Cape Peninsula University of Technology

The possible therapeutic effects of vindoline on testicular and epididymal function in diabetes-induced oxidative stress male Wistar rats

By

PRISCA KACHEPE

Thesis submitted in fulfilment of the requirements for the degree
Master of Science (Biomedical Technology)

In the Faculty of Health and Wellness Sciences

At the Cape Peninsula University of Technology

Supervisor: Professor Oluwafemi Oguntibeju

Co-supervisor: Dr YG Aboua

Bellville

October 2018

DECLARATION

I, Prisca Kachepe, declare that the contents of this thesis represent my own unaided work, and that the 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 is defined as a group of metabolic disorders characterised by chronic hyperglycaemia due to insufficient production and/or action of insulin and is regarded as one of the major sources of morbidity, mortality and economic burden to the modern society. A large body of scientific evidence support the fact that oxidative stress is elevated in diabetic conditions. Oxidative stress plays a significant role in the development of secondary complications of diabetes including diabetes-linked male sexual dysfunction. The management of sexual dysfunction as a secondary complication of diabetes relies on the management of the underlying diabetic condition. Glycaemic control and increased antioxidant protection are therefore necessary in the management of diabetes-induced oxidative stress male infertility.

Pharmacological management of diabetes in form of various antihyperglycaemic, synthetic drugs has improved the outlook of diabetic patients; however, they are expensive, harbour unfavourable adverse effects and some have done little to prevent secondary complications of diabetes including diabetes-induced male sexual dysfunction. In addition to this, access to basic technologies for the management of diabetes mellitus and its secondary complications is still a challenge in low resource areas. Because of these challenges, there is a need to search for alternative remedies such as medication from natural products which are more affordable, well tolerated by the human body and are easily accessible. Medicinal plants are therefore viewed as an easily accessible and potent source of antioxidants capable of scavenging free radicals and fighting diabetes-induced oxidative stress. This study therefore investigated the effects of vindoline; an alkaloid extractable from *Cantharanthus roseus* in ameliorating diabetes-induced oxidative stress effects in testicular and epididymal tissues using male Wistar rats.

Forty-eight (48), 6-week old male Wistar rats weighing between 190-230g with a conventional microbial status were divided into 6 groups, n=8, and used for this research

project. Group 1 was the normal control, group 2 comprised non-diabetic rats treated with vindoline, and group 3 was the non-diabetic group of rats treated with glibenclamide- the standard drug for the treatment of diabetes. Group 4 was the diabetic control, group 5 comprised diabetic rats treated with vindoline and group 6 was the diabetic group of rats treated with glibenclamide. Diabetes was induced in group 4, group 5 and group 6 rats by subjecting them to 10% fructose water over a period of 2 weeks and thereafter, administering a single intraperitoneal injection of 40 mg/kg b.w streptozotocin (STZ). Fasting blood glucose levels were measured 72 hours after STZ injection and hyperglycaemia was confirmed where fasting blood glucose levels were more than 18mmol/l.

The diabetic control (group 4) had higher fasting blood glucose levels, lower body weights as well as lower testicular and epididymal weights in comparison to the normal control (group 1). Additionally, the extent of lipid peroxidation in testicular and epididymal tissues of the diabetic control (group 4) was higher in comparison to that of the normal control (group 1). The diabetic control had lower testicular and epididymal antioxidant enzyme activities (superoxide dismutase and catalase) and lower oxygen radical absorption capacity (ORAC) in comparison to the normal control. Ferric reducing antioxidant power (FRAP) in testicular and epididymal tissues of the diabetic control (group 4) were not significantly different from those of the normal control (group 1).

Treatment of diabetic rats with vindoline (group 5) for 5 weeks significantly reduced fasting blood glucose levels although the extent of reduction could not restore diabetic blood glucose levels to near-normal levels. Overall, treatment of diabetic rats with vindoline was able to minimise testicular oxidative stress as reflected by reduction in testicular malondialdehyde (MDA) levels. Furthermore, results of this study showed an increase in both testicular and epididymal catalase activities, an increase in epididymal SOD, an increase in testicular ORAC as well as an increase in both testicular and epididymal FRAP levels after 5 weeks of treating diabetic rats with vindoline (group 5). Epididymal lipid peroxidation levels, epididymal ORAC levels and testicular SOD levels of diabetic rats treated with vindoline (group 5) were

however not significantly different from those of the diabetic control (group 4). Treatment of diabetic rats with vindoline or glibenclamide could not restore total body weights and testicular weights of group 5 and group 6 rats respectively, to near-normal levels. Furthermore, epididymal weights and testicular SOD activity of diabetic rats treated with vindoline (group 5) were not significantly different from those of the normal control (group 1).

In conclusion, findings from this study demonstrated that treatment with vindoline could have protective effects against diabetes-induced oxidative stress in both testicular and epididymal tissues of male Wistar rats. Vindoline can therefore be considered a potential agent for the management of diabetes-induced oxidative stress male sexual dysfunction. Further studies with advanced technologies are however recommended to study the possible efficacy of vindoline in ameliorating diabetes-induced oxidative stress male sexual dysfunction. Furthermore, studies on the dose-dependent effects and long-term effects of vindoline administration on male reproductive function as well as the overall safety of treatment with vindoline are necessary.

Keywords: Diabetes mellitus, Oxidative stress, Vindoline, *Catharanthus roseus,* Testicular tissue, Epididymal tissue.

ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude to:

- The Almighty God for granting me the opportunity to persue this Masters Degree, his love, protection, gift of knowledge and the grace to successfully finish this degree against all odds.
- My supervisor Professor Oluwafemi Oguntibeju for his dedication, efforts, guidance and encouragement. His input cannot be overlooked and I could not have made it without him.
- My co-supervisor Dr Yapo G. Aboua for his support and guidance.
- The Oxidative Stress Laboratory Manager Mr Fanie Rautenbach for his technical assistance during the practical aspects of this research. His input is highly appreciated.
- Mr Godfrey Kaperezo for encouraging me to study towards this Masters degree, his constant support throughout the course of this study and inspiring me to be a success.
- My parents Mr and Mrs Kachepe for their encouragement, helping me with the kids and always reminding me that I can do all wonderful things through Christ who strengthens me.
- Miss Medline Goboza for her advice and guidance on how I can successfully complete my Masters degree.
- My brother, Mr Perfect Kachepe and young sisters Tendai Kachepe and Kudzai Kachepe for their encouragement and always reminding me that I am their role model.

DEDICATION

To my two daughters

Chloe Dadiso Kaperezo and Salome Ruvarashe Kaperezo.

TABLE OF CONTENTS

CHAPTER THREE: RESEARCH DESIGN AND METHODOLOGY

LIST OF FIGURES

LIST OF TABLES

GLOSSARY

Terms/Acronyms/Abbreviations	Definition/Explanation
AGEs	Advanced Glycation End-products
AGIs	Alpha-glucosidase inhibitors
AMP	Adenosine monophosphate
BCA	Bicinchoninic Acid
BHT	Butylated hydroxyl toulene
C. roseus	*Catharanthus roseus*
cAMP	cyclic adenosine 3'- 5' monophosphate
CoQ10	Coenzyme Q-10
Cu·	Copper
Cu-Zn SOD	Copper-zinc superoxide dismutase
DNA	Deoxyribonucleic acids
DPP-4	Di-peptidyl peptidase- 4
EC-SOD	Extracellullar superoxide dismutase
Fe	Iron
FRAP	Ferric reducing antioxidant power
GIP	Glucose-dependant insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GPx	Glutathione peroxidase
GSH	Reduced glutathione
GSSG	Oxidised glutathione
H_2O_2	Hydrogen peroxide
kATP	Potassium adenosine triphosphate channels
LPO	Lipid peroxidation
MDA	Malondialdehyde
Mn-SOD	Manganese superoxide dismutase
MtDNA	Mitochondrial deoxyribonucleic acids

ORAC	Oxygen radical absorbance capacity
PHGPx	Phospholipid hydroperoxides glutathione peroxidase
PPAR	Peroxisome Proliferator Activator Receptor
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxygen species
SGLT2-inhibitors	Sodium-glucose co-transporter inhibitor
SOD	Superoxide dismutase
SRC	Standard Rat Chow
Std	Standard
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TBARS	Thiobarbituric Acid Reactive Substances
TZDs	Thiozolinediones
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1. Background

Diabetes mellitus is a potentially devastating, incurable yet treatable lifelong metabolic disorder whose prevalence is rapidly increasing (Al-Attar & Zari, 2010; Bommer et al., 2018). In the last few decades, the worldwide prevalence of diabetes has increased enormously due to a multitude of factors including general population growth, urbanisation, aging and an increase in obesity cases as a consequence of physical inactivity (Tiwari et al., 2013). In 2014, the global prevalence of diabetes amongst adults aged 18 and above was 422 million, in comparison to 108 million in 1980. These results show that the global age-standardized (adults aged 18 and above) prevalence of diabetes rose from 4.7% to 8.5% between the years 1980 and 2014 (World Health Organisation, 2016).

Diabetes has become one of the major sources of morbidity, mortality and economic burden to the modern day world (World Health Organisation, 2014; da Rocha et al., 2016; Ogurstova et al., 2017). In the year 2000, the prevalence of diabetes mellitus in the World Health Organization African region was estimated to be 7.02 million (World Health Organisation, 2008). Amongst these, 113 100 people died as a result of diabetes-related causes, 561 600 people suffered permanent disability while 6 458 400 experienced temporary disability as a consequence of diabetes mellitus (Kirigia et al., 2009). In 2015, the worldwide economic burden of diabetes was estimated to be USD1.31 trillion, a value that accounted for approximately 1.8% of the global gross domestic product with an estimated two thirds of these costs attributed to direct medical costs for the treatment of diabetes mellitus and the remaining third related to loss of productivity (Bommer et al., 2017).

The incidence of diabetes has been shown to rise in association with the increasing incidence of infertility amongst men of reproductive age (Agbaje et al., 2007). Diabetes has been established as one of the risk factors for the development of male sexual dysfunction.

Multiple studies using both diabetic men and animal models demonstrate that diabetes mellitus has detrimental effects on male reproductive function (La-Vignera et al., 2012; Ukwenya et al., 2013; Ghosh et al., 2014; Giribabu et al., 2014; Jain & Jangir, 2014; Ding et al., 2015) . A large body of scientific evidence support the fact that oxidative stress is elevated in diabetic conditions as a consequence of the overproduction of reactive oxygen species (ROS) without adequate neutralization of these harmful chemical entities by the body`s antioxidant system (Rochette et al., 2014; Asmat et al., 2015; Chikezie et al., 2015). This oxidative stress plays a pivotal role in the development of diabetes secondary complications (Giacco & Brownlee, 2010). Multiple studies indicate that diabetes-induced oxidative stress has a negative impact on male reproductive capacity (Chatterjee et al., 2012; Suresh et al., 2013; Singh et al., 2013; Ukwenya et al., 2013, Giribabu et al., 2014; Hajizadeh et al., 2014; Shah & Khan, 2014; Maresch et al., 2018). Diabetes-induced oxidative stress can compromise spermatozoal quality, alter testicular morphology, alter spermatogenesis, reduce testosterone levels, reduce libido and promote retrograde ejaculation (La-Vignera et al., 2012; Jain & Jangir, 2014; Oliveira et al., 2015; Elabbady et al., 2016). Oxidative stress generated in diabetic conditions is also responsible for the oxidation of spermatozoal lipids, proteins and deoxyribonucleic acids (DNA). Peroxidative damage of spermatozoal membrane lipids impairs spermatozoal motility while DNA oxidation disrupts the spermatozoal genome (Bucak et al., 2010; Tvrdá et al., 2011). The oxidation of proteins on the other hand, results in site-specific amino acid modifications, altered electric charge and increased susceptibility of spermatozoa to proteolysis (Tvrdá et al., 2011). The collective effect of diabetes-induced oxidative stress may therefore precipitate infertility in male diabetic patients.

The management of sexual dysfunction as a secondary complication of diabetes relies on the management of the underlying diabetic condition (Tremellen, 2008). The maintainence of blood glucose homeostasis and increase in antioxidant levels in diabetic patients are therefore important in the management of diabetes-induced oxidative stress male infertility (Singh et al., 2013; Chaudhury et al., 2017). Consumable and behavioural therapies capable

of countering the effects of diabetes-induced oxidative stress may be useful in reducing the extent of diabetic complications (Tiwari et al., 2013) including diabetes-linked male infertility.

Lifestyle management is the major therapeutic option for diabetes and involves regular exercise and weight control measures amongst others (American Diabetes Association, 2017). Besides lifestyle management, pharmacological management of diabetes in form of insulin therapy and the use of hypoglycaemic drugs have improved the outlook of diabetic patients; however, synthetic antidiabetic drugs are expensive, harbour unfavourable side effects and in some cases have done little to prevent the development of diabetes-related secondary complications (Siddiqui et al., 2013; Chaudhury et al., 2017). In addition to this, lack of access to insulin and basic technologies crucial for the management of diabetes remain a key impediment to the successful treatment of diabetes with oral hypoglycaemic agents and insulin being reported as generally available in only a minority of low income countries (World Health Organisation, 2016).

Because of these challenges in the management of diabetes, the search for alternative remedies such as medications from natural products is paramount (Saxena & Kishore, 2004). An antioxidant capable of reducing oxidative stress could be useful in the management of oxidative stress-induced male infertility (Bansal & Bilaspuri, 2011). Due to their hypoglycaemic and antioxidant potential, a variety of medicinal plants have been used in the management of diabetes mellitus (Singh et al., 2013). Medicinal plants are a rich source of bioactive constituents that can effectively lower blood glucose levels (Rizvi & Mishra, 2013). Some of their phytochemicals are viable antioxidants capable of ameliorating oxidative stress-induced diabetic complications such as testicular and epididymal oxidative stress as seen in diabetes (Oliveira et al., 2015). In view of this, the use of medicinal plants can be a source of new hope, as an affordable and easily accessible prevention and treatment method with fewer side effects.

Catharanthus roseus (L.) G. Don Linn *(C. roseus)* is an ornamental shrub commonly known as the Madagascar periwinkle, as it is native to Madagascar (Tiong et al., 2013). *C. roseus* extracts are a viable source of natural antioxidants and hence might be exploited for nutraceutical applications (Rasool et al., 2011; Patharajan & Abirami, 2014). Phytochemical analysis of *C. roseus* shows the presence of alkaloids, terpenoids, steroids, flavonoids as well as some other useful secondary metabolites (Aslam et al., 2010). Previous studies have demonstrated the antihyperglycaemic and antioxidant activities of various extracts from *C. roseus* (Rasineni et al., 2010; Vega-Vila et al., 2012; Al-Shaqha et al., 2015). Vindoline is an intermediate, semi-synthetic, vinca alkaloid, extractable from *C. roseus* that has been reported to exhibit antidiabetic properties in diabetes-induced animal models by increasing glucose-stimulated insulin release, lowering blood glucose levels, inhibiting Kv 2.1 potassium channels, decreasing the potassium ion outward current, lowering glycated haemoglobin, reducing triglyceride levels and inhibiting H^+/K^+ ATPases (Sertel et al., 2011; Tiong et al., 2013; Yao et al., 2013). Despite the available few studies on the antidiabetic and antioxidant properties of vindoline, no specific research has reported on the effects of vindoline on reproductive functions of diabetic men or diabetes-induced animal models.

1.2. Research Problem

Oxidative stress is one of the predominant causes of diabetes secondary complications amongst which is male sexual dysfunction and infertility (Giacco & Brownlee, 2010). High blood glucose levels as seen in diabetic conditions promote the generation of ROS species and in the absence of an appropriate compensatory response from the antioxidant system, loss of equilibrium in favour of oxidizing species occurs and this phenomenon is known as oxidative stress (Asmat et al., 2015). The impairment of testicular and epididymal antioxidant response in diabetic men affects male fertilizing potentials (Mallick et al., 2007; Hamden et al., 2008). Glycaemic control and increased antioxidant protection are therefore crucial in the management of diabetes-induced male infertility (Singh et al., 2013). Although the use of hypoglycaemic agents has improved the outlook of diabetic patients, many of them harbour serious adverse effects, are expensive, require expertise (Modak et al., 2007) and in some

cases have done little to control secondary complications of diabetes including diabetes-linked male infertility (Gurib-Fakim, 2006). As such, the management of diabetes without side effects remains a challenge and the search for alternative remedies such as medication from natural products is paramount (Saxena & Kishore 2004). Medicinal plants are regarded as easily accessible sources of antioxidants capable of scavenging free radicals and fighting diabetes-induced oxidative stress. Additionally, medicinal plants have proven to be clinically effective and relatively less toxic as compared to synthetic hypoglycaemic agents currently in use for the treatment of diabetes mellitus (Bhatt et al., 2013).

1.3. Aim

To investigate the possible therapeutic effects of vindoline on testicular and epididymal function, using diabetes-induced oxidative stress male Wistar rats as an experimental model.

1.4. Objectives

This study was designed to evaluate the effects of vindoline on testicular and epididymal function of both normal and diabetic male Wistar rats. The objectives of this study include the following:

i. To determine if treatment with 10% fructose water for 2 weeks and a single STZ dose can induce diabetes mellitus associated with oxidative stress in adult male Wistar rats.
ii. To investigate whether treatment with vindoline has an effect on the body, testicular and epididymal weights of diabetes-induced oxidative stress male Wistar rats.
iii. To investigate the effects of vindoline on lipid peroxidation in testicular and epididymal tissues of diabetic and non-diabetic rats as expressed by malondialdehyde (MDA) biomarkers.
iv. To assess the effect of vindoline on testicular and epididymal antioxidant enzyme activities such as superoxide dismutase and catalase, using diabetic and non-diabetic rats as experimental models.

v. To investigate if treatment with vindoline can reduce, modulate or inhibit the induced oxidative stress effects in testicular and epididymal tissues as represented by ORAC and FRAP assays.

1.5. Research Questions

i. Is treatment with 10% fructose water for 2 weeks and a single intra-peritoneal injection of STZ (40mg/kg b.w.) sufficient to destroy pancreatic β-cells thereby causing persistent hyperglycaemia?

ii. What are the effects of vindoline on the body, testicular and epididymal weights of diabetes-induced male Wistar rats and non-diabetic controls?

iii. What could be the potential impact of vindoline on lipid peroxidation, as expressed by MDA levels in testicular and epididymal tissues of diabetic and non-diabetic rats?

iv. How would antioxidant enzymes such as superoxide dismutase and catalase differ between diabetic rats treated with vindoline and untreated groups?

v. Does treatment with vindoline reduce, modulate or inhibit the induced oxidative stress effects in testicular and epididymal tissues as expressed by ORAC (Oxygen Radical Absorbance Capacity) and FRAP (Ferric Reducing Antioxidant Power) assay results?

CHAPTER 2
LITERATURE REVIEW

2.1. Diabetes

The rapidly increasing incidence of diabetes is becoming a serious threat to human health in all parts of the world (Malviya et al., 2010). Diabetes is defined as a group of metabolic disorders characterized by chronic hyperglycaemia due to insufficient production or action of insulin (American Diabetes Association, 2009). In a healthy person, blood glucose levels are regulated by several hormones, primarily insulin which is produced by the pancreas. Insulin allows glucose to move out of the blood into the cells throughout the body where it is used for fuel. In diabetic conditions, the production of insulin is not enough or the target cells are resistant to the available insulin or both. As such, blood glucose cannot move into cells effectively and hence remain at high levels in the blood; a phenomenon known as hyperglycaemia. This not only starves cells of glucose for fuel, but also harms organs like the brain and other tissues by exposing them to high blood glucose levels (Siddiqui et al., 2013).

2.2. Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (T1DM) is characterized by processes of autoimmune mediated destruction of the β-cells of the pancreas (Tomita, 2017). Because patients with T1DM require insulin for survival, T1DM is alternatively known as insulin-dependent diabetes mellitus (Asmat et al., 2015). Markers of immune destruction e.g. insulin autoantibodies and antibodies to glutamic acid decarboxylase are present in patients with T1DM (Taplin & Baker, 2008). T1DM causes a state of intracellular hypoglycaemia and extracellular hyperglycaemia (Asmat et al., 2015). Intracellular hypoglycaemia causes glycogenolysis whereby glycogen molecules are broken down to individual glucose molecules and gluconeogenesis whereby glucose is generated from non-carbohydrate sources such as amino acids, lactate and glycerol. When the body uses fat as an alternative source of energy, ketones accumulate in the blood making it acidic. This phenomenon is known as diabetic acidosis and it may lead to a coma and eventually death (Gillespies, 2006). On the other hand, extracellular

hyperglycaemia leads to a hyperglycaemic coma and osmotic diuresis (Ozougwu et al., 2013).

2.3. Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM) is the most common type of diabetes mellitus accounting for almost 90-95% of all diabetic cases (Rubino, 2008). T2DM is characterized by excessive hepatic glucose production, decreased insulin secretion as well as insulin resistance (Guillasseau et al., 2008). Unlike patients with T1DM, risk of ketoacidosis is infrequent and T2DM patients do not need insulin for survival. As such T2DM is alternatively known as non-insulin-dependent diabetes mellitus (Asmat et al., 2015). There is convincing data to indicate genetic components associated with T2DM insulin resistance (Ali, 2013; Corbi et al., 2017; Ajabnoor et al., 2018). Besides genetic inheritance, T2DM may be acquired through leading a sedentary lifestyle, obesity as well as an increase in age (Bertoglia et al., 2017). Women with prior gestational diabetes are also at increased risk for the development of T2DM (Herath et al., 2017).

Overweight and obesity aggravate insulin resistance and glucose intolerance through several pathways including hormonal imbalances such as increasing levels of leptin, glucagon and cytokines such as interleukin 6 and tumour necrosis factor-α as well as decreasing levels of adiponectin (Makki et al., 2013; Rodriguez et al., 2014). Because obesity and overweight are major contributors to insulin resistance, insulin sensitivity may be increased by increasing physical activity, weight reduction and/or pharmacological treatment of hyperglycaemia (American Diabetes Association, 2011).

2.4. Diabetes-induced oxidative stress

Hyperglycaemia promotes the generation of ROS as a consequence of increased production of mitochondrial ROS (Busik et al., 2008; Savu et al., 2011), non-enzymatic glycation of proteins (Nawule et al., 2006) and glucose auto-oxidation (Chetyrkii et al., 2011). In the absence of an appropriate compensatory response from the antioxidant system, the system

becomes overwhelmed by the oxidative effects of ROS and when the balance between oxidants and antioxidants derail for whatever reason, loss of equilibrium in favour of oxidizing radicals as seen in most cases of diabetes often leads to a phenomenon known as oxidative stress (Chikezie et al., 2015).

Diabetes-induced oxidative stress activates stress sensitive pathways that lead to the activation of the protein-kinase C isoforms β, δ and α, increase in the formation of advanced glycation end-products (AGEs) and activation of their pathways, increase in glucose flux through the polyol pathway, overactivity of the hexosamine pathway as well as decreased antioxidant defences (Giacco & Brownlee, 2010; Ceriello, 2011; Safi et al., 2014). Altogether, these metabolic pathways converge on the elevation of ROS, ultimately worsening both insulin secretion and insulin action and leading to overt T2DM (Yan, 2014).

2.5. Physiological Effects of ROS on Testicular and Epididymal Function

While spermatozoa are vulnerable to oxidative stress, physiological (low) levels of free radicals can contribute to successful spermatogenesis (Saleh et al., 2002). ROS are vital in the transduction of signals during spermatogenic biochemical cascades including processes of spermatozoal maturation, capacitation, hyperactivation, acrosome reaction, sperm-oocyte fusion, maintenance of fertilizing ability and stabilization of the mitochondrial capsule in the mid-piece (Kothari et al., 2010; Bansal & Bilaspuri, 2011). Hence, the concentration of free radicals and ROS need to be kept in a state of homeostasi, at levels appropriate to ensure their physiological function while preventing pathological damage. Figure 2.1 shows the physiological and pathophysiological roles of ROS on spermatozoa.

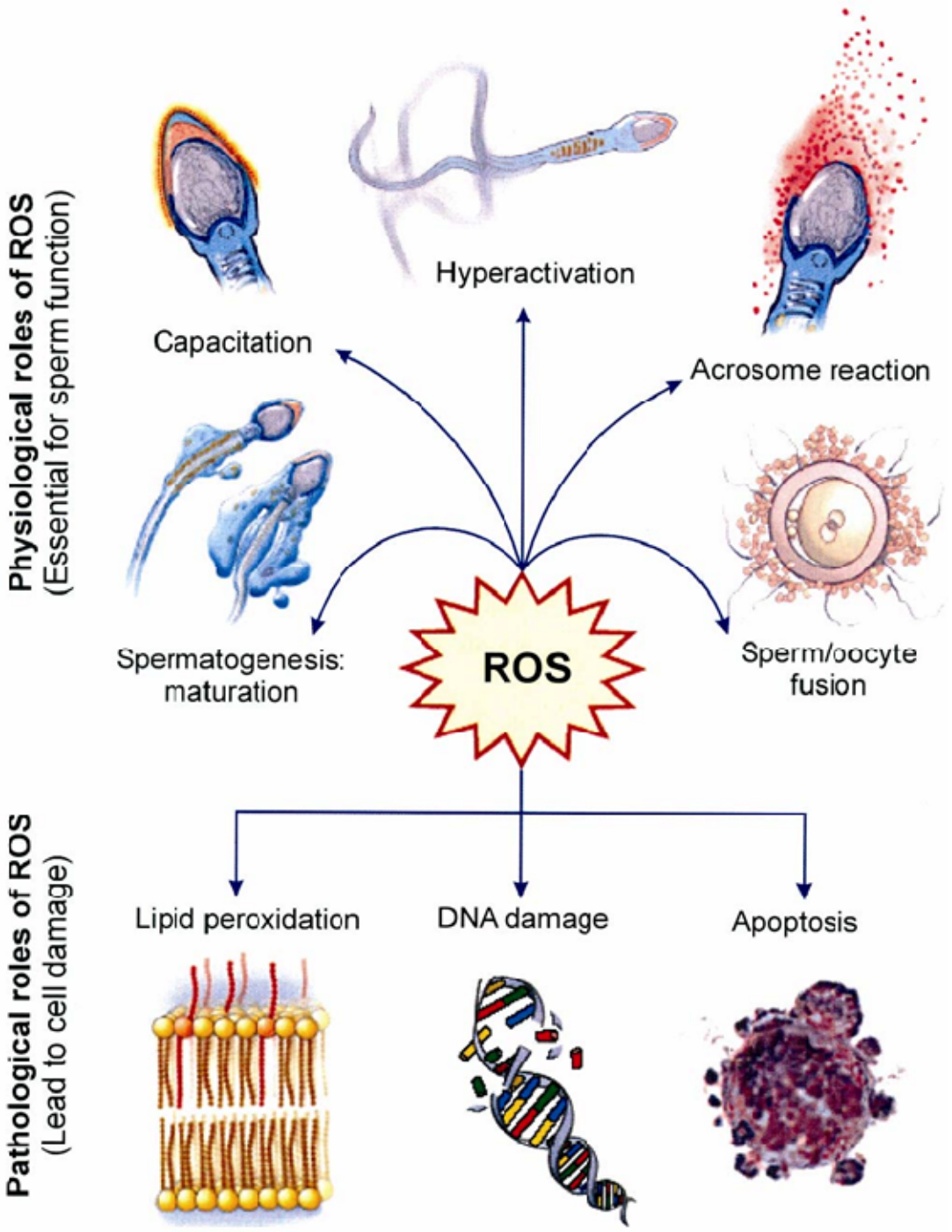

Figure 2.1: Physiological and pathological roles of ROS (Kothari et al., 2010). Physiological roles of ROS include processes of spermatozoal maturation, capacitation, hyperactivation, acrosome reaction and sperm-oocyte fusion. Pathological roles of ROS include spermatozoal membrane lipid peroxidation, DNA damage and apoptosis.

2.5.1. Maturation

During the process of spermatozoal maturation, ROS such as hydrogen peroxide (H_2O_2) contribute to the development of a keratinous-like protective coat called the membranous capsule around the mitochondria by oxidizing a phospholipid selenoenzyme called phospholipid hydroperoxide glutathione peroxidase (PHGPx), to form a chemical intermediate capable of forming a selenadisulfide bond with reduced protein thiol groups of the capsule (Roveri et al., 2001). When the mitochondrial capsule is assembled, chromatin undergoes condensation and motility is acquired for the capacitation of spermatozoa. The importance of this mitochondrial capsule lies in the fact that deletion of spermatic mitochondrial capsule selenoprotein seriously affects spermatozoal motility despite having normal sperm morphology (Baker & Aitken, 2004).

2.5.2. Capacitation

Capacitation is the penultimate process in spermatozoal maturation that ensures that only fertile spermatozoal cells are able to reach, bind and fertilize the oocyte (Baker & Aitken, 2004; O'Flaherty et al., 2015). During capacitation, spermatozoa physiologically produce controlled amounts of ROS for the regulation of downstream events such as the activation of protein kinase A and increase in cyclic adenosine 3', 5'- monophosphate (AMP) with subsequent phosphorylation of its substrates (O'flaherty et al., 2004) leading to the phosphorylation of MEK-like proteins and threonine-glutamatetyrosine, and finally tyrosine phosphorylation of fibrous sheath proteins (de Lamirande & O'flaherty 2008; Kothari et al., 2010; Chen et al., 2013). Physiological levels of ROS have also been hypothesized to enhance spermatozoal capacitation by activating tyrosine kinase, stimulating adenylyl cyclase and inhibiting the activities of PTPases. By inhibiting PTPase activity, ROS prevent the dephosphorylation and deactivation of phospholipase A2 which cleaves the secondary fatty acids from the triglycerol backbone of the membrane phospholipid thereby increasing membrane fluidity (Calamera et al., 2003; Khosrowbeygi & Zarghami, 2007; Kothari et al.,

2010). The increase in cAMP also promotes the hyperactivation of spermatozoa (Agarwal et al., 2014).

