male factor infertility in an equal access, no cost medical system. *Urology*, 58(6):1021-1024.

Czabotar, E.P., Lessene, G., Strasser, A., Adams, J.M., 2014. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. *Nature Reviews Molecular Cell Biology*, 15:49-63.

Da Silva, N., Pietrement, C., Brown, D., Breton, S., 2006. Segmental and cellular expression of aquaporins in the male excurrent duct. *Biochimica et Biophysica Acta,* 1758:1025-1033.

Dacheux, J.L., Belleannee, C., Guyonnet, B., Labas, V., Teixeira-Gomes, A.P., Ecroyd, H.,Dacheux, J.L., Castella, S., Gatti, L.J., Dacheux, F., 2005. Epididymal cell secretory activities and the role of the proteins in boar sperm epididymis. *Theriogenology*, 63(2):319-341.

Danial, N.N., & Korsmeyer, S.J., 2004. Cell death: oxidative stress as a mediator of apoptosis. *Immunology Today*, 15(1):7-10.

De Hoff, P.L., Brill, L.M., Hirsh, A.M., 2009. Plant lectins: the ties that bind in root symbiosis and plant defense. *Molecular Genetics and Genomics,* 282:1-15.

De Kretser, D.M., Temple-Smith, P.D., Kerr, J.B., 1982. Anatomical and functional aspects of the male reproductive organs. In Bandhauer, K., and Fricks, J., (eds). *Handbook of Urology*. Berlin, Springer-Verlag, 1-131.

de La Rochebrochard, E., Mouzon, J., Thepot, F., Thonneau, P., 2006. French National IVF Registry Association: Fathers over 40 and increased failure to conceive: the lessons of in vitro fertilization in France. *Fertility and Sterility*, 85:1420-1424.

de Lamirande, E., & O'Flaherty, C., 2008. Sperm activation: role of reactive oxygen species and kinases. *Biochimica et Biophysica* Act, 1784:106-115.

de Lamirande, E., Jiang, H., Zini, A., Kodama, H., Gagnon, C., 1997. Reactive oxygen species and semen physiology. *Reviews of Reproduction,* 2:48-54.

de Lamirande, E., Leclerc, P., & Gagnon, C., 1997. Capacitation as a regulatory event that primes spermatozoa for the acrosome reaction and fertilization. *Molecular Human Reproduction*, 3(3):175-194.

de Lamirande, E., & Gagnon, C., 1993. A positive role for the superoxide anion in triggering hyperactivation and capacitation of human spermatozoa. *International Journal of Andrology*, 16:21-25.

De-Eknamkul, W., & Potduang, B., 2003. Biosynthesis of β-sitosterol and stigmasterol in *croton sublyratus* proceeds via a mixed origin of isoprene units. *Phytochemistry,* 62:389-398.

Delaney, C.A., Dunger, A., Di Matteo, M., Cunningham, J.M., Green, M.H., Green, I.C., 1995. Comparison of inhibition of glucose-stimulated insulin secretion in rat islets of Langerhans by streptozotocin and methyl andethyl nitrosoureas and methanesulphonates. Lack of correlation with nitric oxide-releasing or O6-alkylating ability. *Biochemical Pharmacology,* 50: 2015-2020.

Dennery, P.A., 2004. Role of Redox in Fetal Development and Neonatal Diseases. *Antioxidants & Redox Signaling*, 6(1):147-153.

Desai, N.R., Mahfouz, R., Sharma, R., Gupta, S., Agarwal, A., 2010. Reactive oxygen species levels are independent of sperm concentration, motility, and abstinence in a normal, healthy, proven fertile man: a longitudinal study. *Fertility and Sterility*, 94:1541-1543.

Devasagayam, T.P., Tilak, J.C., Boloor, K.K., Sane, K.S., Ghaskadbi, S.S., Lele, R.D., 2004. Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. *Journal of the Association of Physicians of India,* 52:794-804.

Dohle, G.R., 2010. Guidelines on male infertility. *European Association of Urology*, http://uroweb.org/wp-content/uploads/15_Male_Infertility_LR-II.pdf

Dohle, G.R., Colpi, G.M., Hargreave, T.B., Papp, G.K., Jungwirth, A., Weidner, W., 2005. European Association of Urology guidelines on male infertility. *European Urology,* 48:703-11.

Dolan, M.E., 1997. Inhibition of DNA repair as a means of increasing the antitumor activity of DNA active agents. *Advanced Drug Delivery Reviews*, 26:105-118.

Donaldson, M.S., 2004. Nutrition and cancer: A review of the evidence for an anti-cancer diet. *Nutrition Journal,* 3:19-25.

Drewes, S.E., & Khan, F., 2004. The African potato (*Hypoxis hemerocallidea*) a chemical-historical perspective. *South African Journal of Science,* 100:425–430.

Drewes, S.E., Elliot, E., Khan, F., Dlamini, J.T., Gcumisa, M.S., 2008. *Hypoxis* hemerocalidea- not merely a cure for benign prostate hyperplasia. *Journal of Ethnopharmacology,* 119:593-598.

Droge, W., 2002. Free radicals in the physiological control of cell function: Review. *Physiological Reviews*, 82:47-95.

Du Plessis- Stoman, D., Downing, T.G., Van De Venter, M., Govender, S., 2009. Traditional herbal medicines; potential degradation of sterols and sterolins by microbial contaminants. *South African Journal of Sciences,* 105:147-150.

Du, X.L., Edelstein, D., Dimmeler, S., Ju, Q., Sui, C., & Brownlee, M., 2001. Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. *Journal of Clinical Investigation*, 108(9):1341-1348.

Dufrane, D., van Steenberghe, M., Guiot, Y., Goebbels, R.M., Saliez, A., Gianello, P., 2006. Streptozotocin-induced diabetes in large animals (pigs/primates): role of GLUT2 transporter and beta-cell plasticity. *Transplantation*, 81:3645.

Duru, N.K., Morshedi, M., & Oehninger, S., 2000. Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. *Fertility and Sterility*, 74:1200-1207.

Eisenegger, C., Haushofer, J., & Fehr, E., 2011. The role of testosterone in social interaction. *Trends in Cognitive Sciences,* 15(6):263-271.

Ellem, S.J., & Risbridger, G.P., 2009. The dual, opposing roles of estrogen in the prostate. *Steroid Enzymes and Cancer*, 1155:174-186.

Ellerby, L.M. & Bredesen, D.E. 2000. Measurement of cellular oxidation reactive oxygen species and antioxidant enzymes during apoptosis. *Methods in Enzymology*, 322: 413-421.

Ellerman, D.A., Brantúa, S., Martínez, S.P., Cohen, D.J., Conesa, D., Cuasnicú, P.S., 1998. Potential contraceptive use of epididymal proteins: immunization of male rats with epididymal protein DE inhibits sperm fusion ability. *Biology of Reproduction*, 59(5):1029-1036.

English, P., & Williams, G., 2004. Hyperglycaemic crises and lactic acidosis in diabetes mellitus. *Postgraduate Medical Journal*, 80(943):253-261.

Erasto, P., Adebola, P.O., Grierson, D.S., Afolayan, A.J., 2005. An ethnobotanical study of plants used for the treatment of diabetes in the Eastern Cape province. *South Africa African Journal of Biotechnology,* 4:1458-1460.

Erlwanger, K.H., & Cooper, R.G., 2008. The effects of orally administered crude alcohol and aqueous extracts of African potato (*Hypoxis hemerocallidea*) corm on the morphometry of viscera of suckling rats. *Food and Chemical Toxicology,* 46:136-139.

Fatani, A.J., Al-Rejaie, S.S., Abuohashish, H.M., Al-Assaf, A., Parmar, M.Y.,Ahmed, M.M., 2015. Lutein Dietary Supplementation Attenuates Streptozotocin-induced testicular damage and oxidative stress in diabetic rats. *BMC Complementary and Alternative Medicine,* http://doi.10.1186/s12906-015-0693-5

Fasinu, P.S., Gutmann, H., Schiller, H., Bouic, P.J., Rosenkranz, B., 2013. The potential of *Hypoxis hemerocallidea* for herb–drug interaction. *Pharmaceutical Biology, Early Online*, http://dio:10.3109/13880209.2013.7963933

Fierro, R., González-Marquez, H., Ortiz, R., Chevier, J., Foliguet, B., 2013. Fluorometric Viability Assessment of Capacitated and Acrosome-Reacted Boar Spermatozoa by Flow Cytometry. *Optics and Photonics Journal*, 3:40-44.

Flesch, F.M., & Gadella, B.M., 2000. Dynamics of the mammalian sperm plasm membrane in the process of fertilization. *Biochimica et Biophysica Acta,* 1469(3)197-235.

Ford, W.C., 2004. Regulations of sperm function by reactive oxygen species. *Oxford Journals, Medicine, Human Reproduction*, 10(5):387-399.

Fouchecourt, S., Metayer, S., Locatelli, A., Dacheux, F., Dacheux, J.L., 2000. Stallion epididymal fluid proteome: qualitative and quantitative characterization; secretion and dynamic changes of major proteins. *Biology of Reproduction,* 62:1790-1803.

Fouquet, J., Kann, M., Soues, S., Melki, R., 2000. ARP1 in Golgi organisation and attachment of manchette microtubules to the nucleus during mammalian spermatogenesis. *Journal of Cellular Science*, 113(5):877-886.

Fraczek, M., & Kurpisz, M., 2005. The redox system in human semen and peroxidative damage of spermatozoa. *Postepy Higieny Medycyny Doswiadczalnej (Warszawa) (online)*, 59:523-534.

Friederich, M., Hansell, P., & Palm, F., 2009. Diabetes, oxidative stress, nitric oxide and mitochondria function. *Current Diabetes Reviews*, 5:120-144.

