



Cape Peninsula  
University of Technology

**Antidiabetic and profertility mechanisms of  
aqueous extract of *Basella alba* in male Wistar  
rats**

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BY

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## DECLARATION

I, Dennis Seyi Arokoyo, declare that the content of this thesis represent my own work, and that the thesis has not been previously submitted for any academic qualification or examination. Furthermore, it represents my own opinion and not necessarily those of the Cape Peninsula University of Technology.

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Signed

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Date

## ABSTRACT

The use of medicinal plants in the management of various health problems date back to the ancient times. However, only in recent years, researchers are starting to focus on the use of natural plant products as alternative treatment in disease control. *Basella alba* (*Ba*), commonly called Ceylon or Indian spinach is one of such medicinal plants, widely cultivated and consumed mostly as vegetable.

Studies have established many beneficial effects of *Ba*, including androgenic effects as well as antidiabetic effects which have been described in rats following oral administration of the leave extract. However, the actual mechanisms underlying the antihyperglycemic effect of *Ba* have not been reported in any study and little or no research details are yet available on the potential beneficial effects of *Ba* in reproductive dysfunction resulting from diabetes mellitus. This study was aimed at investigating the mechanisms underlying the antidiabetic effect of *Ba* and the possibility of a role for the plant in correcting diabetic-induced reproductive dysfunctions in male Wistar rats.

The first part of the study involved comparing of three different solvent extracts of *Ba* leaves namely ethyl acetate, methanolic and aqueous extracts for their antioxidant potentials, after which the aqueous extract was selected for further use in the experiments. Animal experimentation involved male rats (n=40) aged 8-10 weeks, randomly divided into four equal groups as follows: Healthy Control, Diabetic Control, Healthy Treatment and Diabetic Treatment. Diabetes was induced via a single intraperitoneal injection of streptozotocin (55mg/kg) and all animals subsequently received treatment via gavage (Rats in Control groups received 0.5ml/100g normal saline daily and treatment groups received 200mg/kg plant extract daily) for a period of four weeks. Fasting blood sugar and body weights were recorded weekly throughout the study. Animals were sacrificed upon completion of the treatment and blood samples and tissues collected for further analysis which included computer aided sperm analysis, Luminex<sup>®</sup> technology and enzyme-linked immunosorbent hormonal assays, inflammatory cytokine assays, analysis of oxidative stress markers and Histopathological analysis.

The single intraperitoneal injection of a high streptozotocin dose resulted in hyperglycaemia, weight loss, subnormal sperm parameters, negative balance of inflammatory cytokines and endogenous antioxidants and degenerative changes in the

pancreas, testes and epididymis as observed in the diabetic control rats. Oral administration with the aqueous extract of *Ba* for four weeks in diabetic treatment rats led to a significant reduction in blood sugar and improvement of sperm parameters by modulating the production of gonadal hormones, *in vivo* antioxidants and inflammatory cytokines. There was also significant recovery of normal islet histology and reduction in testicular and epididymal degeneration in the diabetic treatment rats when compared to their diabetic control counterparts. It was concluded from the findings of this study that the antidiabetic and profertility effects of *Ba* are largely dependent on the modulation of *in vivo* production of antioxidants, gonadal hormones and inflammatory cytokines, probably stimulated by one or more phytochemical component(s) that can be isolated in the aqueous extract of the plant.

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# DEDICATION

This dissertation is dedicated to

**God Almighty**

And

**Me**

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## GLOSSARY

### Abbreviations

•OH  
AGEs  
ANOVA  
*Ba*  
CASA  
CAT  
CDs  
CPUT  
Cu-SOD  
DM  
DNA  
DPPH  
EDTA  
FBS  
FRAP  
FSH  
GPx  
GR  
GSH  
GSSG  
H&E  
IDDM  
IL  
LH  
MCP-1  
Mn-SOD  
nDNA  
NIDDM  
O<sub>2</sub>  
O<sub>2</sub><sup>-•</sup>

### Definitions

Hydroxyl radical  
Advance glycation end products  
Analysis of variance  
*Basella alba*  
Computer aided sperm analysis  
Catalase  
Conjugated dienes  
Cape Peninsula University of Technology  
Copper superoxide dismutase  
Diabetes mellitus  
Deoxyribonucleic acid  
1, 1-Diphenyl-2-picrylhydrazyl  
Ethylene-diaminetetraacetic acid  
Fasting blood sugar  
Ferric reducing antioxidant power  
Follicle stimulating hormone  
Glutathione peroxidase  
Glutathione reductase  
Reduced Glutathione  
Oxidized Glutathione  
Hematoxylin and Eosin  
Insulin dependent diabetes mellitus  
Interleukin  
Luteinizing hormone  
Monocyte chemotactic protein 1  
Manganese superoxide dismutase  
Nuclear Deoxyribonucleic Acid  
Non-insulin dependent diabetes mellitus  
Oxygen  
Superoxide anion

|        |   |
|--------|---|
| ORAC   | Oxygen radical absorbance capacity          |
| OS     | Oxidative stress                            |
| PKC    | Protein kinase C                            |
| RAGE   | Receptors of advance glycation end products |
| REC    | Research Ethics Committee                   |
| ROS    | Reactive oxygen species                     |
| SB     | SpermBlue                                   |
| SEM    | Standard error of mean                      |
| SOD    | Superoxide dismutase                        |
| SPSS   | Statistical package for social sciences     |
| SRC    | Standard rat chow                           |
| STZ    | Streptozotocin                              |
| T1DM   | Type 1 diabetes mellitus                    |
| T2DM   | Type 2 diabetes mellitus                    |
| TBARS  | Thiobarbituric acid reactive substances     |
| TEAC   | Trolox equivalent antioxidant capacity      |
| WHO    | World Health Organization                   |
| Zn-SOD | Zinc superoxide dismutase                   |

# Chapter I

## Introduction

Medicinal plants have been used in the management of different types of health problems for many decades (Sofowora, 1993), but in recent years, an increasing number of research studies are focused on the use of these natural plant products as alternative for disease control in developing countries. About 85,000 plant species have been reported worldwide to possess medicinal properties, but till date, only a few of these have been scientifically validated (Anand *et al.*, 2017).

*Basella alba* is one of such medicinal plants, wildy cultivated and consumed mostly as vegetable. It belongs to the plant family, *Basellaceae*, fast growing and said to originate from heat tolerant tropical Asian countries like India and Indonesia (Grubben and Denton, 2004). It is widely cultivated in the tropics as a perennial plant and as an annual vegetable in the warmer temperate regions. *Basella alba* has thick, heart-shaped leaves which are semi-succulent, mild flavoured and mucilaginous in texture. It is a branched, climbing plant and is commonly called Ceylon spinach, Malabar spinach or Indian spinach (Roshan *et al.*, 2012).

Diabetes mellitus (DM) is a chronic, complicated metabolic anomaly characterized by high blood glucose, which often result from defects in insulin secretion, insulin action, or both and associated with severe disturbances of carbohydrate, fat, and protein metabolism (American Diabetic Association, 2011). According to the World Health Organization (WHO), the incidence of DM, which was reported to affect 177 million people worldwide as at the year 2000, is on the rise, and projected to affect about 300 million by 2025 (WHO, 2002). However, by year 2011, the International Diabetic Federation reported that, 366 million people were already affected by DM worldwide and the figure is projected to rise to 522 million by 2030 (Whiting *et al.*, 2011). DM remains a major global health concern that is yet to have any scientifically proven cure and the numerous challenges associated with the currently available conventional treatment options underscore the need to continue the search for alternatives.



**Figure 1.1: Creeping *Basella alba* (Courtesy: Forest and Kim Starr - Plants of Hawaii - Image licensed under a Creative Commons Attribution 3.0 License, permitting sharing and adaptation with attribution)**

Studies have established many beneficial medicinal effects of *Basella alba*, including the antidiabetic effect described in rats after oral administration of the aqueous extract of the leave (Bamidele *et al.*, 2014). The flowers have being reported to be useful as antidote for certain poisons (Duke and Ayensu, 1985). It has also being used as a safe laxative for pregnant women and in relieving labour pains (Duke and Ayensu, 1985). The aqueous leave extract of *Basella alba* have been reported to correct anaemia and enhance general health (Bamidele *et al.*, 2010) and the plant is traditionally believed to stimulate good libido (Kuetete and Efferth, 2010). The methanolic extract was found to enhance testosterone secretion from fractions of testes and Leydig cells extracted from normal adult male albino rats (Nantia *et al.*, 2011). Furthermore, a recent literature survey reported that the extracts of *Basella alba* possess analgesic (Anandarajagopal *et al.*, 2011), anti-inflammatory (Chaitanya, 2012), antimicrobial (Oyewole and Kalejaiye, 2012), gastro-protective (Kumar *et al.*, 2012) and CNS depressant (Anandarajagopal *et al.*, 2011) activities. However, the actual mechanisms underlying these effects of the plant are still unclear in most cases and explanations are at best, speculative. The inference of an antidiabetic effect of *Basella alba* as described by Bamidele and co-authors (2014) was purely based on the ability of the plant's extract to lower blood sugar. The mechanism by which *Basella alba* induces this glycaemic control is yet unknown and it is not clear if this blood sugar lowering effect in diabetic subjects translates into reversal of any of the systemic complications of DM.

This study was aimed at investigating some possible molecular and cellular basis of the antihyperglycaemic effect of the plant extract and to determine the possibility of *Basella alba* ameliorating some reproductive dysfunction caused by DM.