2.5.3. Hyperactivation

Increased generation of cAMP via capacitation causes hyperactivation of spermatozoa, which is essential for successful fertilization and is considered a subcategory of capacitation (Miraglia et al., 2010). Research shows that the superoxide anion and other ROS such as nitric oxide and H_2O_2 play an essential role in triggering spermatozoal Hyperactivation thereby providing spermatozoa with the necessary propulsion to penetrate the cumulus oophorous of the oocyte (Kothari et al., 2010; du Plessis et al., 2015). During hyperactivation, spermatozoa become highly motile, exhibit high amplitude and an overall increase in side-to-side head displacement. Spermatozoal motility becomes non-linear with an asymmetric flagella movement. As such, hyperactive spermatozoa are capable of penetrating the oocyte with a strong propulsive force (Suarez, 2008; Kothari et al., 2010).

2.5.4. Acrosome Reaction

After successful propulsion of hyperactivated spermatozoa past the cumulous oophorous, the capacitated spermatozoa must bind to the zona pellucida (ZP), which is a glycoprotein layer that surrounds the oocyte (Sanocka &Kurspisz, 2004; Itach et al., 2012). Capacitated spermatozoa must initiate the exocytotic release of proteolytic enzymes on the ZP (de Lamirande & O'Flaherty, 2008). Activities of ROS have been shown to increase spermatozoal affinity for the ZP. *In vitro* activation of the acrosome reaction was observed when physiological concentrations of the superoxide radical, H_2O_2, and nitric oxide were added to the seminal plasma (Bansal & Bilaspuri, 2011). These ROS activate adenylyl cyclase which triggers cAMP to initiate the exocytotic release of proteolytic enzymes thereby creating a pore in the extracellular matrix of the ZP (O'Flaherty et al., 1999; Herrero et al., 2003).

2.5.5. Sperm-oocyte Fusion

After penetration of the ZP, a high degree of spermatic membrane fluidity is essential for successful fertilization of the oocyte (Agarwal et al., 2014). The mechanism by which ROS increase membrane fluidity occurs during the processes of capacitation and acrosome reaction (Wathes et al., 2007; Kothari et al., 2010). By inhibiting PTPase activity, ROS prevent the dephosphorylation and deactivation of phospholipase A2 which cleaves the secondary fatty acids from the triglycerol backbone of the membrane phospholipid thereby increasing membrane fluidity (Calamera et al., 2003; Khosrowbeygi & Zarghami 2007).

2.6. Pathophysiological effects of ROS on Testicular and Epididymal Function

Although ROS are crucial for processes of spermatozoal maturation, capacitation, hyperactivation, acrosome reaction and sperm-oocyte fusion, their uncontrolled production (oxidative stress) produces deleterious effects on spermatozoal biomolecules leading to male infertility (Kothari et al., 2010; Bansal & Bilaspuri, 2011; Tvrdá et al., 2011). In cases where the antioxidant defence system is overwhelmed by the high levels of ROS, pathological effects result (Chitra et al., 2001). Both current and previous literature have failed to set limits that demarcate between physiological and pathological levels of ROS making the management of ROS pathological effects a difficulty (Kothari et al., 2010).

Experiments using diabetic patients and experimentally-induced diabetic animal models demonstrate that both T1DM and T2DM have detrimental effects on male fertility potentials (Agbaje et al., 2007, Mangoli et al., 2013; Ding et al., 2015; Singh et al., 2016). Diabetes-induced ROS attack testicular and epididymal tissues leading to reduced fertilizing abilites of spermatozoa. In testicular tissues, diabetes-induced ROS may disrupt the capacity of the germinal epithelium to differentiate into normal spermatozoa as well as reduce normal steroidogenic capacity of the leydig cells (Naughton et al., 2001). Diabetes-induced oxidative stress can alter testicular morphology, reduce testosterone levels, reduce libido and promote retrograde ejaculation (La-Vignera et al., 2012; Jain & Jangir, 2014; Oliveira et al., 2015; Elabbady et al., 2016). Diabetes-induced oxidative stress can impair the gonadal antioxidant

system thereby leading to testicular and epididymal oxidative damage and hindering successful spermatogenesis (Mallick et al., 2007; Hamden et al., 2008). Low levels of superoxide dismutase and catalase antioxidant enzymes have been reported in diabetic conditions and this has been associated with testicular germinal cell apoptosis (Chatterjee et al., 2012). ROS as seen in diabetes mellitus can also oxidize spermatozoal lipids, DNA and protein molecules thereby altering cell signalling pathways and enzymatic systems and producing irreparable spermatozoal cell alterations, cell death and necrosis (Tvrdá et al., 2011). Figure 2.2 below shows the impact of diabetes-induced oxidative stress on male reproductive function.

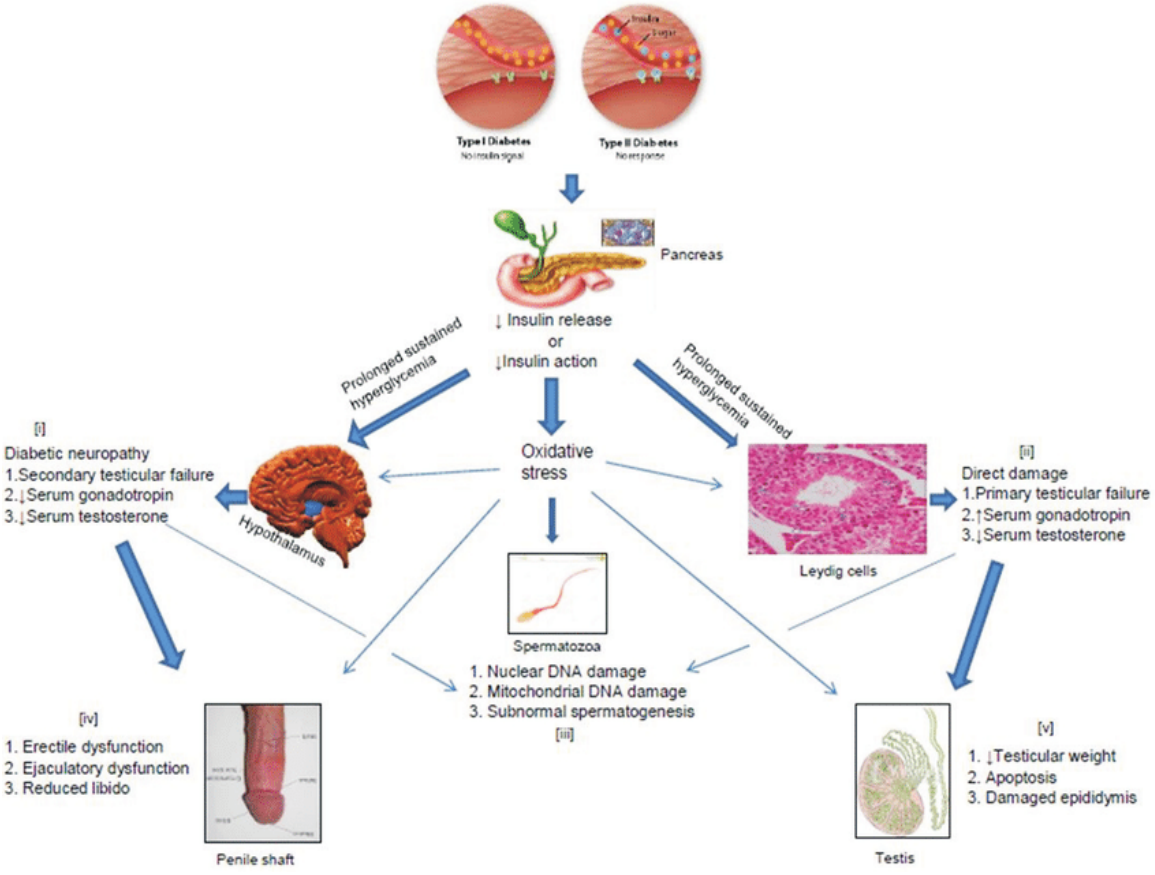

Figure 2.2: The impact of diabetes-induced oxidative stress on male reproductive function (Arokoyo et al., 2017).

2.6.1. Lipid Peroxidation

Diabetes is characterised by high levels of ROS (Asmat et al., 2015). Research shows that high levels of testicular and epididymal ROS can oxidize spermatozoal membrane lipids, a phenomenon known as lipid peroxidation (LPO) (Sanocka & Kurspisz, 2004; Kothari et al., 2010; Tvrdá et al., 2011). LPO is the key mechanism for ROS-induced spermatozoal damage that leads to male infertility. It impairs spermatozoal membrane function by inactivating membrane bound receptors and enzymes, decreasing the fluidity of spermatozoal membranes and increasing non-specific permeability to ions (Sanocka & Kurspisz, 2004). The susceptibility of spermatozoal membranes to LPO lies in the fact that the lipid membranes are largely composed of polyunsaturated fatty acids (PUFA) (Agarwal & Saleh, 2002; Bansal & Bilaspuri, 2011). The presence of the double bond in PUFA makes spermatozoal membranes prone to oxidative attack (Bucak et al., 2010).

Oxidative attack on spermatozoal lipids initiate the LPO cascade, which is an autocatalytic, self-propagating reaction capable of precipitating cellular dysfunction associated with loss of membranal function and integrity (Bansal & Bilaspuri, 2010; Agarwal et al., 2014). The LPO cascade can be divided into 3 phases namely the initiation phase, propagation phase and termination phase (Tremellen, 2008; Kothari et al., 2010). Before the cascade can be initiated, ROS energetic enough to initiate the cascade must be generated. The superoxide free radical is the predominant ROS generated within the spermatozoa. It can react by itself via dismutation to generate H_2O_2. Depending on the availability of transition metals such as iron (Fe) or copper (Cu), the superoxide free radical and H_2O_2 can undergo the Haber-Weiss reaction to generate the highly pernicious hydroxyl radical (OH•) (Badade & Samant, 2011). Alternatively, in the presence of a reducing agent such as ferrous ions or ascorbate, OH• radicals can be generated from H_2O_2, via the Fenton reaction. The presence of OH• radicals mark the initiation phase as these radicals are energetic enough to initiate the LPO cascade (Sikka, 2001; Hazout et al., 2008; Chen et al., 2013).

The initiation phase is characterized by hydrogen abstraction of PUFA by potent initiators of the LPO cascade such as the OH• radicals (Ayala et al., 2014). Thereafter, the propagation

phase follows which is characterized by the formation of lipid alkyl radicals which rapidly react with molecular oxygen leading to the formation of lipid peroxyl radicals. Lipid peroxyl radicals are energetic enough to abstract a hydrogen atom from PUFA leading to the formation of another lipid radical and lipid hydroperoxides (Ogbuewo et al., 2010). Because the peroxyl and alkyl radicals are generated in a cyclical fashion, they continue to oxidize lipid membranes indefinitely until one of the substrates is consumed or until the LPO cascade is terminated by a radical-to-radical reaction to form a stable product during the termination phase (Agarwal & Saleh, 2002; Sanocka & Kurspisz, 2004). Because LPO is self-propagating, approximately 60% of PUFA can be lost from the spermatozoal lipid membranes leading to increased non-specific permeability to ions, inactivation of membrane bound receptors and enzymes and subsequent loss of fluidity (Agarwal et al., 2014).

2.6.2. DNA damage

ROS have the potential to damage both spermatozoal nuclear DNA and mitochondrial DNA (mtDNA), however, research shows that mtDNA is more susceptible to oxidative stress because it is naked (Kumar et al., 2009). Human spermatozoal nuclear chromatin is highly condensed and organized. It is packed into nucleosomes and coiled into solenoids (Arpanahi et al., 2009). During spermatogenesis, the chromatin is modified by replacing histones with transition proteins and protamines (Miller et al., 2010). The strands of DNA are condensed by the protamines and form the basic packaging unit of spermatozoal chromatin known as the toroid which is further compacted by intra- and inter- molecular disulphide bridges (Bennetts & Aitken, 2005). This intricate compaction and organisation shields spermatozoal chromatin from oxidative damage, conferring resistance against ROS-induced DNA damage (Agarwal et al., 2014). However, some areas of poor compaction and incomplete protamination do exist and these are vulnerable to the effects of oxidative stress. These areas suffer base modifications, deletions, base-free sites, DNA crosslinks, frame-shift mutations, point mutations as well as chromosomal rearrangements (Agarwal et al., 2003; Schulte et al., 2010; Spiropoulos et al., 2002).

In contrast to the highly organized and protaminated nuclear DNA, mtDNA is not bound by histones making it more vulnerable to the effects of ROS (Sawyer & Aitken, 2001). Mitochondrial genome mutations can result in the production of excessive amounts of ROS that can disturb the formation of morphologically and functionally mature spermatozoal cells thus leading to male factor infertility (Saalu, 2010). MtDNA mutations may also cause a defect in mitochondrial energy metabolism (Chinney & Turnbull, 2000); hence, lower levels of mutant mtDNA may compromise sperm motility (Spiropoulos et al., 2002). González-Marín et al, (2012) reported that in humans, approximately 80% of structural chromosome aberrations are of paternal origin. Spermatozoal DNA damage leads to apoptosis, poor fertilization rates, high frequency of miscarriages and morbidity in offspring (Chen et al., 2013).

2.6.3. Apoptosis

Apoptosis is a natural physiological process by which the body gets rid of old, senescent cells including abnormal germ cells thereby preventing their overgrowth during spermatogenesis (Agarwal & Allamaneni, 2006). Apoptosis is a non-inflammatory response to tissue damage characterised by a series of morphological and biochemical modifications that promote controlled cell death (Majai et al., 2006; Davidovich et al., 2014). High levels of ROS promote spermatozoal apoptosis thereby playing an important role in the development of male infertility (Agarwal & Allamaneni, 2006).

Mitochondria are important generators of spermatozoal ROS and their activity become enhanced when spermatozoal cells become apoptotic. As such, the generation of ROS by mitochondria is one of the early signs that spermatozoal cells have engaged the intrinsic apoptotic cascade (Koppers et al., 2011). High levels of ROS as seen in diabetic situations are capable of disrupting the inner and outer mitochondrial membranes thereby stimulating the release of cytochrome C that in turn signals the release of caspases 3 and 9 which induces apoptosis (Agarwal et al., 2008). In most cases, increased spermatozoal damage by ROS is associated with higher levels of cytochrome C and caspases 3 and 9 indicating that

ROS-induced apoptosis is a significant male factor of infertility (Agarwal et al., 2008). A study by Said et al, (2004) revealed that the HOCI radical, formed from H_2O_2 and chloride ions, is associated with high levels of spermatozoal apoptosis.

2.6.4. Oxidative damage to proteins

The structure and function of proteins is susceptible to oxidative damage by ROS (Tvrdá et al. 2011). Proteins have many side chain targets that are susceptible to the oxidative effects of ROS e.g. cysteine, methionine, and tyrosine (Tiwari et al., 2013). By producing free radicals, diabetic hyperglycaemia can lead to protein glycation and oxidative degeneration (Asmat et al., 2015). ROS attack on proteins result in site-specific amino acid modifications, fragmentation of the peptide chain, altered electric charge, aggregation of cross-linked reaction products, and increased susceptibility to proteolysis (Tvrdá et al. 2011). High levels of protein carbonyls have been reported in a variety of cells and plasma in diabetic patients (Pandey et al., 2010). Oxidative damage to protein may also alter the structure and function of antioxidant enzymes. Altered enzymes cannot perform their antioxidative role hence ROS accumulate without detoxification leading to the enhancement of oxidative stress in diabetic conditions (Maritim et al., 2003).

2.7. Testicular and Epididymal Antioxidants

ROS are necessary for spermatozoa to achieve functional competence; hence ROS should be maintained at concentrations not deleterious to spermatozoa (Saleh et al., 2002). An antioxidant is defined as any substance whose availability, even in minute concentrations inhibits or delays the oxidation of a substrate (Somogyi, 2007). Antioxidants are capable of disposing, scavenging and suppressing the formation of ROS or oppose their actions (Bansal & Bilaspuri, 2011). The testes, epididymis, seminal plasma and spermatozoa contain an elaborate array of both enzymatic and non-enzymatic antioxidants to ensure spermatogenic and steroidogenic functions are not impacted by oxidative stress (Fujii et al., 2003; Garrido et al., 2004a; Aitken & Roman, 2008). These antioxidant defence systems are of great importance as peroxidative damage is currently regarded as one of the most important

causes of impaired testicular function (Aitken & Roman, 2008). Besides classifying antioxidants as either enzymatic or non-enzymatic, antioxidants can further be subdivided into fat-soluble or water-soluble antioxidants. Fat soluble antioxidants are located in cellular membranes and lipoproteins e.g. Vitamin E, β-carotene and coenzyme Q-10 while water-soluble antioxidants are found in extracellular and intracellular fluids e.g. Vitamin C, glutathione peroxidase, superoxide dismutase and catalase (Ogbuewo et al., 2010).

2.7.1. Enzymatic Antioxidants

There are three main types of enzymatic antioxidants in spermatozoa namely superoxide dismutase, catalase and glutathione peroxidase antioxidant enzymes. These play a significant role in decomposing ROS in spermatozoal cells (Tremellen, 2008).

2.7.1.1. Superoxide Dismutase (SOD)

These are metal-containing enzymes that catalyse the conversion of 2 superoxide radicals into molecular oxygen and H_2O_2 which is less toxic than the superoxide radical. In this manner, SOD protects spermatozoa against oxygen toxicity (Agarwal et al., 2004; Aitken & Roman, 2008). The anti-lipoperoxidative defence system in human spermatozoa relies almost entirely on the dimeric copper-zinc isoform of SOD (Cu-Zn SOD). This isoform is localized in the cytosol and inter-membranal space of spermatozoa. In addition to the Cu-Zn SOD, there are 2 other SOD isoforms namely the manganese SOD (Mn-SOD) and extracellular SOD (EC-SOD) which are located in the mitochondria and extracellular space respectively (Mruk et al., 2002;; Badade & Samant, 2011).

2.7.1.2. Catalase

Catalase is a common enzymatic antioxidant found in most living organisms that are exposed to oxygen. It plays a significant role against oxidative stress-generated complications such as is seen in diabetes mellitus. H_2O_2 molecules generated during energy metabolism are powerful oxidants that must be rapidly eliminated from the cell before they induce oxidative damage to cellular lipids, DNA and protein molecules (Tiwari et al., 2013).

Catalase regulates H_2O_2 metabolism by enzymatically processing it into oxygen and water molecules thereby neutralising it and completing the reaction started by SOD (Asmat et al., 2015). Catalase is usually located in cellular organelles called peroxisomes and is efficient at an optimum temperature of 37^0C. Its turnover is among the highest in comparison to other enzymes with reports stating that a single catalase molecule is capable of converting millions of H_2O_2 molecules into water and molecular oxygen per second (Badade & Samant, 2011).

2.7.1.3. Glutathione peroxidase

Glutathione peroxidase (GPx) constitutes a family of enzymatic antioxidants involved in the reduction of hydroperoxides using glutathione as an electron donor (Tremellen, 2008). GPx enzymes are present within spermatozoa, seminal plasma, the testis, seminal vesicles, prostate as well as the epididymis (Vernet et al., 2004). In differentiating spermatozoa, GPx is mostly located in the mitochondrial region, nucleus and acrosomal domains (Vaisberg et al., 2005). GPx enzymatically reduce lipid hydroperoxides into their corresponding alcohols and detoxifies H_2O_2 into water molecules (Badade & Samant, 2011).

In the testicular context, the phospholipid hydroperoxides glutathione peroxidase (PHGPx) isoform is highly expresses in both leydig and spermatogenic cells. Baek et al, (2007) and Foresta et al, (2002) reported a direct relationship between male fertility and PHGPX levels. In addition to protecting spermatozoal membranes against peroxides, GPx also permits the regeneration of spermatozoal membrane lipid molecules (Ogbuewo et al., 2010). Scientific research has correlated a reduction in spermatozoal and seminal plasma GPx activity with male factor infertility (Giannattasio et al., 2002; Garrido et al., 2004b).

Continued GPx activity depends on the reduction of glutathione by an enzyme called glutathione reductase (Labuschagne et al., 2013). Inhibition of glutathione reductase affect GPx activity by reducing the availability of reduced glutathione thereby exposing spermatozoa to the effects of oxidative stress (Williams & Ford, 2004). In this manner, the

co-ordinated activities of GPx, glutathione reductase and glutathione (as an electron donor) protect spermatozoa against oxidative attack (Dinesh et al., 2012).

2.7.2. Non-enzymatic antioxidants

While the testicles clearly do possess highly specialized antioxidant defence enzymes such as SOD, catalase and GPx, they also rely on small molecular weight, non-enzymatic antioxidants (Aitken & Roman, 2008). The antioxidative function of these non-enzymatic molecular antioxidants is particularly important in the extracellular space where enzymatic antioxidants are absent or are present in minute quantities (Ogbuewo et al., 2010). There are benefits associated with treating susceptible individuals with exogenous, small molecular weight, non-enzymatic antioxidants. Non-enzymatic antioxidants such as Vitamin C, Vitamin E, zinc, selenium, glutathione, cysteine, melatonin, coenzyme Q-10, carotenoids and carnitine can be acquired from consuming certain fruits and vegetables (Aitken & Roman, 2008; Lampiao, 2012).

2.7.2.1. Vitamin C (Ascorbic Acid)

Vitamin C is an important water-soluble, non-enzymatic, chain-breaking antioxidant capable of neutralizing OH• and superoxide radicals as well as H_2O_2 in extracellular areas of the body e.g. seminal plasma (Agarwal et al., 2004; Ogbuewo et al., 2010). The concentration of Vitamin C in seminal plasma is reported as being 10× greater than that in blood plasma. Vitamin C can prevent LPO, recycle oxidized Vitamin E and protect against H_2O_2 -induced DNA damage (Agarwal et al., 2007; Badade & Samant, 2011). As such, Vitamin C plays a significant role (up to 65%) in combatting oxidative stress in seminal plasma (Sharma & Agarwal, 1996).

2.7.2.2. Vitamin E

Vitamin E, also known as α-tocopherol, is a potent lipophilic, non-enzymatic antioxidant which is vital in the protection of mammalian spermatozoa against the effects of oxidative stress (Asadi et al., 2017). The structure of Vitamin E is characterized by the presence of a

benzene ring and a long hydrocarbon chain, making it hydrophobic in nature (Ogbuewo et al., 2010). As such, α-tocopherol is located in biological membranes and lipoproteins where it traps and scavenge ROS such as the superoxide radical, OH• radical and H_2O_2 (Agarwal et al., 2004). Because it can directly quench free radicals generated during the LPO cascade such as the peroxyl and alkoxyl radicals, Vitamin E is considered a major chain-breaking antioxidant and appears to have a dose-dependent effect (Bolle et al., 2002; Badade & Samant, 2011).

2.7.2.3. Glutathione

Glutathione is a tripeptide molecule (glutamyl-cysteine-glycine) that is an important cofactor of GPx, an enzymatic antioxidant that catalyses the reduction of toxic H_2O_2 and other hydroperoxides (Uysal & Bucak, 2007). On the other hand, reactions with ROS oxidize reduced glutathione (GSH) into oxidised glutathione (GSSG). However, once oxidized, glutathione is reduced in a redox cycle involving glutathione reductase and an electron acceptor (Nistiar et al., 2009). On that note, testicular and epididymal GSH and GPx act as reducing agents thereby protecting lipid constituents against peroxidative damage and preserving sperm viability and motility (Lanzafame et al., 2009; Mora-Esteves & Shin, 2013).

2.7.2.4. Selenium

In the testis, most of the selenium is incorporated within PHGPx, where it forms part of the catalytic centre of this antioxidant enzyme (Ogbuewo et al., 2010). PHGPx protects liposomes and biomembranes from LPO and exhibits GPx activity on phosphatidylcholine hydroperoxides. PHGPx is able to react with hydroperoxides of fatty acids esterified in the phospholipids and use protein thiol groups as donor substrates (Hermesz & Ferencz, 2009; Said et al., 2010). Selenium deficiency is often associated with reduced testicular mass, destabilization of the spermatozoal midpiece and affects the overall morphology of spermatozoa (Garrido et al., 2004b).

2.7.2.5. β-carotene

β-carotene is a non-enzymatic antioxidant that occurs naturally in apricots and carrots (El-Demerdash et al., 2004). It is known to possess pro-vitamin A activity and antioxidative properties (Vardi et al., 2009). Like tocopherol, β-carotene is a lipid-soluble antioxidant, a characteristic that enables it to easily permeate through biological membranes; however, its antioxidant strength is weaker than that of α-tocopherol and will only be used as an antioxidant after all other antioxidant defences have been exhausted (Ogbuewo et al., 2010). Because of its non-polar nature, β-carotene is located in spermatozoal membranes and lipoproteins and is effective in reducing lipid-derived free radicals and quenching singlet oxygen molecules (El-Demerdash et al., 2004; Vardi et al., 2009; Ogbuewo et al., 2010).

2.7.2.6. Cysteine

Cysteine (N-acetyl-cysteine) is a naturally occurring low-molecular weight amino acid which functions as a seminal antioxidant and precursor of intracellular glutathione (glutamyl-cysteine-glycine) (Uysal & Bucak, 2007; Mistry et al., 2012). The cysteine subunit of glutathione provides and exposes a sulfhydryl group that directly scavenges free radicals (Badade & Samant, 2011; Tvrdá et al. 2011). By scavenging free radicals, cysteine protects spermatozoal membrane lipids and proteins against peroxidative damage (Hendin et al., 1999).

2.7.2.7. Zinc

Zinc (Zn) is a potent, non-enzymatic antioxidant and a main component of free radical-inhibiting enzymes such as Cu-Zn SOD (Asadi et al., 2017). Zn also serves as a catalyst that prevents LPO by relocating and transferring metals including iron and copper from catalytic sites (Sikka, 2001).

2.7.2.8. Carnitine

L-carnitine or 3-aminobutyric acid is a naturally-occurring, vitamin-like, non-enzymatic antioxidant (Lenzi et al., 2004), that is found in high concentrations within the epididymis where its concentration has been reported as being 2000 times higher than in whole blood (Enomoto et al., 2002; Arduini et al., 2008). It prevents LPO by scavenging free radicals (Lenzi et al., 2004). Apart from its antioxidant properties, research also acknowledges the antiapoptotic effects of carnitine, its ability to promote spermatozoal maturation as well as its function as an energy provider within the male reproductive tract (Ng et al., 2004; Steibar et al., 2004; Dokmeci, 2005).

2.7.2.9. Melatonin

Melanin (N-acetyl-5-methoxytryptamine) is a pineal hormone that is crucial in the protection against testicular oxidative stress (Aitken & Roman, 2008). The antioxidant effects of melatonin on the testis are supported by findings by, Mogulkoc et al, (2006), who reported that rats that underwent pinealoctomy suffered significant testicular oxidative damage. Additionally, unlike other free-radical scavengers that undergo one electron oxidation; when melatonin acts as an antioxidant, it undergoes a two electron oxidative process making it readily attractive to free radicals, as well as giving it an added advantage of not redox-cycling and inadvertently generating free radicals (Aitken & Roman, 2008; Asadi et al., 2017).

2.7.2.10. Cytochrome C

Cytochrome C is a small molecular mass, non-enzymatic antioxidant that can effectively scavenge free radicals such as H_2O_2. It is also a powerful activator of apoptosis that increases the protective capacity of testicular tissue by eliminating damaged germ cells (Liu et al., 2006).

2.7.2.11. Coenzyme Q-10

Coenzyme Q-10 (CoQ10) alternatively known as ubiquinone, is a potent lipophilic, naturally occurring, non-enzymatic antioxidant related to low-density lipoproteins that is capable of protecting spermatozoa against LPO (Mazen & Elgris, 2013; Ogbuewo et al., 2013; Asadi et al., 2017). It is also essential in the regeneration and recycling of Vitamin E (Karbownik et al., 2001) and is a cofactor in the mitochondrial respiratory chain and in ATP production (Mazen & Elgris, 2013). Because CoQ10 has energy-promoting properties, it enhances spermatozoal motility and is therefore present in large quantities in the spermatozoal midpiece (Karbownik et al., 2001).

2.8. Management of diabetes mellitus-related sexual dysfunction

Once an individual has been identified as having oxidative stress-related infertility, treatment should be aimed at the identification and amelioration of the underlying cause before considering antioxidant treatment (Tremellen, 2008). Lifestyle behaviours such as smoking, poor diet, alcohol abuse, obesity or psychological stress have all been linked with oxidative stress and eliminating these lifestyle triggers for oxidative stress by making positive lifestyle changes such as a diet high in fruit and vegetables, maintenance of normal weight and a reduction in smoking and alcohol intake can at least have some beneficial effects on spermatozoal health (Tremellen, 2008; Dinesh et al., 2012).