Frei, B., Kim, M.C., & Ames, B.N., 1990. Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proceedings of the National Academy of Sciences of the United States of America*, 87(12):487-499.

Fu, L.L., Zhou, C.C., Yao, S., Yu, J.Y., Liu, B., Bao, J.K., 2011. Plant Lectins: targeting programmed cell death pathways as antitumor agent. *The International Journal of Biochemistry and Cell Biology,* 43:1442-1449.

Gaboriau, D., Howes, E.A., Clark, J., Jones, R., 2007. Binding of sperm proacrosin/ ßacrocsin to zona pellucida glycoproteins is sulphate and stereo dependent synthesis of a novel fertilization inhibitor. *Developmental Biology*, 306:646-657.

Gadea, J., Parrington, J., Kashir, J., Coward, K., 2013. The male reproductive tract and spermatogenesis. *Textbook of Clinical Embryology*, 18.

Gadella, B.M., Rathi, R., Brouwers, J.F., Stout, T.A., Colenbrander, B., 2001. Capacitation and the acrosome reaction in equine sperm. *Animal Reproduction Science*, 68:249-265.

Gaidamashivili, M., & Van Staden, J., 2006. Prostaglandin inhibitory activity by lectin-like proteins from South African plants. *S*outh *Afrcan Journal of Botany,* 71: 661–663.

Galley, H., 2011. Oxidative stress and mitochondrial dysfunction in sepsis. *British Journal of Anaesthesia,* 107(1):57-64.

Gatti, J.L., Castella, S., Dacheux, F., Ecroyd, H., Métayer, S., Thimon, V., Dacheux, J.L., 2004. Post-testicular sperm environment and fertility. *Animal Reproduction Science*, 82-83: 321-339.

Gaur, D.S, Talekar, M., &Pathak V.P., 2000. Effects of smoking on semen quality of infertile men. *Singapore Medical Journal*, 48(2):119-123.

Gericke, N., 2011. Muthi to medicine. *South African Journal of Botany*; 77(4):850-856.

Gharagozloo, P., & Aitken, R.J., 2011. The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy. *Human Reproduction*, 26(7):1628–40.

Ghareeb, D.A., & Sarhan, E.M., 2014. Role of oxidative stress in male fertility and idiopathic infertility: causes and treatment: Review. *Journal of Diagnostic Techniques and Biomedical Analysis,* http://dx.doi.org/10.4172/jdtba.10001077

Ghazarian, H., Idoni, B., & Oppenheimer, S.B., 2011. A glycobiology review: carbohydrates, lectins and implications in cancer therapeutics. *Acta Histochemica,* 113:236-247.

Ghlissi, Z., Atheymen, R., Boujbiha, M.A., Sahnoun, Z., Ayedi, F.M., Zeghal, K., Feki, A.E., Hakim, A., 2013. Antioxidant and androgenic effects of dietary ginger on reproductive function of male diabetic rats. *International Journal of Food Sciences and Nutrition,* http://dx.doi.10.3109/09637486.2013.812618

Ghorbani, R., Mokhtari, T., Khazaei, M., Salahshoor, M.R., Jalili, C., Bakhtiari, M., 2014. The effect of walnut on the weight, blood glucose and sex hormones of diabetic male rats. *International Journal of Morphology, 32(3)*:833-838

Ghosh, S., Sinha- Hikim, A.P., & Russell, L.D., 1991. Further observations of stage- specific effects seen after short- term hypophysectomy in the rat. *Tissue cell*, 23:613-630.

Giron, M.D., Salto, R., Gonzalez, Y., Giron, J.A., Nieto, N., Periago, J.L., Saurez, M.D., Hortelano, P., 1999. Modulation of hepatic and intestinal glutathione S-transferases and other antioxidant enzymes by dietary lipids in streptozotocin diabetic rats. *Chemosphere*, 38:3003–3013.

Glenn, D.R., McClure, N., & Lewis, S.E., 2003. The hidden impact of diabetes on male sexual dysfunction and fertility. *Human Fertility* (Camb), 6(4):174-179.

Green, K., Brand, M.D., & Murphy, M.P., 2004. Prevention of mitochondrial oxidative damage as therapeutic strategy in diabetes. *Diabetes*, 53(1):110-118.

Griffin, J.E., & Wilson, J.D., 1998. Disorders of the testes and the male reproductive tract. In: Wilson J.D., (ed). *Williams Textbook of Endocrinology*. Saunders, Philadelphia, PA, 9:819-875.

Griffeth, R.J., Carretero, J., & Burks, D.J., 2013. Insulin receptor substrate 2 is required for testicular development. *PLoS ONE*, 8(5):62103.

Grivaeu, J.F., Dumont, E., Renard, B., Callegari, J P., Lannou, D.L., 1995. Reactive oxygen species, lipid peroxidation and enzymatic defense systems in human spermatozoa. *Journal of Reproduction and Fertility*, 103:17-26.

Guneli, E., Tugyan, K., Ozturk, H., Gumustekin, M., Cilaker, S., Uysal, N., 2008. Effect of melatonin on testicular damage in streptozotocin-induced diabetes rats. *European Journal of Surgical Research*, 40:354-336.

Gupta, N. P., & Kumar, R., 2002. Lycopene therapy in idiopathic male infertility – a preliminary report. *International Urology and Nephrology*, 34:369-372.

Gupta, R., Sharma, M., Lakshmy, R., Prabhakaran, D., Reddy, K.S., 2009. Improved method of total antioxidant assay*. Indian Journal of Biochemistry and Biophysics*, 46(1):126-129.

Guzdek, A., Nizankowska, E., & Allison, A., 1996. Cytokine production in human and rat Hypoxidaceae (Monocotyledones). *Bulletin De La Societe Royale De Botanique* macrophages and dicatechol Rooperol and esters. *Biochemical Pharmacolology*, 1152(7): 991-998.

Halliwell, B., 1984. Tell me about free radicals, doctor: a review. *Journal of the Royal Society of Medicine*, 82:747-752.

Halliwell, B., 2007. Biochemistry of oxidative stress. *Biochemical Society Transactions*, 35:1147-1150.

Halliwell, B., 2011. Free radicals and antioxidants - quo vadis? *Trends in Pharmacological Sciences,* 32:125-130.

Hamilton, B.E., & Ventura, S.J., 2006. Fertility and abortion rates in the United States, 1960–2002. *International Journal of Andrology,* 29:34-45.

Hampl, R., Drábková, P., Kanďár, R., Stěpán, J., 2012. Impact of oxidative stress on male infertility. *Ceska Gynekol*, 77:241-245.

Handel, M.A., & Schimenti, J.C., 2010. Genetics of mammalian meiosis: regulation, dynamics and impact on fertility. *Natural Review Genetics*, 11:124-136.

Hansen, J.C., & Deguchi, Y., 1996. Selenium and fertility in animals and man- a review. *Acta Veterinaria Scandinavica,* 37:19-30.

Hauser, R., 2006. The environment and male fertility: recent research on emerging chemicals and semen quality. *Seminars in Reproduction Medicine*, 24:156-67.

Heldt, H.W., 2005. A large diversity of isoprenoids has multiple functions in plant metabolism. In: *Plant Biochemistry*. Elsevier Academic Press. London, http://www.oalib.com/references/12051085

Hemachand, T., & Shaba, C., 2003. Functional role of sperm surface glutathione S-transferases and extracellular glutathione in the haploid spermatozoa under oxidative stress. *FEBS letters,* 538:14-18.

Hermo, L., Oko, R., & Morales, C.R., 1994. Secretion and endocytosis in the male reproductive tract: a role in sperm maturation. *Internacional Review of Cytology*, 154:106-189.

Hermo, L., Pelletier, R.M., Cyr, D.G., Smith, C.E., 2010a. Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 1: background to spermatogenesis, spermatogonia, and spermatocytes. *Microscopic Research Technology*, 73(4):278.

Hermo, L., Pelletier, R.M., Cyr, D.G., Smith, C.E., 2010b. Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 2: changes in spermatid organelles associated with development of spermatozoa. *Microscopic Research Technology*, 73(4):279-319.

Hess, R.A., 1999. Spermatogenesis: Overview. *Encyclopaedia of Reproduction*, 4:540-545.

Holmström, K., & Finkel, T., 2014. Cellular mechanisms and physiological consequences of redox-dependent signalling. *Nature Reviews Molecular Cell Biology,* 15(6):441-421.

Hosokawa, M., Dolci, W., & Thorens, B., 2001. Differential sensitivity of GLUT1 and GLUT2-expressing beta cells to streptozotocin. *Biochemical and Biophysical Research Communications*, 289:1114-1117.

Hostetmann, K., Martson, A., Ndjoko, K., Wofender, J.L., 2000. The potential of African plants as a source of drugs. *Current Organic Chemistry,* 4:973-1010.

Hruska, K.S., Furth, P.A., Seifer, D.B., Sharara, F.I., Flaws, J.A., 2000. Environmental factors in infertility. *Clinical Obstetrics and Gynecology*, 43:821-829.

Huhtaniemi, I., 2010. How are communication signals read in the male reproductive system? Robaire, B., & Chan, P., (eds). *Handbook of Andrology*. The American Society of AndrologyAllen Press Inc, Lawrence, 3-7.

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.B., 1996. *Zulu medicinal plants—An inventory*.University of Natal Press, Pietermaritzburg, South Africa.

Irvine, D.S., Twigg, J.P., Gordon, E.L., Fulton, N., Milne, P.A., Aitken, R.J., 2000. DNA integrity in human spermatozoa: relationships with semen quality. *Journal of Andrology*, 21:33-44.