The objectives were manifold and included the following:

- To first determine the *in vitro* antioxidant capacities of aqueous extract of *Basella alba* leave and compare these to those of organic solvents of extraction.
- To determine the influence of the aqueous extract of *Basella alba* on *in vivo* oxidative stress markers and markers of inflammation in diabetic and non-diabetic male Wistar rats.

- To investigate whether or not the antidiabetic properties of aqueous extract of *Basella alba* translate to improved reproductive functions in male Wistar rats.
- To determine the effect of aqueous extract of *Basella alba* on anthropometric and microscopic analysis of pancreas and gonadal tissues in diabetic male Wistar rats.

The thesis is written in article-based format with each article addressing topical aspects of the study objectives and presented in separate chapters. Chapter one is the introduction which contains general background information about the subject matter, justification and the aim and objectives of the study. Chapter two contains a comprehensive review of relevant literature including a review article on the 'Male reproductive complications of DM and possible medicinal plant remedies' which has been accepted for publication in the Research Journal of Health Sciences (AJOL). Chapter three is a comparative study of the antioxidant properties of three different types of *Basella alba* leave extracts entitled 'Antioxidant and tyrosinase inhibiting activities of ethyl acetate, methanolic and aqueous extracts of *Basella alba* leaves'. This is currently being peer-reviewed for possible publication with International Journal of Applied Research in Natural Products. The next three chapters subsequent to this contain three separate original articles from this study; Chapter four, titled 'Reproductive parameters in streptozotocin-Induced diabetic male Wistar rats: Beneficial role of *Basella alba* Aqueous leave extract' is a demonstration of the profertility potentials of *Basella alba*. The result of this study was presented orally at the 44th conference of the Physiology Society of Southern Africa (PSSA) in Cape Town, South Africa and also at the conference of the Polish Society of Andrology (18<sup>th</sup> Andrology Day) in Gdansk, Poland in August and September of 2016 respectively. The full original article is at the moment undergoing peer-review for possible publication in the Journal of Kerman University of Medical Sciences. Chapter five is the second original article titled 'Modulation of inflammatory cytokines and islet morphology as therapeutic mechanisms of *Basella alba* in streptozotocin induced diabetic rats'. This has been peer-reviewed and accepted for publication in Toxicological Research (PubMed). The third original article from this study is presented in chapter six, titled 'Antioxidant activities of *Basella alba* aqueous leave extract in blood, pancreas and gonadal tissues of diabetic male Wistar rats'. This has also been reviewed and accepted for publication in Pharmacognosy Research (PubMed). All the five articles in the various chapters as

mentioned are presented in the exact final format submitted to (or accepted by) the various journal bodies. Chapter seven is the last chapter of this thesis and contains a general discussion of all the main findings of the study, conclusion and a brief analysis of key areas identified for further studies as a result of questions generated from the findings reported in this present study.

## Chapter II

### Literature review

DM is a disease that is primarily characterized by persistent, prolonged hyperglycaemia due to severe alteration in carbohydrate, protein and lipid metabolism which alters the normal functioning of organs and tissues of the body and result in multisystemic dysfunction (Alves *et al.*, 2013). The Saunders Comprehensive Veterinary Dictionary described 'diabetes mellitus' as a broad term that denote a 'complex group of syndromes that have in common a disturbance in the oxidation and utilization of glucose, which is secondary to a malfunction of the Beta cells of the pancreas, whose function is the production and release of insulin'. The pathological basis underlying the occurrence of this metabolic abnormality is the absence or insufficiency of insulin action which can result from several causes (American diabetic association, 2004). The metabolic disturbance in DM is not limited to glucose balance alone due to the fact that insulin is not only involved in carbohydrate metabolism, but also in the metabolism of proteins and fat (Newsholme and Dimitriadis, 2001). Insulin is a protein hormone that is released from the Beta-islet cells of endocrine pancreas in response to glucose (Asmat *et al.*, 2016), and failure of this glycaemic response, to certain extent, forms part of the events that culminate in the different types of DM.

#### 2.1 Classification of diabetes mellitus

Most cases of DM can be classified under one or the other of two major classes of the diseases, namely; Type I and Type II DM. Generally, type II DM (T2DM) is said to account for 85-90% of the diabetes incidences, while type I DM (T1DM) is seen in about 10-15% of cases (Dlamini *et al.*, 2017). However, other categories have been identified in a more detailed etiopathological classification (American diabetic association, 2004) as follows:

(a) *Type I or Insulin dependent DM (IDDM)*: This occurs as a result of absolute insulin deficiency secondary to an immune-mediated Beta-islet cell destruction and treatment usually requires insulin supplementation.

- (b) *Idiopathic DM*: This type of DM is equally insulin dependent but the etiology is unknown. Features are similar to those of IDDM and it can be inherited.
- (c) *Type II or Non-insulin dependent DM (NIDDM)*: In this type of DM, there is 'relative rather than absolute insulin deficiency' and is usually associated with insulin resistance and/or insulin secretory defect. This is the commonest form of DM and etiology can be multifactorial in most cases.
- (d) *Specific types of DM*: Some cases of DM may not particularly fit into any of the above type are better described by the unique etiology. Prominent under this category is the gestational DM (GDM) which describes glucose intolerance that begins for the first time during pregnancy. The condition may or may not persist after termination of the pregnancy. Other types of DM in this category include; drug or chemical-induced diabetes, endocrinopathies, diseases of exocrine pancreas, genetic defects of Beta cells or insulin action, infections etc.

## **2.2 Features of DM**

The features of DM are largely dependent on a number of factors including the level and duration of hyperglycaemia. Generally, symptoms of significant hyperglycaemia usually include polyuria, polyphagia, polydipsia and weight loss, however, some life-threatening acute complication including high risk of certain infections, ketoacidosis and non-ketotic hyperosmolar syndrome may further accompany uncontrolled hyperglycaemia (American diabetic association, 2012). Chronic complications of DM affect virtually all systems of the body and are mostly consequences of microvascular and cardiovascular anomalies (Mard-Soltani *et al.*, 2012). Majority of these systemic complications are believed to be induced via the generation of free radicals and oxidative stress and include nephropathy, coronary artery diseases, retinopathy, neuropathy, stroke (Asmat *et al.*, 2015) and a wide range of male reproductive dysfunction (Sexton *et al.*, 1997; Baccetti *et al.*, 2002; Scarano *et al.*, 2006; Agbaje *et al.*, 2007; Ricci *et al.*, 2009; Mallidis *et al.*, 2011; Schoeller *et al.*, 2012; La Vignera *et al.*, 2012 and Alves *et al.*, 2013).

## **2.3 Oxidative stress in DM**

The production of reactive oxygen/nitrogen species (ROS/RNS) like superoxide anion ( $O_2^{\cdot-}$ ), nitric oxide ( $\cdot NO$ ) and hydrogen peroxide ( $H_2O_2$ ) in the human body is an unavoidable aspect of metabolism (Nowotny *et al.*, 2015). These reactive species are toxic to body tissues and are therefore constantly removed or detoxified with the aid of enzymatic [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)] and non-enzymatic [ascorbic acid (vitamin C), b-carotene, a-tocopherol (vitamin E), vitamin A, glutathione (GSH)] antioxidant systems of the body (Mates *et al.*, 1999). The activities of these endogenous antioxidants are constantly moderated as the level of ROS changes in tissues so as to maintain a healthy oxidant/antioxidant balance. “An imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage” is called oxidative stress (Kumawat *et al.*, 2013). In DM, the rate of generation of ROS (superoxide anion and hydrogen peroxide) is said to increase in response to uncontrolled hyperglycaemia (Ceriello *et al.*, 2000) and coupled with the compromised defense system, there is an imbalance which favors ROS/RNS. DM causes cytotoxicity by altering the normal redox state via production of peroxides and free radicals thereby causing tissue damage (Kumawat *et al.*, 2013). Oxidative stress have been hypothesized to activate all the mechanisms by which chronic hyperglycaemia induce tissue damage (Giacco and Brownlee, 2010). These includes the stimulation of high glucose flux into the polyol pathway, production of advanced glycation end-products (AGEs), up-regulation of the receptor for advanced glycation end products (RAGE) and activation of the hexosamine pathway and protein kinase C (PKC) isoforms (Giacco and Brownlee, 2010).

### **2.3.1 Role of antioxidants in the prevention of oxidative stress**

As mentioned above, two major categories of antioxidants (enzymatic and non-enzymatic) are endogenously available and function mainly to scavenge free radicals and prevent damaging oxidative stress. The mechanism of these antioxidants' actions can be described as either chain-breaking or preventive with regards to the ROS/RNS generating reactions (Somogyi *et al.*, 2007). The mitochondria being the main source of

ATP production in all mammalian cells, is the major site of superoxide generation (Cadenas and Sies, 1998) due to premature 'leak' of electrons which attaches to molecular oxygen to form superoxide anion radical ( $O_2^{\cdot-}$ ) (Valko *et al.*, 2004). Superoxide radicals are involved in the Haber-Weiss reaction which is a complex of many reactions including the Fenton reaction to generate other reactive species like the hydroxyl radical ( $\cdot OH$ ) from hydrogen peroxide (Liochev and Fridovich, 2002). SOD, CAT and GPx are major enzymatic antioxidants that help to prevent the accumulation of reactive radicals  $O_2^{\cdot-}$ ,  $H_2O_2$  and  $\cdot OH$  by catalyzing their detoxification as illustrated in figure 2.1.

Glutathione is a very important non-enzymatic antioxidant that is very abundant in cytosol, nuclei and mitochondria where the ratio of its reduced to oxidized form (GSH/GSSG) is a good measure of the oxidative stress status of the organism (Valko *et al.*, 2007). Glutathione has multiple key roles in preventing oxidative stress as shown in figure 2.1, which includes acting as carrier for amino acids in plasma, cofactor for detoxifying enzymes, scavenging free radicals and regeneration of other important antioxidants like vitamins C and E into their active forms after use (Valko *et al.*, 2007).