High blood glucose levels as seen in diabetic conditions promote the generation of ROS and in the absence of an appropriate compensatory response from the antioxidant system, oxidative stress results, which is the main cause of diabetes secondary complications (Maritim et al., 2003) including sexual dysfunction (Van belle et al., 2011; Oliveira et al.,

2015). By increasing the production of ROS and LPO in testicular and epididymal tissues, diabetes may contribute to male infertility.

The first step in the management of diabetes-induced male infertility must therefore be the management of the underlying diabetic condition. By controlling blood glucose levels and increasing antioxidant capacity, levels of oxidative stress can be reduced (Singh et al., 2013). Although the use of synthetic hypoglycaemic agents has improved the outlook of diabetic patients, many of them harbour serious adverse effects, are expensive, require expertise (Modak et al., 2007) and some of them have done little to control secondary complications of diabetes. To date, the management of diabetes without side effects remains a challenge and the search for alternative remedies such as medication from natural products is paramount (Saxena & Kishore, 2004).

2.9. Pharmacological Management of Diabetes

Because T1DM is characterised by the absence or inadequate production of insulin, treatment is focused on introducing insulin so as to control blood glucose levels. As such, exogenous insulin therapy is the main pharmacological treatment option in the management of T1DM (Sheeja et al., 2010). With regards to T2DM, besides diet modulation and physical exercise to reduce weight and abdominal fat; the use of hypoglycaemic agents with different modes of action such as biguanides, incretin mimetics, sulfonylureas, meglitinides, thiozolinedione, bromocriptine, α- glycosidase inhibitors and sodium-glucose cotransporter-inhibitors are used for the treatment of type 2 diabetic patients (Siddiqui et al., 2013). Figure 2.3 outlines mechanisms of action of various antidiabetic drugs for the management of T2DM.

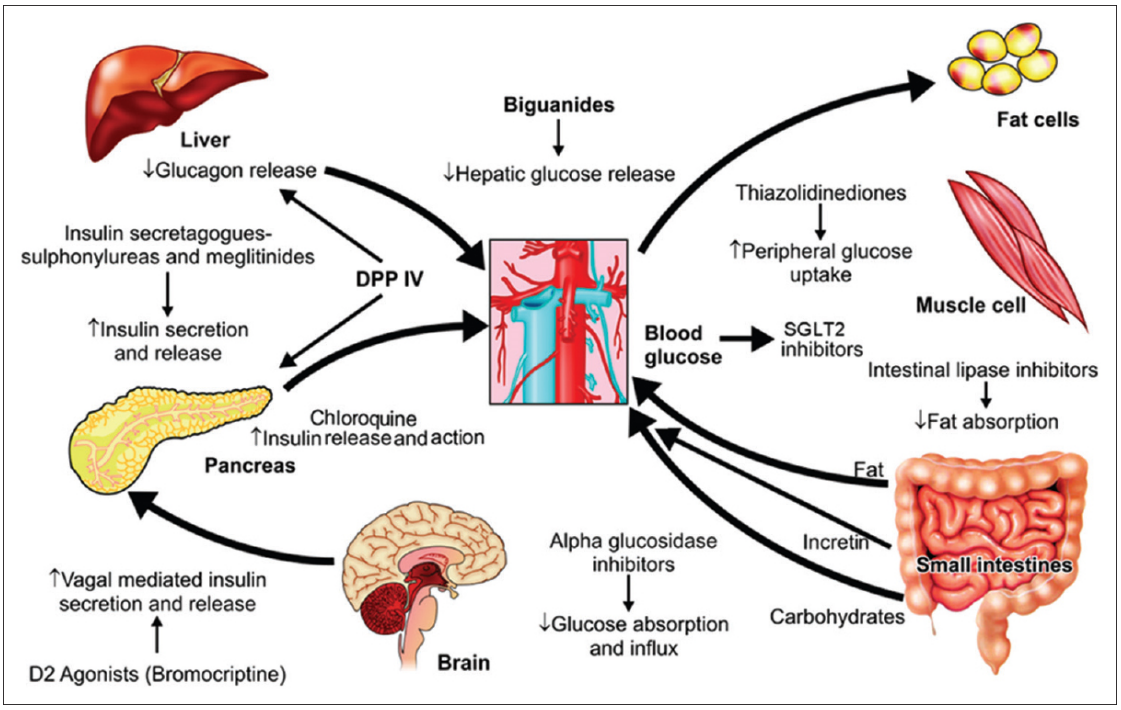

Figure 2.3: Mechanism of action of various antidiabetic drugs for the management of T2DM (Evans et al., 2016)

2.9.1. Insulin Therapy

Exogenous insulin is the mainstay of T1DM treatment and can also be used in cases where T2DM progresses to a state of β-cell failure with little or no insulin production (Sheeja et al., 2010; Chaudhury et al., 2017). A variety of beef, pork and pork/beef insulin were previously used as insulin regimens; however recombinant human insulin is now used almost exclusively especially in the United States of America (Khardori, 2008). Insulin is administered by means of subcutaneous injections, insulin pumps or insulin pens in basal and bolus schemes (Schaschkow et al., 2016). There are 4 main categories of insulin namely rapid-acting, short-acting, intermediate-acting and long-acting insulin. The difference between these insulin categories is based on their onset of action, peak-action and duration of action (Khardori, 2008).

Rapid-acting insulin e.g. Lispro and glulisine are absorbed more quickly and have a rapid onset of action (within 15 minutes), peaks 30-90 minutes after injection and have a short duration of action (4-5 hours). Short-acting insulin, e.g. regular insulin, reaches blood within

30 minutes, peaks 2-4 hours after injection and is active for 4-8 hours. Intermediate-acting insulin e.g. Neutral Protamine Hagedorn have a slower onset of action as compared to regular insulin and reaches blood 2-6 hours following injection. Peak action occurs 4-14 hours after dosage and its effects last for 14-20 hours. Long acting insulin e.g. glargine have no peak action and produces stable insulin levels for more than 24 hours (Sheeja et al., 2010).

2.9.1.1. Challenges of Insulin Therapy

Despite the fact that insulin therapy can significantly lower levels of glycated haemoglobin several patient, physician and health-care system barriers negatively influence its usage (Linetzky et al., 2016). With regards to patient barriers, factors such as injection phobia, fear of hypoglycaemic events, perceived lack of efficacy, mentality that insulin therapy may have a negative impact on their lifestyle as well as fear of gaining weight, may make patients reluctant to initiate insulin therapy (Peyrot et al., 2005). Besides the fact that insulin therapy is a time consuming and expensive treatment option, physicians also fear for the safety of their patients especially the possibility of weight gain and hypoglycaemic events (Oish et al., 2011; Ross et al., 2011). Sometimes physicians are reluctant to initiate insulin therapy for some patients because of the fear that those patients may fail to comply due to inadequate patient understanding of how complex insulin regimens are (Philis-Tsimikas, 2013). Healthcare system barriers such as limited access to medical care and out-of-pocket expenditures may also limit the consideration of insulin therapy (Wallace & Mathews, 2000; McEwen et al., 2009).

2.9.2. Biguanides (Metformin)

Across all age groups, T2DM is managed by use of metformin, a drug classified as a biguanide (Chaudhury et al., 2017). Metformin is the first line oral drug of choice in the management of T2DM because of its effectiveness in reducing levels of blood glucose with minimum risk of hypoglycaemia (Siddiqui et al., 2017). It has been reported that metformin is capable of delaying the progression of T2DM as well as reducing mortality amongst these

patients (Viollet et al., 2012). Metformin decreases hepatic glucose synthesis and sensitizes the liver to the effects of insulin by activating insulin receptor expression and enhancing tyrosine kinase activity (Chaudhury et al., 2017). It activates AMP-activated protein kinases in the liver thereby promoting hepatic glucose uptake and inhibiting gluconeogenesis by influencing activities of mitochondrial enzymes (Viollet et al., 2012).

2.9.2.1. Challenges of Metformin

Although metformin has a relatively safe profile in comparison to most other hypoglycaemic agents, it is contraindicated in patients with advanced renal insufficiency (Inzucchi et al., 2014). The use of metformin has also been reported to cause gastrointestinal disturbances in form of diarrhoea, dyspepsia and nausea in an estimated 30% of subjects (Valeron & de Pablos-Velasco, 2013). Research has also revealed that metformin is highly efficient when there is adequate insulin production; however, its efficacy decreases as T2DM progresses to a state of β-cell failure with little or no insulin production (Chaudhury et al., 2017).

2.9.3. Sulfonylureas

By blocking potassium ATP channels (kATP channels); sulfonylureas increase the secretion of insulin by the pancreas thereby lowering levels of glucose in the blood (de Wet & Proks, 2015). This class of drugs binds to specific sites on the kATP channel complex thereby inhibiting the activity of this channel and leading to cell membrane depolarisation and a cascade of activities that promote the secretion of insulin (Siddiqui et al., 2013).

2.9.3.1. Challenges of Sulfonylureas

Hypoglycaemia is the major drawback with regards to the usage of sulfonylureas (Valeron & de Pablos-Velasco, 2013); hence, the use of long-acting sulfonylureas is discouraged in elderly patients as well as patients with issues of alcohol abuse and poor nutritional status (Siddiqui et al., 2013). This class of drugs must also be used with caution in patients using β-blockers as these prolong the effects of sulfonylureas (May & Schindler, 2016). Minor side

effects associated with this class of drugs include hypersensitivity reactions, headaches, dizziness and gaining of weight (Chaudhury et al., 2017).

2.9.4. Meglitinides

Meglitinides (e.g. repaglinide and nateglinide) are non-sulfonylureas insulin-secretagogues that control postprandial hyperglycaemia by binding to the sulfonylureas receptor in the β-cells of the pancreas in the same way as sulfonylureas (Mendoza et al., 2013); however, the binding of meglitinides is weaker than that by sulfonylureas hence meglitinides are regarded as short-acting insulin secretagogues giving them flexibility in terms of administration (Dornhorst, 2001). Although meglitinides have a rapid onset of action, its duration of action is relatively short (Brickle et al., 2006). However, because the risk of hypoglycaemia is less than that of sulfonylureas, meglitinides are more preferable in the management of diabetic patients with chronic kidney disease and those who cannot tolerate sulfonylureas and metformin (Siddiqui et al., 2013).

2.9.4.1. Challenges of Meglitinides

Higher levels of blood glucose must first be reached before meglitinides can stimulate the secretion of insulin by the β- cells of the pancreas making them less effective in the management of diabetes as compared to sulfonylureas (Chaudhury et al., 2017). In addition, the need for multiple dosages may reduce compliance in patients using meglitinides (Inzucchi, 2002).

2.9.5. Incretin mimetics

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are naturally occurring, short-lived incretin hormones that play an important role in the maintenance of glycaemic control by enhancing the synthesis and release of insulin from pancreatic β-cells and decreasing the release of glucagon from pancreatic β-cells (Siddiqui et

al., 2013). Because GIP and GLP-1 are rapidly degraded by the dipeptidyl peptidase-4 (DPP-4) enzyme, they have short half-lives (Meneilly et al., 2000; Drucker, 2006).

The incretin effect is responsible for the secretion of 50-70% of total insulin after oral glucose intake (Nauck & Meier, 2016). In T2DM conditions, the incretin effect is reduced or absent since the insulinotropic action of GIP is lost in these patients (Holst et al., 2011). Two drug classes namely the GLP-1 agonists (e.g. exenatide and liraglutide) and DPP-4 inhibitors (e.g. sitagliptin, saxagliptin, linagliptin, alogliptin and vidagliptin) are currently in use (Messori et al., 2014; Andreozzi et al., 2016). These drugs improve glycaemic control while reducing weight and systolic blood pressure in T2DM patients. As such, insulin mimetics are finding increased usage in the management of patients with T2DM (Maruthur et al., 2016; Chaudhury et al., 2017).

2.9.5.1. Challenges of Incretin Mimetics

Treatment with GLP-1 agonists such as exenatide has been associated with gastric discomfort that in many cases has led to discontinuation of medication (Valeron & de Pablos-Velasco, 2013). Liraglutide has been associated with nausea and minor hypoglycaemia (Marre et al., 2009). On the other hand, usage of DPP-4 inhibitors has been associated with upper respiratory tract infections, nasopharyngitis and headaches (Pathak & Bridgeman, 2010). Acute pancreatitis was also reported in a fraction of patients treated by sitagliptin as monotherapy or in combination with metformin (Chaudhury et al., 2017).

2.9.6. Thiozolinediones

Thiozolinediones (TZDs) e.g. rosiglitazone, pioglitazone and troglitazone (Sanchez et al., 2006) are insulin sensitizers that act as Peroxisome Proliferator Activator Receptor (PPAR)-agonists thereby reducing insulin resistance in peripheral tissues such as the muscle and

adipose tissues (Chiarelli & Di-Marzio, 2008). They decrease hepatic gluconeogenesis and facilitate increased glucose uptake by tissues of the liver, muscle and adipose tissue (Yoon et al., 2009). TZDs have also been reported to reduce inflammatory cytokines and increase levels of adiponectin thereby improving β-cell integrity and function. In this manner, TZDs improve insulin sensitivity and pancreatic β-cell function (Powers & D'Alessio, 2011; Chaudhury et al., 2017).

2.9.6.1. Challenges of Thiozolinediones

Hepatotoxicity has been reported in an estimated 2% of patients using TZDs as early as 35 days and as late as 8 months after onset of therapy. As such monthly monitoring of transaminase and bilirubin levels is crucial in the first 8 months of therapy (Mahler & Adler, 1999). Additionally, the risk of peripheral oedema and dilutional anaemia has been reported among patients using TZDs; hence, limiting its use in patients with renal disease (Mahler & Adler, 1999; Mudaliar et al., 2003; Horita et al., 2015). The usage of pioglitazone has been associated with an increased risk for weight gain, oedema, heart failure, bladder cancer and distal bone fractures in post-menopausal women (Valeron & de Pablos-Velasco, 2013).

2.9.7. Alpha -glucosidase inhibitors (AGIs)

Retarding the absorption of glucose by inhibiting enzymes that hydrolyse carbohydrates is an effective therapeutic approach in the management of T2DM and impaired glucose tolerance (Kim et al., 2008; Van de Laar, 2008). Alpha-glucosidases are membrane-bound enzymes localised in the epithelium of small intestines that catalyse the breakdown of complex dietary carbohydrates into absorbable monosaccharaides such as glucose (Kumar et al., 2011). AGIs slow down the breakdown of carbohydrates into glucose by inhibiting α-glucosidase enzymes, thereby reducing post-prandial glucose levels and suppressing post-prandial hyperglycaemia (Bukhari et al., 2017).

2.9.7.1. Challenges of AGIs

Major side effects associated with the usage of AGIs come as a consequence of undigested carbohydrates reaching the colon where they are fermented by bacteria. Patients using AGIs suffer from abdominal discomfort, bloating and flatulence (Dabhi et al., 2013). Because of these gastrointestinal side effects, compliance has become a problem amongst AGI users. On the other hand, AGIs are relatively expensive in comparison to sulfonylureas and the need for 3 daily dosages might affect compliance amongst AGI users. (Van de Laar, 2008).

2.9.8. Sodium-glucose co-transporter-inhibitors

With the growing knowledge concerning the importance of the kidneys in maintaining glucose homeostasis, the development of a new set of drugs that target the kidneys in the treatment of diabetes has emerged (Nauck, 2014). Sodium-glucose co-transporters (SGLT2) facilitate the reabsorption of glucose in the kidneys (Chao & Henry, 2010). Inhibiting SGLT2 decreases renal reabsorption of glucose resulting in enhanced urinary excretion of glucose and subsequent reduction in levels of glucose and glycated haemoglobin in plasma (Ferrennini & Solini, 2012; Nauck, 2014). Because SGLT2-inhibitors enhance urinary excretion of glucose, its usage is associated with weight loss. As such SGLT2-inhibitors are viewed as an attractive therapeutic alternative for T2DM patients who are not benefiting from metformin monotherapy especially if weight is part of the underlying treatment consideration. In addition to this, the mechanism of action of SGLT-2 inhibitors does not rely upon insulin sensitivity or β-cell dysfunction making it possible to use this drug with other antidiabetic agents including insulin (Nauck, 2014).

2.9.8.1. Challenges of SGLT2-inhibitors

Side effects following the usage of SGLT2-inhibitors are related to high levels of glucose in urine. Patients on SGLT2-inhibitors experience genital mycotic infections and urinary tract infections (Nauck, 2014). In May of 2015, the Food and Drug Administration organisation warned that treatment with SGLT2-inhibitors may increase the risk of ketoacidosis in both T1DM and T2DM patients (Food and Drug Administration, 2015).

2.9.9. Bromocriptine

Bromocriptine is a sympatholytic, D2-dopamine agonist used in the treatment of T2DM (DeFronzo, 2011). This class of drugs is derived from an ergot alkaloid and has been in clinical use for over 30 years (Colao et al., 2006; Kvernmo et al., 2006). Glucose and energy metabolism is tightly controlled by the central nervous system (CNS) through sympathetic pathways (Sandoval et al., 2008). By activating CNS dopaminergic pathways, dopamine agonist therapy with bromocriptine regulates the production of hepatic glucose and integrates

information on fuel availability through hormonal signals such as insulin, GLP-1, leptin and ghrelin (Via et al., 2010). Because diabetic patients have impaired response to these pathways; they present with elevated hepatic glucose production, insulin resistance and β-cell dysfunction.

The modulation of neurotransmitter action in the brain by bromocriptine has been shown to improve insulin sensitivity as well as glucose tolerance using obese and diabetic animals as experimental models (Pijl et al., 2000). Quick release-bromocriptine may be used as monotherapy or in combination with insulin and other hypoglycaemic agents in the management of T2DM patients with mild hyperglycaemia characterised by glycated haemoglobin levels close to 7.5% (Nasser, 2011; Siddiqui et al., 2013). The addition of bromocriptine in poorly controlled T2DM patients treated by sulfonylureas and metformin alone decreases glycated haemoglobin levels by 0.5-0.7 (DeFronzo, 2011).

2.9.9.1. Challenges of Bromocriptine Therapy

Drawbacks associated with the use of bromocriptine include high rates of nausea, considerable cost as well as inadequate information with regards to the efficacy and safety of bromocriptine (Nasser, 2011; Garber et al., 2013).

2.10. Medicinal plants and diabetes mellitus-linked male reproductive dysfunction

Herbal medicine is the oldest and most popular form of healthcare practice known to humanity and has been practiced by all cultures in all ages throughout the history of civilisation (Chikezie et al., 2015). Because medicinal plants are more compartible with the human body, their usage is usually associated with lesser side effects and are culturally acceptable. As such, medicinal plants have remained the mainstay of more than 70% of the world population particulary in developing countries where a large population might not have access to basic conventional therapies (Acharya & Shrivastava, 2008; Sen et al., 2010).

Before the introduction of synthetic antidiabetic drugs, diabetes was treated with medicinal plants (Piero et al., 2012); however, the use of medicinal plants for the treatment of diabetes

in developed countries declined during the early part of the 20th century following the introduction of conventional antidiabetic oral agents and insulin therapy. Recently however, there has been increased interest in the usage of medicinal plants because of the high cost, advese effects and secondary failure rates associated with pharmacological and diabetic agents (Gurib-Fakim, 2006; Yatoo et al., 2017).

The use of plant-derived medicines is based on the premise that plants are a rich source of safer, well-tolerated substances that are relatively less expensive and globally competitive (Sen et al., 2010). Medicinal plants are viewed as an easily available, potent source of antioxidants capable of scavenging free radicals and fighting diabetes-induced oxidative stress and have proven to be clinically effective and relatively less toxic than existing antidiabetic orthodox drugs (Sen et al., 2010; Bhatt et al., 2013). Piero et al, (2012) acknowledged that medicinal plants have been used in the management of diabetic complications and results of several other studies support the idea that medicinal plants have significant beneficial effects towards the management of diabetes-induced fertility problems (Oliveira et al., 2015). As such, the use of medicinal plants can be a source of new hope in the management of diabetes-associated male infertility as an affordable treatment method with fewer side effects.

2.11. *Catharanthus roseus*

Catharanthus roseus (L.) G. Don *(C. roseus)* is an important medicinal plant that belongs to the family Apocynaecea (Vega-Vila et al., 2012). *C. roseus* has previously been known as *Vinca Rosea* and is commonly known as the Madagascar periwinkle since it is native to Madagascar (Tiong et al., 2013). This plant presents as an erected, procumbent, evergreen subshrub with white to dark pink flowers that grows to a height of 1 metre (Frode & Medeiros, 2008; Gajalakshmi et al., 2013). The leaves of *C. roseus* are glossy and dark green in colour, oblong to elliptic in shape (Aslam et al., 2010), 1.0-3.5 cm broad, have a pale midrib and a short petiole of approximately 1.0 – 1.8 cm in length arranged in opposite pairs (Gajalakshmi et al., 2013). *C. roseus* blooms throughout the year and is propagated by seedlings or

cuttings (Aslam et al., 2010). Flowers of *C. roseus* have 5 petals that range from white to dark-pink in colour with a darker red centre (Gajalakshmi et al., 2013). Figure 2.4 shows the plant presentation of *Catharanthus roseus*. On the basis of floral colour, *C. roseus* has been classified into 2 classes namely the pink- flowered *'Rosea'* and white-flowered 'Alba.' (Jaleel & Panneerselvam, 2007). Besides the 2 classifications for *C. roseus*, horticulturists have developed more than 100 different varieties of *C. roseus* with colours ranging from white to pink to purple (Aslam et al., 2010).

Although *C. roseus* originates from Madagascar, it is now distributed around the world due to its ability to survive in a variety of habitats and its use as an ornamental plant (Van Bergen & Snoeijer, 1996). *C. roseus* has been cultivated in countries such as South Africa, India (Akhtar et al., 2007), China, Mexico (Patel et al., 2012) and Malaysia (Ong et al., 2011) where it has been utilised as a medicinal plant in the treatment of various ailments (Li et al., 2004; Aslam et al., 2010).

Figure 2.4: *Catharanthus roseus* plant presentation (Robertson, 2008)

Traditionally, *C. roseus* has been used to relieve muscle pain, CNS depression, wasp stings, manage nose bleeding, mouth sores, sore throats and bleeding gums as well as in the

treatment of memory loss, hypertension, gastritis, cystitis, diarrhoea and hyperglycaemia (Dessisa, 2001). In Southern and Central America, *C. roseus* was used as a homemade remedy for easing lung congestion and inflammation while the flower extracts were useful in the treatment of eye irritations and infections by inhabitants of the Caribbean (Aslam et al., 2010).

C. roseus has been extensively investigated from ancient times for its pharmacological effects and phytochemical components (Gajalakshmi et al., 2013). Scientific research has confirmed *C. roseus* as a medicinal plant with antihelminthic activity, antitumour activity, antimutagenic activity, antihypertensive activity, antifungal activity, antimitotic activity, antinflammatory activity, antihypercholesterolemic activity, antidiuretic activity, cardiotonic activity, CNS depressant activity, antimalarial activity, antibacterial activity ,antiviral activity as well as antidiabetic activities (Aslam et al.,2010; Gajalakshmi et al., 2013) and qualitative analysis of phytochemical constituents of aqueous and ethanolic extracts of *C. roseus* have revealed the presence of alkaloids, tannins, flavonoids, terpenoids, glycosides, phenols, steroids, saponins, antioxidants and many other secondary metabolites (Patharajan & Abirami, 2014).

2.12. Antidiabetic and antioxidant activities of *C. roseus*

C. roseus has been traditionally used in the management of diabetes in various settings as it was believed to promote the production of insulin and increase the usage of sugars derived from food in cases of diabetes (Singh et al., 2001). Extensive research has been conducted to determine the efficacy of *C. roseus* as an antidiabetic and antioxidant agent and results have demonstrated that *C. roseus* has excellent antidiabetic and antioxidant properties (Rasineni et al., 2010; Vega-vila et al., 2012; Patharajan & Abirami, 2014; Al-Shaqha et al., 2015; Nisar et al., 2017). The antidiabetic and antioxidant activities of *C. roseus* can be attributed to the presence of a variety of hypoglycaemic phytochemical constituents as well as free- radical scavengers and antioxidants within this plant's extracts (Singh et al., 2014).

2.13. Vindoline

C. roseus produces more than 130 different alkaloids, some of which exhibit strong and important pharmacological properties making it an economically valuable medicinal plant (Vega-Vila et al., 2012; Almagro et al., 2015). Amongst the many alkaloids derived from *C. roseus* is vindoline ($C_{25}H_{32}N_2O_6$; Molecular Weight 456.539 grams/mole); which is an intermediate, semi-synthetic monomeric vinca alkaloid that is used as a precursor to vincristine and vinblastine; drugs currently in use for their antineoplastic properties (Verma et al., 2007; Zhu et al., 2015). Figure 2.5 outlines the chemical structure of vindoline.

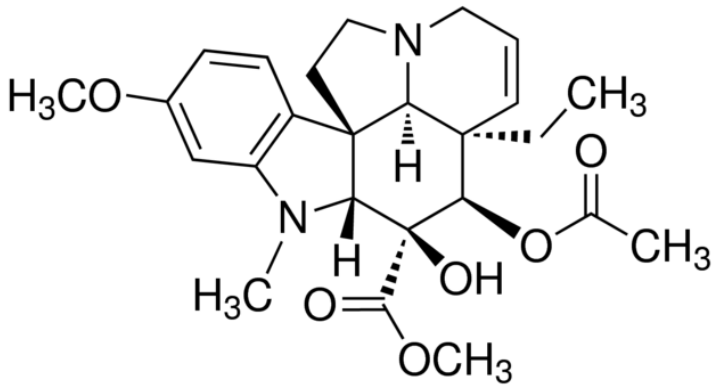

Figure 2.5: Chemical Structure of vindoline (Merck, 2018)

Vindoline can be extracted from the aerial parts of *C. roseus* and is primarily used in the treatment of cancer because of its ability to repress cell growth by altering microtubular dynamics thereby provoking apoptosis (Nirmala et al., 2011; Chandrasekaran et al., 2014). Besides the antimitotic effects of vindoline, research has demonstrated the possible efficacy of vindoline in the treatment of diabetes mellitus. Vindoline has been reported to exhibit antidiabetic properties in diabetes-induced animal models by increasing glucose-stimulated insulin release, lowering blood glucose levels, inhibiting Kv2.1 potassium channels, decreasing the K^+ outward current, lowering glycated haemoglobin, reducing triglyceride levels and inhibiting H^+/K^+ ATPases (Sertel et al., 2011; Tiong et al., 2013; Yao et al., 2013).

Diabetes-induced male reproductive dysfunction is a consequence of oxidative stress produced in diabetic conditions. Because of the revealed antidiabetic and antioxidant properties of vindoline, it is therefore envisaged that this alkaloid could be useful in the

management of diabetes-induced male infertility. This research therefore sought to assess the antioxidant and antidiabetic activities of vindoline on testicular and epididymal tissues in STZ-induced diabetic and non-diabetic rats.

CHAPTER THREE
RESEARCH DESIGN AND METHODOLOGY

3.1. Type of study

This research was a laboratory-based experimental study.

3.2. Animal Requirements

Forty-eight, 6 week old, male Wistar rats, *Rattus Norvegicus*, 190-230g body mass, with a conventional microbial status were obtained from the Primate Unit and Delft Animal care, South African Medical Research Council, and used for this study.

3.3. Place of study

Animal feeding and sacrifice was done at the South African Medical Research Council in Parrow, Western Cape, South Africa; while laboratory analysis of samples obtained from the rats was carried out at the Oxidative Stress Research Centre of the Cape Peninsula University of Technology, Bellville; South Africa.

3.4. Ethical Consideration

Ethical approval was sought and obtained from the Research Ethics Committee, (CPUT/HW-REC 2017/A1), Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology. All animals received humane care and were treated with respect according to the principles of Laboratory Animal Care of the National Society of Medical Research and the National Institute of Health Guide for the Care and Use of Laboratory Animals of the National Academy of Sciences (National Institutes of Health, Publication Number 80-23, Revised 1978).

3.5. Source of vindoline

Vindoline used for this study was commercially purchased from Best of Chemicals; United States of America.

3.6. Experimental Design

The experiment comprised 6 groups of rats; each group with 8 rats. The first 3 groups (groups 1, 2 and 3) were normal, non-diabetic male Wistar rats while the last 3 groups (groups 4, 5 and 6) were type 2 diabetes-induced male Wistar rats.

3.7. Induction of diabetes

Induction of T2DM followed the modified method of Wilson and Islam (2012), Kumar et al, (2014) and Mohamed et al, (2016). Diabetic groups (Groups 4, 5 and 6) received distilled water and 10% fructose water for the first 2 weeks of the study. At the end of 2 weeks, the administration of 10% fructose water was terminated and the rats were fasted overnight prior to the administration of a single intraperitoneal injection of STZ solution (Sigma, United States of America) at 40 mg/kg b.w. dissolved in sodium citrate at pH 4.4. Three days after STZ injection, drops of blood were collected from the tails and blood glucose levels were measured using a portable glucometer (Accu-Chek, Roche, Germany). Diabetes status was confirmed where glucose levels were above 18 mmol/l.

3.8. Animal feeding

All rats were fed with standard rat chow (SRC) purchased from AQUANUTRO Company (Mamelsbury, South Africa).