Irvine, S., Cawood, E., & Richardson, D., 1996. Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. *Biomedicine Journal*, 312:467-71.

Isidori, A., Latini, M., & Romanelli, F., 2005.Treatment of male infertility.*Contraception,* 72:314-8.

Islam, M.S., & Loots du, T., 2009. Experimental rodent models of type 2 diabetes: A review. *Methods and Findings of Experimental and Clinical Pharmacology*,31:249-261.

Jager, A.K., Hutchings, A., & Van Staden, J., 1996. Screening Zulu medicinal plants for prostaglandin- synthesis inhibitors. *Journal of Ethnopharmacoloy*, 52:95-100.

Jannsen, Y.M., Van-Houton, B., Borm, P.J., Mossuran, B.T., 1993. Cell and tissue responses to oxidative damage. *Laboratory Investigation*, 69:261-265.

Johnson, M.D., 1998. Genetic risks of intracytoplasmic sperm injection in the treatment ofmale infertility: recommendations for genetic counseling and screening. *Fertility and Sterility,* 70(3):397-411.

Johnson, F., & Giulivi, C., 2005. Superoxide dismutases and their impact upon human health. *Molecular Aspects of Medicine*, 26:340-352.

Juanchi, X., Albarran, G. & Negron-Mendoza, A., 2000. Radiolysis of cyanocobalamin (vitamin B12). *Radiation Physics and Chemistry*, 57:337-339.

Jungwirth, A., Diemer, T., Dohle, GR., Giwercman, A., Kopa, Z., Tournaye, H., Krausz, C., 2012. Guidelines in male infertility. *European Association of Urology*,61(1):159-63.

Kahn, S.M., Hyrb, D.J., Nakhla, A.M., Romas, N.A., Rosner, W., 2002. Sex- hormone binding globulin is synthesized in target cells. *Journal of Endocrinology*, 175(1):113-120.

Kao, S.H., Chao, H.T., Chen, H.W., Hwang, T.I., Liao, T.L., Wei, Y.H., 2008. Increase of oxidative stress in human sperm with lower motility. *Fertility and Sterility*, 89:1183-1190.

Karbownik, M., Gitto, E., Lewinski, A., Reiter, R.J., 2001. Induction of lipid peroxidation in hamster organs by the carcinogen cadmium: melioration by melatonin. *Cell Biology and Toxicology*, 17:33-40.

Katerere, D., & Eloff, J.N., 2008. Anti-bacterial and anti-oxidant activity of *Hypoxis hemerocallidea* (*Hypoxidaceae*): can leaves be substituted for corms as conservation strategy? *South African Journal of Botany,* 74:613-616.

Kavishankar, G.B., Lakshmidevi, N., Mahadeva Murthy, S., Prakash, H.S., Niranjana, S.R., 2011. Diabetes and medicinal plants- A review. *International Journal of Pharmaceutical and Biomedical Sciences,* 2(3):65-80.

Kemal Duru, N., Morshedi, M., & Oehninger, S., 2000. Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. *Fertility and Sterility*, 74:1200-1207.

Khaki, A., Fathiazad, F., Nouri, M., Khaki, A.A., Maleki, N.A., Khamnei, H.J., Ahmadi, P., 2010. Beneficial effects of quercetin on sperm parameters in streptozotocin-induced diabetic male rats. *Phytotheraphy Research,* 24:1285-1291.

Kiasalari, Z., Khalili, M., & Aghaei, M., 2009. Effect of withania somnifera on levels of sex hormones in the diabetic male rats. *Iranian Journal of Reproductive Medicine,* 7(4):163-168.

Kim, E.D., Lipshultz, L.I., 7 Howards, S.S., 2002. Male infertility.In: Gillenwater, J.Y., Grayhack, J.T., Howards, S.S., &Mitchell, M.E., (eds).*Adult and pediatric urology*. Pa: Lippincott Williams & Wilkins, Philadelphia, 4(2):1683–1758.

Kitzmiller, J.L., Buchanan, T.A., Kjos, S., Combs, C.A., Ratner, R.E., 1996. Pre-conception care of diabetes, comgenital malformations and spontaneous abortions. *Diabetes Care,* 19:514-541.

Klinefelter, G.R., & Hess, R.A., 1998.Toxicology of the male excurrentducts and accessory sex glands in the male .*In Reproductive and Development Toxicology*, Korach, K.S., (ed). Basel Ltd., New York, 553-591.

Koppenol, W., Moreno, J., Pryor, W., Ischiropoulos, H., Beckman, J. S., 1992. Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. *Chemical Research in Toxicology*, 5:834-842.

Koslov, D.S., & Andersson, K.E., 2013. Physiological and pharmacological aspects of the vas deferens—an update: review. *Frontiers in Pharmacology*, 4:101.

Kothari, S., Thompson, A., Agarwal, A., & du Plessis, S.S., 2010. Free radicals: Their beneficial and detrimental effects on sperm function. *Indian Journal of Experimental Biology*, 48:425-435.

Kovalski, N.N., de Lamirande, E., &Gagnon, C., 1992. Reactive oxygen species generated by human neutrophils inhibit sperm motility: protective effects of seminal plasma and scavengers. *Fertility and Sterility*, 58:809-816.

Krinsky, N.I., & Yeum, K.J., 2003. Carotenoid–radical interactions. *Biochemistry and Biophysics Research Community*, 305:754–760.

Kumar, P.J., & Clark, M., 2002. Textbook of Clinical Medicine. Pub: Saunders, London, 1099-1121.

Kumar, T. R., Doreswamy, K., Shrilatha, B., Muralidhara, 2002. Oxidative stress associated DNA damage in testis of mice: Induction of abnormal sperms and effects on fertility. *Mutation Research,* 513:103-111.

Kumar, V., Fausto, N., Abbas, A.K., Cotran, R.S., Robbins, S.L., 2005. *Robbins and Cotran Pathologic Basis of Disease*. Pa.: Saunders, Philadelphia, 7:1194-1195.

Kwon, Y.K., & Hecht, N.B., 1991. Cytoplasmic protein binding to highly conserved sequences in the 3' untranslated region of mouse protamine 2 mRNA, a translationally regulated transcript of male germ cells. *Proceedings of the National Acadamy of Sciences of the United States of America,* 88:3584-3588.

Lampiao, F., 2012. Free radicals generation in an in vitro fertilization setting and how to minimize them. *World Journal of Obstetrics and Gynecology*, 1:29-34.

Laporta, O., Funes, L., Garzón, M.T., Villalain, J., Micol, V., 2007a. Role of membranes on the antibacterial and anti-inflammatory activities of the bioactive compounds from *Hypoxis* rooperi corm extracts. *Archives of Biochemistry and Biophysics,* 467:119-131.

Laporta, O., Perez-Fons, L., Mallavia, R., Caturla, N., Micol, V., 2007b. Isolation, characterisation and anti-oxidant capacity assessment of the bioactive compounds derived from *Hypoxis* rooperii corm extract (African potato). *Food Chemistry*, 101:1425-1437.

Lasserre, A., Barrozo, S., Tezón, J.G., Miranda, P.V., Vazquez-Levin, M.H., 2001. Human epididymal proteins and sperm function during fertilization: an update. *BiologicalResearch,* 34(3-4):65-178.

Lazari, M.F.M., Lucas, T.F.G., Yasuhara, F., Gomes, G.R.O., Siu, E.R., Royer, C., Fernandes, S.A.F., Porto, C.S., 2009. Estrogen receptors and function in the male reproductive system. *Arquivos Brasileiros de Endocrinologia and Metabologia,* 53(8):923-933.

Leclerc, P., de Lamirande, E., & Gagnon, C., 1998. Interaction between Ca^{2+}, cyclic 3',5' adenosine monophosphate, the superoxide anion and tyrosine phosphorylation pathway in the regulation of human sperm capacitation. *Journal of Andrology,* 19:434-443.

Lee, B.J., Lin, Y.C., Huang, Y.C., Ko, Y.W., Hsia, S., Lin, P.T., 2012. The Relationship between Coenzyme Q10, Oxidative Stress, and Antioxidant Enzymes Activities and Coronary Artery Disease. *The Scientific World Journal,* http://doi:10.1100/2012/792756

Lee, J., Richburg, J.H., Yonkin, S.C., & Bockelheide, K., 1997. The Fas system is a key regulator of germ cell apoptosis in the testis. *Endocrinology*, 138:2081-2088.

Lehninger, A.L., 2005. *Lehninger principles of biochemistry,* 4thedn., New York: W.H Freeman.

Leistner, O., 2000. Seed plants of southern Africa: families and genera. *National botanical institute, Hypoxidaceae (liliidae-asparagales)*, 1:621-623.

Lewin, A., & Lavon, H., 1997. The effect of coenzyme Q10 on sperm motility and function.*Molecular Aspects of Medicine*, 18:213-219.

Li, Y., & Schellhorn, H.E., 2007. New developments and novel therapeutic perspectives for vitamin C. Critical Review. *Journal of Nutrition,* 137:2171-2184.

Li, P., Zhang, Li., Tian, X., Xing, J., 2014. Docosahexaenoic acid has an anti-diabetic effect in streptozotocin-induced diabetic mice. *International Journal of Clinical and Experimental Medicine*, 7(9):3021-3029

Lie, P.P., Chan, A.Y., Mruk, D.D., Lee, W.M., Cheng, C.Y., 2010. Restricted Arp3 expression in the testis prevents blood-testis barrier disruption during junction restructuring at spermatogenesis. *Proceeding of the National Academy of Sciences of the United States of America,* 107:11411-11416.

Liu, H.K., McCluskey, J.T., Mc Clenghan, N.H., Flatt, P.R., 2007. Streptozotocin-resistant BRIN-BD11 cells possess wide spectrum of toxin tolerance and enhanced insulin-secretory capacity. *Endocrine,* 32:20-29.