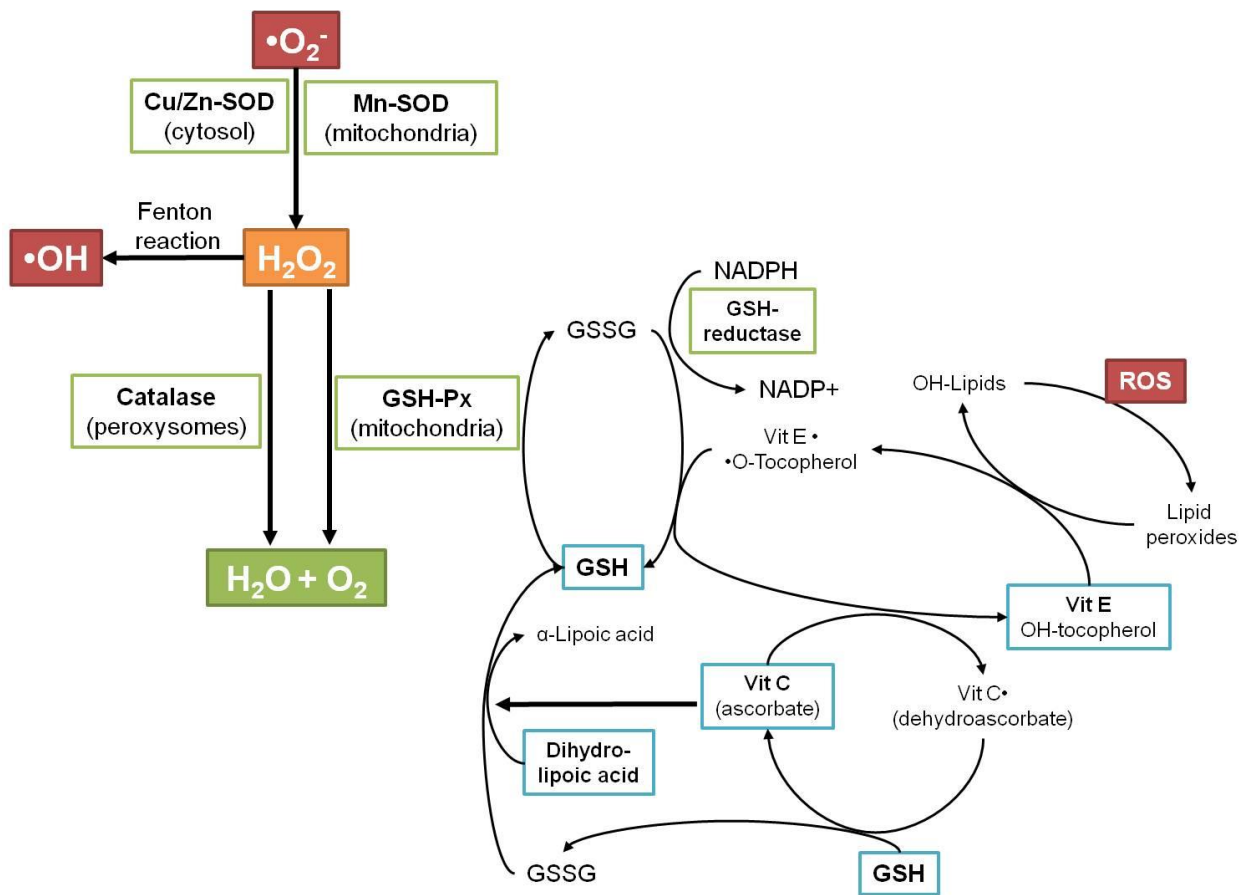


Figure 2.1: Role of enzymatic and non-enzymatic antioxidants in biological systems (Courtesy: Maria-Luisa and Fernández-Mejía, 2013)

## **2.4 Management of DM**

The management of diabetes entails a comprehensive and multifaceted approach and the success is largely dependent on the patient's understanding of the disease process. It includes modification of diet and life style, diabetes education, control of blood glucose and careful regulation of drug use among other measures (Imam, 2012). In the last nine decades, the management of DM has been centered upon enhancing the control of blood glucose through life style modification and the use of pharmacologically active substances, targeted at reducing the risk of systemic complications (Chatterjee and Davies, 2015). Insulin therapy and oral antihyperglycaemic agents are the two major modalities of drug treatment commonly used in the management of T1DM and T2DM respectively. Despite their high level of efficacy, both of these conventional methods of medication are associated with disturbing long-term adverse effects, thereby necessitating the continuous search for alternative therapy (Anand *et al.*, 2017).

### **2.4.1 Mechanism of insulin action**

Insulin is a peptide hormone produced by Beta-islet cells of the endocrine pancreas and the primary function is to ensure glucose uptake from blood into the various tissues of the body (Stryer and Lubert, 1995). Like any other peptide hormone, insulin exerts its physiological actions in target organs by binding to specific receptors that are located on the surface of cell membrane (Catt and Dufau, 1977; Lloyd *et al.*, 1991). The insulin receptor (IR) is trans-membrane in nature and made up of two covalently bound protein dimers containing an Alpha subunit which is extracellular ligand binding and an intracellular Beta subunit that is tyrosine kinase active (Harrington *et al.*, 2012) (Figure 2.2). After binding to the Alpha subunit, insulin is capable of generating a cascade of signals (Phosphorylation and dephosphorylation reactions) that eventually lead to the actions on glucose, protein and lipid metabolism by activating the tyrosine-specific kinase in the Beta subunit (Kahn, 1985).

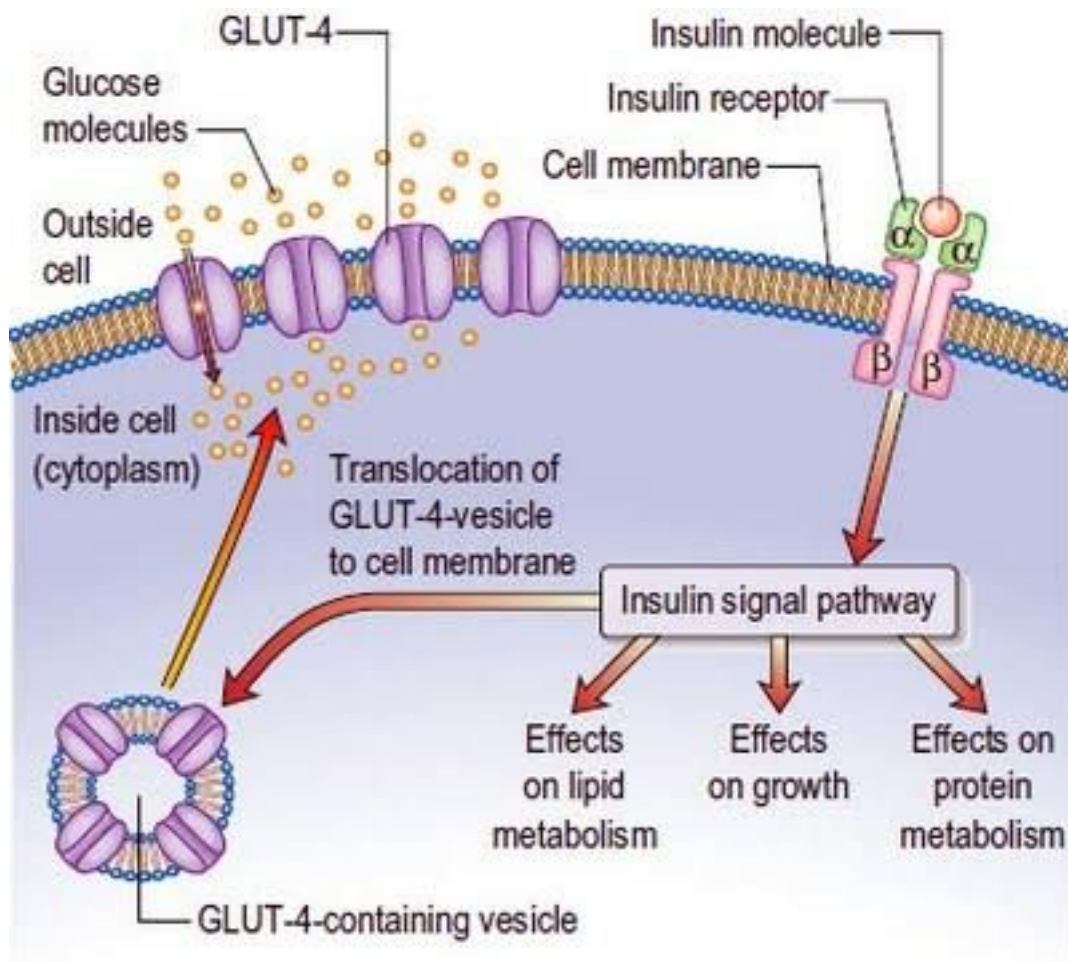


Figure 2.2: Mechanism of insulin action (Courtesy: Chhabra, 2012)

As illustrated in figure 2.2, the cascade of phosphorylation and dephosphorylation reactions serve as a physiological stimuli which lead to the translocation of vesicles containing GLUT4 glucose transporter to the cell surface of adipose tissues and skeletal muscles for the uptake of glucose into the cell cytoplasm (Huang *et al.*, 2007). It is important to note that the GLUT4 transporter is just one of the fourteen known members of the glut protein family which is also a subclass of a larger group of membrane transporters called the Major Facilitator Superfamily (MFS) (Thorens and Mueckler, 2010). Each of these transport proteins are species and tissue specific and can sometimes be substrate specific as is the case with GLUT5 which is specific for fructose transport (Douard and Ferraris, 2008). Glut4 is an insulin-responsive, transmembrane, glucose transporter and is abundant in adipocytes and skeletal muscle cells (Olson, 2012).