Table 3.1: Composition of rat diets

Nutrient	Amount g/kg	
Protein	160	Minimum
Moisture	120	Maximum
Lipids	25	Minimum
Fibre	60	Maximum
Phosphorus	7	Minimum
Calcium	18	Maximum

3.9 Animal Treatment

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 Guideline for the Care and Use of Laboratory Animals of the National Academy of Sciences (National Institutes of Health publication no. 80-23, revised 1978). They were accommodated individually in plastic cages at the animal facility of the South African Medical Research Council and supplied with SRC and distilled water *ad libitum*. They were subjected to standard laboratory conditions of 22 ± 2°C and 12 hour light/dark cycles at humidity levels of 55 ± 5%.

Rats from the 6 different groups were subjected to different treatments as follows:

- Group 1 comprised normal non-diabetic rats (n=8) and was considered the normal control. Rats from this group were treated with the vehicle, castor oil; 5 times a week for 5 weeks, fed on SRC and had access to distilled water *ad libitum*.
- Group 2 comprised normal, non-diabetic rats (n=8), treated with vindoline (20 mg/kg/b.w), 5 times a week for 5 weeks, using castor oil as the vehicle for the administration of vindoline. The rats were fed on SRC and had access to distilled water *ad libitum*
- Group 3 comprised normal, non- diabetic rats (n=8) treated with glibenclamide (50 mg/kg/b.w), 5 times a week for 5 weeks. Glibenclamide is the standard drug for the treatment of diabetes. This group of rats was fed on SRC and allowed access to distilled water *ad libitum.*
- Group 4 comprised untreated-diabetic rats (n=8) and was considered the diabetic control. Rats from this group were treated with the vehicle, castor oil, fed on SRC and allowed free access to distilled water *ad libitum.*
- Group 5 was the diabetic group of rats (n=8), treated with vindoline (20 mg/kg/b.w.), using castor oil as the vehicle for administration. This group of rats was fed on SRC and allowed free access to distilled water *ad libitum*.

- Group 6 was the diabetic group of rats (n=8), treated with glibenclamide (50 mg/kg/b.w.), 5 times a week for 5 weeks. Rats from this group were fed on SRC and allowed access to distilled water *ad libitum*.

3.10. Sample collection and preparation

Body weights were measured from the onset of the study and monitored throughout the feeding period until sacrifice. 72 hours after STZ injection, drops of blood were collected from the tails and blood glucose levels were measured using a portable glucometer (Accu-Chek, Roche, Germany). Diabetes status was confirmed where glucose levels were above 18 mmol/l. On completion of the treatment periods, rats were fasted overnight in preparation for sacrifice and sample collection. Fasting blood glucose levels were measured after 4 hours of fasting. Rats were anaesthetised and euthanized using isoflurane gas at 2% with 1% oxygen during laparotomy. Following complete anaesthesia, blood was collected from the abdominal vena cava using 5ml syringes connected to a 23 gauge hypodermic needle and collected into appropriate tubes containing sodium fluoride/potassium oxalate. The epididymis and testes were excised, weighed and used for this research project. Testicular and epididymal tissue samples were snap-frozen in liquid nitrogen and stored at -80°C until ready for biochemical analysis.

3.11. Biochemical Experiments

All laboratory investigations were carried out at the Oxidative Stress Research Laboratory of the Cape Peninsula University of Technology, in Bellville; Western Cape Province of South Africa.

3.12. Buffer solutions

Fresh 50mM sodium phosphate buffer with 0.5% v/v Triton X-100 at pH 7.5 was used for homogenising all tissue samples. Thereafter, 50mM sodium phosphate buffer without Triton X-100 at pH 7.5 was used for diluting samples for purposes of running all the biochemical analyses.

3.13. Preparation of testicular and epididymal homogenates

Using a balance, 100 mg of each tissue (testicular/ epididymal) was weighed and added to 1ml of the 50Mm sodium phosphate buffer with Triton X-100 (pH 7.5) in a glass homogeniser on ice water. The tissues were homogenised in potter-elvehjam for five strokes on ice water and the homogenates were transferred to new tubes and sonicated on ice. The homogenates were centrifuged for 15 minutes at 15 000rpm at a temperature of 4^0C. The supernatant was transferred to newly marked tubes and stored at -80^0C until needed for protein determination and biochemical assays.

3.14. Total Protein Determination Assay

The Bicinchoninic Acid (BCA) protein assay is a detergent compatible formulation that is based on BCA for the colorimetric detection and quantitation of total protein concentration. It is a combination of the biuret reaction (characterised by the reduction of Cu^{2+} to Cu^{+} by protein in an alkaline medium) and the colorimetric detection of the produced Cu^{+} using a unique reagent containing BCA. The chelation of two molecules of BCA with a single Cu^{+} ion results in a water-soluble purple-coloured complex with a strong absorption at 562nm.

3.14.1. Total Protein Determination Assay Methodology

Total protein was quantified in accordance to the manufacturer's instruction assay kit supplied by Sigma Aldrich. The BCA working reagent was prepared by mixing 50 parts of BCA Reagent A with 1 part of BCA reagent B [(50:1) Reagent A: Reagent B]. 100ml of Reagent A was therefore mixed with 2ml of Reagent B. Protein standards were prepared by serial dilutions of the BSA standard protein as outlined in the table below.

Table 3.2: Serial dilutions of the BSA standard

Standard (Std)	Buffer	BSA	Final BSA Concentration
Standard 5	325µl	325 µl	1000µg/ml
Standard 4	325µl	325µl of Std 5	500µg/ml
Standard 3	325µl	325µl of Std 4	250µg/ml
Standard 2	325µl	325µl of Std 3	125µg/ml
Standard 1	400µl	100µl of Std 2	25µg/ml
Blank	400µl	0	0

Using a 96-well plate, 25µl of the blank, each standard and unknown testicular and epididymal samples were pipetted into each well in triplicates. 200µl of the BCA working reagent was added into each well using a multiple channel pipette. The contents of each well were thoroughly mixed on a plate shaker for 30 seconds and the microplate was allowed to cool at room temperature. Absorbance was then measured at 562nm on a microplate reader (Thermo Electron Corporation, Multiskan spectrum, United States of America).

The BCA assay has a broad working range of 20- 2000µg/ml, and protein concentration of the unknown samples were determined and reported with reference to standards of a common protein. In this assay; Bovine Serum Albumin (BSA) was used as the common protein. A series of BSA dilutions were prepared and assayed alongside the unknowns. Protein concentrations of the unknowns were quantified using the standard curve generated from BSA and results were expressed as µg/ml.

3.15. TBARS ASSAY

Malondialdehyde (MDA), a meta-stable end-product of lipid peroxidation (LPO) can be assayed through the thiobarbituric acid reactive substances (TBARS) Assay which is a simple and useful diagnostic tool for the measurement of LPO for *in-vitro* and *in-vivo* systems. The TBARS assay involves the reaction of MDA at 90^0C under acidic conditions to produce a pink-coloured chromogen that strongly absorbs at a wavelength of 532nm.

3.15.1. TBARS Assay Methodology

Briefly, 100µl of each testicular/epididymal homogenate was mixed with 12.5µl of butylated hydroxytoluene (BHT) ethanol and 100µl of 0.2M O-PA in a 2ml eppindoff tube. 12µl of thiobarbituric acid in 0.1M NaOH was added to the same mixture and the tubes were vortexed and incubated in a 90^0C water-bath for 45 minutes. The tubes were then cooled on ice water. 1000µl of butanol and 100µl of NaCl were added into each tube and the tubes were vortexed. Using a 96-well plate, 300µl of butanol/each prepared testicular and epididymal samples were pipetted into each well in triplicates and the absorbance of the

pink-coloured chromogen was measured spectrophotometrically at 532nm on a microplate reader (Thermo Electron Corporation, Multiskan spectrum, United States of America). Results were expressed in µmol/g.

3.16. SOD ASSAY

The following SOD activity assay is a modification of the methodology outlined by Ellerby and Bredesen, 2000. SOD is one of the most important antioxidant enzymes that catalyses the dismutation of the superoxide radical into the less destructive H_2O_2 and molecular oxygen. The SOD assay uses 6 hydroxydopamine (6-HD, Sigma Cat H8523) and diethylenetriaminepentaacetic acid (DETAPAC, Sigma Cat CD6518) to generate superoxide anions. However, in the presence of SOD, the superoxide anion concentrations are reduced, yielding less colorimetric signal. The kinetics of the autoxidation of 6-HD is monitored at 490nm at 25^0C for ± 4 minutes.

3.16.1. SOD Assay Methodology

In a 15ml tube, 1.6mM 6-HD was freshly prepared by mixing 10ml of distilled water and 50µl of perchloric acid for 15 minutes. 10 ml of this solution was measured, and to this, 4mg of 6-HD was added. The tube was labelled as 6-HD, stored on ice and used as soon as possible. 0.1mM DETAPAC solution was prepared by dissolving 2mg of DETAPAC in 50 ml of the 50mM sodium phosphate buffer *without* Triton X-100 and mixing the solution. This solution was stored at -20^0C and used as soon as possible. Tubes were labelled and into each tube, 50µl of the 50mM sodium phosphate buffer and 50µl of the blank/ testicular/ epididymal homogenates were added making up a 2× dilution of the samples' original concentrations. Using a 96-well plate; 12µl of the blank/diluted testicular and epididymal samples was added into each respective well in triplicates. Using a multiple-channel pipette, 15µl of the 6-HD solution and 170µl of the DETAPAC solution was added into each well making a total volume of 197µl for each well. The absorbance was measured immediately at 490nm for 4min at 1min intervals on a microplate reader (Thermo Electron Corporation, Multiskan spectrum,

United States of America). The activity of SOD was calculated from a linear calibration curve and expressed as µmol/mg protein.

3.17. CATALASE ASSAY

Catalase activity was determined by measuring the amount of substrate (H_2O_2) remaining after sample addition (Aebi, 1984). This assay is based on the enzymatic detoxification of H_2O_2 by catalase into molecular oxygen and water molecules. The rate of H_2O_2 disintegration into water molecules and molecular oxygen is proportional to the concentration of catalase in the sample. The catalase-containing sample is incubated with a known amount of H_2O_2 and the rate of H_2O_2 disintegration is proportional to the concentration of catalase present in the sample.

3.17.1. Catalase Assay Methodology

Determination of Catalase activity followed the methodology outlined by Ellerby and Bredesen, (2000). H_2O_2 solution was prepared by adding 34µl of the H_2O_2 stock solution to 10ml of the 50mM sodium phosphate buffer at pH 7.5. Testicular and epididymal tissue homogenates were diluted 5× by adding 10µl of each homogenate to 40µl of the 50mM sodium phosphate buffer. Using a 96 well plate, 10µl of the blank and each diluted unknown tissue sample were pipetted into each well in triplicates. 170µl of the 50mM sodium phosphate buffer was added to each well using a multiple channel pipette. The loaded 96 well plate was carried to the spectrophotometer where 75µl of the H_2O_2 solution was added to each well using a multiple channel pipette. The plate was immediately loaded onto the microplate reader (Thermo Electron Corporation, Multiskan spectrum, United States of America) and absorbance was measured at 240nm. Catalase activity results were expressed as µmol/mg of protein.

3.18. FRAP ASSAY

The Ferric Reducing Antioxidant Power (FRAP) assay is used to rank the reducing power and antioxidant potential of a wide range of biological samples. It is an endpoint assay that

uses antioxidants as reducing agents in a redox-linked colorimetric method employing an easily reduced oxidant, Fe^{3+}. The reduction of colourless Fe^{3+} to blue Fe^{2+} can be monitored spectrophotometrically at 593nm where absorbance readings are related to the reducing power of the electron-donating antioxidants present in the sample.

3.18.1. FRAP Assay Methodology

This FRAP assay methodology is a modification of the methodology outlined by Benzi and Strain, (1996). Iron chloride solution ($FeCl_3$) was prepared by dissolving 0.053g of $FeCl_3$ in 10ml of distilled water. TPTZ solution was prepared by dissolving 0.0279g of TPTZ in 9ml of 0.1M hydrochloric acid. The FRAP reagent was prepared by adding 90ml of the 50mM sodium phosphate buffer solution (pH 7.5) with 9ml of the already prepared $FeCl_3$ solution, 9ml of the TPTZ solution and 18ml of distilled water. Ascorbic acid (AA) was diluted into different standard solutions as shown on the table below.

Table 3.3: Preparation of FRAP standards

Standard	Ascorbic Acid	Distilled Water
Blank	0	1000
Standard 1	50µl	950µl
Standard 2	100µl	900µl
Standard 3	250µl	750µl
Standard 4	500µl	500µl
Standard 5	1000µl	0

Using a 96-well microplate; 10µl of the blank, each standard and respective testicular and epididymal samples were pipetted into each well in triplicates. 300µl of the FRAP working reagent was added into each well using a multiple channel pipette and the plate was incubated for 30 minutes at 37^0C. The plate was then loaded into the spectrophotometer (Thermo Electron Corporation, Multiskan spectrum, United States of America) where absorbance was measured at 593nm. Results were compared to a standard curve and expressed in µmol/g.

3.19. ORAC ASSAY

Oxygen Radical Absorbance Capacity (ORAC) is a kinetic assay that measures fluorescein and antioxidant decay over time in biological fluids, cells and tissue samples. The ORAC assay provides a comprehensive analysis of test sample antioxidant activity by measuring hydrogen atom transfer. It measures loss of fluorescein florescence over time due to peroxyl radical formation during the time-dependant, thermal decomposition of AAPH (2,2- azobis-2-methyl-propanimidamide, dihydrochloride) at 37^0C. The peroxyl radicals can oxidise fluorescein to generate a product without fluorescence. Antioxidants however suppress this reaction by hydrogen atom transfer thereby inhibiting the oxidative degradation of the fluorescein signal.

In this assay, Trolox (6-hydroxy-2, 5, 7, 8- tetramethylchroman-2 carboxylic acid), a water soluble Vitamin E analog, served as a positive control by inhibiting fluorescein decay in a dose-dependent manner. The concentration of the antioxidant in the unknown sample was proportional to the intensity of the fluorescence generated throughout the course of the assay and the dynamics of the signal inhibition were expressed as the area under the curve generated by Trolox.

3.19.1. ORAC Assay Methodology

The following ORAC methodology is a modification of the methodology by Rautenbach and co-workers, (2010). Testicular and epididymal samples were deproteinated by mixing 50µl of each homogenised tissue with 50µl of perchloric acid making a 2× dilution of the original sample concentration. The mixture was vortexed and centrifuged at 14 000rpm for 1minute at 4^0C. 50µl of each supernatant was then added to 200µl of the 75mM ORAC buffer solution at pH 7.5 making an additional 5× dilution of the tissue samples. Fluorescein was prepared by adding 10µl of fluorescein to 2ml of the ORAC buffer in a 2.5ml tube. 300µl of this mixed solution (10µl fluorescein + 2ml ORAC buffer) was then pipetted into a 15ml tube and 15ml of the ORAC buffer solution was added into that tube. AAPH was prepared by

adding 0.150g of AAPH to 6ml of the 75Mm ORAC buffer in a 15ml tube. Standard Trolox was diluted as shown in the table below.

Table 3.4: Preparation of ORAC standards

Standard	Buffer	Trolox
Blank	750µl	0
Standard 1	625µl	125µl
Standard 2	500µl	250µl
Standard 3	325µl	325µl
Standard 4	250µl	500µl
Standard 5	125µl	625µl

Using a 96-well plate, 12µl of the blank, each standard and deproteinated testicular and epididymal homogenates were added into each well in triplicates. Using a multiple channel pipette, 138µl of fluorescein was added into each well. The loaded 96-well plate was carried to the reading machine where 50µl of the AAPH solution was added into each well using a multiple-channel pipette. The 96-well plate was then loaded into the machine where results were generated over a period of 2 hours. The regression equation ($Y=a+bX+cX^2$) was used to determine ORAC values where Y= Trolox concentration in µM and X= net area under the fluorescence decay curve. Results were reported in Trolox equivalents per millilitre/µmol. The area under the curve (AUC) was calculated as follows:

$$AUC= (0.5 +f_2 / f_1 +f_3 / f_1 +f_4 / f_1 +\ldots\ldots\ldots+ f_i / f_1) \times CT.$$

Where f_1 is the initial reading at cycle 1, f_i is the reading at cycle I and CT is the cycle time in minutes.

3.20. Statistical analysis

Data was expressed as mean ± standard error of the mean (Mean ± SEM). One-way analysis of variance (ANOVA) was used to test for significance between the different experimental groups. The Bonferroni Multiple Comparison analysis was used to compare the differences between multiple groups. Statistical analysis of two groups was performed using the unpaired student's t-test. Differences were considered significant at $p<0.05$. GraphPad PRISM 5 software package and Microsoft Excel 2010 were used for all statistical evaluations and graphical representations.

CHAPTER FOUR
RESULTS

4.1. Initial blood glucose levels in diabetic and non-diabetic groups before treatments.

Diabetes was induced by subjecting rats to be used as group 4, 5 and 6 subjects to 10% fructose water for 2 weeks and thereafter administering a single intraperitoneal injection of 40mg/kg b.w of STZ. Fasting blood glucose levels of diabetes-induced male Wistar rats were measured 72 hours after STZ injection. Initial fasting blood glucose levels of normal, non-diabetic rats (used as group 1, 2 and 3 subjects) and diabetic rats (used as group 4, 5 and 6 subjects) are presented in Figure 4.1. The average fasting blood glucose levels of diabetic rats was significantly higher in comparison to fasting blood glucose levels of normal, non-diabetic rats (29.27 ± 0.7882 mmol/l versus 11.21 ± 0.9771 mmol/l), $p<0.05$.

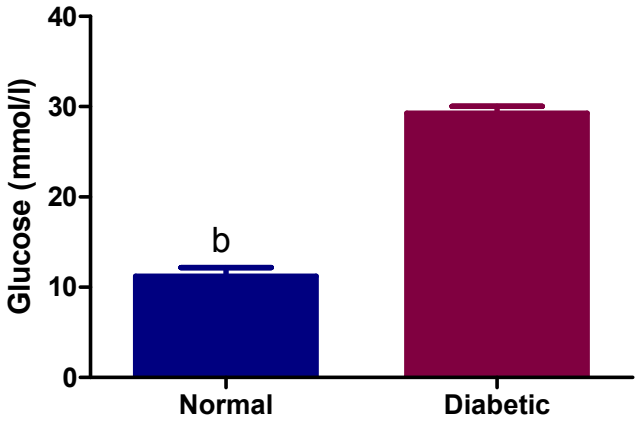

Figure 4.1: Fasting blood glucose levels of normal, non-diabetic rats and diabetic male Wistar rats before subjection to various treatments. Data is presented as mean ± SEM. (b) indicates a significant difference of normal fasting blood glucose levels when compared to the diabetic group, $p<0.05$, mmol/l: millimoles per litre.

4.2. Evaluation of blood glucose levels after subjecting rats to various treatments.

Figure 4.2 shows fasting blood glucose levels of non-diabetic and diabetic rats after being subjected to various treatments for 5 weeks. The difference in fasting blood glucose levels between the normal control (group 1) and non-diabetic rats treated with vindoline (group 2)

(10.56 ± 0.3380 mmol/l versus 10.03 ± 0.9161 mmol/l) and the difference between fasting blood glucose levels of group 1 and group 3 (non-diabetic rats treated with glibenclamide) (10.56 ± 0.3380 mmol/l versus 10.18 ± 0.2944 mmol/l) was not statistically significant ($p>0.05$). The difference in final fasting blood glucose levels between groups 2 and 3 (10.03 ± 0.9161 mmol/l versus 10.18 ± 0.2944 mmol/l) was also non-significant, $p>0.05$. The average fasting blood glucose level of the diabetic control (group 4) was significantly higher in comparison to fasting blood glucose levels of the normal control (group 1), (31.94 ± 0.5438 mmol/l versus 10.56 ± 0.3380 mmol/l), $p<0.05$. Diabetic rats treated with vindoline (group 5) and diabetic rats treated with glibenclamide (group 6) had significantly higher fasting blood glucose levels ($p<0.05$ when compared to the normal control (group 1), (27.15 ± 1.472 mmol/l versus 10.56 ± 0.3380 mmol/l) and (29.23 ± 1.335 mmol/l versus 10.56 ± 0.3380 mmol/l) respectively). An insignificant difference ($p>0.05$), wasnoted between the final fasting blood glucose levels of the diabetic control (group 4) and diabetic rats treated with glibenclamide (group 6), (31.94 ± 0.5438 mmol/l versus 29.23 ± 1.335 mmol/l). The average fasting blood glucose levels of diabetic rats treated with vindoline (group 5), were however significantly lower in comparison to that of the diabetic control (group 4), (27.15 ± 1.472 mmol/l versus 31.94 ± 0.5438 mmol/l). There was no significant difference between final fasting blood glucose levels of group 5 and group 6 after 5 weeks of subjection to different treatments (27.15 ± 1.472 mmol/l versus 29.23 ± 1.335 mmol/l).

Experimental Groups

Group 1: Untreated, non-diabetic rats (normal control)

Group 2: Non-diabetic rats treated with 20 mg/kg b.w. vindoline

Group 3: Non-diabetic rats treated with 50 mg/kg b.w. glibenclamide

Group 4: Untreated, diabetic rats (diabetic control)

Group 5: Diabetic rats treated with 20 mg/kg b.w. vindoline

Group 6: Diabetic rats treated with 50 mg/kg b.w. glibenclamide

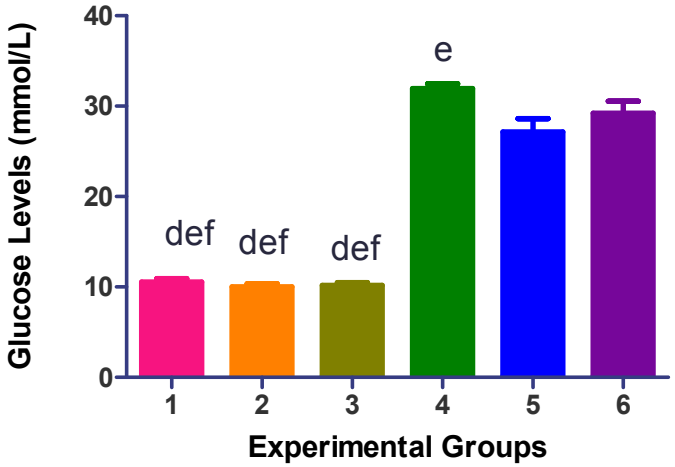

Figure 4.2: Fasting blood glucose levels of various groups after subjection to various treatments. Data is presented as mean ± SEM. (d) indicates significant difference of groups when compared to group 4 at $p<0.05$; (e) indicates significant difference of groups when compared to group 5 at $p<0.05$ and (f) indicates significant difference of groups when compared to group 6 at $p<0.05$, mmol/l; millimoles per litre.

4.3. Evaluation of body weights after subjecting rats to treatments.

Figure 4.3 shows final body weight measurements of non-diabetic and diabetic male Wistar rats after being subjected to various treatments for 5 weeks. The mean final body weight of non-diabetic rats treated with vindoline (group 2) was significantly higher in comparison to that of the normal control (group 1), (338.4 ±13.10g versus 293.0 ± 8.251g). There was no significant difference between final body weights of the non-diabetic control (group 1) and non-diabetic rats treated with glibenclamide (group 3), (293.0 ± 8.251g versus 310.0 ± 10.41g). Additionally, the difference in final body weights between groups 2 and 3 was not significant (338.4 ±13.10g versus 310.0 ± 10.41g), $p>0.05$. Final body weights of the diabetic control (group 4), were significantly lower in comparison to those of the normal control (group 1), (243.1 ± 11.88g versus 293.0 ± 8.251g), $p<0.05$. The final body weights of non-diabetic rats treated with vindoline (group 2) were significantly higher ($p<0.05$) in comparison to mean final body weights of the following diabetic groups; group 4 (338.4 ± 13.10g versus 243.1 ± 11.88g), group 5 (338.4 ± 13.10g versus 241.0 ± 9.417g) and group 6 (338.4 ± 13.10g versus 255.0 ± 5.586g). The difference in final body weights between the diabetic control (group 4) and diabetic rats treated with vindoline (group 5); (243.1 ± 11.88g versus 241.0 ± 9.417g)

and the difference between final body weights of the diabetic control (group 4) and diabetic rats supplemented with glibenclamide (group 6); (243.1 ± 11.88g versus 255.0 ± 5.586g) was not statistically significant, ($p>0.05$). There was no significant difference in final body weights between groups 5 and 6 (241.0 ± 9.417g versus 255.0 ± 5.586g), $p>0.05$.

Experimental Groups

Group 1: Untreated, non-diabetic rats (normal control)

Group 2: Non-diabetic rats treated with 20 mg/kg b.w. vindoline

Group 3: Non-diabetic rats treated with 50 mg/kg b.w. glibenclamide

Group 4: Untreated, diabetic rats (diabetic control)

Group 5: Diabetic rats treated with 20 mg/kg b.w. vindoline

Group 6: Diabetic rats treated with 50 mg/kg b.w. glibenclamide

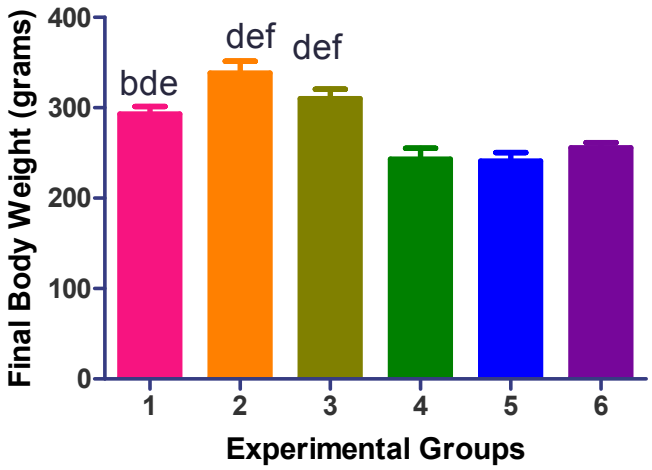

Figure 4.3: Final body weights of various groups after subjection to various treatments. Data is presented as mean ± SEM. (b) indicates significant difference of groups when compared to group 2 at $p<0.05$, (d) indicates significant difference of groups when compared to group 4 at $p<0.05$; (e) indicates significant difference of groups when compared to group 5 at $p<0.05$ and (f) indicates significant difference of groups when compared to group 6 at $p<0.05$.

4.4. Effect of vindoline on testicular and epididymal weights

Figure 4.4 compares testicular weights of type 2 diabetes-induced rats and non-diabetic rats after 5 weeks of subjection to various treatments. The differences in testicular weights between the normal control (group 1) and non-diabetic rats treated with vindoline (group 2),

(2.647 ± 0.09365g versus 2.941 ± 0.06671g) and the difference between testicular weights of the normal control (group 1) and non-diabetic rats treated with glibenclamide (group 3), (2.647 ± 0.09365g versus 2.906 ± 0.08967g) were not significantly different, $p>0.05$. There was no significant difference between testicular weights of groups 2 and 3 (2.941 ± 0.06671g versus 2.906 ± 0.08967g), $p>0.05$. The average testicular weight of the diabetic control (group 4) was significantly lower in comparison to that of the normal control (group 1), (2.123 ± 0.04656g versus 2.647 ± 0.09365g), $p<0.05$. Diabetic rats treated with vindoline (group 5) and diabetic rats treated with glibenclamide (group 6) had significantly lower testicular weights ($p<0.05$), in comparison to testicular weights of the normal control (group 1); (2.299 ± 0.06905g versus 2.647 ± 0.09365g) and (2.275 ± 0.06370g versus 2.647 ± 0.09365g), respectively. There was no significant difference ($p>0.05$), between testicular weights of group 4 and group 5 (2.123 ± 0.04656g versus 2.299 ± 0.06905g). Testicular weights of diabetic rats treated with glibenclamide (group 6) were not significantly different in comparison to testicular weights of group 4, (2.275 ± 0.06370g versus 2.123 ± 0.04656g). The difference in testicular weights between group 5 and group 6 was not statistically significant (2.299 ± 0.06905g versus 2.275 ± 0.06370g), $p>0.05$.

Figure 4.5 compares epididymal weights of diabetes-induced rats and non-diabetic rats after being subjected to various treatments for 5 weeks. The difference in epididymal weights between the normal control (group 1) and non-diabetic rats treated with vindoline (group 2) was non-significant, (1.047 ± 0.02988g versus 1.050 ± 0.02712g), $p>0.05$. Epididymal weights of non-diabetic rats treated with glibenclamide (group 3) were not statistically different in comparison to those of the normal control (group 1), (1.066 ± 0.01710g versus 1.047 ± 0.02988g), $p>0.05$. The difference between epididymal weights of groups 2 and 3 was statistically insignificant, (1.050 ± 0.02712g versus 1.066 ± 0.01710g), $p>0.05$. The differences between epididymal weights of the diabetic control (group 4) and diabetic rats treated with vindoline (group 5), (0.9202 ± 0.007020g versus 0.9720 ± 0.004497g) and the difference between epididymal weights of the diabetic control (group 4) and diabetic rats treated with glibenclamide (group 6), (0.9202 ± 0.007020g versus 0.9562 ± 0.005837g) were

not significant, p>0.05. Epididymal weights of group 5 and group 6 were not statistically different (0.9720 ± 0.004497g versus 0.9562 ± 0.005837g), p>0.05. Epididymal weights of non-diabetic groups (group 1, group 2 and group 3) were significantly higher than epididymal weights of the diabetic control (group 4); (1.047 ± 0.02988g versus 0.9202 ± 0.007020g), (1.050 ± 0.02712g versus 0.9202 ± 0.007020g) and (1.066 ± 0.01710g versus 0.9202 ± 0.007020g), respectively.