Loghmani, E., 2005. Diabetes Mellitus Type and Type 2.In: Stang, J., & Story, M., (eds). *Guidelines for Adolescent Nutrition Services,* http://www.epi.umn.edu/let/pubs/adol_book.shtm

Luetjens, C.M., Wistabu, J., Weiner, G., Nieschlag, E., 2007. The Leydig cell a target for hormonal contraception. In: Payne, A.H., and Hardy, M.P., (eds). *The leydig cell in Health and Disease*. Human Press, New York, 415-445.

Lutz, W., 2006. Fertility rates and future population trends: will Europe's birth rate recover or continue to decline? *International Journal of Andrology,* 29:25-33.

Madhukar, D., & Rajender, S., 2009. Hormonal Treatment of Male Infertility: Promises and Pitfalls. *Journal of Andrology,* 30(2):95-112.

Maiorino, M. & Ursini, F., 2002.Oxidative stress, spermatogenesis and fertility. *Biological Chemistry*, 383(59):1-7.

Makker, K., Agarwal, A., & Sharma, R., 2009. Oxidative stress & male infertility. *Indian Journal of Medical Research*, 129:357-67.

Mallidis, C., Agbaje, I.M., Rogers, D.A., Glenn, J.V., Pringle, R., Atkinson, A.B., Steger, K., Stitt, A.W., McClure, N., 2009. Advanced glycation end products accumulate in the reproductive tract of men with diabetes. *International Journal of Andrology*, 32:295-305

Manach, C., Scalbert, A., Morand, C., Remesy, C., Jimenez, L., 2004. Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, 79:727-747.

Mancuso, C., Barone, E., Guido, P., Miceli, F., Di Domenico, F., Perluigi, M., Preziosi, P., 2012. Inhibition of lipid peroxidation and protein oxidation by endogenous and exogenous antioxidants in rat brain microsomes in vitro. *Neuroscience Letters*, 518(2):101-105.

Margis, R., Dunand, C., Teixeira, F.K., Margis-Pinheiro, M., 2008. Glutathione peroxidase family – an evolutionary overview. *FEBS Journal*, 275:3959-3970.

Mariod, A., Matthäus, B., & Hussein, I.H., 2008. Antioxidant properties of methanolic extractsfrom different parts of Sclerocarya birrea*. International Journal of Food Science and Technology,* 43:921-926.

Maritim, A.C., Moore, B.H., Sanders, R.A., Watkins, J.B., 1999. Effects of melatonin on oxidative stress in streptozotocin-induced diabetic rats. *International Journal of Toxicology,* 18:161-166.

Matés, J.M., Perez-Gomez, C., & Nunez De Castro, I., 1999. Antioxidant enzymes and human diseases. *Clinical Biochemistry,* 32:595-603.

Matsumoto, A.M., & Bremner, W.J., 2012. Testicular disorders. In: Melmed S, Polonsky KS, Larsen, P.R., Kronenberg, H.M., (eds). *Williams textbook of endocrinology*, 12th edn. Philadelphia: Saunders/Elsevier.

Mawhinney, M., & Mariotti, A., 2013. Physiology, pathology and pharmacology of the male reproductive system. *Periodontology 2000*, 61:232–251.

Mayo Clinic Medical Information, 2005. Drugs and supplements. Vitamin E. http://www.mayoclinic.com/health/vitamin-e/NS_patientvitamin-e

Mazur, A., &Booth, A., 1998. Testosterone and dominance in men.*Behavoural Brain Sciences*, 21:353-397.

Mendiola, J., Torres-Cantero, A.M., Moreno-Grau, J.M., Ten, J.,Roca, M.,Moreno-Grau, S., Bernabeu, R., 2008. Exposure to environmental toxins in males seeking infertility treatment: a case-controlled study. *Reproductive Biomedicine Online,* 16(6):842–850.

Middendorff, R., Müller, D., Mewe, M., Mukhopadhyay, A.K., Holstein, A.F., Davidoff, M.S., 2002. The tunica albuginea of the human testis is characterizedby complex contraction and relaxation activitiesregulated by cyclic GMP. *Journal of Clinical Endocrinology Metabolism,* 87:3486-3499.

Miller, A.L., 1996. Antioxidant Flavonoids: Structure, Function and Clinical Usage. *Alternative Medicine Review,* 1:103-111.

Mills, E., Cooper, C., Seely, D., Kanfer, I., 2005a. African herbal medicines in the treatment of HIV: *Hypoxis* and Sunderlandia. An overview of evidence and pharmacology. *Nutrition Journal*, 4:19-24.

Mills, E., Foster, B.C., van Heeswijk, R., Phillips, E., Wilson, K., Leonard, B., Kosuge, K., Kanfer, I., 2005b. Impact of African herbal medicines on antiretroviral metabolism. *AIDS,* 19(1):95-97.

Momoh, A.R., Idonije, B.O., Nwoke, E.O., Osifo, U.C., Okhai, O., Omoroguiwa, A., Momoh, A.A*.,* 2011. Pathogenic bacteria-a probable cause of primary infertility among couples in Ekpoma. *Journal of Microbiology and Biotechnology Research,* 1(3):66-71.

Montaner, A.D., Park, M.K., Fischer, W.H., Craig, A.G., Chang, J.P., Somoza, G.M., Mooradian, A.D., Morley, J.E., Korenman, S.G., 1987. Biological actions of androgens. *Endocrine Review*, 8:1-28.

Moore, H.D., 1996. The influence of the epididymis on human and animal sperm maturation and storage. *Human Reproduction*, 11:103-110.

Mora-Esteves, C., & Shin, D., 2013. Nutrient supplementation: improving male fertility fourfold. *Seminars in Reproductive Medicine*, 31:293-300.

Moreno, S.G., Brielmeier, M., & Bornkamm, G.W., 2003. Testis-specific expression of the nuclear form of phospholipid hydroperoxide glutathione peroxidise (PHGPx). *Journal of Biology and Chemistry,* 384:635-643.

Mortensen, A., Skibsted, L.H., & Truscott, T.G., 2001. The interaction of dietary carotenoids with radical species. *Archives of Biochemistry and Biophysics*, 385(1):13-19.

Mosby, N., 1996. *Biochemistry: A case oriented approach*. Chapter 10, Cholesterol. Montgomery and Conray, 6:335-355.

Murphy, M.P., 2014. Antioxidants are therapies: can we improve on nature? *Free Radical Biology and Medicine*, 66:20-23.

Musabayane, C.T., Xozwa, K., & Ojewole, J.A., 2005. Effects of *Hypoxis hemerocallidea* (Fisch. & C.A. Mey.) Hypoxidaceae corm (african potato) aqueous extract on renal electrolyte and fluid handling in the rat. *Renal Failure*, 27(6):763-770.

Moure, A., Cruz, J.M., Franco, D., Dominques, J.M., Sineiro, J., Dominguez, H., Numez, M.J., Parajo, C.J., 2001. Natural antioxidants from residual sources. *Food Chemistry,* 72:145-171.

Nair, V.D., & Kanfer, I., 2006a. High-performance liquid chromatographic method for quantitative determination of hypoxoside in African potato (*Hypoxis hemerocallidea*) and in commercial products containing the plant material and/or its extracts.*Journal of Agricultural and Food Chemistry,* 54:2816-2821.

Nair, V.P., & Kanfer, I., 2006b. Determination of β-sitosterol and stigmastanol in oral dosage forms using high performance liquid chromatography with evaporative light scattering detection. *Journal of Pharmaceutical Biochemical Analysis,* 41:731-737.

Nair, V.P., Dairam, A., Agbonon, A., Arnason, J.T., Foster, B.C., Kanfer, I., 2007. Investigation of the Antioxidant Activity of African Potato (*Hypoxis hemerocallidea*). *Journal of Agriculture and Food Chemistry*, 55:1707-1711.

Naraghi, M.A., Abolhasani, F., Kashani, I., Anarkooli, I.J., Hemadi, M., Azami, A., Barbarestani, M., Aitken, R.J., Shokri, S., 2010. The effects of swimming exercise and supraphysiological dose of nandrolone decanoate on the testis in adult male rats: Atransmission electron microscope study. *Folia Morphol*ogica, 69(3):138-146.

National Institutes of Health, 1995. *Diabetes in America*, 2nd edn. Bethesda, MD: National Institutes of Health, (NIH Publication no. 95-1468.)

Navarro-Casado, L., Juncos-Tobarra, M.A., Cháfer-Rudilla, M., de Onzoño, L.Í., Blázquez-Cabrera, J.A., Miralles-García, J.M., 2010. Effect of experimental diabetes and STZ on male fertility capacity. Study in rats. *Journal of Andrology*, 31:584-92

Ndong, T.A., Wadouachi, A., Sangwan-Norreel, B.S., Sangwan, R.S., 2006. Efficient in *vitro* regeneration of fertile plants from corm explants of *Hypoxis* hemerocallidea landrace gaza-the African potato. *Plant cell Reports,* 25:265-273.

Nelli, G.B., Anand S.K., & Kilari, E.K., 2013. Antidiabetic effect of α-mangostin and its protective role in sexual dysfunction of streptozotocin induced diabetic male rats. *Systems Biology in Reproductive Medicine*, 59:319-328.

Nguyen, L.A., He, H., & Pham-Huy, C., 2006. Chiral drugs: An overview. *International Journal of Biomedical Sciences (IJBS),* 2:85-100.

Nieschlag, E., & Behre, H.M., 2004. *Testosterone. Action, Deficiency, Substitution*. Springer Verlag, Berlin, 3:747.