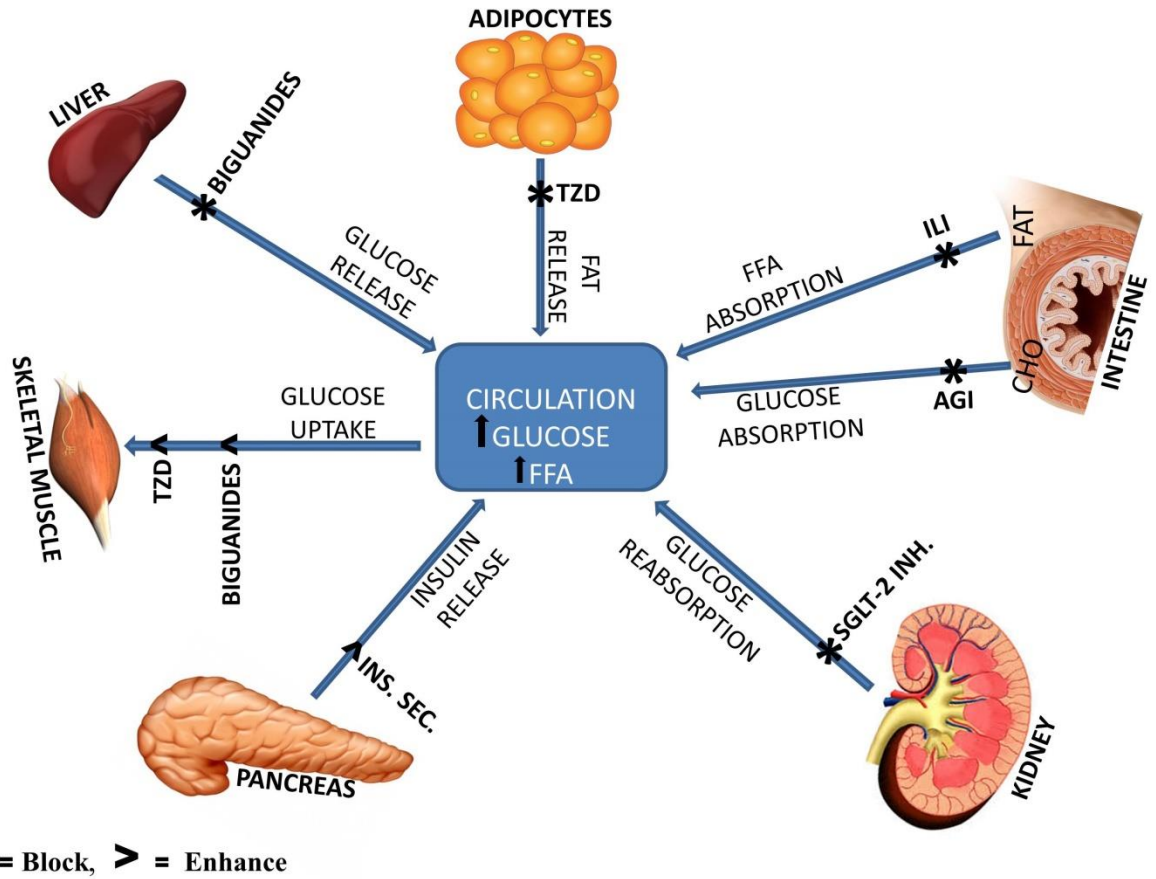
#### **2.4.2 Mechanisms of action of oral antihyperglycemic agents**

The oral treatment options for DM which are usually effective only for T2DM are generally based on counteracting the eight known pathophysiological mechanism that lead to hyperglycemia in DM (Chatterjee and Davies, 2015). In contrast to what obtains in T1DM, the pathological basis of the hyperglycemia in T2DM involves complex interplay of a number of constitutional and environmental factors that causes both insulin resistance and subnormal insulin release from the Beta-islet cells of pancreas (Yale, 2005). An in-depth knowledge of the pathogenesis of T2DM is very instrumental to the understanding of the mechanism of action of the various classes of oral antidiabetic agents (Cheng and Fantus, 2005). According to Chatterjee and Davies (2015), the eight main pathophysiological derangements in T2DM include:

- Lower insulin release from Beta-islet cells of the pancreas.
- Increased secretion of glucagon from Alpha-islet cells of the pancreas.
- Increased renal glucose reabsorption.
- Increased hepatic breakdown of glycogen into glucose.
- High circulating free fatty acid (FFA) due to increased lipolysis.
- Reduced incretin action in the small intestine.
- Insulin resistance in the brain and neurotransmitter dysfunction.

- Reduced uptake of glucose in peripheral tissues especially skeletal muscle, adipocytes and liver.

Figure 2.3 is a pictorial illustration of some of these pathologies in T2DM that culminates into hyperglycemia and the role played by the various classes of drugs in reversing them.



**Figure 2.3: Mechanisms of action of oral antihyperglycaemic agents in T2DM**

[TZD = Thiazolidinediones, FFA = Free fatty acid, CHO = Carbohydrates, ILI = Intestinal lipase inhibitor, AGI = Alpha-glucosidase inhibitors, SGLT-2 INH = Sodium-glucose cotransporter-2 inhibitors, INS. SEC = Insulin secretagogues].

Briefly, the mechanisms employed by the common categories of oral antihyperglycaemic agents are as follows:

*Biguanides:* This group of drugs acts by preventing hepatic gluconeogenesis as well as enhance glucose uptake by skeletal muscles (Yale, 2005; Cheng and Fantus, 2005). Metformin is a very effective member of this group and can be used together with any other antidiabetic therapy (Chatterjee and Davies, 2015).

*Insulin secretagogue:* This set of drugs stimulates insulin secretion from the Beta-islet cells of pancreas. There are two subclasses of the group namely Sulfonylureas (Gliclazide) and Non-sulfonylureas (repaglinide), with the difference being in the acute nature of the action of the latter subclass (Cheng and Fantus, 2005).

*Intestinal lipase inhibitor:* These drugs act by inhibiting intestinal fat absorption through selective inhibition of gastric and pancreatic lipases, thereby reducing serum level of free fatty acid and causing weight loss (Guerciolini, 1997). Orlistat is a common example and the use of these drugs in the management of diabetes is limited to obese patients (Cheng and Fantus, 2005).

*Thiazolidinediones (TZDs):* TZDs increase target organ sensitivity to insulin, enhance peripheral glucose uptake, and also affect hepatic gluconeogenesis (Yale, 2005). Rosiglitazone is a common example of TZD and their effect is better seen on adipose tissues where the receptors are more abundant (Cheng and Fantus, 2005).

*Alpha-glucosidase inhibitors:* These antidiabetics inhibit carbohydrate absorption from the intestine by reversibly antagonizing the actions of a number of alpha glucosidase enzymes like maltase (Van de Laar, 2008). They cause a delay in intestinal glucose absorption and are not suitable for use as single drug treatment of DM (Cheng and Fantus, 2005). Acarbose is a common example.

*Sodium-glucose cotransporter-2 inhibitors (SGLT-2):* This is a relatively new class of antidiabetic drugs which act by inhibiting the function of Sodium-glucose co-transporter-2 in the proximal convoluted tubule of the kidneys, thereby preventing glucose reabsorption and facilitating its renal excretion (Kalra, 2014). A typical example is

Dapagliflozin and their efficacy is dependent on insulin action and the extent of hyperglycaemia (Kalra, 2014).

### **2.4.3 Current innovations in the management of DM**

The treatment modalities currently available for T1DM and T2DM as earlier discussed are more or less palliative and require prolonged or even lifelong administration. This makes the treatment processes tiring for patients, and expectedly, nonadherence to medication is a common finding among diabetic patients which worsens the outcome of diabetic treatment (Jaam *et al.*, 2017). Therefore, the investigation of new modalities of diabetes treatment has remained a major area of medical research interest and a number of the current innovations are aimed at achieving curative treatment. The cellular basis of treatment of diabetes is currently aimed at possible ways of replacing the dysfunctional insulin producing Beta-islet cells by transplantation of either islet cells (Paty *et al.*, 2002) or whole pancreas from healthy donors (Abdulazeez, 2015). This is frequently limited by the challenge of scarcity of donor organs, and researchers are now shifting attention to the possibility of regenerating Beta islet cells through earlier established knowledge of stem cell technology (McCall *et al.*, 2009). There has been research evidence from animal model that the full mass of pancreatic Beta cells can be generated from a small piece of pancreatic tissue (Bonner-Weir *et al.*, 1993). Researchers have also considered liver, small intestine and embryonic stem cells as potential sources of insulin producing Beta cells via transdifferentiation, with some results in experimental animals (Krause *et al.*, 2001; Jiang *et al.*, 2002; Trounson, 2013).