Experimental Groups

Group 1: Untreated, non-diabetic rats (normal control)

Group 2: Non-diabetic rats treated with 20 mg/kg b.w. vindoline

Group 3: Non-diabetic rats treated with 50 mg/kg b.w. glibenclamide

Group 4: Untreated, diabetic rats (diabetic control)

Group 5: Diabetic rats treated with 20 mg/kg b.w. vindoline

Group 6: Diabetic rats treated with 50 mg/kg b.w. glibenclamide

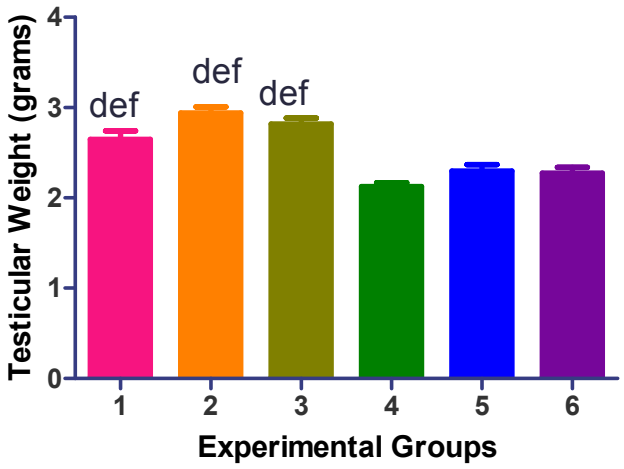

Figure 4.4: Testicular weights of various groups after subjection to various treatments. Data is presented as mean ± SEM. (d) indicates significant difference of groups when compared to group 4 at $p<0.05$; (e) indicates significant difference of groups when compared to group 5 at $p<0.05$ and (f) indicates significant difference of groups when compared to group 6 at $p<0.05$.

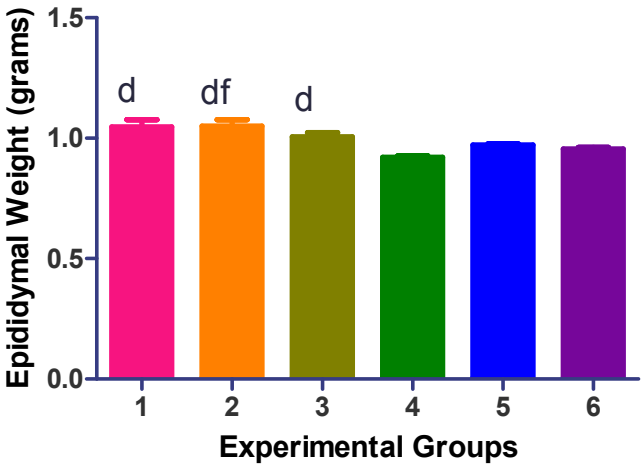

Figure 4.5: Epididymal weights of various groups after subjection to various treatments. Data is presented as mean ± SEM. (d) indicates significant difference of groups when compared to group 4 at $p<0.05$ and (f) indicates significant difference of groups when compared to group 6 at $p<0.05$.

4.5. Effects of vindoline on testicular and epididymal lipid peroxidation levels

Figure 4.6 demonstrates a non-significant difference ($p>0.05$), between testicular MDA levels of the normal control (group 1) and non-diabetic rats treated with vindoline (group 2), (0.2847 ± 0.01239 µmol/g versus 0.2770 ± 0.02008 µmol/g). The difference between testicular MDA levels of the normal control (group 1) and testicular MDA levels of non-diabetic rats treated with glibenclamide (group 3), was statistically insignificant (0.2847 ± 0.01239 µmol/g versus 0.2905 ± 0.001540 µmol/g), $p>0.05$. There was no significant difference between testicular MDA levels of groups 2 and 3 (0.2770 ± 0.02008 µmol/g versus 0.2905 ± 0.001540 µmol/g), $p>0.05$. Testicular MDA levels of the diabetic control (group 4) were significantly higher in comparison to testicular MDA levels of the normal control (group 1), (0.3890 ± 0.01046 µmol/g versus 0.2847 ± 0.01239 µmol/g), $p<0.05$. Diabetic rats treated with vindoline (group 5) and diabetic rats treated with glibenclamide (group 6) had significantly lower testicular MDA levels in comparison to testicular MDA levels of the diabetic control (group 4); (0.2654 ± 0.02747 µmol/g versus 0.3890 ± 0.01046 µmol/g) and (0.2877 ± 0.01934 µmol/g versus 0.3890 ± 0.01046 µmol/g) respectively. The difference in testicular MDA levels between groups 5 and 6 (0.2654 ± 0.02747 µmol/g versus 0.2877 ± 0.01934 µmol/g) was statistically insignificant ($p>0.05$).

Figure 4.7 demonstrates the effect of various treatments on epididymal MDA levels in adult male Wistar rats. No significant difference ($p>0.05$) was observed between epididymal MDA levels of the normal control (group 1) and non-diabetic rats treated with vindoline (group 2) (0.1913 ± 0.2290 µmol/g versus 0.1410 ± 0.01164 µmol/g). Figure 4.7 also shows a non-significant difference ($p>0.05$) between lipid peroxidation levels of the normal control (group 1) and non-diabetic group of rats treated with glibenclamide (group 3), (0.1913 ± 0.2290 µmol/g versus 0.2115 ± 0.01492 µmol/g). Epididymal MDA levels of the diabetic control (group 4) were significantly higher in comparison to those of the normal control (group 1), (0.4846 ± 0.03188 µmol/g versus 0.1913 ± 0.2290 µmol/g) $p<0.05$. No significant difference was observed between epididymal MDA levels of the diabetic control (group 4) and diabetic rats treated with vindoline (group 5), (0.4846 ± 0.03188 µmol/g versus 0.3756 ± 0.03689 µmol/g), $p>0.05$. The difference between epididymal lipid peroxidation levels of the diabetic control (group 4) and diabetic rats treated with glibenclamide (group 6) was not significant (0.4846 ± 0.03188 µmol/g versus 0.4380 ± 0.04474 µmol/g). The difference between epididymal MDA levels of groups 5 and 6 (0.3756 ± 0.03689 µmol/g versus 0.0.4380 ± 0.04474 µmol/g) was insignificant, $p>0.05$. Overall, epididymal MDA levels of non-diabetic groups (Groups 1, 2 and 3) were significantly lower in comparison to epididymal MDA levels of diabetic groups (Groups 4, 5 and 6).

Experimental Groups

Group 1: Untreated, non-diabetic rats (normal control)

Group 2: Non-diabetic rats treated with 20 mg/kg b.w. vindoline

Group 3: Non-diabetic rats treated with 50 mg/kg b.w. glibenclamide

Group 4: Untreated, diabetic rats (diabetic control)

Group 5: Diabetic rats treated with 20 mg/kg b.w. vindoline

Group 6: Diabetic rats treated with 50 mg/kg b.w. glibenclamide

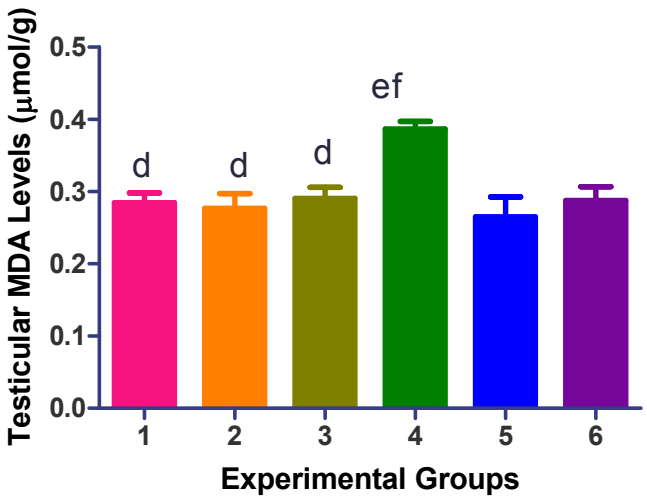

Figure 4.6: Testicular MDA levels of various groups after subjection to various treatments. Data is presented as mean ± SEM. (d) indicates significant difference of groups when compared to group 4 at $p<0.05$, (e) indicates significant difference of groups when compared to group 5 at $p<0.05$ and (f) indicates significant difference of groups when compared to group 6 at $p<0.05$, µmol/g: micromoles per gram.

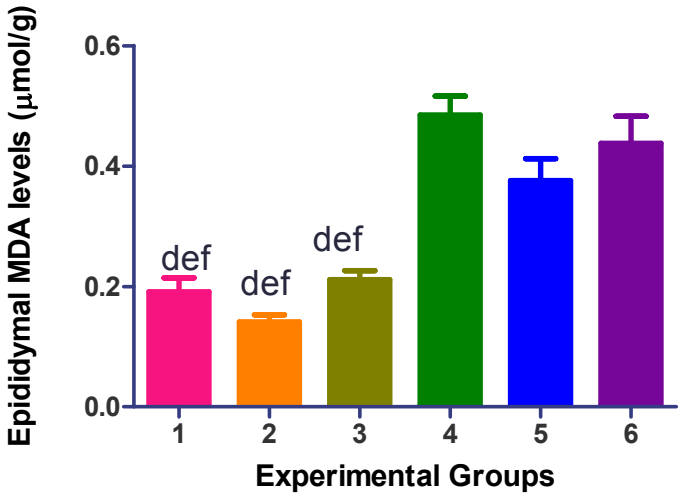

Figure 4.7: Epididymal MDA levels of various groups after subjection to various treatments. Data is presented as mean ± SEM. (d) indicates significant difference of groups when compared to group 4 at $p<0.05$; (e) indicates significant difference of groups when compared to group 5 at $p<0.05$ and (f) indicates significant difference of groups when compared to group 6 at $p<0.05$. µmol/g: micromoles per gram.

4.6. Effects of vindoline on testicular and epididymal SOD activities

Figure 4.8 compares testicular SOD activities between non-diabetic and diabetic rats after 5 weeks of subjection to various treatments. The difference in testicular SOD activity between non-diabetic rats supplemented with vindoline (group 2) and the normal control (group 1) was

not significant (1.041 ± 0.03497 µmol/mg versus 1.063 ± 0.007962 µmol/mg), p>0.05. There was no significant difference between testicular SOD activities of the normal control (group 1) and non-diabetic rats treated with glibenclamide (group 3), (1.063 ± 0.007962 µmol/mg versus 0.9725 ± 0.0347 µmol/mg), *p*>0.05. The difference between testicular SOD activities of groups 2 and 3 (1.041 ± 0.03497 µmol/mg versus 0.9725 ± 0.0347 µmol/mg) was also insignificant, *p*>0.05. The average SOD activity in testicular tissues of the diabetic control (group 4) were significantly lower in comparison to that of the normal control (group 1) (0.8313 ± 0.3629 µmol/mg versus 1.063 ± 0.007962 µmol/mg), p<0.05. No significant difference (p>0.05) was observed between testicular SOD activities of the diabetic control (group 4) and diabetic rats treated with vindoline (group 5), (0.8313 ± 0.3629 µmol/mg versus 0.8900 ± 0.01505 µmol/mg), p>0.05. The difference between average testicular SOD activities of the diabetic control (group 4) and diabetic rats treated with glibenclamide (group 6) was not significant (0.8313 ± 0.3629 µmol/mg versus 0.9517 ± 0.0446 µmol/mg), p>0.05. There was no significant difference between testicular SOD activities of groups 5 and 6 (0.8900 ± 0.01505 µmol/mg versus 0.9517 ± 0.0446 µmol/mg), (p>0.05). Testicular SOD activities of all non-diabetic groups (Groups 1, 2 and 3) were significantly higher (p<0.05), in comparison to testicular SOD activities of the diabetic control (group 4); (1.063 ± 0.007962 µmol/mg versus 0.8313 ± 0.3629 µmol/mg), (1.041 ± 0.03497 µmol/mg versus 0.8313 ± 0.3629 µmol/mg) and (0.9725 ± 0.0347 µmol/mg versus 0.8313 ± 0.3629 µmol/mg) respectively. SOD activity in testicular tissues of diabetic groups 5 and 6 were not significantly different from testicular SOD levels of the normal control (group 1),

Figure 4.9 compares epididymal SOD activities in diabetic and non-diabetic male Wistar rats after 5 weeks of subjection to various treatments. The difference in epididymal SOD activity between the normal control (group 1) and non-diabetic rats supplemented with vindoline (group 2) was not significant (2.117 ± 0.1476 µmol/mg versus 2.286 ± 0.2281 µmol/mg), p>0.05. Epididymal SOD activities of non-diabetic rats treated with glibenclamide (group 3) were not statistically different from epididymal SOD activities of the normal control (group 1), (1.841 ± 0.1348 µmol/mg versus 2.117 ± 0.1476 µmol/mg), p>0.05. No significant difference

was observed between epididymal SOD activities of groups 2 and 3 (2.286 ± 0.2281 ɥmol/mg versus 1.841 ± 0.1348 ɥmol/mg), p>0.05. Epididymal SOD activities of the diabetic control (group 4), were significantly lower (p<0.05) in comparison to those of the normal control (group 1), (0.9938 ± 0.06395 ɥmol/mg versus 2.117 ± 0.1476 ɥmol/mg). The difference in epididymal SOD activities between the diabetic control (group 4) and diabetic rats treated with glibenclamide (group 6) was insignificant (0.9938 ± 0.06395 ɥmol/mg versus 1.104 ± 0.06542 ɥmol/mg), p>0.05. However, epididymal SOD activities of group 5 subjects were significantly higher in comparison to those of the diabetic control (group 4), (1.513 ± 0.089 µmol/mg versus 0.9938 ± 0.06395 µmol/mg), p<0.05.

Experimental Groups

Group 1: Untreated, non-diabetic rats (normal control)

Group 2: Non-diabetic rats treated with 20 mg/kg b.w. vindoline

Group 3: Non-diabetic rats treated with 50 mg/kg b.w. glibenclamide

Group 4: Untreated, diabetic rats (diabetic control)

Group 5: Diabetic rats treated with 20 mg/kg b.w. vindoline

Group 6: Diabetic rats treated with 50 mg/kg b.w. glibenclamide

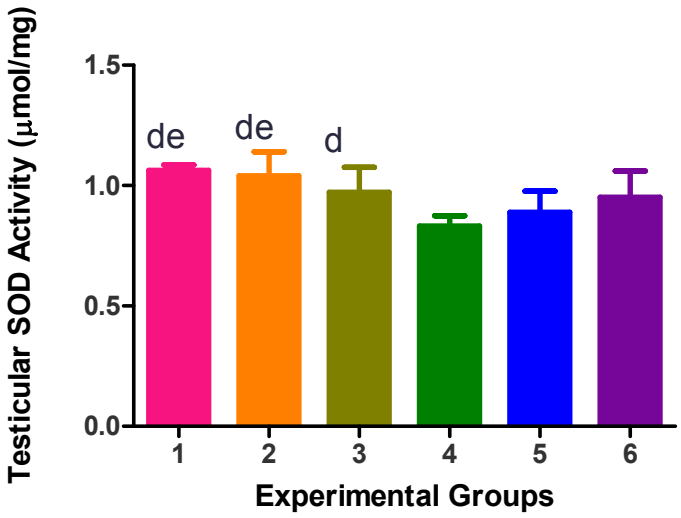

Figure 4.8: Testicular SOD activity of various groups after subjection to various treatments. Data is presented as mean ± SEM. (d) indicates significant difference of groups when compared to group 4 at *p*<0.05 and (e) indicates significant difference of groups when compared to group 5 at *p*<0.05. µmol/mg: micromoles per milligram.

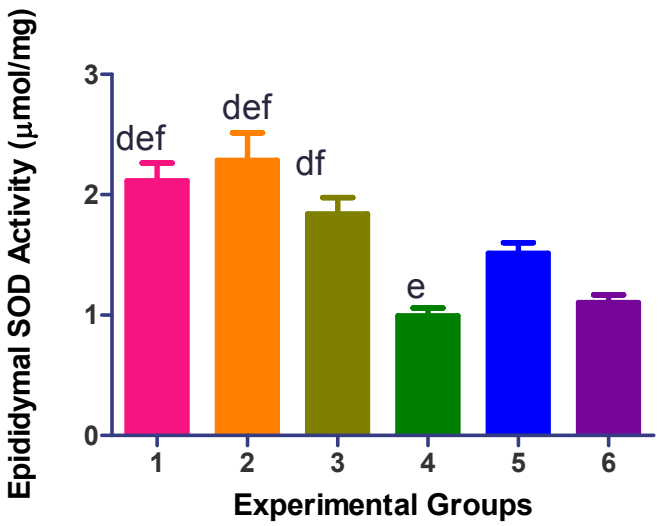

Figure 4.9: Epididymal SOD activity of various groups after subjection to various treatments. Data is presented as mean ± SEM. (d) indicates significant difference of groups when compared to group 4 at $p<0.05$; (e) indicates significant difference of groups when compared to group 5 at $p<0.05$ and (f) indicates significant difference of groups when compared to group 6 at $p<0.05$. µmol/mg: micromoles per milligram.

4.7. Effects of vindoline on testicular and epididymal catalase activity

According to Figure 4.10, non-diabetic rats treated with vindoline (group 2) had higher testicular catalase activity in comparison to that of the normal control (group 1), (38.69 ± 0.9186 µmol/mg versus 30.69 ± 0.9186 µmol/mg), $p<0.05$. The difference between testicular catalase activities of non-diabetic rats treated with glibenclamide (group 3) and the normal control (group 1) was not significant (24.77 ± 1.588 µmol/mg versus 30.69 ± 0.9186 µmol/mg), $p>0.05$. The diabetic control (group 4) had significantly lower testicular catalase activity in comparison to that of the normal control (group 1), (23.65 ± 0.994 µmol/mg versus 30.69 ± 0.9186 µmol/mg), $p<0.05$. Testicular catalase activity of diabetic rats treated with glibenclamide (group 6) were not statistically different from testicular catalase activity of the diabetic control (group 4), (19.08 ± 1.486 µmol/mg versus 23.65 ± 0.994 µmol/mg), $p>0.05$. Testicular catalase activities of group 5 were significantly higher in comparison to those of the diabetic control, (32.34 ± 1.589 µmol/mg versus 23.65 ± 0.994 µmol/mg). Furthermore, testicular catalase activities of group 5 were significantly higher in comparison of those of group 6 (32.34 ± 1.589 µmol/mg versus 19.08 ± 1.486 µmol/mg), $p>0.05$.

According to Figure 4.11, the difference between epididymal catalase activity of the normal control (group 1) and non-diabetic rats supplemented with vindoline (group 2); (62.66 ± 0.5878 ɥmol/mg versus 63.54 versus 1.462 ɥmol/mg) and the difference between epididymal catalase activities of the normal control (group 1) and non-diabetic rats treated with glibenclamide (group 3); (62.66 ± 0.5878 ɥmol/mg versus 61.65 versus 2.396 ɥmol/mg), were not significant, $p>0.05$. The difference in epididymal catalase activities between groups 2 and 3 was also insignificant (63.54 ± 1.462 ɥmol/mg versus 61.65 ± 2.396 ɥmol/mg), $p>0.05$. Epididymal catalase activity of the diabetic control (group 4) was significantly lower in comparison to that of the normal control (group 1), (27.73 ± 1.131 ɥmol/mg versus 62.66 ± 0.5878 ɥmol/mg), $p<0.05$. Epididymal catalase activities of diabetic rats treated with vindoline (group 5) and diabetic rats treated with glibenclamide (group 6), were significantly higher in comparison to that of the diabetic control (group 4); (39.79 ± 1.587 ɥmol/mg versus 27.73 ± 1.131 ɥmol/mg) and (38.60 ± 0.9332 µmol/mg versus 27.73 ± 1.131 µmol/mg) respectively, $p<0.05$. The difference in epididymal catalase activity between groups 5 and 6 was statistically insignificant (39.79 ± 1.587 ɥmol/mg versus 38.6 ± 0.9332 ɥmol/mg), $p>0.05$. Overall, epididymal catalase activities of diabetic groups (4, 5 and 6) were significantly lower, ($p<0.05$), in comparison to those of the non-diabetic groups (1, 2 and 3).

Experimental Groups

Group 1: Untreated, non-diabetic rats (normal control)

Group 2: Non-diabetic rats treated with 20 mg/kg b.w. vindoline

Group 3: Non-diabetic rats treated with 50 mg/kg b.w. glibenclamide

Group 4: Untreated, diabetic rats (diabetic control)

Group 5: Diabetic rats treated with 20 mg/kg b.w. vindoline

Group 6: Diabetic rats treated with 50 mg/kg b.w. glibenclamide

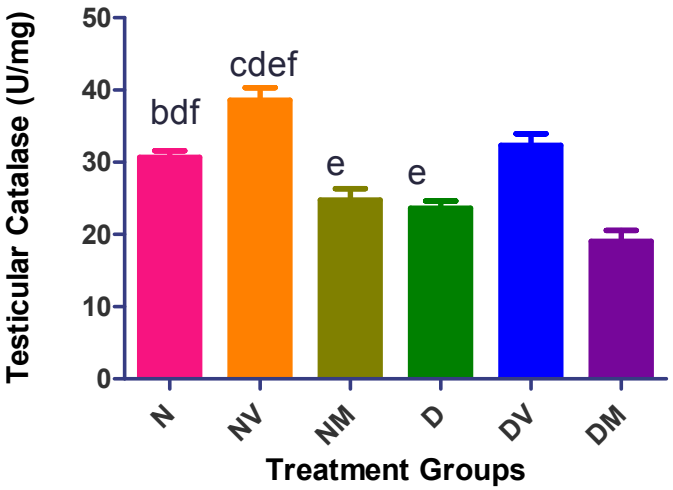

Figure 4.10: Testicular catalase activity of various groups after subjection to various treatments. Data is presented as mean ± SEM. (b) indicates significant difference of groups when compared to group 2 at $p<0.05$, (c) indicates significant difference of groups when compared to group 3 at $p<0.05$; (d) indicates significant difference of groups when compared to group 4 at $p<0.05$; (e) indicates significant difference of groups when compared to group 5 at $p<0.05$ and (f) indicates significant difference of groups when compared to group 6 at $p<0.05$. µmol/mg: micromoles per milligram.

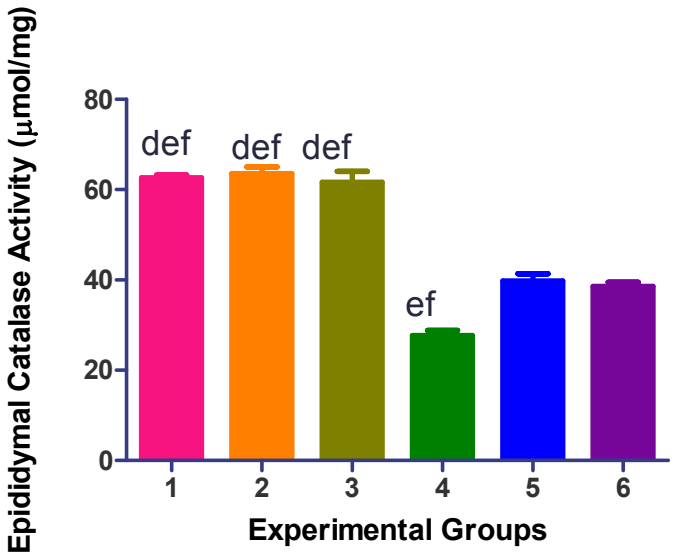

Figure 4.11: Epididymal catalase activity of various groups after subjection to various treatments. Data is presented as mean ± SEM. (b) indicates significant difference of groups when compared to group 2 at $p<0.05$, (d) indicates significant difference of groups when compared to group 4 at $p<0.05$; (e) indicates significant difference of groups when compared to group 5 at $p<0.05$ and (f) indicates significant difference of groups when compared to group 6 at $p<0.05$. µmol/mg: micromoles per milligram.

4.8. Effects of vindoline on testicular and epididymal FRAP levels

Figure 4.12 illustrates testicular FRAP levels of diabetic and non-diabetic rats after being subjected to various treatments. The difference in testicular FRAP levels between the normal control (group 1) and non-diabetic rats treated with vindoline (group 2) was statistically insignificant, (3.285 ± 0.1319 µmol/g versus 2.374 ± 0.2127 µmol/g), $p>0.05$. There was no significant difference between testicular FRAP concentrations of the normal control (group 1) and non-diabetic rats treated with glibenclamide (group 3); (3.285 ± 0.1319 µmol/g versus 3.483 ± 0.2617 µmol/g). The difference between testicular FRAP levels of non-diabetic rats treated with vindoline (group 2) and testicular FRAP levels of non-diabetic rats treated with glibenclamide (group 3) was not significant, (2.374 ± 0.2127 µmol/g versus 3.483 ± 0.2617 µmol/g), $p>0.05$. The difference in testicular FRAP concentration between the diabetic control (group 4) and the normal control (group 1) was not significant (2.566 ± 0.1273 µmol/g versus 3.285 ± 0.1319 µmol/g), $p<0.05$. FRAP concentration in testicular tissues of diabetic rats treated with vindoline (group 5) were significantly higher in comparison to those of the diabetic control (group 4); (4.524 ± 0.4045 µmol/g versus 2.566 ± 0.1273 µmol/g), $p<0.05$. The mean testicular FRAP concentration of group 6 subjects was significantly higher in comparison to that of the diabetic control (3.941 ± 0.3147 µmol/g vesus 2.566 ± 0.11273 µmol/g), $p<0.05$. The average testicular FRAP concentrations of group 5 and group 6 were not statistically different, (4.524 ± 0.4045 µmol/g versus 3.941 ± 0.3147 µmol/g), ($p>0.05$).

Figure 4.13 compares epididymal FRAP levels between diabetic and non-diabetic rats subjected to various treatments for 5 weeks. The differences in epididymal FRAP levels between the normal control (group 1) and non-diabetic rats supplemented with vindoline (group 2), (3.007 ± 0.05471 µmol/g versus 2.402 ± 0.2198 µmol/g) and the difference in epididymal FRAP concentration between the normal control (group 1) and non-diabetic rats fed on glibenclamide (group 3), (3.007 ± 0.05471 µmol/g versus 3.371 ± 0.2077 µmol/g) were not statistically significant $p>0.05$. Epididymal FRAP levels of group 2 were however lower than those of group 3 (2.402 ± 0.2198 µmol/g versus 3.371 ± 0.2077 µmol/g). The difference between epididymal FRAP levels of the diabetic control (group 4) and normal

control (group 1) was statistically insignificant, (2.708 ± 0.04741 µmol/g versus 3.007 ± 0.05471 µmol/g), p>0.05. Diabetic rats treated with vindoline (group 5) and diabetic rats treated with glibenclamide (group 6) had significantly higher epididymal FRAP activities p<0.05, in comparison to the diabetic control (group 4); (2.708 ± 0.04741 µmol/g versus 3.702 ± 0.2126 µmol/g) and (2.708 ± 0.04741 µmol/g versus 4.372 ± 0.1498 µmol/g), respectively. The difference between epididymal FRAP concentrations of groups 5 and 6 was not significant (3.702 ± 0.2126 µmol/g versus 4.372 ± 0.1498 µmol/g), p>0.05.

Experimental Groups

Group 1: Untreated, non-diabetic rats (normal control)

Group 2: Non-diabetic rats treated with 20 mg/kg b.w. vindoline

Group 3: Non-diabetic rats treated with 50 mg/kg b.w. glibenclamide

Group 4: Untreated, diabetic rats (diabetic control)

Group 5: Diabetic rats treated with 20 mg/kg b.w. vindoline

Group 6: Diabetic rats treated with 50 mg/kg b.w. glibenclamide

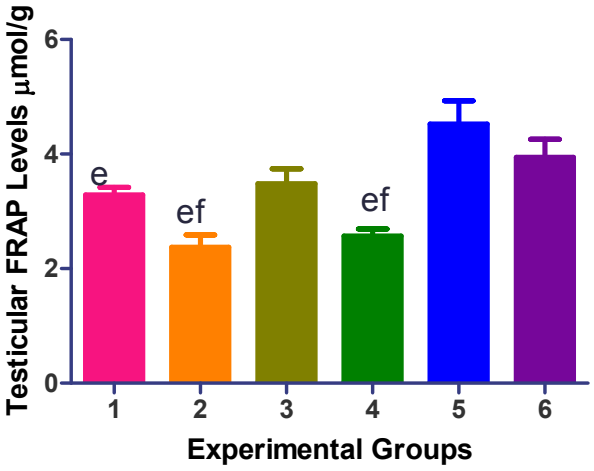

Figure 4.12: Testicular FRAP concentrations of various groups after subjection to various treatments. Data is presented as mean ± SEM. (e) indicates significant difference of groups when compared to group 5 at *p*<0.05 and (f) indicates significant difference of groups when compared to group 6 at *p*<0.05. µmol/g represents micromoles per gram.