Nilsson, S., Mäkelä, S., Treuter, E., Tujague, M., Thomsen, J., Andersson, G.,Enmark, E.,Pettersson, K.,Warner, M.,Gustafsson, J.A., 2001. Mechanisms of estrogen action. *Physiological Reviews*, 81(4):1535-1565.

Nukatsuka, M., Yoshimura, Y., Nishida, M., Kawada, J., 1990. Allopurinol protects pancreatic beta cells from the cytotoxic effect of streptozotocin: in vitro study. *Journal of Pharmacobiodynamics* 13: 259-262.

O'Donnel, L., Mc Lachlan, R.I., Wreford, N.G., Robertson, D.M., 1994. Testosterone promotes the conversion of round spermatids between stages vii and viii of the rat spermatogenic cycle. *Endocrinology*, 135(4):2608-2614.

O'Donnell, L., Robertson, K.M., Jones, M.E., Simpson, E.R., 2001. Estrogen and Spermatogenesis. *Endocrine Reviews,* 22(3):289-318.

O'Flaherty, C.M., Beorlegui, N.B., & Beconi, M.T., 1999. Reactive oxygen species requirements for bovine sperm capacitation and acrosome reaction. *Theriogenology*, 52:289-301.

O'Hara, L., Welsh, M., Saunders, P.T.K., & Smith, L.B., 2011. Androgen receptor expression in the caput epididymal epithelium is essential for development of the initial segment and epididymal spermatozoa transit. *Endocrinology,* 152(2):718-729.

Oates, P.J., &Mylari, B.L., 1999. Aldose reductase inhibitors: Therapeutic implications for diabetic complications. *Expert Opinion on Investigational Drugs,* 8:2095-2119.

Ochsendorf, F.R., Ozdemir, K., Rabenau, H., Fenner, T., Doer, H.W., 1999. Chlamydia IgA antibodies in seminal plasma are Chlamydia trachomatis specific and associated with an inflammatory response. *Journal of the European Academy of Dermatology and Venerology*, 12:143-152.

Oh, H., Ko, E.K., Jun, J.Y., Oh, M.H., Park, S.U., Kang, K.H., Lee, H.S., Kim, Y.C., 2002. Hepatoprotective and free radical scavenging activities of prenylflavonoids, coumarin, and stilbene from *Morus alba*. *Planta Medica,* 68:932-934.

Ojewole, J.A., 2002. Anti-inflammatory properties of *Hypoxis hemerocallidea* corm (African potato) extracts in rats. *Methods and Findings of Experimental and Clinical Pharmacology,* 24(10):685-687.

Ojewale, J.A., 2006. Antinociceptive, anti-inflammatory and antidiabetic properties of*Hypoxis hemerocallidea* Fisch & C.A. Mey (Hypoxidaceae) corm ['African Potato'] aqueous extract in mice and rats. *Journal of Ethnopharmacology*, 103:126-134.

Ojewale, J.A., Olaniyan, O.T., Faduyile, F.A., Odukanmi, O.A., Oguntola, J.A., Dare, B.J., 2014. Testiculo protective effects of ethanolic rootsextract of *pseudocedrela kotschyi* on alloxaninduced testicular damage in diabetic rats. *British Journal of Medicine & Medical Research,* 4(1):548-563.

Ojewale, J.A., Olayiwola, G., & Nyinawumunti, A., 2009. Bronchorelaxant property of 'African potato' (*Hypoxis* hemerocallidea corm) aqueous extract in *vitro*. *Journal of Smooth Muscle Research,* 17:166-171.

Ojewole, J.A., Kamadyaapa, D.R., & Musabayane, C.T., 2006. Some in vitro and in vivo cardiovascular effects of *Hypoxis* hemerocallidea Fisch & CA Mey (Hypoxidaceae) corm (African potato) aqueous extract in experimental animal models. *Cardiovascular Journal of South Africa*, 17:166-171.

Okubo, K., Amano, M., Yoshiura, Y., Suetake, H., Aida, K., 2000. A novel form of gonadotropin-releasing hormone in the medaka, Oryzias latipes. *Biochemical and Biophysical Research Communications*, 276:298-303.

Okyar, A., Can, A., Akev, N., Baktir, G., Sütlüpinar, N., 2001. Effect of Aloe vera leaves on blood glucose level in type I and type II diabetic rat models. *Phytotherapy Research,* 15:157-161.

Olivares, A., Méndez, J.P., Cárdenas, M., Oviedo, N., Palomino, M.A., Santos, I., Perera-Marín, G., Gutiérrez-Sagal, R., Ulloa-Aguirre, A., 2009. Pituitary-testicular axis function, biological to immunological ratio and charge isoform distribution of pituitary LH in male rats with experimental diabetes. *General and Comparative Endocrinology,* 161:304-312.

Olooto, W.E., Amballi, A.A., & Banjo, T.A., 2012. A review of Female Infertility; important etiological factors and management. *Journal of Microbiology and Biotechnology Research*, 2(3):379-385.

Olson, L.M., Zhou, X., & Schreiber, J.R., 1995. Cell specificlocalization of apolipoprotein E messenger ribonucleicacid in the testis and epididymis of the rat. *Biology Reproduction,* 52:1003-1011.

Oluwule, O.O., Sukati, N.A., Dlamini, P.S., Sibandze, F.G., 2007. Some Swazi phytomedicines and their constituents. *African Journal of Biotechnology*, 6(3):267–272.

Orgebin-Crist, M.C., 1962. Recherches expérimentales sur la durée de passage des spermatozoïdes dans l'épididyme du taureau. *Annales De Biologie, Animale, Biochimie, Biophysique,* 2:51-108.

Orgebin-Crist, M.C., 1967. Sperm maturation in rabbit epididymis. *Nature*, 216(5117):816-818.

Owen, D.H., & Katz, D.F., 2005. A review of the physical and chemical properties of human semen and the formulation of a semen simulant. *Journal of Andrology*, 26:459-469

Owira, P.M., & Ojewole, J.A., 2009. African Potato' (*Hypoxis hemerocallidea* corm): A Plant-Medicine for Modern and 21st Century Diseases of Mankind?–A Review. *Phytotherapy Research,* 23:147-152.

Ozmen, B., Caglar, G.S., & Koster, F., 2007. Relationship between sperm DNA damage, induced acrosome reaction and viability in ICSI patients. *Reproductive Biomedicine Online*, 15:208-214.

Patki, P., Woodhouse, J.,Hamid, R., Craggs, M., Shah, J., 2008. Effects of spinal cord injury on semen parameters. *Journal of Spinal Cord Medicine,* 31:27-32.

Paulson, J.R., 1977. The structure of histone-depleted metaphase chromosomes. *Cell,* 12:817-828.

Peeker, R., Abramsson, L., & Marklund, S.L., 1997. Superoxide dismutase isoenzymes in human seminal plasma and spermatozoa. *Molecular Human Reproduction*, 3:1061-1066.

Pegel, K.H., 1997. The importance of sitosterol and sitosterolin in human and animal nutrition.*South African Journal of Sciences,* 93:263-268.

Peppa, M., Uribarri, J., & Vlassara, H., 2004. The role of advanced glycation end products in the development of atherosclerosis. *Current Diabetes Reports,* 4:31-36.

Peters, M.A., Mol, J.A., Van Wolferen, M.E., Oosterlaken-Dijksterhuis, M.A., Teerds, K.J., Van Sluijs, F.J., 2003. Expression of the insulin-like growth factor (IGF) system and steroidogenic enzymes in canine testis tumors. *Reproduction Biology and Endocrinology*, 1:22.

Pfeifer, H.M., Conrad, D., Roethlein, A., Kyriakopoulos, M., Brielmeier, G.W., Bornkamm, D.B., 2001. Identification of a specific sperm nuclei selenoenzyme necessary for protamine thiol cross-linking during sperm maturation. *FASEB*, 15:1236-1238.

Pham-Huy, L.A., He, H., & Pham-Huy, C., 2008. Free Radicals, Antioxidants in Disease and Health: A Review. *International Journal of Biomedical Sciences*, 4(2):89-96.

Phipps, S.M., Sharaf, M.H., & Butterweck, V., 2007. Stimuli to the revision process-Assessing Antioxidant Activity in Botanicals and Other Dietary Supplements. *Pharmacopeial Forum*, 33 (4):810-814.

Pirinccioglu, A.G., Alyan, O., Kizil, G., Kangin, M., Beyazit, Z., 2012. Evaluation of oxidative stress in children with congenital heart defects. *Pediatrics International,* 54:94-98.

Pop-Busui, R., Sima, A., & Stevens, M., 2006. Diabetic neuropathy and oxidative stress. *Diabetes Metabolism Research Reviews*, 22:257-273.

Prins, G.S., & Korach, K.S., 2008. The role of estrogens and estrogen receptors in normal prostate growth and disease. *Steroids*, 73(3):233-244.

Pugliese, A.G., Toma-Barberan, F.A., Truchado, P., Genovese, M.I., 2013. Flavonoids, proanthocyanins, vitamin C and antioxidant activity of Theobroma grandiflorum (Cupuassu) pulp and seeds. *Journal of Agriculture and Food Chemistry,* 61(11):2720-2728.

Rabbani, S.I., Devi, K., & Khanam, S., 2009. Inhibitory effect of glimepiride on nicotinamide–streptozotocin induced nuclear damage and sperm abnormality in diabetic Wister rats*. Indian Journal of experimental Biology*, 47:804-810.

Rahimi, R., Nifkfar, S., & Larijani, B., 2005. A review on the role of antioxidants in the management of diabetes and its complications. *Biomedical Pharmacotherapy,* 59:365-373.

Rahman, K., 2003. Garlic and aging: new insights into an old remedy. *Ageing Research Revelation,* 2:39-56.