One of the most recent innovations in the treatment of T1DM is the consideration of insulin gene therapy. This is aimed at either preventing immune destruction of Beta cells, generation of surrogate Beta cells from native non-Beta cells, or stimulation of insulin production from other cells via genetic manipulations (Handorf *et al.*, 2016). All these innovative treatment options however come with their advantages and disadvantages and therefore, the search for that ideal treatment option has continued. Significant attention has been given to to the use of medicinal plants as alternative treatment of DM worldwide, even though herbal formulations with proven therapeutic

antidiabetic effect are yet to be available (Kavishankar *et al.*, 2011). The antidiabetic properties of most of the plants so used are frequently linked with phytochemical components that exert antioxidant activities (Abdolahnejad *et al.*, 2009; Kavishankar *et al.*, 2011; Wankeu-Nya *et al.*, 2013; Long *et al.*, 2015). At present, there is a renewed interest in the study of medicinal plants as therapeutic agents for DM especially in Asian and African communities, due to the believe that plant remedies offer multiple health benefits and are associated with less adverse effects (Anand *et al.*, 2017). More definitive studies of these plants are however required to establish their standard use in the management of DM.

## **2.5 Complications of DM**

DM is frequently associated with complications especially when blood glucose is poorly controlled. The prevention of most complications of DM is largely dependent upon constant maintenance of blood glucose within the normal range (Chase *et al.*, 1989). In the United States of America, about 57.9% of all diabetes cases are said to be associated with at least one form of systemic complication and three or more complications are seen in 14.3% of patients (Mitka, 2007). The complications of DM can be categorized into metabolic acute complications like hypoglyceamia, hyperosmolar non-ketotic coma and ketoacidosis or systemic late complications which can manifest in any of the body systems (Asmat *et al.*, 2016). The systemic complications of DM that affect the male reproductive system are of particular interest in this study.

A review of recently published articles on some of the male reproductive complications of DM and common medicinal plants that may be useful in the management of these complications was carried out as part of this literature review. The review article has already been accepted for publication in the Research Journal of Health Sciences (AJOL) and is presented in this chapter in the accepted format:

## **2.6 Male Reproductive Complications of Diabetes Mellitus and Possible Medicinal Plant Remedies – A review**

**Running title:** Medicinal Plants Alleviate Reproductive complications of Diabetes

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## **Abstract**

**Objective:** Male reproductive dysfunction and subsequent infertility are major complications that are becoming increasingly associated with Diabetes Mellitus (DM). Due to frequent failure in treatment with orthodox antidiabetic drugs, there has been a massive shift of attention to alternative therapies. The antidiabetic potential of a large number of medicinal plants have been investigated both *in-vitro* and in experimental animal models. These specific plants were predominantly used because of the antioxidant capacity of their bioactive phytoconstituents. This review focuses on reproductive dysfunctions commonly suffered by male diabetic patients and medicinal plants that have been tested and reported for their roles in ameliorating such dysfunctions.

**Method:** All original journal articles and reviews cited on PubMed between 2005 and 2015 in English language were considered for this review.

**Results and conclusion:** This review reestablished the fact that male infertility is a common complication of poorly managed diabetes mellitus. It also highlighted the fact that the numerous challenges associated with the use of orthodox drugs in management of the disease makes medicinal plant therapy inevitable. However, the full potentials of these medicinal plants at correcting reproductive complications of the disease are still to be realized and more specific studies are required in this field for improved therapeutic outcomes.

**Keywords:** Diabetes, Complications, Medicinal Plants, Erectile dysfunction, Remedies.

## ***Introduction***

In 2012, the American Diabetes Association defined Diabetes Mellitus (DM) as a group of metabolic diseases characterized by hyperglycaemia and resulting from defects in insulin secretion, insulin action, or both (1). Two major etiopathogenetic classes of DM namely, type 1 or Insulin Dependent DM (IDDM) and type 2 or Non-Insulin Dependent DM (NIDDM) are widely recognized (1). Insulin Dependent Diabetic Mellitus is caused by absolute insulin deficiency due to autoimmune destruction of the pancreatic  $\beta$ -islet cells, while NIDDM is usually as a result of a combination of insulin resistance and inadequate compensatory insulin release response (1). The main feature of DM is chronic hyperglycaemia which results in long-term damage, dysfunction, and failure of different organs, involving virtually all systems of the body. Diabetes is a major public health challenge, increasingly affecting millions of people across all age groups worldwide (2). If not well managed, it results in a wide range of complications including neuropathy, retinopathy, cardiovascular diseases, renal diseases and reproductive dysfunction (2).

A large number of studies, both in diabetic humans and animal models associated DM with impotence, erectile dysfunction, retrograde ejaculation and hypogonadism (2). Despite this, the disease was not directly linked with male infertility until recently when findings revealed that diabetes induces subtle molecular alterations which are very paramount to spermatogenesis and sperm physiology (3). The role of DM in the incidence of male infertility is poorly reported and available data is characterized with controversies and inconsistencies (4). However, due to the conflicting reports on the influence of diabetes on the endocrine control of spermatogenesis, it was concluded that, DM may affect male reproductive function at multiple levels and the individual anomalies may not have any significant effect on reproductive function (5).

There has been a major upsurge in the number of research publications on DM in the last decade with special attention towards finding alternative plant remedies for the troublesome disease. Often, the attention is fixed on the ability of these plant agents to restore glycaemic control and reverse hyperglycaemia with little recourse to the correction of the disease complications. This review focuses on the reported male

reproductive complications of DM and the medicinal plant materials that have been investigated for their efficacies in correcting such complications.

### ***Materials and Methods***

This review considered all original journal papers and reviews cited on PubMed from 2005 to 2015. Few selected, exceptionally relevant publications outside this period were also reviewed. Only the studies published in English language were considered and keywords such as; 'Diabetes mellitus, definition and complications, medicinal plant treatment, diabetes mellitus and male reproductive dysfunction' were used for the search.

### ***Male Reproductive dysfunctions in diabetes mellitus***

Diabetes mellitus is not routinely investigated in cases of male infertility due to the scanty and conflicting research findings on the subject matter, but this may start to change in the wake of recent reports. La Vignera *et al.* (4) reported 1.2% prevalence of DM in the male counterparts of couples that were treated for infertility, 35.1% infertility cases among men with type 2 DM, and 51% subfertility among DM patients in general. These reported cases of infertility might be attributable to the following reported male reproductive complications of DM.

*Morphologic effects:* DM causes a significant reduction in testicular weight and total number of Leydig cells in the testes (6, 8). This may be linked with the reported low testosterone levels and histological changes induced by DM in the testes (6). Hyperglycaemia was also recently reported to induce apoptotic damage and cause morphologic impairment in the testes (8). This could further explain the testicular changes observed in diabetic subjects (see Fig. 1 [ii] & [v]). Histologically, a regression in the absolute weight of the caput, corpus and caudal regions of the epididymis, accompanied with reduction in tubule and lumen size, as well as increased interstitial stroma has been observed in the testes of Streptozotocin (STZ)-induced diabetic Wistar rats (9).

*Effects on spermatogenesis and sperm parameters:* Several studies conducted in both diabetic humans and animal models revealed that DM may have a deleterious effect on the process of spermatogenesis as suggested by reports of subnormal sperm parameters (3, 4, 10). Testosterone, the major hormone that drives spermatogenesis, has been found to be decreased in diabetic men due to impaired Leydig cell function (11). There are contradictory and inconsistent reports with regards to the exact impact DM has on semen quality (3). For instance, Delfino *et al.* (12) reported that there was no difference in sperm concentration of semen samples from diabetic men when compared to non-diabetic controls, whereas, Amaral *et al.* (11) reported a deterioration of all sperm parameters including sperm density in diabetic men. In a separate study on diabetic and non-diabetic human semen, Agbaje *et al.* (5) found a positive association between DM and sperm nuclear and mitochondrial DNA damage, in spite of zero effect on the conventional semen parameters (see Fig. 1 [iii]). The controversy deepened recently when a study, designed to evaluate the isolated effect of high glucose concentration on human semen reported that 'high glucose levels per se have no adverse effect on sperm function *in vitro*' (13). However, it can be deduced from the overwhelming majority of literature reports that spermatogenesis and consequently, at least one or more of the parameters determining semen quality and fertility (namely: sperm count, sperm concentration, motility, kinetics, morphology, semen fructose/glucose level, semen pH and semen volume) are adversely affected by DM (4, 5, 11, 14).

*Effects on sexual behaviour:* The processes controlling male libido, penile erection and ejaculation are largely autonomic, and DM is known to be complicated by autonomic neuropathy (2). One of the most reported reproductive complications of DM in men is Erectile Dysfunction (ED) (15). It simply means the inability to achieve and/or sustain penile erection long enough for adequate sexual relations (11). The prevalence of ED among diabetic men ranges from 20% to 85% and the etiology can be from either one or a combination of any of vascular, neurological and endocrine anomalies (15). Moore and Wang (16) reported that ED affects 32% of IDDM and 46% NIDDM patients and is found to be the presenting complaint in about 12% of all diabetic patients (16). Another report, however, places the prevalence of ED in NIDDM at 75% (17), thus, emphasizing the variability of occurrence in different populations of diabetic patients. The

pathophysiology of ED in diabetic patients is multifactorial and includes: end organ damage by Advanced Glycation End-products (AGEs), increased oxygen free radicals, decreased smooth muscle in the corpus cavernosum due to increased apoptosis, impaired nitric oxide synthesis, nitric oxide-cyclic guanosine monophosphate (NO-cGMP) pathway dysfunction and effects of comorbid conditions, among others (16, 18, 19) (see Fig. 1 [iv]). All these causes of ED were recently reported to culminate in up-regulation of the Transforming Growth Factor  $\beta$ 1/Smad (TGF- $\beta$ 1/Smad) signaling pathway which is a final common pathway of tissue insult (20).