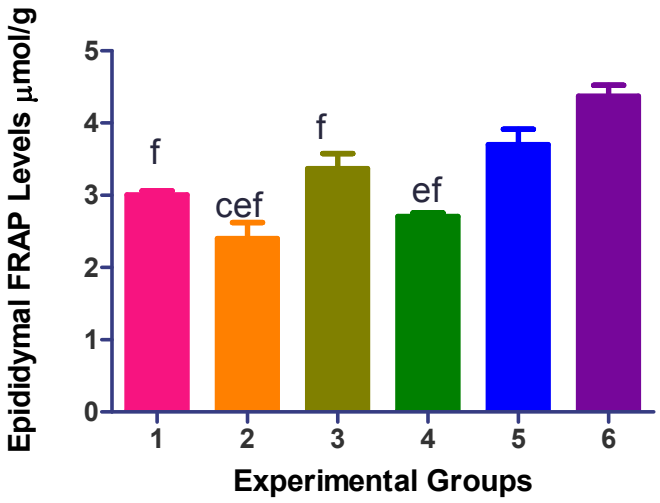

Figure 4.13: Epididymal FRAP concentrations of various groups after subjection to various treatments. Data is presented as mean ± SEM. (c) indicates significant difference of groups when compared to group 3 at $p<0.05$; (e) indicates significant difference of groups when compared to group 5 at $p<0.05$ and (f) indicates significant difference of groups when compared to group 6 at $p<0.05$. µmol/g represents micromoles per gram

4.9. Effect of vindoline on testicular and epididymal ORAC levels of Wistar rats

Figure 4.14 illustrates the differences in testicular ORAC levels between diabetes-induced and non-diabetic Wistar rats after subjection to various treatments for 5 weeks. Testicular ORAC levels of non-diabetic rats treated with vindoline (group 2) were significantly higher ($p<0.05$), in comparison to those of the normal control (group 1), (5.109 ± 0.1298 µmol TE/g versus 3.710 ± 0.4902 µmol TE/g). The difference between testicular ORAC levels of non-diabetic rats treated with glibenclamide (group 3) and the normal control (group 1), (3.892 ± 0.3946 µmol TE/g versus 3.710 ± 0.4902 µmol TE/g) was non-significant, $p>0.05$. Testicular ORAC levels of group 2 were significantly higher than those of group 3, (5.109 ± 0.1298 µmol TE/g versus 3.892 ± 0.3946 µmol TE/g), $p<0.05$. Treatment of diabetic rats with vindoline (group 5) resulted in significantly higher testicular ORAC levels in comparison to the diabetic control (group 4), (3.143 ± 0.08254 µmol TE/g versus 1.926 ± 0.1577 µmol TE/g), $p<0.05$. The mean testicular ORAC value for group 6 was however not statistically different from that of the diabetic control (group 4), (3.009 ± 0.1354 µmol TE/g versus 1.926 ± 0.1577 µmol TE/g) $p>0.05$. The difference between mean testicular ORAC values of

groups 5 and 6 (3.143 ± 0.08254 μmol TE/g versus 3.009 ± 0.1354 μmol TE/g) was also non-significant, p>0.05).

Figure 4.15 illustrates epididymal ORAC levels of diabetic and non-diabetic Wistar rats after being subjected to various treatments for 5 weeks. Using the Bonferroni's Multiple Comparison Test that compares all pairs of columns, epididymal ORAC levels of all non-diabetic groups (1, 2 and 3) were significantly higher in comparison to those of all diabetic groups (4, 5 and 6). Epididymal ORAC levels of non-diabetic rats treated with vindoline (group 2) were not statistically different from epididymal ORAC levels of the normal control (group 1); (7.209 ± 0.1479 μmol TE/g versus 6.663 ± 0.2992 μmol TE/g), p>0.05. The difference between epididymal ORAC levels of the normal control (group 1) and non-diabetic rats treated with glibenclamide (group 3), was not significant (6.663 ± 0.2992 μmol TE/g versus 7.109 ± 0.1370 μmol TE/g), p>0.05. There was no significant difference between epididymal ORAC levels of groups 2 and 3 (7.209 ± 0.1479 μmol TE/g versus 7.109 ± 0.1370 μmol TE/g), p>0.05. The difference in epididymal ORAC levels between the diabetic control (group 4) and diabetic rats treated with vindoline (group 5); (3.809 ± 0.4244 μmol TE/g versus 4.571 ± 0.2455 μmol TE/g), the difference between epididymal ORAC levels of groups 4 and 6 (3.809 ± 0.4244 μmol TE/g versus 3.896 ± 0.2300 μmol TE/g) and the difference between epididymal ORAC levels of group 5 and group 6; (4.571 ± 0.2455 μmol TE/g versus 3.896 ± 0.2300 μmol TE/g) were statistically insignificant (p>0.05).

Experimental Groups

Group 1: Untreated, non-diabetic rats (normal control)

Group 2: Non-diabetic rats treated with 20 mg/kg b.w. vindoline

Group 3: Non-diabetic rats treated with 50 mg/kg b.w. glibenclamide

Group 4: Untreated, diabetic rats (diabetic control)

Group 5: Diabetic rats treated with 20 mg/kg b.w. vindoline

Group 6: Diabetic rats treated with 50 mg/kg b.w. glibenclamide

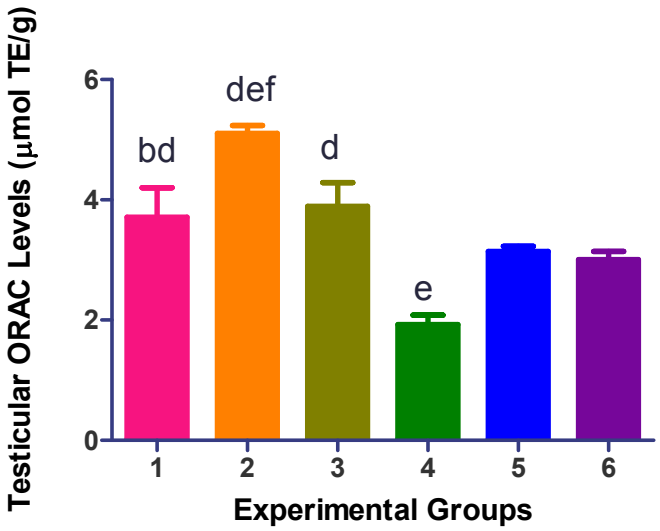

Figure 4.14: Testicular ORAC levels of various groups after subjection to various treatments. Data is presented as mean ± SEM. (b) indicates significant difference of groups when compared to group 2 at $p<0.05$; (d) indicates significant difference of groups when compared to group 4 at $p<0.05$; (e) indicates significant difference of groups when compared to group 5 at $p<0.05$ and (f) indicates significant difference of groups when compared to group 6 at $p<0.05$.

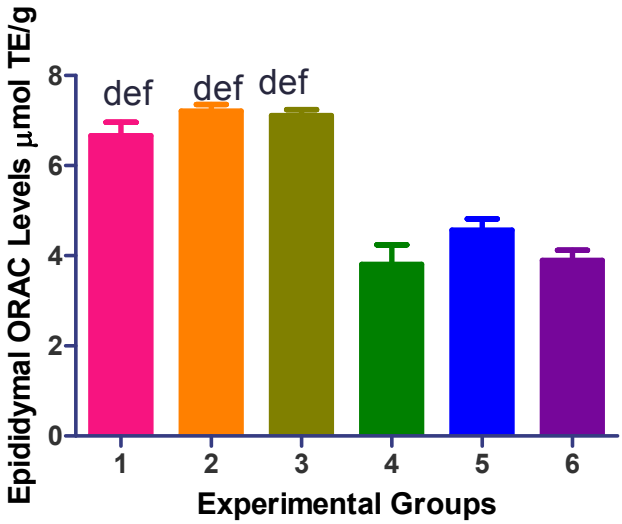

Figure 4.15: Epididymal ORAC levels of various groups after subjection to various treatments. Data is presented as mean ± SEM. (d) indicates significant difference of groups when compared to group 4 at $p<0.05$; (e) indicates significant difference of groups when compared to group 5 at $p<0.05$ and (f) indicates significant difference of groups when compared to group 6 at $p<0.05$.

CHAPTER FIVE

DISCUSSION

Previous research support the fact that diabetes mellitus contributes significantly to the generation of ROS and oxidative stress that leads to impairment of male reproductive function particularly in testicular and epididymal tissues (Ding et al., 2015). Medicinal plants are viewed as an easily available, potent source of antioxidants capable of scavenging free radicals and fighting diabetes-induced oxidative stress and have proven to be clinically effective and relatively less toxic than existing antidiabetic orthodox drugs (Sen et al., 2010; Bhatt et al., 2013). The World Health Organisation has therefore encouraged frontiers of scientific evaluation of diverse plant species for possible antidiabetic efficacy (Chikezie et al., 2015).

The present study sought to add scientific knowledge pertaining to the possible antidiabetic and antioxidant activities of vindoline (a bioactive ingredient from *C. roseus*), particularly in testicular and epididymal tissues. It follows scientific studies by Tiong et al, (2013) and Yao et al. (2013) who reported on the antidiabetic and antioxidant properties of vindoline. Tiong et al, (2013) reported that vindoline could enhance glucose uptake in β-TC6 and C2C12 cells in a dose-dependent manner and alleviate H_20_2-induced oxidative stress in β-TC6 cells thereby showing its potential as an effective antioxidant. Additionally, Yao et al, (2013) reported that vindoline was able to enhance glucose-stimulated insulin release in a glucose- and dose-dependent manner by inhibiting Kv2.1 potassium channels and reducing the voltage-dependent outward potassium currents. They reported that 20mg/kg b.w treatment with vindoline improved glucose homeostasis as reflected by its ability to increase the concentration of plasma insulin, protect pancreatic β-cells, decrease glycated haemoglobin levels, reduce blood glucose levels, reduce plasma triglyceride concentration and improve oral glucose tolerance test results. The present study investigated the antidiabetic potential of vindoline, its potential to reduce diabetes-induced oxidative stress and improve antioxidant

capacity in testicular and epididymal tissues using diabetes-induced adult Wistar rats as an experimental model.

5.1. Induction of diabetes before various treatments.

An ideal animal model of T2DM is expected to resemble as much as possible diabetic conditions seen in humans affected with T2DM. The administration of high energy diet and a single low dose STZ have been demonstrated to induce insulin resistance in animal models and partially destroy pancreatic β-cells leading to insufficient insulin secretion and closely mimicking the symptoms observed in human T2DM patients (Wilson & Islam, 2012).

With regards to the present study, the possible therapeutic effect of vindoline against diabetes-induced oxidative stress in testicular and epididymal tissues was investigated using 10% fructose water and STZ- induced diabetic male Wistar rats as an animal model. Diabetes was induced in male Wistar rats to be used as group 4, 5 and 6 subjects by feeding them on 10% fructose water for 2 weeks and thereafter administering a single intraperitoneal injection of STZ (40mg/kg b.w). Hyperglycaemia was observed 72 hours after STZ administration and this was confirmed by high fasting blood glucose levels in diabetes-induced rats of groups 4, 5 and 6. As such, a T2DM experimental model was successfully created in rats to be used as groups 4, 5 and 6 subjects. These diabetes-induced rats were then subjected to various treatments with the aim of evaluating the possible therapeutic effects of vindoline against diabetes-induced oxidative stress effects in testicular and epididymal tissues.

5.2. Evaluation of final body weights, testicular and epididymal weights after treatments

This research project compared final body weights, epididymal weights and testicular weights of diabetic and non-diabetic rats subjected to various treatments for 5 weeks. Final body weights, epididymal weights and testicular weights of the diabetic control (group 4) were significantly lower in comparison to those of the normal control (group 1). These findings are

in agreement with results from previous studies by Abbasi et al, (2013) and Korejo et al, (2016) who reported reduced body weights, testicular weights and epididymal weights in diabetic animal models in comparison to their normal controls. Insufficient insulin secretion and dysfunction in the insulin signalling pathways as seen in diabetic conditions result in disturbed glucose homeostasis. This prompts the body to use alternative sources of energy such as lipids and proteins instead of glucose by gluconeogenesis (Gannon & Nuttall, 2006). Diabetes is therefore usually associated with increased protein degradation, decreased protein synthesis and increased gluconeogenesis (Perry et al., 2016), that ultimately leads to rapid organ and body weight loss. Final body weights of non-diabetic treated groups (group 2 and group 3) were also higher than those of the diabetic control (group 4) mainly due to undisturbed glucose metabolism and no muscle atrophy in these non-diabetic groups. As such, rats from non-diabetic groups (1, 2 and 3) were able to maintain high body weights in comparison to the diabetic control (group 4).

Non-diabetic rats treated with vindoline (group 2) had significantly higher body weights in comparison to the normal control (group 1) showing the possible enhancing and protective effects of vindoline in terms of promoting increase in body weights in non-diabetic conditions. However, treatment of non-diabetic rats with vindoline (group 2) or glibenclamide (group 3) for 5 weeks did not have a significant impact on both testicular and epididymal weights in comparison to the normal control (group 1).

Results of this study demonstrated that treatment of diabetic male Wistar rats with 20mg/kg b.w vindoline (group 5) or 50mg/kg b.w glibenclamide (group 6) for 5 weeks did not have a significant impact in terms of increasing final body weights and testicular weights of diabetes-induced, male Wistar rats when compared against the diabetic control (group 4). Vindoline and glibenclamide might have not been effective in reducing gluconeogenesis as seen in diabetic conditions. Diabetes is associated with increased gluconeogenesis which might precipitate organ and body weight loss (Gannon & Nuttall, 2006; Perry et al., 2016). Alternatively, failure of vindoline and glibenclamide to significantly improve body weight gain

and testicular weights in T2DM-induced rats of this study might have been due to a short treatment period or an insufficient dosage.

Treatment of diabetic rats with 20mg/kg b.w. vindoline for 5 weeks slightly increased epididymal weights in comparison to the diabetic control (group 4) although the increase was not statistically significant ($p>0.05$). The average epididymal weight of diabetic rats treated with vindoline was however not significantly different from the mean epididymal weight of the normal control. It can therefore be concluded that the administration of vindoline was able to normalise epididymal weights in type 2-diabetic rats treated with vindoline for 5 weeks.

5.3. The effect of vindoline on blood glucose and LPO levels in various treated groups.

There is a close relationship between chronic hyperglycaemia, oxidative stress and LPO in the progression of diabetes mellitus (Maritim et al., 2003), and diabetes-induced sexual dysfunction is considered one of the most prevalent diabetic complications where oxidative stress plays an important role in its pathogenesis. Results of this study demonstrated high blood glucose levels and high oxidative stress as reflected by MDA levels in both testicular and epididymal tissues of the diabetic control. Final blood glucose levels of diabetic rats treated with vindoline remained significantly higher than those of the normal control. The inability of vindoline to restore blood glucose levels in these diabetic rats might have been due to a shorter feeding period, an insufficient dosage or simply the fact that vindoline is incapable of restoring fasting blood glucose levels in T2DM conditions to normal. In contrast, non-diabetic rats had normal fasting blood glucose levels after 5 weeks of treatment with vindoline and glibenclamide, mainly due to absence of diabetic pathology.

Testicular MDA levels of the diabetic control were significantly higher in comparison to those of normal control. This finding is in-line with previous studies by Nelli et al, (2013) and Ostovan et al, (2016) who reported high testicular lipid peroxidation in diabetic rats. Treatment of diabetic rats with vindoline (group 5), was however able to reduce testicular

MDA levels to normal. These results therefore show that vindoline has the potential to restore normal oxidative balance in diabetic testicular tissues.

Epididymal MDA levels of the diabetic control were also significantly higher in comparison to those of the normal control. This finding is also in line with previous studies by Nelli et al, (2013) and Ostovan et al, (2016) who reported high epididymal lipid peroxidation levels in epididymal tissues of diabetic rats. Although there was a significant reduction in epididymal MDA in diabetic rats treated with vindoline (group 5) when compared against the diabetic control (group 4), vindoline could not restore diabetic epididymal MDA to levels equal to those of the normal control (group 1). As such, it can be concluded that treatment with vindoline for 5 weeks can significantly reduce oxidative stress levels in epididymal tissues of T2DM- induced rats but not necessarily restore them to normal. Treatment of non-diabetic rats with vindoline (group 2) did not produce a significant difference in epididymal MDA levels when compared to the normal control (group 1).

5.4. Evaluation of the effect of vindoline on testicular and epididymal SOD activity.

Oxidative stress and LPO depletes antioxidant defence systems in diabetic conditions and a dysfunctional antioxidant system cannot protect tissues against ROS. As such, free radicals continue to accumulate leading to enhanced oxidative stress (Maiti et al., 2017). SOD is an endogenous antioxidant enzyme that detoxifies the superoxide anion into the less reactive H_2O_2 molecules via dismutation (Sheng et al., 2014). H_2O_2 is further detoxified by activities of glutathione peroxidase or catalase. Catalase is a haem-containing antioxidant enzyme that catalyses the detoxification of H_2O_2 into water and non-ROS (Birben et al., 2012). SOD therefore acts as the primary defence against superoxide radicals by preventing further free radical generation. Decreased activities of the SOD and catalase antioxidant enzymes in various tissues of diabetic rats clearly demonstrate that these antioxidant enzymes are easily inactivated by diabetes-induced ROS and lipid peroxides (Maritim et al., 2003).

No significant difference was observed in both testicular and epididymal SOD activity after 5 weeks of treating non-diabetic rats with vindoline. The relatively normal levels of blood glucose, low MDA levels and normal catalase and SOD activity in both testicular and epididymal tissues of non-diabetic rats treated with vindoline (group 2) may be due to the absence of diabetic pathology in these rats. Non-diabetic rats used as group 2 subjects were not pre-exposed to 10% fructose water or STZ. As such, glucose levels in these rats remained normal, minimum levels of free radicals were produced and there was less oxidative stress in testicular and epididymal tissues of these rats. As a result of low oxidative stress, antioxidant enzyme activity was not altered and high SOD activity was maintained.

Testicular SOD activity in the diabetic control (group 4) was significantly lower in comparison to that of the normal control (group 1). This finding is in line with previous studies by Xu et al, (2014), Afifi et al, (2015) and Fatani et al. (2015) who reported significantly low testicular SOD activities in diabetes-induced rats. Chronic hyperglycaemia in diabetic conditions promotes free radical production precipitating oxidative stress that is capable of altering antioxidant enzymes in various ways such that antioxidant enzymes' activities are reduced. This leads to the accumulation of free radicals without adequate detoxification thereby enhancing testicular and epididymal oxidative stress. High oxidative stress due to chronic hyperglycaemia in diabetic rats might have altered SOD antioxidant enzymes leading to reduced SOD activity in testicular tissues of the diabetic control. Although the difference between testicular SOD activity of diabetic rats treated with vindoline (group 5) and the diabetic control (group 4) was statistically insignificant, there was no significant difference between testicular SOD activity of group 5 and the normal control. This shows that treatment of diabetic rats with vindoline has the potential to normalise testicular SOD activity in diabetic conditions.

Epididymal SOD activity in diabetic rats treated with vindoline (group 5) was significantly higher than that reported for the diabetic control (group 4). This shows the possible efficacy of vindoline in improving epididymal SOD activity in diabetic rats. Chronic hyperglycaemia in

diabetic rats decreased epididymal SOD activity, however, administration of vindoline was able to improve and significantly increase epididymal SOD activity although to levels not as high as those recorded for the normal control (group 1).

5.5: The effect of vindoline on testicular and epididymal catalase activity.

Testicular and epididymal catalase activities of the diabetic control (group 4) were significantly lower in comparison to that of the normal control (group 1). These findings are in agreement with previous reports by Nelli et al, (2013), Afifi et al, (2015) and Fatani et al, (2015) who reported low testicular and epididymal catalase activities in diabetes-induced animal models. Diabetes mellitus is associated with high levels of ROS which subsequently attack amino acids that are the building blocks of antioxidant enzymes leading to the modification and alteration of antioxidant enzyme activity. As such, diabetes is usually associated with low antioxidant activities (Maritim et al., 2003).

Testicular catalase activity in non-diabetic rats treated with vindoline (group 2) was significantly higher in comparison to that of the normal control (group 1). This shows the potential efficacy of vindoline in enhancing testicular catalase activity in non-diabetic subjects. Additionally, treatment of diabetic rats with vindoline (group 5) increased testicular catalase activity to levels near normal. These findings show the potential of vindoline in restoring testicular catalase levels in diabetic conditions.

Treatment of non-diabetic rats with vindoline (group 2) or glibenclamide (group 3) did not have a significant impact on epididymal catalase activity. However, treatment of diabetic rats with vindoline (group 5) and glibenclamide (group 6) significantly increased epididymal catalase levels in diabetes-induced rats although they could not restore epididymal catalase activity to normal. These results therefore show that vindoline can potentially improve epididymal catalase activity in diabetes-induced animal models but not necessarily restore it to normal.

5.6. Assessment of vindoline on testicular and epididymal total antioxidant capacity

A number of assays are available to determine the total antioxidant capacity of biological and phytochemical compounds. The current study used the FRAP and ORAC assays to measure the effects of vindoline on testicular and epididymal total antioxidant capacity in type 2 diabetic rats and non-diabetic rats.

FRAP summarises the overall antioxidant power of a sample based on its ferrous ion reducing power by antioxidant vitamins and enzymes (Kiran et al., 2016). Results of this study showed no significant difference between testicular and epididymal FRAP levels of the diabetic control (group 4) in comparison to that of the normal control (group 1). Testicular and epididymal FRAP levels of diabetic rats treated with vindoline (group 5) and glibenclamide (group 6) were significantly higher in comparison to those of the diabetic control (group 4). Treatment of diabetic rats with vindoline (group 5) and glibenclamide (group 6) for 5 weeks increased levels of ferric ion reducing antioxidants possibly as a defence against diabetes-induced oxidative stress in testicular and epididymal tissues of diabetes-induced Wistar rats.

ORAC is an oxidation-inhibition based method that measures the ability of a specimen's antioxidants to inhibit the oxidation of a fluorescent probe derived from peroxyl radicals by hydrogen atom transfer (Sahari & Berenji, 2015). The association between antioxidants and free radicals result in delayed fluorescent decay and high ORAC levels. In diabetic conditions, the ROS-scavenging power of antioxidants becomes weakened resulting in diabetes-induced oxidative stress (Houcher et al., 2007).

Testicular and epididymal tissues of the non-diabetic control (group 1) had higher ORAC values in comparison to the diabetic control (group 4). The absence of diabetic pathology in the normal control (group 1) was responsible for the high ORAC levels seen in this group. Poor glycaemic control in the diabetic control led to the accumulation of ROS and reduced antioxidant capacity as reflected by low ORAC levels.

Treatment of non-diabetic rats with vindoline (group 2) significantly increased testicular ORAC levels, showing the potential of vindoline to enhance testicular antioxidant capacity in normal subjects. However, treatment of non-diabetic rats with vindoline for 5 weeks did not have a significant impact on epididymal ORAC levels of group 2 as they remained high and within the normal range. Group 2 subjects had excellent glycaemic control, low levels of ROS and optimum antioxidant activity hence high testicular and epididymal ORAC levels.

Treatment of diabetic rats with vindoline (group 5) significantly increased testicular ORAC levels to near-normal levels. This shows the potential of vindoline to restore testicular ORAC capacity in T2DM conditions. However, treatment of diabetic rats with vindoline did not significantly increase epididymal ORAC levels in type 2 diabetic rats. Group 5 epididymal ORAC levels remained significantly lower than that of the normal control after 5 weeks of treatment with vindoline, due to diabetic pathology.

Overall, treatment of non-diabetic and diabetic rats with vindoline (group 2 and group 5 respectively) significantly increased testicular ORAC levels in comparison to the normal control (group 1) and the diabetic control (group 4), respectively. This shows the potential of vindoline in enhancing testicular ORAC capacity in non-diabetic rats and its potential in improving testicular ORAC capacity in type 2 diabetic conditions. In contrast, administration of vindoline could not significantly improve epididymal ORAC levels in both non-diabetic rats and diabetic rats (group 2 and group 5 respectively). Results obtained in this study therefore show that treatment with vindoline for 5 weeks could not significantly enhance epididymal ORAC capacity in both non-diabetic (group 2) and T2DM conditions (group 5).

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

Investigations performed in this study focused on using vindoline for supplementation and therapeutic treatments under normal and diabetes-induced oxidative stress conditions in testicular and epididymal tissues of normal and T2DM-induced male Wistar rats. This study investigated if treatment of adult male Wistar rats with 10% fructose water for 2 weeks and a single low dose intraperitoneal injection with STZ could induce sustainable chronic hyperglycaemia that closely mimics human T2DM. Furthermore, this study evaluated the possible efficacy of vindoline; an alkaloid extractable from *C. roseus,* in reducing fasting blood glucose levels, reducing testicular and epididymal oxidative stress and increasing antioxidant enzyme activity and total antioxidant capacity in T2DM-induced male Wistar rats.

Diabetes-induced male Wistar rats demonstrated chronic hyperglycaemia, low body weights, low testicular and epididymal weights, high levels of testicular and epididymal oxidative stress and reduced oxygen radical absorption capacity in both testicular and epididymal tissues. Short-term treatment of diabetic rats with vindoline for 5 weeks significantly reduced fasting blood glucose levels after 5 weeks of treatment. However, fasting blood glucose levels of the diabetic rats remained significantly higher in comparison to fasting blood glucose levels of normal subjects. There is therefore a need to investigate the antihyperglycaemic effects of vindoline on a long-term.

Most results obtained in this study showed that treatment of diabetic rats with vindoline reduced lipid peroxidation levels, significantly increased antioxidant enzyme activities (as demonstrated by SOD and catalase activity assays) and increased the total antioxidant capacity as reflected by FRAP and ORAC assays in testicular and epididymal tissues. As such, findings of this study demonstrate the possible antihyperglycaemic effects as well as the antioxidant potential of vindoline treatment on testicular and epididymal tissues of diabetic and non-diabetic animal model.

Functional data such as spermatozoal motility and spermatozoal counts could not be performed due to lack of proper infrastructure. This limited our study to interpretation of oxidative stress biomarkers, antioxidant enzyme activities and total antioxidant capacity assays. There is therefore a need to investigate the possible efficacy of vindoline in ameliorating diabetes-induced oxidative stress in testicular and epididymal tissues using more advanced technologies. Furthermore, there is a need to investigate the long-term effects of vindoline on testicular and epididymal function in T2DM conditions. Studies on the safety and dose-dependent effects of vindoline on male reproductive function in diabetic conditions are also necessary.

REFERENCES

Abbasi, Z., Tabatabaei, S.R.F., Mazaheri, Y., Barati, F. & Morovvati, H. 2013. Effects of sesame oil on the reproductive parameters of diabetes mellitus -induced male rats . *The World's Journal of Men's Health*, (31(2): 141-9.

Acharya, D. & Shrivastava, K. 2008. Indigenous herbal Medicine: Tribal formulations and traditional herbal practices. Aavishkar Publishers Distributors. Jaipur, Indian 440.

Aebi, H, 1984. Catalase *in vitro*. *Methods Enzymology*, 105: 121-6.

Afifi, M., Almagharabi, O.A. & Kadasa, N.M. 2015. Ameliorative effect of zinc oxide nanoparticles on antioxidants and sperm characteristics in STZ diabetic rat testes. *Biomed Research International*, Article ID 153573: 6 pages.

Agarwal, A. & Allamaneni, S. 2006. Oxidative stress and human reproduction. In: Oxidative stress, disease and cancer. Singh, K.K. (eds). New York: Imperial College Press, London, UK. Chapter 23, pp 687- 703.

Agarwal, A., Hanafy, T. & Said, M. 2003. Role of sperm chromatin abnormalities and DNA damage in male infertility. *Human Reproduction Update*, 9 (4): 331–45.

Agarwal, A. & Saleh, R.A. 2002. Role of oxidants in male fertility: Rationale, significance and treatment. *Urologic Clinics of North America,* 29 (4): 817-27.

Agarwal, A., Makker, K. & Sharma, R. 2008. Clinical relevance of oxidative stress in male factor infertility: An update. *American Journal of Reproductive Immunology*, 59(1): 2-11.

Agarwal, A., Nallela, K.P., Allamaneni, S.S.R. & Said, T.M. 2004. Role of antioxidants in treatment of male infertility: an overview of the literature. *Reproductive Biomedicine Online,* 8: 616-27.

Agarwal, A., Prabakaran, S.A. & Sikka, S. 2007. Clinical relevance of Oxidative stress in patients with male factor infertility: Evidence-based analysis. *AUA Update series,* 26:1-12.

Agarwal, A., Virk, G., Ong, C. & du Plessis, S.S. 2014. Effect of oxidative stress on male reproduction. *World Journal Men's Health*, 32(1): 1-17.

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

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

Ajabnoor, M.A.M., Ahmed, A.A.E., Al-Ama M.N.A.H., Alshali, K.Z. & Ajabnoor, G.M.A. 2018. The covariant CDKN2A rs10811661 (C/T) gene polymorphism is associated with increased risk of type 2 diabetes mellitus in a Saudi Arabian population. *Middle East Journal of Medical Genetics*, 7(1): 19-25.

Al-Attah, A.M. & Zari, T.A. 2010. Influences of crude extract of tea leaves*, camellia sinensis*, on streptozotocin diabetic albino mice. *Saudi Journal of Biological Sciences*,17(4):295-301.

Ali, O. 2013. Genetics in type 2 diabetes*. World Journal of Diabetes,* 4(4): 114-23.