Rains, J.L., & Jain, S.K., 2011. Oxidative stress, insulin signaling, and diabetes*. Free Radical Biology and Medicine,* 50(5):567-575.

Ramos-Lobo, A.M., Buonfiglio, D.C., & Cipolla-Neto, J., 2015. Streptozotocin-induced diabetes disrupts thebody temperature daily rhythm in rats. *Diabetology & Metabolic Syndrome*, http://dx.doi.10.1186/s13098-015-0035-2

Rautenbach, F., Faber, M., Laurie, S., Laurie, R., 2010. Antioxidant capacity and antioxidant content in roots of 4 sweetpotato varieties. *Journal of Food Science*, 75(5):400-405.

Ravi, K., Rajasekaran, S., & Subramanian, S., 2005. Antihyperlipidemic effect of Eugenia jambolana seed kernel on streptozotocin-induced diabetes in rats. *Food and Chemical Toxicology,* 43:1433–1439.

Raza, H., & John, A., 2012. Streptozotocin-induced Cytotoxicity, Oxidative Stress and Mitochondrial Dysfunctin in Human Hepatoma HepG2 cells. *International Journal of Mileculae Sciences,* 13:5751-5767.

Resnick, H.E., & Howard, B.V., 2002. Diabetes and cardiovascular disease*. Annual Review of Medicine,* 53:245-267.

Rhodes, L., Primka, R.L., Berman, C., Verguilt, G., Gabriel, M., Pierre-Malice, M., Gubelin, B., 1993. Comparison of finasteride (Proscar), a 5-alpha reductase inhibitor and various commercial plant extracts in *in vitro* and *in vivo* 5-alpha reductase inhibition. *Prostate,* 22:43-51.

Ridnour, L.A., Thomas, D.D., Mancardi, D., Espey, M.G., Miranda, K.M., Paolocci, N., 2004. The chemistry of nitrosative stress induced by nitric oxide and reactive nitrogen oxide species. *Biological Chemistry,* 385:1-10.

Riffo, M.S., & Parrago, M., 1996. Study of the acrosome reaction and fertilizing ability of hamster epididymal cauda spermatozoa treated with antibodies against phospholipase A2 and/or lysophosphatidylcholine. *Journal of Experimental Zoology,* 275:81-89.

Robaire, B., Hinton, B.T., & Orgebin-Crist, M.C., 2006. The Epididymis. In: *The physiology of reproduction*, Knobil and Neil, J., (eds). Elsevier, New York, European Urology Association, 1071-114

Robaire, B., Syntin, P., &J ervis, K., 2000. The coming of age of the epididymis. In: *Testis, Epididymis and Technologies in the Year 2000*, Jégou, B., Pineau, C., & Saez, J., (eds), Springer: Hildenberg, New York, EUA, 229-262.

Roberts, K.P., 2010. What are the components of the male reproductive system.Robaire, B., and Chan, P., (eds). *Handbook of Andrology*. The Society of Andrology, Allen press Inc, Lawrence, 1-5.

Rolo, A.P., & Palmeira, C.M., 2006. Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. *Toxicology and Applied Pharmacology,* 212:167-178.

Romero, A.C., Olvera Hernández, E.G., Cerón, T.F., Chávez, A.A., 2013. Oxidative stress and chronic degenerative disease: A role for antioxidants. *The Enogenous Antioxidant,* 49:52.

Roveri, A., Ursini, F., Flohe, L., Maiorino, M., 2001. PHGPx and spermatogenesis. *Biofactors,* 14:213-222.

Rowley, W.R., & Bezold, C., 2012. Creating public awareness: state 2025 diabetes forecasts. *Population Health Management*, 15(4):194-200.

Russell, L.D., Saxena, N.K., & Turner, T.T., 1989. Cytoskeletal involvement in spermiation and sperm transport. *Tissue Cell,* 21:361-379.

Rybicki, E.P., Chikwamba, R., Koch, M., Rhodes, J.I., Groenewald, J.H., 2012. Plant-made therapeutics: an emerging platform in South Africa. *Biotechnology Advances,* 30(2):449-459.

Saalu, L.C., 2010. The incriminating role of reactive oxygen species in idiopathic male infertility: an evidence based evaluation. *Pakistan Journal of Biological Sciences*, 13:413-422.

Safarinejad, M.R., 2009. Efficacy of coenzyme Q10 on semen parameters, sperm function and reproductive hormones in infertile men. *Journal of Urology*, 182:237–48.

Safarinejad, M.R., 2012. The effect of coenzyme Q10 supplementation on partner pregnancy rate in infertile men with idiopathic oligoasthenoteratozoospermia: an open-label prospective study. *International Urology and Nephrology*, 44:689–700.

Salway, J.G., 2004. *Metabolism at a glance,* 3rd edn. Alden, Mass: Blackwell Publications.

Sanchez, E.E.T., Marquette, M.L., Brown, D.B., Ansari, N.H., 2006. The effect of oxidative stress on human sperm morphology. *Fertility and Sterility*, 86(1):444.

Sanocka, D., & Kurpisz, M., 2004. Reactive oxygen species and sperm cells. *Reproductive Biology and Endocrinology*, http://dx.doi.org/10.1186%2F1477-7827-2-12

Santen, R.J., & Bardin, C.W., 1973. Episodic luteinizing hormone secretion in man: pulse analysis, clinicalinterpretation, physiologic mechanisms. *Journal of Clinical Investigations*, 52:2617.

Saraswathi, R., & Devaraj, S.N., 2013. Oxidative stress in skeletal muscle impairs mitochondrial function in alloxan indiced diabetic rats: Role of alpha lipoic acid. *Biomedicine and preventative Nutrition,* 3(3):213-219.

Sathishsekar, D., & Subramanian, S., 2005. Beneficial effects of Momordica charantia seeds in the treatment of STZ-induced diabetesin experimental rats. *Biological and Pharmaceutical Bulletin*, 28:978-983.

Sato, T., Iwaki, M., Shimogaito, N., Wu, X., Yamagishi, S.I., Takeuchi, M., 2006. TAGE (toxic AGEs) theory in diabetic complications. *Current Molecular Medicine,* 6(3):351-358.

Satorius, G.A., & Nieschlag, E., 2010. Paternal age and reproduction. *Human Reproduction Update*, 16(1):65-79.

Sawyer, D.E., Mercer, B.G., Wiklendt, A.M., Aitken, R.J., 2003. Quantitative analysis of gene specific DNA damage in human spermatozoa. *Mutatation Research*, 529:21-34.

Scarano, W.R., Messias, A.G., Oliva, S.U., Klinefelter, G.R., Kempinas W.G., 2006. Sexual behaviour, sperm quantity and quality after short-term streptozotocin-induced hyperglycaemia in rats. *International Journal of Andrology,* 29(4):482-488.

Schally, A.V., Arimura, A., Baba, Y., Nair, R.M., Matsuo, H., Redding, T.W., Debeljuk, L.,1971. Isolation and properties of the FSH andLH-releasing hormone. *Biochemical and Biophysical Research Communications*, 43:393-399.

Schoeller, E.L., Schon, S., & Moley, K.H., 2012. The effects of type 1 diabetes on the hypothalamic, pituitary and testis axis. *Cell Tissue Research*, 349:839-847.

Schroeter, H., Boyed, C., & Spencer, J.P., 2002. MAPK signalling in neurodegeneration: influences of flavonoids and nitric acid. *Neurobiology Aging,* 23:861-880.

Schulte, R.T., Ohl, D.A., Sigman, M., Smith, G.D., 2010. Sperm DNA damage in male infertility: etiologies, assays, and outcomes. *Journal of Assisted Reproduction Genetics*, 27:3-12.

Seiler, P., Cooper, T.G., & Nieschlag, E., 2000. Sperm number and condition affect the number of basal cells and their expression of macrophage antigen in the murine epididymis. *International Journal of Andrology,* 23:65-76.

Sentman, C.L., Shutter, J.R., Hockenbery, D., Kanagawa, O., Korsmeyer, S. J., 1991. Bcl-2 inhibits multiple forms of apoptosis but not negative selection in thyocytes. *Cell*, 67:879-888.

Serra, C., Tangherlini, F., Rudy, S., Lee, D., Toraldo, G., Sandor, N.L.,Zhang, A.,Jasuja, R.,Bhasin, S., 2013. Testosterone improves the regeneration of old and young mouse skeletal muscle. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 68: 17-26.

Sharma, R.K., & Agarwal, A., 1996. Role of reactive oxygen species in male infertility.*Urology*, 48:835-850.

Sharpe, R.M., McKinnel, C., Kivlin, C., Fisher, J.S., 2003. Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. *Reproduction*, 125(6)769-784.

Shaw, J.E., Sicree, R.A., & Zimmet, P.Z., 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*, 87:4-14.

Shokri, S., Aitken, R.J., Abdolvahhabi, M., Abolhasani, F., Mohammad Ghasemi, F., Kashani, I., Ejtemaeimehr, S., Ahmadian, S., Minaei, B., Naraghi, M.A., Barbarestani, M.,2010.Exercise and supraphysiological dose of nandrolone deconoate increase apoptosis in spermatogenic cells.*Basic Clinical Pharmacology and Toxicology,* 106:324-330.

Shum, W.W., Da Silva, N., Brown, D., Breton, S., 2009. Regulation of luminal acidification in the male reproductive tract via cell-cell crosstalk. *The Journal of Experimental Biology*, 212:1753-1761.

Siemons, L.J., & Mahler, C.H., 1987. Hypogonadotropic hypogonadism in hemochromatosis: recovery of reproductive function after iron depletion. *Journal of Clinical Endocrinology Metabolism*, 65:585-590.