Ejaculatory dysfunction secondary to autonomic neuropathy syndrome and depletion in serum testosterone level is another common sequela of DM (21). This dysfunction actually describes a range of anomalies from premature ejaculation to total failure of ejaculation (such as anejaculation or retrograde ejaculation), and DM is a common cause (22). Pontes and colleagues (21) conducted an extensive study on the role of testosterone supplementation in diabetic male Wistar rats that exhibited reduced sexual activity and failure of ejaculation. They reported only 33.3% sexual activity and ejaculation in diabetic rats as compared to 89.0% in the control group. Ejaculatory behaviour improved to about 62.5% after three weeks of testosterone supplementation, confirming their conclusion that DM induces ejaculatory dysfunction by causing low serum testosterone levels (21).

Other disturbances in sexual function common in DM which are probably neurologic and psychological in etiology include: reduced libido and Retrograde Ejaculation (RE) (11). In RE, the semen is released into the male urinary bladder rather than the female genital tract during sexual intercourse. This is due to bladder neck dysfunction from DM-induced autonomic neuropathy (21).

*Endocrinological effects:* The role of abnormal hormone regulation in diabetic induced male reproductive dysfunction is multifaceted. Diabetic neuropathy can affect the hypothalamus or anterior pituitary leading to secondary testicular failure exhibited by low serum levels of gonadotropins and testosterone (23). A direct effect on the Leydig cells will usually result in primary testicular failure which in contrast to secondary failure, presents with low serum testosterone and high gonadotropin level (24) (see Fig. 1 [i] &

[ii]). The direct effect of insulin interaction with the testes and spermatozoa has also been suggested as a possible basis for the reproductive complications of DM, since both testes and spermatozoa themselves produce insulin (24). The possibility of insulin playing an important role in spermatogenesis was re-emphasized by the report that the process was impaired in both diabetic men and knockout mice (2). However, it is not clear if this effect is induced via testicular insulin insufficiency or through the systemic effects of DM.

A study recently described an association between diabetic-induced functional hypercortisolism and hypogonadism. The researchers conducted a retrospective clinical comparative study between fifteen diabetic patients with hypercortisolism and late onset hypogonadism and another group of fifteen non-hypercortisolic diabetic patients also with late onset hypogonadism. All sexual parameters were found to be significantly worse in the hypercortisolic group and it was suggested that 'the dysregulated hypothalamic-pituitary-adrenal axis has an impairing influence on sexual function in diabetes mellitus-associated late-onset hypogonadism' (25).

### ***Medicinal plants in the treatment of diabetes-induced male reproductive dysfunction***

The management of DM as a disease entity is very elaborate and multidisciplinary in approach. Insulin therapy and/or use of oral antihyperglycemic agents have been central in the disease management. However, the numerous challenges of these drugs, ranging from cost, dosing, administration techniques, to side effects among others, have necessitated a wanton shift of research attention to alternative therapy in medicinal plants.

Many medicinal plants have been used empirically in the treatment of male infertility from diverse etiologies (26), but only a few have been investigated for their specific roles in correcting or ameliorating reproductive dysfunctions secondary to DM.

## ***Animal studies***

*Effects on the morphology of reproductive organs:* Methanol extract of *Amaranthus spinosus* (spiny pigweed) stem was found to increase testicular weight significantly in STZ-induced diabetic rats after fifteen days of oral administration (27). Extracts of *Dracaena arborea* (Tree Dracaena) root barks also partially corrected diabetes-induced morphological impairment of the testes and led to increased testicular weight in experimental rats even without having any significant effect on blood glucose after three weeks of oral treatment (7). This effect of *D. arborea* was attributed to the antioxidant and androgenic properties of saponins, phenols, flavonoids and phytosterols found in both aqueous and ethanol extracts of the plant's root (7). *Allium sativum* (Garlic) has similar positive effect on testicular weight and number of Leydig cells in diabetic rats and also ameliorated reproductive complications of STZ-induced diabetes in pretreated rats (6). The ethyl acetate fraction of *Eugenia jambolana* (Jambul tree) seed, when given orally for sixty days to experimental diabetic rats resulted in a significant recovery in weights of testes and all accessory reproductive organs (28). Mallick *et al.* (29) investigated the effectiveness of a herbal mixture, MTEC, comprising of root of *Musa paradisiaca* (Banana), seeds of *Tamarindus indica* (Indian date) and *Eugenia jambolana* (Jambul) and leave of *Coccinia indica* (Baby watermelon) in ratio 2:2:1:1, in correcting reproductive dysfunction in male diabetic rats. MTEC was reported to improve all testiculo-somatic indices that were earlier deteriorated by STZ injection. *Quercetin*, which is a flavonoid that is also found abundantly in onions, prevented all histological abnormalities that are usually associated with DM in STZ treated Wistar rats (30). Additionally, Long *et al* (8) evaluated the effect of pharmaceutical product, scutellarin, an active component of *Erigeron breviscapus* (fleabane) in type 2 diabetes rat models. Scutellarin was found to significantly inhibit the formation of apoptotic cells and morphological damage in the testes caused by DM (8).

*Effects on spermatogenesis and sperm parameters:* *Amaranthus spinosus*, *Eugenia jambolana* and MTEC increased sperm count significantly in diabetic rats (27, 28, 29). *Quercetin* reportedly restored epididymal sperm count, sperm motility and sperm viability in STZ-induced diabetic rats (30). While both methanol and aqueous extracts of

*Zingiber officinale* (Ginger) root were found to significantly enhance sperm parameters and sexual indices (31).

*Effects on erectile function:* Icariside II (ICA II), an active component of *herba epimedii* (*horny goat weed*) was investigated and found to improve erectile function in STZ-induced diabetic rats with ED. ICA II achieved this by increasing the neuronal and endothelial Nitric Oxide Synthase (nNOS and eNOS) as well as the Vascular Endothelial Growth Factor (VEGF) in penile tissues. It also down-regulated the TGF- $\beta$ 1/Smad2 pathway and decreased apoptotic index in the corpus cavernosum of diabetic rat after twelve weeks of oral treatment (32). S-Allyl Cysteine (SAC), a bioactive component of garlic significantly improved erectile function in diabetic rats after four weeks of treatment, owing to its ability to reduce formation of Reactive Oxygen Species (ROS) in penile tissue (33). Furthermore, caffeine had similar effect after eight weeks of treatment, acting via a different mechanism (34). Likewise, Cyanidin-3-O- $\beta$ -D-glucopyranoside (C3G); concentrated materials from mulberry fruit was reported to protect and improve erectile function in STZ-induced diabetic male Sprague-Dawley rats (35).

*Hormonal effects:* Both serum and testicular testosterone levels in diabetic rats were significantly increased after fifteen days of oral administration of *Amaranthus spinosus* stem and sixty days of oral treatment with ethyl acetate extract of *Eugenia jambolana* seed (27, 28). *Cinnamomum zeylanicum* (Cinnamon) and *Zingiber officinale*, when administered individually or combined increased serum testosterone, luteinizing hormone and follicle stimulating hormone, as well as enhanced spermatogenesis in STZ-induced diabetic rats (31, 36). The use of both plants appears to produce synergistic effect since these hormonal effects were greater with combined administration. MTEC and *Quercetin* are other substances reported to increase serum testosterone and enhance testicular antioxidative capacity in diabetic rats (29, 30).

*Curculigo orchioides* (black musli) is one of the few medicinal plants whose Rhizome extract was specifically reported for their role in enhancing libido in experimental diabetic animals (37). The exact mechanism underlying this effect was not ascertained,

however, modulation of the actions and blood levels of reproductive hormones may play a part.

### ***Human studies***

In contrast to the array of reports on scientific studies investigating the efficacy of numerous medicinal plants in the treatment of reproductive dysfunctions in experimentally induced DM in animals, only a very few human studies are available in this respect.

Many plants have been used empirically in the traditional settings to alleviate various reproductive dysfunctions especially poor libido, sexual asthenia and erectile dysfunction (26), but these have not been specifically investigated in diabetic patients. *Curculigo orchoides* is a herb known for its potent antioxidant and adaptogenic properties and used in ayurvedic medicine as a sexual tonic in diabetic men (37). This has been confirmed in diabetic animal models (37), but no report of any scientific study in diabetic humans is available.

A summary of some studies involving medicinal plants that have been reported for their efficacies in treating reproductive complications of DM is presented in table 1.

### ***Discussion***

This review clearly re-emphasizes the possibility of occurrence of male infertility as a complication of DM especially when poorly managed. It may further justify the recent equitable distribution of the etiology of infertility between both sexes, rather than the usual stigmatization of the female partner alone (26), since DM is not gender biased. As mentioned earlier, the complications of DM cuts across most systems of the body, but the reproductive effects are topical owing to the sensitive nature and cultural implications of infertility in many societies (38).

From many of the studies reviewed, it is obvious that the increased generation of oxidative stress is central in the pathophysiology of most of the reproductive

dysfunctions seen in DM (2, 3, 16, 19) (see Fig.1). Oxidative stress has been linked directly or indirectly to the sustained hyperglycaemia characteristic of the disease and therefore a large number of plants investigated for antidiabetic properties were so used because of their antioxidant or antihyperglycaemic potential (6-8, 28, 30).

It is noteworthy, that a very large number of medicinal plants have been reported to be useful in the treatment of male reproductive dysfunctions and infertility (26). However, only a few of these have been investigated for their efficacy in the treatment of male reproductive dysfunctions secondary to DM. Likewise, a large fraction of medicinal plants known for their antidiabetic potentials has not been specifically investigated for the correction of the reproductive complications of DM. These aspects of diabetic research cannot be discarded especially when some plants like *Dracaena arborea* were found to improve sexual function in diabetic rats without any significant effect on blood glucose (7). This implies that the correction of diabetic complications is not always dependent on antihyperglycaemic effect of a substance.