Almagro, L., Fernandez-Perez, F. & Pedreno, M.A. 2015. Indole alkaloids from *Catharanthus roseus*: Bioproduction and their effect on human health. *Molecules*, 20(2): 2973-3000.

Al-Shaqha, W., Khai, M., Salam, N., Azzi, A. & Chaudhary, A. 2015. Antidiabetic potential of *Catharanthus roseus* Linn and its effects on the glucose transport gene (GLUT-2 and GLUT-4) in streptozotocin induced diabetic Wistar rats. *BMC complementary and AlternatIve Medicine,* (ISMR), 15:379.

American Diabetes Association, 2009. Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 32(1): s62-7.

American Diabetes Association. 2009. Clinical, practice recommendations. *Diabetes care,* 32(1): 51-2.

American Diabetes Association. 2011. Standards of medical care in diabetes. *Diabetes care*, 34: 511-61.

American Diabetes Association. 2017. *Diabetes Care*, 40(1): S33-S43.

Andreozzi, F., Raciti, G.A., Nigro, C., Mannino, G.C., Procopio, T., Davalli, A.M., Beguinot, F., Sesti, G., Miele, C. & Folli, F. 2016. The GLP-1 receptor agonist exenatide and liraglutide activate glucose transport by an AMPK-dependent mechanism. *Journal of Transitional Medicine,* 14(1): 229.

Arduini, A., Bonomini. M., Savica, V., Amato, A. & Zammit, V. 2008. Carnitine in metabolic disease: Potential for pharmacological intervention. *Pharmacology Therapeutics*, 120(2): 149-156.

Arpanahi, A., Brinkworth, M., Iles, D., Krawetz, S.A., Paradowska, A., Plattsa, E., Saida, M., Steger, K., Tedder, P. & Miller, D. 2009. Endonuclease-sensitive regions of human spermatozoal chromatin are highly enriched in promoter and CTCF binding sequences. *Genome Research*, 19(8): 1338-49.

Arokoyo, D.S., Oyepipo, I.P., Du Plessis S.S. & Aboua Y.G. 2017. Male reproductive complications of diabetes mellitus and possible medicinal plant remedies: A review. *Research Journal of Health Sciences,* 5(3): 126-36.

Asadi, N., Bahmani, M., Kheradmand, A. & Rafieian- Kopaei, M. 2017. The impact of oxidative stress on testicular function and the role of antioxidants in improving it: A review. *Journal of Clinical and Diagnostic Research,* 11(5): IE01-IE05.

Aslam, J., Khan, S.H., Siddiqui, Z.H., Fatima, initial, Maqsoud, M., Bhat, M.A., Nasim, S.A., Ilah, A., Ahmad, I.Z., Khan, S.A., Mujib, A. & Sharma, M.P. 2010. Catharanthus roseus (L.) G. Don.: An important drug: Its implications and production. *International Journal of Comprehensive Pharmacy*, 4(12):1- 16.

Asmat, U., Abad, K. & Ismail, K. 2015. Diabetes mellitus and oxidative stress_ a concise review. *Saudi Pharmaceutical Journal,* 24(5): 547-53.

Ayala, A., Munoz, M.F. & Arguelles, S. 2014. Lipid peroxidation, production, metabolism and signalling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal.*Oxidative Medicine and Cellular Longevity, 2014*: ID 360438.

Badade, Z.G. & Samant, P.M. 2011. Role of oxidative stress in male infertility. *Journal of Biomedical Sciences and Research*, 3(2): 385-91.

Baek, I.J., Seo, D.S., Yon, J.M., Lee, S.R., Jin, J., Nahm, S.S., Jeong J.H., Chao, Y.K., Kang, J.K., Lee, B.J., Yun, Y.W. & Nam, S.Y. 2007. Tissue expression and cellular localisation of phospholipid hydroperoxides glutathione peroxidase (PHGPx) mRNA in male mice. *Journal of Molecular Histology,* 38(3):237-44.

Baker, M.A. & Aitken, R.J. 2004. The importance of redox regulated pathways in sperm cell biology. *Molecular and Cellular Endocrinology,* 38(3): 216:47.

Bansal, A. & Bilaspuri, G.S. 2011. Effect of ferrous sulphate and ascorbic acid on motility, viability and lipid peroxidation of cross-bred cattle bull spermatozoa. *The Animal Consortium*, 2(1): 100-04.

Bennetts, L.E. & Aitken, R.J.2005. A comperative study of oxidative DNA damage in mammalian spermatozoa. *Molecular reproduction and development*, 71(1): 77-87.

Benzi, I.F.F. & Strain, J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power: the FRAP assay. Analytical Biochemistry, 239(1):70-6.

Bertoglia, M.P., Gormaz, J.G., Libuy, M., Sanhueza, D., Gajardo, A. & Srur, A. 2017. The population impact of obesity, sedentary lifestyle, tobacco and alcohol consumption on the prevalence of type 2 diabetes: analysis of a health population survey in Chile. *PLoS ONE,* 12(5): E0178092.

Bhatt, I.D., Rawat, S. & Rawal, R. 2013. Antioxidants in medicinal plants. In Biotechnology for Medicinal plants- Micropropagation and improvement: Springer, Berlin, Heidelberg: 295-326.

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S. & Kalayci, O. 2012. Oxidative stress and antioxidant defence*. The World Allergy Organisation Journal*, 5(1):9-19.

Bolle, P., Evandri, M.G. & Saso, L. 2002. The controversial efficacy of vitamin E for human male infertility. *Contraception*, 65(4): 313-15.

Bommer, C., Heesemann, E., Sagalova, V., Manne G.J.R., Barnighausen, T. & Vollmer, S. 2017. The global economic burden of diabetes in adults aged 20-79: A Cost-of-illness study. The *Lancet Diabetes & Endocrinol*ogy, 5(6): 423-30.

Bommer, C., Sagalova, V., Heesemann, E., Manne, G.J., Atun, R., Barnighausen, T., Davies, J. & Vollmer, S. 2018. Global economic burden of diabetes in adults: projections from 2015 to 2030. *Diabetes care*, 41(5):963-70.

Brickle, J.F. 2006. Meglitinide analogues: a review of clinical data focused on recent trials. *Diabetes and Metabolism*, 32(2):113-20.

Bucak, M.N., Atessahin, A. & Yuce, A. 2010. Effect of antioxidants and oxidative stress parameters on ram semen after the freeze thawing process. *Small Ruminant Research*, 75(3):128-34.

Bukhari, D.A.M., Siddiqui, M.J., Shamsudin, S.H., Rahman, M.M., So'ad, S.Z.M. 2017. α-glucosidase inhibitory activity of selected Malaysian plants. *Journal of Pharmacy and Bioallied Sciences*, 9(3): 164-70.

Busik, J.V., Mohr, S. & Grant, M.B. 2008. Hyperglycaemia-induced reactive oxygen species toxicity to endothelial cells is dependent on paracrine mediators. *Diabetes*, 57(7): 1952-65.

Calamera, J., Buffone, M., Ollero, M., Alvarez, J. & Doncel, G.F. 2003. Superoxide dismutase content and fatty acid composition in subsets of human spermatozoa from normozoospermic, asthenozoospermic and polyzoospermic semen samples. *Molecular Reproduction and Development*, 66(4): 422-30.

Ceriello, A. 2011. Diabetic complications: from oxidative stress to inflammatory cardiovascular disorders. *Medicographia,* 33:29-34.

Chao, E.C. & Henry, R.R. 2010. SGLT2 inhibition – a novel strategy for diabetes treatment. *Nature Reviews Drug Discovery*, 9(7):551- 59.

Chatterjee, K., Ali, K.M., De, D., Bera, T.K., Jana, K., Maiti, S., Ghosh, A., Samanta, R. & Ghosh, D. 2012. Diabetes induced testicular dysfunction amelioration by ethyl acetate fraction of hydromethanolic extract of root of *Musa paradisiaca L.* in streptozotocin-induced diabetic rats. *Asian Pacific Journal of Tropical Disease,* 2(1): 233- 41.

Chaudhury, A., Duvoor, C., Dendi, V.S.R., Kraleti, S., Chada, A., Ravilla, R., Marco, A., Shekhawatt, N.S., Montales, M.T., Kuriakose, K., Sasapu, A., Beebe, A., Patil, Initial, Musham, C.K., Lohani, G.P. & Mirza, W. 2017. Clinical review of antidiabetic drugs: implications for type 2 diabetes mellitus management. *Frontiers in Endocrinology*, 8:6.

Chen, S.J., Allam, J.P., Duan, Y.G. & Haidi, G. 2013. Influence of reactive oxygen species on human sperm functions and fertilizing capacity including therapeutical approaches. *Archives of Gynaecology Obstetrics*, 288(1):191-9.

Chetyrkii, S., Mathias, M., Pedchenko, V., Sanchez, O.A., Macdonald, W.H., Hachey, D.L., Madu, H., Stec, D., Hudson, B. & Vaziyan, D.L. 2011. Glucose autoxidation induces functional damage to proteins via modification of critical arginine residues. *Biochemistry*, 50(27): 6102-12.

Chiarelli, F. & Di-Marzio D. 2008. Peroxisome proliferator activated receptor-gamma agonist and diabetes. Current Evidence. *Vascular Health and Risk Management*, 4(2): 297-304.

Chikezie, P.C., Ojiako, O.A. & Nwufu, K.C. 2015. Overview of anti-diabetic medicinal plants: the Nigerian Research Experience. *Journal of Diabetes & Metabolism,* 6:546.

Chikezie, P.C., Ojiako, O.A. & Ogbuji, A.C. 2015. Oxidative stress in diabetes mellitus. *Integrative Obesity and Diabetes, 1(3): 71-9.*

Chinney, P.F. & Turnbull, D.M. 2000. Mitochondrial DNA mutations in the pathogenesis of human disease. *Molecular Medicine Today,* 6(11):425- 32.

Chitra, K.C., Sujatha, R., Latchoumycandane, C. & Mathur, P.P. 2001. Effect of Lindane on antioxidant enzymes in epididymis and epididymal sperm of adult rats. *Asian Journal of Andrology,* 3(3): 205-8.

Colao, A., Di Sarno, A., Guerra, E., De Leo, M., Mentone, A. & Lombardi, G. 2006. DRUG INSIGHT: Cabergoline and bromocriptine in the treatment of hyperglycaemia in men and women. Nature Clinical Practice *Endocrinolology & Metabolism*, 2(4): 200-10.

Corbi, S.C.T., Bastos, A.S., Nepomuceno, K., Cirelli, T., Santos, R.A., Takahash,i C.S., Rocha, C.S., Orrico, S.R.P., Morelli, C.V.M. & Caminaga, R.M.S. 2017. Expression profile of genes potentially associated with adequate glycaemic control in patients with type 2 diabetes mellitus. *Journal of Diabetes Research, 2017*: ID 2180819 *http://doi.org/10.1155/2017/2180819.*

da Rocha F.J., Ogurstova, K., Linnenkamp, U., Guariguata, L., Seuring, T., Zhang, P., Cavan, D & Makaroff, L.E. 2016. IDF diabetes atlas estimates of 2014 global health expenditures on diabetes. *Diabetes Research and Clinical Practice,* 117: 48-54.

Dabhi, A.S., Bhatt, N.R. & Shah, M.J. 2013. Voglibose: An Alpha Glucosidase Inhibitor. *Journal of Clinical and Diagnostic Research*, 7(12): 3023-7.

Davidovich, P., Kearney, C.J. & Martin, S.J. 2014. Inflammatory outcomes of apoptosis, necrosis and necroptosis. *Biological Chemistry*, 395(10): 1163-71.

De Lamirande, E. & O'flaherty, C. 2008. Sperm Activation; role of reactive oxygen species and kinases. *Biochim et Biophysica Acta (BBA)-Proteins and Proteomics*, 1784(1):106-15.

de Wet, H. & Proks, P. 2015. Molecular action of sulphonylureas on kATP channels: a real partnership between drugs and nucleotides. *Biochemical Society Transactions*, 43(5): 901-7.

DeFronzo R.A. 2011. Bromocriptine: a smpatholytic, D2-Dopamine agonist for the treatment of type 2 diabetes mellitus. *Diabetes Care,* 34(4): 789- 94.

Dessisa, D. 2001. Preliminary economic evaluation of medicinal plants in Ethiopia: trade, volume and price. In Medhin, Z. & Abbe, D. (eds). Proceedings of the national workshop on biodiversity conservation and sustainable use of medicinal plants in Ethiopia. Addis Ababa: Ethipia: 176- 88.

Dinesh, V., Shamsi, M.B. & Dada, R. 2012. Supraphysiological free radical levels and their pathogenesis in male infertility. *Reproductive System & Sexual Disorders: Current Research,* 1:114.

Ding, G.l., Liu, Y., Liu, M.E., Pan, J.X., Guo, M.X., Sheng, J.Z. & Huang, H.F. 2015. The effects of diabetes on male fertility and epigenetic regulation. *Asian Journal of Androl*ogy, 17(6): 948-53.

Dokmeci, D. 2005. Oxidative stress, male infertility and the role of carnitines. *Folia Medica (Plovdiv),* 47(1): 26-30.

Dornhorst, A. 2001. Insulinotropic meglitinides analogues. *The Lancet* 358(9294):1709-16.

Drucker, D.J. 2006. The biology of incretin hormones*. Cell metabolism*, 3 (3): 153-65.

du Plessis., S.S., Agarwal, A., Halabi, J. & Tvrda, E. 2015. Contemporary evidence on the physiological role of reactive oxygen species in human sperm function. *Journal of Assisted Reproduction and Genetics,* 32(4): 509-20.

Elabbady, A., Hashad, M.M., Kotb, A.F., Ghanem, A.E. 2016. Studying the effect of type 2 diabetes mellitus on prostate related parameters: - a prospective single institutional study. *Prostate International*, 4(4):156-9.

El-Demerdash, F.M., Yousef, M.I., Kedwany, F.S. & Baghdadi, H.H. 2004. Cadmium-induced changes in LPO, blood haematology, biochemical parametres and semen quality of male rats: protective role of vitamin B and β-carotene. *Food and Chemical Toxicology,* 42 (10):1563-71.

Ellerby, L.M, Bredesen, D.E. 2000. Measurement of cellular oxidation, reactive oxygen species, and antioxidant enzymes during apoptosis. *Methods of Enzymology,* 322: 413-21.

Enomoto, A., Wempe, M.F., Tsuchida, H., Shin, H.J., Cha, S.H., Anzai, N., Goto, A., Sakamoto, A., Niwa, T. & Kanai, Y. 2002. Molecular identification of a novel carnitine transporter specific to human testis. *Journal of Biological Chemistry*, 277 (39): 36262-71.

Evans, J.L., Bakan, B., Chuang, E. & Rushakoff, R.J. 2016. Oral and injectable (non-insulin) pharmacological agents for type 2 diabetes. In: Carbohydrate metabolism and diabetes mellitus. De Groot L.J, Chrousos G, Dungan K et al., eds. Chapter 15:1-19.

Fatani A.J., Al-Rejaie, S.S., Abuohashish, H.A., Al-Assaf, A., Parmar, M.Y. & Ahmed M.M. 2015. Lutein dietary supplementation attenuates STZ-induced testicular damage and oxidative stress in diabetic rats. *BMC Complementary and Alternative Medicine. ISCMR*, 15:204.

Ferrennin, E. & Solini, A. 2012. SGLT2 inhibitors in diabetes mellitus: rationale and clinical prospects. 2012. *Nature Reviews Endocrinology*, 8(8): 495-502.

Food and Drug administration. 2015. FDA drug safety communication: - FDA warns that SGLT2- inhibitors may result in a serious condition of too much acid in the blood. http://www.fda.gov/Drugs/DrugSafety/ucm446845.htm. Accessed 2016 December.

Foresta, C., Flohe, C., Garolla, A., Roveri, A., Ursini, F. & Maiorino, M. 2002. Male fertility is linked to selenoprotein phospholipid hydroperoxide glutathione peroxidase. *Biology of Reproduction*, 67(3): 967-71.

Frode, T.S. & Medeiros, Y.S. 2008. Animal models to test drugs with potential antidiabetic activity. *Journal of Ethnopharmacology*, 115: 173-83.

Fugii. J., Iuchi, Y., Matsuki, S. & Ishii, T. 2003. Cooperative function of antioxidant and redox systems against oxidative stress in male reproductive tissues. *Asian Journal of Andrology,* 5(3): 231-42.

Gajalakshmi, S., Vijayalakshmi, S. & Devi, R.V. 2013. Pharmacological activities of Catharanthus roseus: a perspective review. *International Journal of Pharma- and Bio Sciences,* 4(2): 431-9.

Gannon, M.C. & Nuttall F.Q. 2006. Control of blood glucose in type 2 diabetes without weight loss by modification of diet composition. *Nutrition and Metabolism*, 3(16): PMID 16556307.

Garber, A., Blonde, L., Bloomgarden, Z., Handelsman, Y. & Dagogo-Jack, S. 2013. The role of bromocriptine-QR in the management of type 2 diabetes mellitus: *Expert Panel Recommendations. Endocrine Practice*, 19(1):100-06.

Garrido, N., Meseguer, M., Alvarez, J., Simon, C., Pellicer, A. & Remohi, J. 2004b Relationship among standard semen parametres, glutathione/ peroxidase/ glutathione reductase activity and Mrna expression and reduced glutathione content in ejaculated spermatozoa from fertile and infertile men. *Fertility and Sterility,* 82(3): 1059-66.

Garrido, N., Meseguer, M., Simon, C., Pellicer, A. & Remohi, J. 2004a. Pro-oxidative and antioxidative imbalance in human semen and its relation with male fertility. *Asian Journal of Andrology*, 6(1): 59-65.

Ghosh, A., Jana, K., Ali, K.M., De, D., Chatterjee, K. and Ghosh, D. 2014. Corrective role of Eugenia jambolana on testicular impairment in streptozotocin-induced diabetic male albino rat: An approach through genomic and proteomic study. *Andrologia,* 46(3): 296-307.

Giacco, F. & Brownlee, M. 2010. Oxidative stress and diabetic complications. *Circulation Research*, 107(9): 1058-70.

Giannattasio, A., De Rosa, M., Smeraglia, R., Zarrilli, S., Cimmino, A., Di Rosano, B., Rugeiero, R., Colao, A. & Lombardi, G. 2002. Glutathione peroxidase (GPx) activity in seminal plasma of healthy and infertile males. *Journal of Endocrinological Investigations*, 25(11): 983-6.

Gillespie, K.M. 2006. Type 1 diabetes: Pathogenesis and prevention *Canadian Medical Association Journal*, 175(2):165-170.

Giribabu, N., Kumar, K.E., Rekha, S.S., Muniandy, S. & Salleh, N. 2014. Chlorophytum boviriliarum (Safed Musli) root extract prevents impairment in characteristics and elevation of oxidative stress in sperm of streptozotocin induced adult male diabetic rats. *BMC Complementary and Alternative Medicine,* 14(1): 291-377.

Gonzalez-Marin, C., Gosalvez, J. & Roy. R. 2012. Types, causes, detection and repair of DNA fragmentation in animal and human sperm cells. *International Journal of Molecular Science*, 13(11):14026-52.

Guerriero, G., Di Finizio, A. & Ciarcia, G. 2003. Oxidative defences in the sea bass. Dicentrarchus Labrax In: Dunn, J. & Swarts, H.M. (eds). Advances in experimental medicine and biology; 68; New York: Kluwer Academic/ Plenum Publisher: 681-8.

Guillausseau, P.J., Meas, T., Virally, M., Michelin, M.L., Medeau, V., Kevorkian, J.P. 2008. Abnormalities in insulin secretion in type 2 diabetes mellitus. *Diabetes and Metabolism*, 34(2): s43-8.

Guirimand, G., Coudavault, V., Pierre, B.S. &Burlat, V. 2010. Biosynthesis and regulation of alkaloids. In Plant Developmental Biology: 139-160. DOI 10.1007/978-3-642-04670-4_8

Gurib-Fakim, A. 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*, 27:1-93.

Hajizadeh, M., Eftekhar, E., Zal, F., Jafarian, A. & Mostafavi-Pour, Z. 2014. Mulberry leaf extract attenuates oxidative stress mediated testosterone depletion in streptozotocin induced diabetic rats. *Iranian Journal of Medical Sciences*, 39(2):123-9.

Hamden, K., Carreau, S., Jamoussi, K., Ayadi, F., Garmazi, F., Mezgenni, N. & Elfeki, A. 2008. Inhibitory effects of 1-Alpha, 25 dihydroxyvitamin D3 and Ajuga iva extract on oxidative stress, toxicity and hypofertility in diabetic rat testes. *Journal of Physiology and Biochemistry* 64(3): 231-9.

Hazout, A., Menezo, Y., Madelerat, P., Yazbeck, C., Selva, J. & Cohen-Bacrie, P. 2008. Cause and clinical implications of sperm DNA damages. *Gynaecologie, Obstetrique and Fertilite*, 36(11): 1109-17.

Hendin, B.N., Kolettis, P.N., Sharma, R.K., Thomas Jnr, A.J. & Agarwal, A. 1999. Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity. *Journal of Urology*, 161(6):1831-4.

Herath, H., Herath, R. & Wickremasinghe, R. 2017. Gestational diabetes mellitus and risk of type 2 diabetes mellitus 10 years after the index pregnancy in Srilankan women_ a community based retrospective cohort study. *PLoS One*, 12(6): e0179647.

Hermesz, E. & Ferencz, A. 2009. Identification of two phospholipid hydroperoxide glutathione peroxidase (GPx4) genes in common carp. *Comparative Biochemistry and Physiology- Part C. Toxicology and Pharmacology*, 150(2):101-6.

Herrero, M.B., de Lamirande, E. & Gagnon, C. 2003. Nitric oxide is a signalling molecule in spermatozoa. *Current Pharmaceutical Design*, 9(5):419-25.

Holst, J.J., Knop, F.K., Visboll, T., Krarup, T. & Madsbad, S. 2011. Loss of incretin effect is a specific, important and early characteristic of type 2 diabetes. *Diabetes care*, 34(2): s251-7.

Horita, S., Nakamura, M., Satoh, N., Suzuki, M. & Seki, G. 2015. Thiozolinediones and edema: recent advances in the pathogenesis of thiozolinediones-induced renal sodium retention. *PPAR Research*: ID646423.

Houcher, Z., Boudiaf, K., Benboubetra, M., & Houcher, B. 2007. Effects of methanolic extract and commercial oil of *Nigella sativa* L. on blood glucose and antioxidant capacity in alloxan-induced diabetic rats. *Pteridines*, 18(1):8-18.

Inzucchi, S.E. 2002. Oral antihyperglycaemic therapy for type 2 diabetes: Scientific review. *Journal of the American Association*, 287(3): 360-72.

Inzucchi, S.E., Lipska, K.J., Mayo, H., Bailey, C.J. & McGuire, D.K. 2014. Metformin in patients with type 2 diabetes and kidney disease.a systematic review. *JAMA*, 312(24): 2668-75.

Itach, S.B., Finklestein, M., Elkovitz, N. & Breitbart, H. 2012. Hyperactivated motility in sperm capacitation is mediated by phospholipase D-dependent actin polymerisation. *Developmental Biology*, 362(2): 154-61.

Jain, G.C. & Jangir, R.N. 2014. Modulation of diabetes mellitus-induced male reproductive dysfunction in experimental animal models with medicinal plants. *Pharmacognosy reviews,* 8(16): 113-21.

Jaleel, C.A. & Panneerselvam, R. 2007. Variations in the antioxidative and indole alkaloid status of different parts of 2 varieties of Catharanthus Roseus: an important folk herb. *Chinese Journal of Pharmacology and Toxicology,* 1(6):487-94.

Karbowinik, M., Gitto, E., Lewnski, A. & Reiter, R.J. 2001. Induction of lipid peroxidation in Hamster organ is by the carcinogen cadmium: Amelioration by melatonin. *Cell Biology and Toxicology*, 17:33-40.

Khosrowbeygi, A. & Zarghami, N. 2007. Fatty Acid Composition of Human Spermatozoa and Seminal Plasma Levels of Oxidative Stress Biomarkers in Subfertile Males. *Prostaglandins Leukotrienes and Essential Fatty Acids, 77(2):117- 21.*

Kim, K.Y., Nam, K.A., Kurihara, H. & Kim, S.M. 2008. Potent α-glucosidase inhibitors from the red alga Grateloupia elliptica. *Phytochemistry,* 69(19): 2820-5.

Kiran, B.S.R., Lakshmi, T.M., Srikuma, R. & Reddy, E.P. 2016. Total antioxidant status and oxidative stress in diabetes mellitus and metabolic syndrome. *International Journal of Pharmaceutical Sciences Review and Research*, 40(1): 271-7.

Kirigia, J.M., Sambo, H.B., Sambo, L.G. & Barry, S.P. 2009. Economic burden of diabetes mellitus in the World Health Organisation African region. *BMC International Health and Human Rights,* 9:6.

Koppers, A.J., Mitchell, L.A., Wang, P., Lin, M. & Aitken, R.J. 2011. Phosphoinositide 3-kinase signalling pathway involvement in the truncated apoptototic cascade associated witj mortility loss ad oxidative DNA damage in human spermatozoa. *Biochemical Journal,* 436(3): 687-98.

Korejo, N.A., Wei Q., Shah, A.H. & Shi F.X. 2016. Effects of concomitant diabetes mellitus and hyperthyroidism on testicular and epididymal histoarchitecture and steroidogenesis in male animals. *Journal of Zhejiang University-SCIENCE*, 17(11):850-63.

Kothari, S., Thompson, A., Agarwal, A. & du Plessis, S. 2010. Free radicals: Their beneficial and detrimental effects on sperm function. *Indian Journal of Experimental Biology,* 48(5): 425-35.

Kumar, R.P., Venkatesh, S., Kumar, M., Tanwar, M., Sharmsi, M.B., Kumar, R., Gupta, N.P., Sharma, R.K., Talwar, P. & Dada, R. 2009. Oxidative stress and sperm mitochondrial DNA mutation in idiopathic oligosthenozoospermic men. *Indian Journal of Biochem Biophys*, 46(2): 172-7.

Kumar, S., Narwal, S., Kumar, V. & Prakash, O. 2011. α-glucosidase inhibitors from plants:-a natural approach to treat diabetes, *Pharmacognosy Reviews,* 5(9): 19-29.

Kvernmo, T., Hartter, S. & Burger, E.A. 2006. A review of the receptor-binding and pharmacokinetic properties of dopamine agonists. *Clinical Therapeutics*, 28(8): 1065-78.

Labuschagne, C.F. & Brenkman, A.B. 2013. Current methods in quantifying ROS and oxidative damage in Caenorhabditis elegans and other model organism of aging. *Ageing Research Reviews*, 12(4): 918- 30.

Lampiao, F. 2012. Free radiacals generation in an *in-vitro* fertilization setting and how to minimize them. *World Journal of Obstetrics and Gynaecology*, 1(3): 29-34.

Lanzafame, F.M., La Vignera, S., Vicari, E. & Cologero, A.E. 2009. Oxidative stress and medical antioxidant treatment in male infertility. *Reproductive Biomedicine Online*, 19(5): 638- 59.

La-vignera S., Condorelli, R., Vicari, E., D'agata, R. & Calogero, A.E. 2012. Diabetes mellitus and sperm parameters. *Journal of Andrology*, 33(2): 145-53.

Lenzi, A., Sgro, P., Salacone, P., Paoli, D., Gillio, B., Lambardo, F. et al. 2004. A placebo-controlled double blind randomised trial of the use of combined L-canitine and L-acetyl-carnitine treatment in men with asthenozoospermia. *Fertility and Sterility,* 81(6): 1578-84.

Li, W.L., Zheng, H.C., Bukuru, J. & de Kimpe, N. 2004. Natural medicines used in the traditional Chinese system for therapy of diabetes mellitus. *Journal of Ethnopharmacology*, 92(1): 1- 21.

Linetzky B, Curtis B, Fretchel G, Montenegro Jr R., Pulido ME. Stempa O, De Lana JM & Gangliardino. 2016. Topic. Challenges associated with insulin therapy progression among patients with type 2 diabetes: Latin American MOSAIC study baseline data. *Diabetology and Metabolic Syndrome*, 8:41.

Liu, Z., Lin, H., Ye, S., Liu, Q.Y. & Meng, Z. 2006. Remakably high activities of testicular cytochrome C in destroying reactive oxygen species and in triggering apoptosis. *Proceedings of the National Academy of Science of the United States of America*, 103(24): 8965-70.

Mahler, R.J. & Adler, M.L. 1999. Type 2 diabetes mellitus update on diagnosis, pathophysiology and treatment. *Journal of Clinical Endocrinol and Metabolism*, 84(4):165-71.

Maiti, R., Karak, P., Misra, D.S. & Ghosh, D. 2017. Diabetes-induced testicular dysfuntion correction by hydromethanolic extract of *Tamarindus indica* Linn seed in male albino rats. *International Journal of Green Pharmarcy,* 11(4).

Majai, G., Pretrovski, G. & Fesus, L. 2006. Inflammation and Apopto-Phagocytic System. *Immunology Letters*, 104(1): 94-101.

Maker, K., Agarwal, A. & Sharma, R. 2009. Oxidative stress and male fertility. *Indian Journal of Research,* 129: 357- 67.

Makki, K., Froguel, P. & Wolowczuk, I. 2013. Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines and chemokines. *ISRN* 2013. PMID 24455420.