Sikka, S.C., 2001. Relative impact of oxidative stress on male reproductive function.*Current Medical Chemistry*, 8:851-862.

Sikka, S.C., 2001. Relative impact of oxidative stress on male reproductive function. *Current Medicinal Chemistry*, 8:851-862.

Sikka, S.C., Rajasekaran, M., & Hellstrom, W.J., 1995. Role of oxidative stress and antioxidants in male infertility. *Journal of Andrology,* 16:464-468.

Simoni, M., Gromoll, J., & Nieschlag, E., 1997. The follicle-stimulating hormone receptor: Biochemistry, molecular biology, physiology and pathophysiology. *Endocrine Review,* 18:739-773.

Singab, A.N., El-Beshbishy, H.A., Yonekawa, M., Nomura, T., Fukai, T., 2005. Hypoglycaemic effect of *Egypytian Morus alba* root bark extract: Effect on diabetes and Lipid

peroxidation of streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*, 100:333-338.

Singh, J., Cumming, E., Manoharan, G., Kalasz, H., Adeghate, E., 2011. Medicinal chemistry of the anti-diabetic effects of Memordica charantia: active constituents and modes of action. *Medical Chemistry,* 5(2):70-77.

Singh, Y., 1999. *Hypoxis*: Yellow stars of horticulture, folk remedies and conventional medicine. *Veld and Flora*, 85:123-125.

Singh, Y., 2007. *Hypoxis* (Hypoxidaceea) in Southern Africa: taxonomic notes. *Southern African Journal of Botany,* 73:360-365.

Smith, B.J., Albrecht, C.F., Liebenberg, R.W., Kruger, P.B., Firestone, M., Gouws, L., Theron, E., Bouic, P.J., Atsebeth, S., Van Jaarsveldt, P.P., 1995. A phase 1 trial of hypoxoside and an oral prodrug for cancer therapy- absence of toxicity. *South African Medical Journal,* 85:865-870.

Smith, T.J., Clifton, D.K., & Steiner, R.A., 2006. Regulation of the neuroendocrine reproductive axis by kesspeptin- GPR54 signaling. *Reproduction*, 131:623-630.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., 1985. Measurement of protein using bicinchoninic acid. *Analytical Biochemistry,* 150(1):76-85.

Smith, L.B., & Walker, W.H., 2014. The regulation of spermatogenesis by androgens. *In Seminars in Cell & Developmental Biology*, 30:2-13.

Soawaros, W., & Panyim, S., 1979. The formation of disulfide bonds in human protamines during sperm maturation. *Experientia*, 35:191-192.

Sooriakumaran, P., Sievert, K.D., Srivastava, A., Tewari, A., 2012. *Applied Anatomy of the Prostate*. *ABC of Prostate Cancer,* 1st edn., Dasgupta, P., & Kirby, R.S., (eds). Blackwell Publishing Ltd.

Souci, S.W., Fachmann, W., & Kraut, H., 2000. Food Composition and Nutrition Tables. Stuttgart: CRC Press.

Soudamani, S., Malini, T., & Balasubramanian, K., 2005. Effects of streptozotocin- diabetes and insulin replacement on the epididymis of prepubertal rats: histological and histomorphometric studies. *Endocrine Research*, 31:81-98.

Southern Africa Developmental community (SADC), 2002. SADC Ministerial Consultative Meeting on Nutrition and HIV/AIDS. SADC, Johannesburg.

Srivastava, S.K., Ramana, K.V., & Bhatnagar, A., 2005. Role of aldose reductase and oxidative damage in diabetes and the consequent potential for therapeutic options. *Endocrine Reviews,* 26(3):380-392.

Steenkamp, V., 2003. Phytomedicines for the prostate. *Fitoterapia,* 74:545-552.

Steenkamp, V., Gouws, M.C., Gulumian, M., Elgorashi, E.E., Van Staden, J., 2006. Studies on antibacterial, anti-inflammatory and antioxidant activity of herbal remedies used in the treatment of benign prostate hyperplasia and prostatitis. *Journal of Ethnopharmacology,* 103:71-75.

Street, R.A., & Prinsloo, G., 2013. Commercially Important Medicinal Plants of South Africa: A Review. *Journal of Chemistry,* http://dx.doi.org/10.1155/2013/205048

Street, R.A., Stirk, W.A., & Van Staden, J., 2008. South African traditional medicinal plant trade- challenges in regulating quality, safety and efficacy. *Journal of Ethnopharmacology*, 119:705-710.

Su, J., Zhang, P., Zhang, J.J., Qi, X.M., Wu, Y.G., Shen, J.J., 2010. Effects of total glucosides of paeony on oxidative stress in the kidney from diabetic rat. *Phytomedicine*, 17(3):254-260.

Sudha, S., Valli, G., Julie, P.M., Arunakaran, J., Govindarajulu, P., Balasubramanian, K., 2000. Influence of streptozotocin-induced diabetes and insulin treatment on the pituitary-testicular axis during sexual maturation in rats. *Experimental and Clinical Endocrinology & Diabetes*, 8(1):14-20.

Suleiman, S.A., Ali, M.E., Zaki, Z.M., el-Malik, E.M., Nasr, M.A., 1996. Lipid peroxidation and human sperm motility: Protective role of vitamin E. *Journal of Andrology*, 17:530-537.

Sullivan, R., 2004. Male fertility markers, myth or reality. *Animal Reproduction science*, 82-83:341- 347.

Sullivan, R., Frenette, G., & Girouard, J., 2007. Epididymosomes are involved in the acquisition of new sperm proteins during epididymal transit. *Asian Journal of Andrology*, 9:483-491.

Sun, J.G., Jurisicova, A., & Casper, R.F., 1997. Deletion of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro. *Biology Reproduction*, 56:602-7.

Suthagar, E., Soudamani, S., Yuvaraj, S., Ismail, K.A., Aruldhas, M.M., Balasubramanian, K., 2009. Effects of streptozotocin (STZ)-induced diabetes and insulin replacement on rat ventral prostate. *Biomedicine & Pharmacotherapy,* 63:43-50.

Szasz, T., Thakali, K., Fink, G.D., Watts, S.W., 2007. A comparison of arteries and veins in oxidative stress: producers, destroyers, function and disease. *Experimental Biology and Medicine,* 232(1):27-37.

Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action of β-cells of the rat pancreas*. Physiological Research*, 50(6):537:546.

Te Velde, E.R., & Pearson, P.L., 2002. The variability of female reproductive ageing. *Human Reproduction Update*, 8:141–54.

The Male Infertility Best Practice Policy Committee of the American Urological Association and the Practice Committee of the American Society for Reproductive Medicine, 2008. Report on optimal evaluation of the infertile male*. Fertility and Sterility*, 86(4):111- 129.

Thomas, S.R., Neuzil, J., & Stocker, R., 1997. Inhibition of LDL oxidation by ubiquinol-10.A protective mechanism for coenzyme Q in atherogenesis? *Molecular Aspects of Medicine*, 18:85-103.

Tiwari, A.K., & Rao, J.M., 2002. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current Science*, 83(1):30-37.

Tiwari, B.K., Pandey, K.B., Abidi, A.B., Rizvi, S.I., 2013. Markers of Oxidative Stress during Diabetes Mellitus. *Journal of Biomarkers,* http://dx.doi.org/10.1155/2013/378790

Tolba E.A., 2014. Protective effect of Mulberry leaf extract on male rat fertility affected with Methotrexate toxicity. *Egyptian Academic Journal of Biological sciences, Physiology and Molecular Biology,* 6(1):115-121.

Tomás, G.D.C., 2013. *Male fertility, Type 2 Diabetes mellitus: effect of White tea*. Masters Dissertation, University of Beira Interior. Available from: https://ubithesis.ubi.pt/bitstream/

Torta, G.J., & Derrickson, B.H., 2012. *Essentials of anatomy and Physiology*. International student edition, 9th edn, New York: Wiley.

Toshimori, K., 2003. Biology of spermatozoa maturation: an overview with an introduction to this issue. *Microscopy Research and Technique,* 61(1):1-6.

Tramer, F., Rocco, F., Micali, F., Sandri, G., Panfili, E., 1998. Antioxidant systems in rat epididymal spermatozoa. *Biology of Reproduction*, 59:753-758.

Treuting, P.M., & Dintzis, S.M., 2011. *Comparative anatomy and histology: A mouse and human atlas*. Elsevier Science, Burlington.

Tsai, W.W., Niessen, S., Goebel, N., Yates, J.R., Guccione, E., Montminy, M., 2013. PRMT5 modulates the metabolic response to fasting signals. *Proceeding of the National Academy of Sciences of the United States of America*, 110:8870-8875.

Tunc, O., Bakos, H.W., & Tremellen, K., 2011. Impact ofbody mass index on seminal oxidative stress. *Andrologia,* 43:121-128.

Turner, T.T., 2008. De Graaf's thread: the human epididymis. *Journal of Andrology*, 29 (3):237-250.

Turner, T.T., Johnston, D.S., & Jelinsky, S.A., 2006. Epididymal genomics and the search for a male contraceptive. *Molecular and Cellular Endocrinology*, 250(1-2):178-183.

Twigg J, Irvine DS, Houston P, Fulton, N.,Michael, L.,Aitken, R.J., 1998. Iatrogenic DNA damage induced in human spermatozoa during sperm preparation: protective significance of seminal plasma. *Molecular Human Reproduction*, 4:439-445.

Twigg, J., Irvine, D.S., & Aitken, R.J., 1998. Oxidative damage to DNA in human spermatozoa does not preclude pronucleous formation at intracytoplamic sperm injection. *Human Reproduction*, 13:1864-1871.