The present review also revealed a striking paucity of information with regards to the exact efficacy of medical plants in the treatment of DM-induced reproductive dysfunction in men, since there were no human studies published within the scope of our review. Virtually all investigations in this field during the time frame of this study were carried out on animal models while a large number of these medicinal plants continued to be used empirically in various traditional settings without proper therapeutic trial. The need to change this cannot be overemphasized.

Erectile dysfunction is one of the most common reproductive dysfunctions in male diabetics and yet poorly investigated. Report from one study indicated a difference in response to therapy when comparing patients of DM-induced ED to other patients of ED in the general population, with the diabetic patients showing poorer response (39, 40). Despite these reports, there was no single human study published within the period captured under this review that considered the effectiveness of medicinal plants in the treatment of DM-induced ED. There is therefore the need to specifically investigate many more of these medicinal plants and carry out an in-depth analysis of their therapeutic efficacies in the management of specific reproductive dysfunctions caused

by DM in both animal models and human diabetic subjects. This will go a long way in instituting standard alternative therapies and improving the living conditions of many diabetic patients.

### ***Conclusion***

The use of medicinal plants in the general management of DM has offered a renewed hope of better therapeutic outcome but this however has not yielded any noticeable reduction in the prevalence of systemic complications, including reproductive dysfunctions in diabetic patients. This study highlighted the paucity of information with regards to the potentials of medicinal plants in correcting male reproductive dysfunctions caused by DM. It was therefore concluded from the review that, the use of medicinal plants in the management of DM-induced male reproductive dysfunction in humans is still largely empirical. Further scientific studies, as well as clinical therapeutic trials in human subjects are required to completely evaluate the role of these natural substances.

### ***Competing interest***

The authors declare that there is no competing interest regarding any aspect of this article.

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**Table 1: Medicinal Plants used in treatment of male reproductive complications due to diabetes mellitus**

| Medicinal plant                             | Complication treated                | Type of study  | Route of administration | Daily Dose and Duration of treatment                       | Mechanism of action                            | Outcome  | References |
|---|-------------------------------------|----------------|-------------------------|--|--|--|------------|
| <i>Amaranthus spinosus</i>                  | Hypogonadism                        | In vivo (Rats) | Oral                    | 250 mg/kg<br>500 mg/kg (15 days)                           | ↑Spermatogenesis                               | ↑Testicular weight<br>↑Sperm count                       | 27         |
| Caffeine                                    | Erectile dysfunction                | In vivo (Rats) | Intragastric            | 10 mg/kg<br>20 mg/kg (8 weeks)                             | ↑Cavernous cGMP<br>↑ICP                        | Improved erectile function                               | 34         |
| <i>Dracaena arborea</i>                     | Testicular dysfunction              | In vivo (Rats) | Oral                    | 100 mg/kg<br>500 mg/kg (3 weeks)                           | ↑Antioxidation                                 | Restores testicular morphology and spermatogenesis       | 7          |
| <i>Eugenia jambolana</i>                    | Testicular dysfunction              | In vivo (Rats) | Oral                    | 20 mg/100 g (60 days)                                      | ↑Antioxidation                                 | ↑Organ weight  | 28         |
| <i>Zingiber officinale</i>                  | Spermatogenesis dysfunction         | In vivo (Rats) | Oral                    | 100 mg/kg (56days)<br>100-300 mg/kg (65 days)              | ↑Antioxidation                                 | ↑Sperm parameters and sex hormones                       | 31,36      |
| <i>Allium sativum</i>                       | Testicular damage                   | In vivo (Rats) | Oral                    | 1 ml/100 mg (3 weeks)<br>(6 weeks)                         | ↑Antioxidation                                 | Restores testicular morphology and serum testosterone    | 6          |
| <i>Musa paradisiaca</i>                     | Testicular dysfunction              | In vivo (Rats) | Oral                    | In MTEC 60 mg/0.5 ml olive oil/100 g twice daily (15 days) | ↑Antioxidation                                 | Improved organo-somatic indices and sperm parameters     | 29         |
| <i>Tamarindus indica</i>                    | Testicular dysfunction              | In vivo (Rats) | Oral                    | In MTEC 60 mg/0.5 ml olive oil/100 g twice daily (15 days) | ↑Antioxidation                                 | Improved organo-somatic indices & sperm parameters       | 29         |
| <i>Coccinia indica</i>                      | Testicular dysfunction              | In vivo (Rats) | Oral                    | In MTEC 60 mg/0.5 ml olive oil/100 g twice daily (15 days) | ↑Antioxidation                                 | Improved organo-somatic indices & sperm parameters       | 29         |
| <i>Herba epimedii</i> (Icariside II)        | Erectile dysfunction                | In vivo (Rats) | Oral                    | 1, 5, and 10 mg/kg (3 months)                              | ↑Expression of eNOS, nNOS and VEGF             | Improved erectile function                               | 32         |
| <i>Cinnamomum zeylanicum</i>                | Spermatogenesis dysfunction         | In vivo (Rats) | Oral                    | 75 mg/kg (56 days)   | ↑Antioxidation                                 | Improved sperm parameters and sex hormones               | 36         |
| <i>Quercetin</i>                            | Spermatogenesis dysfunction         | In vivo (Rats) | Intraperitoneal         | 15 mg/kg (28 days)   | ↑Antioxidation and anti-inflammation           | Improved testicular morphology and sperm parameters      | 30         |
| Scutellarin ( <i>Erigeron breviscapus</i> ) | Testicular damage                   | In vivo (Rats) | Oral                    | 100 mg/kg (2 months)                                       | ↑Antioxidation and improved microcirculation   | Reduced apoptotic and morphological damage in the testes | 8          |
| <i>Curculigo orchioides</i>                 | Oligospermia and sexual dysfunction | In vivo (Rats) | Oral                    | 100 mg/kg<br>200 mg/kg (28 days)                           | ↑Antioxidation and increased anabolic activity | Improved sexual function and sperm count                 | 37         |

KEY: eNOS = Endothelial nitric oxide synthase.

nNOS = Nuclear nitric oxide synthase.

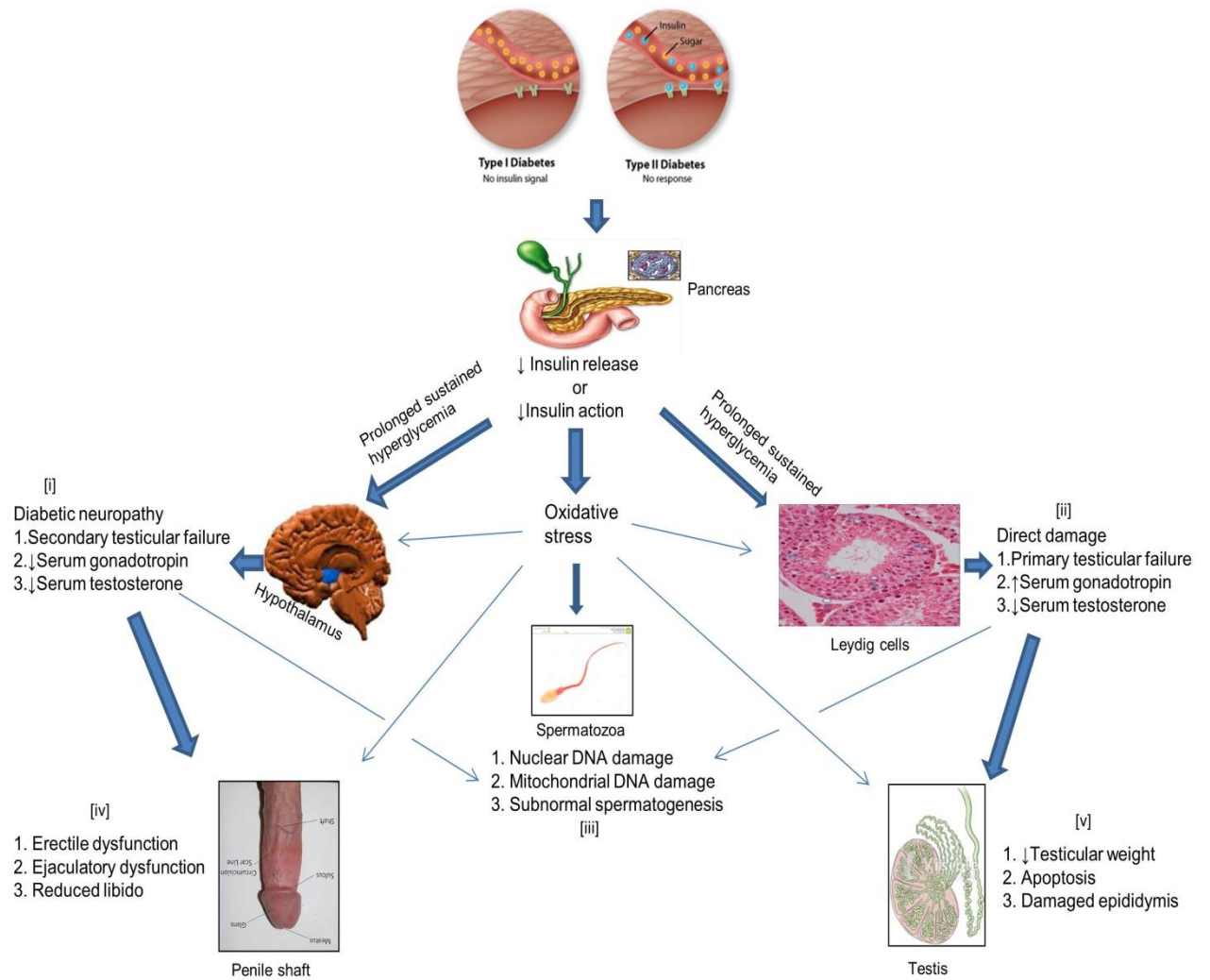
ICP = Intracavernous pressure.