Mallick, C., Mandal, S., Barik, B., Bhattacharya, A. & Ghosh, D. 2007. Protectin of testicular dysfunction by MTEC, a formulated herbal drug in streptozotocin-induced diabetic rats. *Biological and Pharmaceutical Bulletin*, 30(1): 84- 90.

Malviya, N., Jain, S. & Malviya, S. 2010. Antidiabetic potential of medicinal plants. *Acta Poloniae Pharmaceutica N Drug Research,* 67(2): 113-8.

Mangoli, E., Talebi, A.R., Anvari, M. & Pourentezari, R. 2013. Effects of experimentally induced diabetes on sperm parametres and chromatin quality in mice. *Iranian Journal of Reproductive Medicine*, 11(1): 53-60.

Maresch, C.C., Stute, D.C., Alves, M.G., Oliveira, P.F., De Kretser, D.M. & Linn, T. 2018. Diabetes induced hyperglycaemia impairs male reproductive function: a systematic review. *Human Reproduction Update*, 24(1): 86-105.

Maritim, A.C., Sanders, R.A. & Watkins III, J.B. 2003. Diabetes, Oxidative Stress and Antioxidants: A Review. *Journal of Biochemical and Molecular Toxicology,* 17(1): 24-38.

Marre, M., Shaw, J., Brandle, M., Bebakar, W.M.W., Kamaruddin, N.A., Strand, J., Zdravkovic, M., Thi, T.D.L. & Colagiuri.-- 2009. Liraglutide, a once-daily human GLP-1 analogue, added to sulphonylurea over 26 weeks produces greater improvements in glycaemic and weight control compared with rosiglitazone on placebo in subjects with type 2 diabetes (LEAD-ISU), *Diabetic Medicine*, 26(3):268-78.

Maruthur, N.M., Tseng, E., Hutfless, S., Wilson, L.M., Suarez- Cuevo, C. & Berger, Z. et al. 2016. Diabetes medications as monotherapy or metformin- based combination therapy for type 2 diabetes mellitus. A systematic review and meta-analysis. *Annals of Internal Medicine*, 164(11): 740- 51.

May, M. & Schindler, C. 2016. Clinically and pharmacologically relavent interactions of antidiabetic drugs. *Therapeutic Advances in Endocrinology and Metabolism*, 7(2): 69-83.

Mazen, N.F. & Elgris, H.M. 2013. Role of coenzyme Q10 in testicular damage induced by arcrylamide in weaned albino rats: a histological and immunohistochemical study. *Egyptian Journal of Histology,* 36(1): 164-74.

McEwen, L.N., Bilik, D., Johnson, S.L., Halter, J.B., Karter, A.J., Mangione, C.M., Subramanian, U., Waitzfeider, B., Crosson, J.C. & Herman, W.H. 2009. Predictors and impact of intensification of antihyperglycaemic therapy in Type 2 diabetes: Translating research into action for diabetes (TRIAD). *Diabetes Care*, 32(6): 971-76.

Mendoza, R.G., Pioletta, A., Ceja, L.M.J., Sosale, A. & Folli, F. 2013. The role of nateglinide and repaglinide derivatives of meglitinides in the treatment of type 2 diabetes mellitus. *Archives of Medical Sciences,* 9(5):936-43.

Meneilly, G.S., Ryan, E.A., Radziuk, J. et al. 2000. Effect of acarbose on insulin sensitivity in elderly patients with diabetes. *Diabetes Care*, 23: 1162-7.

Messori, A., Fadda, V., Maratha, D., Trippoli, S. & Marinai, C. 2014. Testing the theurapeutic equivalence of alogliptin, linagliptin, saxagliptin, sitagliptin or vildagliptin as monotherapy or in combination with metformin in patients with type 2 diabetes. *Diabetes Therapy*, 5(1): 341-4.

Miller, D., Nrimkworth, M., Iles, D. 2010. Paternal DNA packaging in spermatozoa: more than the sum of its parts? DNA, histones, protamines and epigenetics. *Reproduction*, 139(2): 287-301.

Miraglia, E,. Rullo, M.L., Bosia, A. & Ghigo, I. 2007. Stimulation of the nitric oxide/cyclic guanosine monophosphate signalling pathway elicits human sperm chemotaxis *in-vitro. American Society for Reproductive Medicine*, 87(5): 1059-63.

Miraglia, E., Liussiana, C., Viarisio, D., Racca, C., Cipriani, A., Gazzano, E., Bosia A., Revelli, A. & Ghigo, D. 2010. The pentose phosphate pathway plays an essential role in supporting human sperm capacitation. *Fertility and Sterility*, 93(7): 2437-40.

Mistry, H.D., Pipkin, F.B., Redman, K.W. & Poston, L. 2012. Selenium in reproductive health. *American Journal of Obstetrics and Gynecology*, 206: 21-30.

Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S. & Devasagayan, T.P. 2007. Indian herbs and herbal drugs used for the treatment of diabetes. *Journal of Clinical Biochemistry and Nutrition*. 40(3): 163-73.

Mora-Esteves, C. & Shiri, D. 2013. Nutrient Supplementation: improving male infertility fourfold. *Seminars in Reproductive Medicine*, 31(4): 293-300.

Mruk, D.D., Silvestrini, B., Mo, M.Y. et al. 2002. Antioxidant superoxide dismutase-a review: Its function, regulation in the testis and role in male fertility. *Contraception,* 65: 305-11.

Mudaliar, S., Face, M.D., Anna, R., Chang, M.D. & Henry, R.R. 2003. Thiazolinediones, peripheral edema and type 2 diabetes: Incidence pathophysiology and clinical implications. *Endocrine practice*, 9(5): 406-16.

Nasser, M. 2011. Quick release bromocriptine for treatment of type 2 diabetes. *Diabetes Current Drug Delivery,* 8(5): 511-16.

Nauck, M.A. & Meier, J.J. 2016. The incretin effect in healthy individuals and those with type 2 diabetes: Pathophysiology and response to the therapeutic interventions. *Lancet Diabetes and Endocrinology,* 4(6): 525-36.

Nauck, M.A. 2014. Update on developments with SGLT2 inhibitors in the management of type 2 diabetes mellitus. *Drug Design, Development and Therapy*, 8: 1335-80.

Naughton, C.K., Nangia, A.K. & Agarwal, A. 2001. Pathophysiology of varicoceles in male fertility. *Human Reproduction Update*, 7(5): 473- 81.

Nawule, R.B., Mourya, V.K. & Bhise, S.B. 2006. Non-enzymatic glycation of proteins: A cause for complications in diabetes. *Indian Journal of Clinical Biochemistry*, 43(6): 337-44.

Nelli, G.B., Sankar, A.S.K. & Kilari, E.K. 2013. Antidiabetic effect of α-mangolin and its protective role in sexual dysfuntion of STZ-induced diabetic male rats. *Systems Biology in Reproductive Medicine*, 59(6): 319-38.

Ng, C.M., Blackman, M.R., Wang, C. & Swerdloff, R.S. 2004. The role of carnitine in the male reproductive system. *Annals of the New York Academy,* 1033(1): 177- 88.

Nirmala, M.J., Samundeeswari, A. & Sankar, P.D. 2011. Natural plant resources in anticancer therapy: a review. *International Journal of Plant Biology and Research*, 1(3): 1-14.

Nisar, A., Mamat, A.S., Dzahir, M.I.H.M. & Ahmad M.S. 2017. Antioxidant and total phenolic content of *Catharanthus roseus* using deep eutectic solvent. *Recent Advances in Biology and Medicine,* 3(2017); 7-10.

Nistiar, F., Racz, O., Benacka, R. & Lukacinova, A. 2009. Effect of bioflavonoids on type 1 diabetes onset and antioxidant status in spontaneously diabetic rats of BB strain. *Journal of Central European Agriculture,* 11: 113- 40.

O'flaherty, C. 2015. Redox regulation of mammalian sperm capacitation. *Asian Journal of Andrology*, 17(4): s83-s90.

O'flaherty, C., Rodriguez, P. & Srivastava, S. 2004. L-arginine promotes capacitation and acrosome reaction in cryopreserved bovine spermatozoa. *Biochimica et Biophysica Acta*, 1674(2): 215-21.

O'flaherty, C.M., Beolegui, N.B. & Beconi, M.T. 1999. Reactive oxygen species requirements for bovine sperm capacitation and acrosome reaction. *Theriogenology,* 52(2): 289-301.

Ogbuewo, I.P., Aladi, N.O., Etuk, I.F., Opara, M.N., Uchegbu, M.C., Okoli, I.C. & Iloeje, M.U. 2010. Relavance of oxygen free radicals and antioxidants in sperm production and function. *Research Journal of Verteriary Sciences*, 3(3): 138-64.

Ogurstova, K., da Rocha, F.J.D., Huang, Y., Linnenkamp, U., Guariguata, N.H., Chon, H., Cavan, D, Shaw, J.E. & Makaroff L.E. 2017. IDF diabetes atlas: global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Research and Clinical Practice*, 128:40-50.

Oish, M., Yokohama H., Abe, N., Iwasaki, K., Okuguchi, F., Kawai, K., Sugimto, H., Takamura, H., Takeda, H., Doi, K., Hira, O. & Ikeda, S. 2011. Time and cost of insulin treatment in the care of newly registered type 2 diabetes patients in diabetes clinics across Japan (JDDM22). *Japanese Clinical Medicine*, 2:43-51.

Oliveira, J.S., Silva, A.A.N. & Silva Jnr, V.A. 2015. Phytotherapy in reducing glycaemic index and testicular oxidative stress resulting from induced diabetes: A review. *Brazilian Journal of Biol*ogy, 77(1).

Ong, H.C., Ahmad, N. & Milow, P. 2011. Traditional medicinal plants used by the Temuan villagers in Kampung Tering; Negeri Sembilani; Malaysia. *Journal of Ethnobiology and Ethnomedicine,* 5(3):169-73.

Ostovan, F., Gol, A. & Javadi, A. 2017. Investigating the effects of citrullus colocynthis pulpon oxidative stress in testes and epididymis in STZ – induced diabetic male rats. *International Journal of Reproductive Biomedicine*, 15(1): 41-8.

Ozougwu, J.C., Obimba, K.C., Belonwu, C.D. & Unakalamba, C.B. 2013. The pathogenesis and pathophysiology of Type 1 and Type 2 diabetes mellitus. *Journal of Physiology and Pathophysiology*, 4(4): 46-57.

Pandey, K.B., Mistira, N. & Rizvi, S.I. 2010. Protein oxidation biomarkers in plasma of type 2 diabetes patients. *Clinical Biochemistry*, 43(5): 508-11.

Patel, D.K., Kumar, R., Laloo, O. & Hemalatha, S. 2012. Natural medicines from plant source used for therapy of diabetes melliyus: an overview of its pharmacological aspects. *Asian Pacific Journal of Tropical Diseases,* 2(3): 239-50.

Pathak, R. & Bridgeman, M.B. 2010. Dipeptidyl peptidase-4 (DPP-4) inhibitors in the management of diabetes. *Pharmacy and Theurapeutics,* 35(9): 509-13.

Patharajan, S. & Abirami, S.B. 2014. Antioxidant activity and phytochemical analysis of fractionaed leaf extract of catharanthus roseus. *International Journal of Pharmacognosy*, 1(2): 138-43.

Perry, B.D., Caldow, M.K., Sbaraglia, M., Jerums, G., Garnham, A., Wong, C., Levinger, P., Haq, M.A., Hare, D.l., Price, S.R. & Levinger, I. 2016. Muscle atrophy in patients with type 2 diabetes mellitus: Role of inflammatory pathway, physical activity and exercise. *Exercise Immunology Reviews*, 22: 94-109.

Peyrot, M., Rubin, R.R., Luaritzen, T., Skovland, S.E., Snoek, F.J., Mathews, D.M., Landgraf, R. & Kleinbreil, L. 2005. International DAWN Advisory Panel. Resistance toinsulin therapy among patients and providers: results of the cross-national diabetes Attitudes, Wishes and Needs, (DAWN). *Diabetes Care*, 29(4): 952-3.

Philis-Tsimikas, A. 2013. Initiating basal insulin therapy in type 2 diabetes: practical steps to optimise glycaemic control. *American Journal of Medicine*, 126(9): 521-7.

Piero, N.M., Njagi, M.J., Kibiti, M.C., Ngeranwa, J.J.N., Njue, W.M. & Gathumbi, P.K. 2012. Herbal management of diabetes mellitus: a rapidly expanding research Avenue. *International Journal of Current Pharmaceutical Research,* 4(2): 1- 4.

Pijl, H., Ohashi, S., Mastuda, M., Miyazaki, Y., Mahankali, A., Kumar, V., Pipek, initials, Lozzo, P., Lancaster, J.L., Cincotta, A.H. & de Fronzo, R.A. 2000. Bromocriptine:- a novel approach to the treatment of type 2 diabetes mellitus. *Diabetes Care,* 23(8):1154-61.

Powers A.C & D'Alessio D. 2011. "Chapter 43. Endocrine Pancreas and Pharmacotherapy of Diabetes Mellitus and Hypoglycemia". Brunton LL, Chabner BA,Knollmann BC: Goodman & Gilman's The Pharmacological Basis of Therapeutics. 12th edition. New York: McGraw-Hill.

Rasineni ,K., Bellamkonda, R., Singareddy, S.R. & Desireddy, S. 2010. Antihyperglycaemic activity of *Catharanthus roseus* leaf powder in streptozotocin induced rats. *Pharmacognosy Research,* 2(3): 195-201.

Rasool, A.N., Jaheerunnisa, S., Javaveera, K.N. & Kumar, S. 2011. In vitro callus induction and in-vivo antioxidant activity of *Passiflora foetida* L. leaves. *International Journal of Applied Research in Natural Products*, 4(1).

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.

Rizvi, S.I. & Mishra, N. 2013. Traditional Indian medicines used for the management of diabetes mellitus. *Journal of Diabetes Research*, 2013: ID 712092: 11pages.

Rochette, L., Zeller, M., Cottin, Y., Vergley, C. 2014. Diabetes, oxidative stress and therapeutic strategies. *Biochimica et Biophysica Acta*, 1840(9): 2709-29.

Rodriguez, C.M.V., Villa, M.V., Ocampo, L.G. & Cardona, J.B. 2014. Abdominal obesity and low physical activity are associated with insulin resistance in overweight adolescents: a cross sectional study. *BMC Paediatric*, 14:258.

Ross, S.A., Tildesley, H.D. & Ashkenas, J. 2011. Barriers to effective insulin treatment : the persistanceof poor glycaemic control in type 2 diabetes. *Current Medical Research and Opinion*, 27(3): 13-20.

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

Rubino, F. 2008. Is type 2 diabetes an operable intestinal disease? *Diabetes Care*, 31(2):s290-6.

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(9): 422-31.

Safi, S.Z., Qvist, R., Kumar, S., Batumaile, K. & Ismail, I.S.B. 2014. Molecular mechanisms of diabetic retinopathy, general preventive strategies and novel therapeutic targets. *BioMed Research International,* 2014: ID 801269.

Sahari, M.A. & Berenji, A.S. 2015. Bio-antioxidant activity: their mechanisms and measurement methods. *Applied Food Biotechnology,* 2(1): 3-8.

Said, L., Banni, M., Kerkeni, A., Said, K. & Messaodi, I. 2010. Influence of combined treatment with zinc and selenium on Cardmium induced testicular pathophysiology in rat. *Food and Chemical Toxicology*, 48(10): 2759- 65.

Said, T.M., Paasch, U., Glander, H.J. & Agarwal, A. 2004. Role of caspases in male fertility. *Human Reproduction Update*, 10(1):39-51.

Saleh, R.A., Agarwal, A., Sharma, R.K., Nelson O.D. & Thomas, A.J. Jnr. 2002. Effect of cigarette smoking on levels of seminal oxidative stress in infertile men; a prospective study. *Fertility and Sterility,* 78(3): 491-9.

Sanchez, R.A., Montavon, F., Hatung, T. & Pahler, A. 2006. Thiozolinediones bioactivation :a comparison of the bioactivation potentials of troglitazone, rosiglitazone and pioglitazone using stable isotope-labelled analogue and liquid chromatography tandem mass spectrometry. *Chemical Research in Toxicology*, 19(8): 1106-16.

Sandoval, D., Cota, D. & Seeley, R.J. 2008. The integrative role of CNS fuel-sensing mechanisms in energy balance and glucose regulation. *Annual Review of Physiology*, 70: 513-35.

Sanocka, D. & Kurspisz, M. 2004. Reactive oxygen species and sperm cells. *Reproductive Biology and Endocrinology,* 2:12.

Savu, O., Sunkari V.G., Botusan, I.R., Grunler, J., Nikoshkov, A. & Catrina, S.B. 2011. Stability of mitochondrial DNA against reactive oxygen species generated in diabetes. *Diabetes Metabolism Research and Review*s, 27(5): 470-9.

Sawyer R.S.D. & Aitken, R.J. 2001. Relative susceptibility of mitochondrial and nuclear DNA to damage induced by hydrogen peroxide in two mouse germ cell lines. *Communications in Free Radical Research*, 6(3): 182-4.

Saxena, A. & Kishore, V.N. 2004. Role of selected medicinal Indian plants in the management of type 2 diabetes: a review. *Journal of Alternative and Complementary Medicine*, 10(2): 369-78.

Schaschkow, A., Mura, C., Dal, S., Langois, A., Seyfritz, E., Sookhareea, C., Bietiger, W., Peronet, C., Jeandidier, N., Pinget, M., Sigrist, S. & Maillard, E. 2016. Impact of the type of continuous insulin administration on metabolism in a diabetic rat model. *Journal of diabetes research*, 2016: ID8310516. 10 pages.

Schulte, R.T., Ohl, D.A., Sigman, M. & Smith, G.D. 2010. Sperm DNA damage in male infertility: aetiologies, assays and outcomes. *Journal of Assisted Reproduction and Genetics,* 27(1): 3-12.

Sen, S., Chakraborty, R., Svidhar, C., Reddy, Y.S.R. & De, B. 2010. Free radicals, antioxidants, diseases and phytomedicines: Current status and future prospect. *International Journal of Pharmaceutical Sciences Research*, 3(1): 91- 100.

Sertel, S., Fu, Y., Zu, Y., Rebacz, B., Konk, B., Plinkert, P.K., Kramer, A., Gertsch, J. & Efferth, T. 2011. Molecular docking and pharmacogenomics of vinca alkaloids and their monomeric precursors, vindoline and catharanthine. *Biochemical Pharmacology*, 81(6): 723-35.

Shah, N.A. & Khan, M.R. 2014. Antidiabetic effect of Sida cordata in alloxan induced diabetic rats. *BioMed Research International*, 2014: 671294. http://dx.doi. org/10.1155/2014/671294. PMID: 25114914.

Sharma, R.K. & Agarwal, A. 1996. Role of reactive oxygen species in male infertility. *Urology*, 48: 835-50.

Sheeja, V.S., Harikrisna, M., Reddy, M., Joseph, J. & Reday, O.N. 2010. Insulin Therapy in disease management. *International Journal of Pharmaceutical Sciences Review and Research*, 2:2.

Sheng, Y., Abreu I.A., Cabelli, D.E., Maroney, M.J., Miller, F., Texeira, M. & Valentine, J.S. 2014. Superoxide dismutases and superoxide reductases. *Chemical Reviews*, 114(7):3854-3918.

Siddiqui, A.A., Siddiqui, S.A., Ahmad, S., Siddiqui, S., Ahsan, I. & Siddiqui, K. 2013. Diabetes: Mechanisms, pathophysiology and management: A review. *International Journal of Drug Development Research*, 5(2):1-23.

Sikka, S.C. 2001. Relative impact of oxidative stress on male reproductive function. *Current Medicinal Chemistry,* 8: 851-62.

Singh, K., Devi, S. & Pankaj, P.P. 2016. Diabetes associated male reproductive dysfunction: prevalence, diagnosis and risk factors. *International Journal of Drug Development and Research*, 8: 7-10.

Singh, R., Bhadwaj, P. & Sharma, P. 2013. Antioxidant and Toxicological evaluation of Cassia Sopheira streptozotocin induced diabetic Wistar rats. *Pharmacognosy Research*, 5(4): 225-32.

Singh, S., Singh, P.K. & Singh, R.K. 2014. Antidiabetic and wound healing activity of catharanthus roseus L. in streptozotocin- induced diabetic mice. *American Journal Of Phytomedicine and Clinical Therapeutics,* 2(6): 686-92.

Singh, S.N., Vats, P., Suri, S., Shyam, R., Kumria, M.M., Ranganath, S. & Sridharan, K. 2001. Effect of an antidiabetic extract of Catharanthus roseus on enzymatic activities in streptozotocin induced diabetic rats. *Journal of Ethnopharmacology*, 76(3): 269-77.

Somogyi, S. 2007. Antioxidant measurements. *Physiological Measurements*, 28(4): 41- 55.

Soudamani, S., Yuvaraj, S., Malini, T. & Balasubramanian, K. 2005. Experimental diabetes has adverse effects on the differentiation of ventral prostate during sexual maturation of rats. *Anatomical Record. Part A: Discoveries in Molecular, Cellular and Evolutionary Biology*, 2(87):1281-9.

Soumita, D., Joshi, K.R., Sengupta, P. & Bhattacharya, K. 2013. Unilateral and bilateral cryptorchidism and its effect on the testicular morphology, histology, accessory sex organs, and sperm count in laboratory mice. *Journal of Human Reproductive Sciences*, 6(2):106-10.

Spiropoulos, J., Turnbull, D.M. & Chinney, P.F. 2002. Can mitochondrial mutations cause sperm dysfunction? *Molecular Human Reproduction*, 8(8): 719-21.

Steibar, A., Kerner, J. & Hoppel, C.L. 2004. Carnitine: a nutritional, biosynthetic and functional perspective. *Molecular Aspects of Medicine,* 25(6): 455- 73.

Suarez, J. 2008. Control of hyperactivation in sperm. *Human Reproduction Update*, 14(6): 647-57.

Suresh, S., Prithiviraj, E., Lakshmi, N.V., Ganesh, M.K., Ganesh, L. & Prakash, S. 2013. Effect of Mucuna pruriens (Linn.) on mitochondrial dysfunction and DNA damage in epididymal sperm of streptozotocin induced diabetic rat. *Journal of Ethnopharmacology*, 145(1): 32-41.

Taplin,C.E. & Barker, J.M. 2008. Autoantibodies in type 1 diabetes. *Autoimmunity*,41(1):11-8.

Tiong, S.H., Looi, C.Y., Hazni, H., Arya, A., Paydar, M., Wong, W.F., Chear, S., Mustafa, M.R. & Awang, K. 2013. Antidiabetic and antioxidant properties of alkaloids from Catharanthus roseus (L.) G. Don. *Molecules*, 18(8): 9770-84.

Tiwari, B.K., Pandey, K.B., Abidi, A.B. & Rizvi, S.I. 2013. Markers of oxidative stress during diabetes mellitus: a review. *Journal of Biomarker*, Article 378790: 1-8.

Tomita, T. 2017. Apoptosis of pancreatic β-cells in type 1 diabetes. *Bosinian Journal of Basic Medical Sciences*, 17(3); 183-93.

Tremellen, K. 2008. Oxidative Stress and male fertility- a clinical perspective. *Human Reproduction Update*, 14(3): 243-58.

Tvrdá, E., Kňažická, Z., Bárdos, L., Massányi, P. & Lukáč, N. 2011. Impact of oxidative stress on male fertility- a review. *Acta Vertinaria Hungarica*, 59(4): 465-84.

Ukwenya, V., Ashaolu, O., Adeyemi, D., Obuotor, E., Tijani, A., Biliaminu, A. & Caxton-Martins, E. 2013. Evaluation of antioxidant potential of methanolic leaf extract of Anacardium occidentale on the testes of streptozotocin induced diabetic wistar rats. *European Journal of Anatomy,* 2(17): 72-81.

Uysal, O. & Bucak, M.N. 2007. Effects of oxidised glutathione, bovine serum albumin, cysteine and lycopene on the quality of frozen thawed ram semen. *Acta Veterinaria BMO*, 76(3): 383-90.

Valeron, N. & de Plabos-Velasco, P.L. 2013. Limitations of insulin-dependent drugs in the treatment of type 2 diabetes mellitus. *Mediina Clinica (Barc),* 141(2): 20-25.

Van Belle, T., Coppieters, K. & Von H.M. 2011. Type 1 diabetes: Aetiology, immunology and theurapeutic strategies. *Physiological Reviews*, 91(1): 79-118.

Van Bergen, M.A. & Snoeijer, W. 1996. Revision of *Catharanthus roseus* G. Don. Series of revisions of Apocynaecea XLI; Backhuys Publishers: Leiden, the Netherlands: 32-5.

Van de Laar, F.A. 2008. Alpha glucosidase inhibitors in the early treatment of type 2 diabetes. *Vascular Health Risk Management*, 4(6): 1189-95.

Vardi, N., Parlakpinar, H., Ates, B., Cetin, A. & Otlu, A. 2009. Antiapoptotic and antioxidant effects of β-carotene against methotrexate-induced testicular injury. *Fertility and Sterility,* 92(6): 2028-33.

Vega-Vila, E., Cano-Velasco, Alarcon-Aguilar, F.J., Ortiz, M.C.F., Almanza-Perez, J.C. & Roman-Ramos, R. 2012. Hypoglycaemic activity of aqueous extracts from Catharanthus roseus. *Evidence-Based Complementary and Alternative Medicine*: Article 934258; 7pages.

Verma, A., Laakso, I., Laakso, T.S., Huhtikangas, A. & Riekkola, M.L. 2007. A simplified procedure for indole alkaloid extraction from *Catharanthus roseus* combined with a semi-synthetic production process for vinblastine. *Molecules*, 12(7):1307-15.

Vernet, P., Aitken, R.J. & Drevet, J.R. 2004. Antioxidant strategies in the epidydmis. *Molecular and Celluar Endocrinology*, 216(2): 31-9.

Via, M.A., Chandra, H., Araki, T., Potenza, M.V. & Skamagas, M. 2010. Bromocriptine approved as first medication to target glycaemic control in patients with type 2 diabetes. *Diabetes, Metabolic Syndrome and Obesity*, 3: 43-8.

Viollet, B., Guigas, B., Garcia, N., Leclerc, J., Foretz, M. & Andreeli, F. 2012. Cellullar and molecular mechanisms of metformin: an overview. *Clinical Sciences (London*), 122(6): 253-70.

Wallace, T.M. & Matthews, D.R. 2000. Poor glycaemic control in type 2 diabetes: a conspiracy of disease, suboptimal therapy and attitude. *QJM*, 93(6): 369-74.

Wathes, D.C., Abayasekara, D.R. & Aitken, R.J. 2007. Polyunsaturated fatty acids in male and female reproduction. *Biology of Reproduction*, 77(2):190-201.

Wellen, K.E. & Hotamisligil, G.S. 2005. Inflammation, stress and diabetes. *Journal of Clinical Investigation,* 115(5): 111-9.

Williams, A.C. & Ford, W.C. 2004. Functional significance of the pentose phosphate pathway and glutathione reductase in the antioxidant defence of human sperm. *Biology of Reproduction*, 71(4): 309-16.

Wilson, R.D. & Islam, S. 2012. Fructose-fed streptozotocin-injected rat: an alternative model for type 2 diabetes. *Pharmacology Reports*, 64(1):129-39.

World Health Organisation Diabetes Programme, 2008. http://www.who.int/diabetes/facts/world figures/en/index2.html Geneva: World Health Organisation.

World Health Organisation, 2016. Global report on diabetes. ISBN 97892.

World Health Organisation. 2014. Global status report on non-communicable diseases 2014: attaining the nine global non-communicable diseases targets a shared responsibility. World Health Organisation, 2014.

Xu, Y., Lei, H., Guan, R., Gao, Z., Li, H., Wang, L., Hui, Y., Zhou, F. & Xin, Z. 2014. Prophylactic protective effects and its potential mechanism of *Icarisidell* on STZ-induced spermatogenic dysfunction. *International Journal of Molecular Sciences*, 15(9): 16100-13.

Yan, L.J. 2014. Pathogenesis of chronic hyperglycaemia from reductive stresss to oxidative stress. *Journal of Diabetes Research*, 2014:Article 137919. 11 pages.

Yao, X.G., Chen, F., Li, P., Quan L., Chen L., Gao, Z., Wan P., Hu, L., Jiang H, Shen X. 2013. Natural product vindoline stimulates insulin secretion and efficiently ameliorates glucose homeostasis in diabetic murine models. *Journal of Ethnopharmacology*, 150(1): 285-97.

Yatoo, M.I., Saxena, A., Gopalakshnan, A., Alagawany, M. & Dharma, K. 2017. Promising antidiabetic drugs, medicinal plants and herbs: an update. *International Journal of Pharmacology,* 13(7): 732-45.

Yoon, Y.S., Ruju, D., Lee, M.W., Hong, S. & Koo, S.H. 2009. Adiponectin and thiozolinedione targets CRTC2 to regulate hepatic gluconeogenesis. *Experimental and Molecular Medicine*, 41(8): 577-83.

Zhu, J., Wang, M., Wen, W. & Yu, R. 2015. Biosynthesis and regulation of terpenoid indole alkaloids in *Catharanthus roseus*. *Pharmacognosy Reviews,* 9(17): 24-8.