Umpierrez, G.U., Murphy, M.B., & Kitabchi, A.E., 2002. Diabetic ketoacidosis and hyperglycemic hyperosmolar syndrome. *Diabetes Spectrum*, 15(1):28-36.

Valko, M., Leibfritz, D., Moncola, J., Cronin, M.T.D., Mazura, M., Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry and Cell Biology,* 39:44-84.

Van Langendonckt, A., Casanas-Roux, F., & Donnez, J., 2002. Oxidative stress and peritoneal endometriosis. *Fertility and Sterility,* 77:861-870.

Van Wyk, B.E., 2008. A broad review of commercially important South African medicinal plants. *Journal of Ethnopharmacology,* 119:342-355.

Van Wyk, B.E., Van Oudtshoorn, B., & Gericke, N., 2002. Medicinal Plants of South Africa. Briza Publications, Pretoria, 2:156–157.

Veldhuis, J.D., 1997. Male hypothalamic-pituitary-gonadal axis. In: Lipshultz, L.I., & Howards S.S., (eds). *Infertility in the male*. Mosby-Year Book, St. Louis, MO, 3:23-58.

Verschaeve, l., & Van Staden, J., 2008. Mutagenic and antimutagenic properties of extracts from South African traditional medicinal plants. *Journal of Ethnopharmacology,* 119:575-587.

Verschaeve, L., Mertens, B., Ndhlala, A.R., Anthonissen, R., Gorissen, B., Van Staden, J., 2013. Investigation into genotoxicity of water extracts from *Hypoxis* species and a commercially available *Hypoxis* preparation. *Phytotherapy Research,* 27:350-356.

Viassara, H., & Palace, M.R., 2002. Diabetes and advanced glycation end products. *Journal of Internal Medicine,* 251:87-101.

Vincent, A.M., Russell, J.W., Low, P., Feldman, E.L., 2004. Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocrine Reviews*, 25:612-628.

Von Horsten, H.H., Johnson, S.S., San Francisco, S.K., Hastert, M.C., Whelly, S.M., Cornwall, G.A., 2007. Oligomerization and transglutaminase cross-linking of the cystatin

CRES in the mouse epididymal lumen: Potential mechanism of extracellular quality control. *The Journal of Biological Chemistry*, 282(45):32912-32923.

Walczak-Jedrzejowska, R., Wolski, J.K., & Slowikowska-Hilczer, J., 2013. The Role of Oxidative Stress and Antioxidants in Male Fertility. *Central European Journal of Urology*, 66:60-67.

Wang, X., Sharma, R.K., Sikka, S.C., Thomas, A.J., Falcone, T., Agarwal, A., 2003. Oxidative stress is associated with increased apoptosis leading to spermatozoa DNA damage in patients with male factor infertility. *Fertility and Sterility*, 80: 531-535.

Wang, R.S., Yeh, S., Chen, L.M., Lin, H.Y., Zhang, C., Ni, J., Wu, C.C., di Sant 'Agnese, P.A., deMesy- Bentley, K.L., Tzeng, C.R., Chang, C., 2006. Androgen receptor in sertoli cell is essentialfor germ cell nursery and junctional complex formation in mouse testes. *Endocrinology,* 147:5624-5633.

Wang, R.S., Yeh, S., Tzeng, C.R., Chang, C., 2009. Androgen receptorroles in spermatogenesis and fertility: lessons fromtesticular cell-specific androgen receptor knockoutmice. *Endocrine Reviews*, 30:119-132.

Watkins, P.J., Drury, P.L., & Taylor, K.W., 1990. *An Overview of management. In: diabetes and its Management*. ED Blackwell Scientific Publication, 4:63-66.

Weber, N., Weitkamp, P., & Mukherjee, K.D., 2002. Cholesterol-lowering food additives: lipase catalysed preparation of phytosterol and phytostanol esters. *Food Research Internatinal,* 35:177-181.

Wei, L., Lu, Y., He, S., Jin, X., Zeng, L., Zhang, S., Chen, Y., Tian, B., Mai, G., Yang, G.,Zhang, J.,Wang, L.,Li, H.,Markmann, J.F.,Cheng, J.,Deng, S., 2011. Induction of diabetes with signs of autoimmunity in primates by the injection of multiple-low-dose streptozotocin. *Biochemical and Biophysical Research Communications,* 4(12):373-378.

Weiss, R.B., 1982. Streptozotocin: A review of its pharmacology, efficacy, and toxicity. *Cancer Treatment Report*, 66:427-438.

White, R.B., & Fernald, R.D., 1998. Genomic structure and expression sites of three gonadotropin-releasing hormone genes in one species. *General and Comparative Endocrinology*, 112:17-25.

Wiener-Megnazi, Z., Auslender, R., & Dirnfeld, M., 2012. Advanced paternal age and reproductive outcome. *Asian Journal of Andrology*, 14(1):69-76.

Wiernsperger, N.F., 2003. Oxidative stress as a therapeutic target in diabetes: revisiting the controversy. *Diabetes Metababolism,* 29:579-585.

Wildman, R.P., Wang, D., Fernandez, I., Mancuso, P., Santoro, N., Scherer, P.E., Sowers, M.R., 2013. Associations of testosterone and sex hormone binding globulin with adipose tissue hormones in midlife women. *Obesity*, 21(3):629-636.

Willcox, J.K., Ash, S.L., & Catignani, G.L., 2004. Antioxidants and prevention of chronic disease, Review. *Critical Reviews of Food Sciences and Nutrition,* 44:275-295.

Williams, V.L., Balkwill, K., & Witkowski, E., 2000. Unravelling the commercial market for medicinal plants and plant parts on the Witwatersrand, South Africa.*Economical Botany,* 54:310-327.

Wilt, T.J., Ishani, A., MacDonald, R., Stark, G., Murlow, C.D., Lau, J., 2011. Beta- sitosterols for benign prostate hyperplasia- review. John Wiley and Sons, Ltd. Minnesota.

Winters, S.J., & Moore, J.P., 2004 .Intra-pituitary regulation of gonadotrophs in male rodents and primates. *Reproduction,* 128:13-23.

Wistabu, J., Stukenborg, J., & Luetjens, C.M., 2007. Mammalian spermatozoa.*Functional Development and Embryology,* 1(2):99-117.

World Health Organization, 1994. *Management of diabetes mellitus: standards of care and clinical practice guidelines*. WHO Regional Office for the Eastern Mediterranean, Alexandria, Egypt, WHO-EM/DIN6/E/G

World Health Organisation, 1999. *Definition, diagnosis and classification of Diabetes mellitus and its complications*. Report of WHO study group. Geneva.

World Health Organization, 2000. *WHO Manual for the Standardised Investigation, Diagnosis and Management of the Infertile Male.*Cambridge: Cambridge University Press.

World Health Organization, 2002. *Global Assessment of the State-of-the Science of Endocrine Disruptors*. International Programme on Chemical Safety. Geneva.

http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en

World Health Organization, 2002. Diabetes: the cost of diabetes (Fact sheet No. 236), http://www.who.int/mediacentre/factsheets/fs236/en/

Yanagamachi, R., 1994. Mammalian fertilization. In Knobill, E., & O'Brien, N.J., (eds). *The Physiology of Reproduction*. New York, Raven Press.

Yanagimachi, R.J., 1981. Mechanisms of Fertilization in Mam- mals. In: Mastroianni, L., & Biggers, J.D., (eds), *Fertilization and Embryonic Development in Vitro*. Plenum Press, New York, 81-182.

Yeh, S., Tsai, M.Y., Xu, Q., Mu, X.M., Lardy, H., Huang, K.E., Lin, H., Yeh, S.D., Altuwaijri, S., Zhou, X., Xing, L., Boyce, B.F., Hung, M.C., Zhang, S., Gan, L., Chang, C., 2002. Generation and characterization of androgen receptor knockout (ARKO) mice, in in vivo model for the study of androgen functions in selective tissues. *Proceedings of the National Academy of Sciences of the United States of America,* 99:13498-13503.

Yeung, C.H., Cooper, T.G., De Geyter, M., De Geyter, C., Rolf, C., Kamischke, A., Nieschlag, E., 1998. Studies on the origin of redox enzymes in seminal plasma and their relationship with results of *in vitro* fertilization. *Molecular Human Reproduction*, 4:835-839.

Zhang, C., Liu, J., Pan, H., Yang, X., Bian, K., 2011. Mitochondrial dysfunction induced by excessive ROS/RNS-metabolic cardiovascular disease and traditional Chinese medicines intervention. *Zhongguo Zhong Yao ZaZhi*, 36: 2423-2428.

Zibula, S.M., & Ojewole, J.A., 2000. Hyperglycaemic effects of *Hypoxis* hemerocallidea (Fisch and C.A. Mey) corm 'African potato' methanolic extracts in rats. *Medical Journal of Islamic Academy of Sciences,* 13:75-78.

Zini, A., de Lamirande, E., & Gagnon, C., 1995. Low levels of nitric oxide promote human sperm capititation in vitro. *Journal of Andrology*, 16:424-431.

Zini, A., & Sigman, M., 2009. Are tests of sperm DNA damage clinically useful? Pros and cons. *Journal of Andrology*, 30:219–229.

Zohreh, N., Shahla, R., & Reza, M., 2014. The effects of *Aloe vera* extract on the sperm quality in male diabetic rats. *Bulletin of Environmental, Pharmacology and Life Sciences,* 3(3):223-228.

Zribi, N., Chakroun, N.F., Elleuch, H., Abdallah, F.B., Ben Hamida, A.S., Gargouri, J., Fakhfakh, F.,Keskes, L.A., 2011. Sperm DNA fragmentation and oxidation are independent of malondialdheyde. *Reproductive Biology and Endocrinology*, 9:47.