VEGF = Vascular endothelial growth factor.

MTEC = *Musa paradisiaca*, *Tamarindus indica*, *Eugenia jambolana* and *Coccinia indica*

↑ = Increased

cGMP = Cyclic Guanosine- Monophosphate



**Figure 1: The impact of diabetes mellitus on the male reproductive system**

## Chapter III

### **Antioxidant and tyrosinase inhibiting activities of ethylacetate, methanolic and aqueous extracts of *Basella alba* leaves**

**Running title:** Antioxidant activities of *Basella alba* leave extracts compared

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## Abstract

*Basella alba* is a leafy green plant commonly cultivated and consumed as vegetable in many Asian and tropical African communities. The plant has been scientifically proven to possess a number of beneficial effects including antioxidant effect. This study was aimed at investigating comparatively, the flavonoids content, the antioxidant and antityrosinase activities of ethylacetate, methanol and aqueous extract of *Basella alba* leaves and establish a possible relationship among these parameters. Two flavonoids, namely flavanol and flavonol were quantitatively measured in the extracts and antioxidant capacities assessed using TEAC, ORAC and FRAP assays. The result showed that ethylacetate extract contains much higher concentrations of flavanol and flavonol when compared to both methanol and aqueous extracts which contain negligible amounts of the two flavonoids. Values of antioxidant capacities were as follows; Aqueous (TEAC =  $439.90 \pm 0.07$   $\mu\text{mol TE/g}$ , ORAC =  $1384.40 \pm 90.65$   $\mu\text{mol TE/g}$ , FRAP =  $48.17 \pm 0.00$   $\mu\text{mol AAE/g}$ ), ethylacetate (TEAC =  $61.30 \pm 0.07$   $\mu\text{mol TE/g}$ , ORAC =  $483.09 \pm 35.00$   $\mu\text{mol TE/g}$ , FRAP =  $117.99 \pm 0.01$   $\mu\text{mol AAE/g}$ ) and methanol (TEAC =  $44.20 \pm 0.03$   $\mu\text{mol TE/g}$ , ORAC =  $1286.25 \pm 9.00$   $\mu\text{mol TE/g}$ , FRAP =  $46.64 \pm 0.00$   $\mu\text{mol AAE/g}$ ). All three extracts had inhibitory activity against tyrosinase at effective concentrations of  $193.58 \pm 0.02$ ,  $203.33 \pm 0.02$  and  $222.83 \pm 0.03$   $\mu\text{g/mL}$  for aqueous, methanol and ethylacetate extracts respectively. It was concluded from the results that the aqueous extract of *Basella alba* leaves produced the best antioxidant and antityrosinase activities and the two actions may be directly related.

## Introduction

*Basella alba* (Family: *Basellaceae*) is a climbing perennial herb with deep-green, heart-shaped leaves and bears fleshy dark bluish fruits (Lin et al., 2010). It is commonly cultivated and consumed as vegetable in many Asian and tropical African communities. *Basella alba* have being used traditionally for its diverse health benefits and have been scientifically proven to possess anti-inflammatory, analgesic, antioxidant, antifungal, anticancer, androgenic and red cell membrane-stabilizing properties among others (Moundipa et al., 1999; Arokoyo et al., 2015; Baskaran et al., 2015). Of particular importance to this study is the possibility of a correlation between the antioxidant property of the plant and its reported use in skin care by Bangladeshians for the prevention of freckles (Akhter et al., 2008). This presumption is based on the background knowledge that oxidative stress can contribute significantly to skin aging by stimulating the activities of skin-damaging enzymes like tyrosinase (Popoola et al., 2015).

Tyrosinase (Polyphenol oxidase) is a copper-containing endogenous enzyme which plays a role in the initial steps in melanin biosynthesis that result in the conversion of L-tyrosine to dopaquinone via consecutive hydroxylation and oxidation reactions (Uchida et al., 2014; Pintus *et al.*, 2015). The enzyme is widely distributed in microorganisms, animals and plants. Due to inhibition of enzymatic oxidation, anti-tyrosinase agents have become increasingly significant in cosmetics (Saeio *et al.*, 2011), medicine (Ramanuj *et al.*, 2014) as well as in the agricultural sector (Anand *et al.*, 2014) for the prevention of hyperpigmentation and elongation of the life span of perishable agricultural products (Dao-Mao & Ming-An, 2012). Kojic acid, arbutin and hydroquinones are substances with proven tyrosinase inhibiting activities, but their use in humans have been limited due to the deleterious effects on mammalian cells (Uchida et al., 2014). This necessitated the investigation of many natural substances with antityrosinase activities including *Basella alba*, most of which are medicinal plants with high composition of phenolic compounds like flavonoids (Popoola et al., 2015). Flavonoids are said to be the most abundant phenolic compounds present in all parts of most plants and are capable of exerting potent antioxidant activities in biological systems both in vivo and in vitro (Kumar and Pandey, 2013). Over 6000 biologically

active flavonoids have been identified and of these, flavonols appears to be the most important especially in offering protection against oxidative stress and ultraviolet radiation (Falcone Ferreyra et al., 2012).

The antioxidant activity of plant extracts can be assessed using a wide range of biochemical assays which in essence measures the quantity and potency of antioxidant phytochemicals in the extracted material. A large number of these methods of assessment including trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) are based on colorimetric reactions (Obón et al., 2005). The TEAC method assesses the ability of compounds to scavenge ABTS (2,2'-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid) radical i.e (ABTS<sup>•+</sup>) which has a blue-green colour and a TEAC value given by comparing colour reduction induced by the compound to that induced by a vitamin E analogue, Trolox (Obón et al., 2005). FRAP relies on the ability of antioxidants to reduce complex ferric ion-TPTZ (2,4,6-tri(2-pyridyl)- 1,3,5-triazine) which creates a very intense navy blue colored solution whose absorbance gives an estimate of the amount of iron reduced and correlates with the amount of antioxidants present (Pisoschi and Negulescu, 2011). The oxygen radical absorption capacity (ORAC) assay measures the extent of fluorescence loss from fluorescein as the antioxidants scavenge for peroxy radicals induced by 2,2'-azobis-(2-amidino-propane) dihydrochloride (AAPH) (Pisoschi and Negulescu, 2011). The extent of loss of fluorescence is an indication of the amount of antioxidants present in the substance being assayed.

The use of different solvents for the extraction of bioactive phytochemicals is traditional to science and the extraction yield is largely dependent on polarity of the solvent and extraction conditions (Delfanian et al., 2015). Expectedly, the nature and amount of bioactive substances and antioxidant capacity of any particular plant extract varies with the solvent of extraction. In this present study, *Basella alba* leaves were extracted using three common solvents, ethylacetate (EtOAC), methanol (MeOH) and water (aqueous) with the aim of comparing the antioxidant and antityrosinase capacities of the three types of extract. Additionally, the study is expected to establish whether or not there is a correlation between the antioxidant power and the tyrosinase inhibiting abilities of the extract types.

## Materials and methods

### *Plant material*

*Basella alba* was harvested fresh from humid locations in Osun state, western Nigeria and used for this study. A sample of this was deposited at the Botany Department of the University of Ibadan, Nigeria with the voucher number UIH-22391, following identification and authentication. The leaves were separated from the stem, washed to remove dust and other particles and then air-dried at room temperature. The dried leaf was blended into fine powder using a household electric blender (LOGIK® China).

### *Preparation of plant extracts*

EtOAc and MeOH extracts were prepared by placing 100g of the dry powder of *Basella alba* leaves in a 2L conical flask and then mixed with 1L of organic solvents, ethanol and methanol respectively. These were extracted at room temperature with constant shaking for 48 hours according to the method described by Iloki-Assanga et al., 2015 with slight modifications. The resultant supernatants were filtered and evaporated to dryness using a rotary evaporator (Heidolph 2, Germany) with water bath temperature set at 40 °C. The aqueous extract was prepared by extracting 100g of the leaf powder in 1L of distilled water as described by Iloki-Assanga et al., 2015. Dry extracts were stored at -4 °C and dissolved in dimethyl sulfoxide (DMSO) to a concentration of 1µg/ml prior to use.

### *Determination of flavonoids*

Two subclasses of flavonoids; flavanols and flavonols were analysed in the extracts. Flavanol content was determined using the method described by McMurrough and McDowell (1978). The assay was based on the reaction between 4-dimethylaminocinnamaldehyde (DMACA) and flavanols to form a characteristic light blue colour that was measured spectrophotometrically at 640nm. Catechin hydrate in methanol served as stock standard.

Flavonol was determined according to the method described by Delcour and Janssens (1985), using Quercetin as the standard for measuring flavonols at 360nm. All reagents used in both assays were prepared fresh on the day of the assay.

#### *Analysis of antioxidant capacities*

Three different methods were used to analyze the Antioxidant capacities of the extracts; TEAC was determined according to the method described earlier by Re *et al.*, (1999). The ABTS radical cation scavenging ability of each extract was determined spectrophotometrically at an absorbance of 734nm at 25°C after 30minutes of incubation with ABTS mixture at room temperature. TEAC values were given as micromoles Trolox equivalents per gram ( $\mu\text{M TE/g}$ )

Determination of FRAP was done by a redox-linked colorimetric method as described by Benzie and Strain (1999). Briefly, extract samples were added to FRAP reagent in a 96-well plate and incubated at 37°C for 30minutes. Absorbance was recorded at 593nm and the change in absorbance measured after 30minutes of incubation was directly related to the electron donating antioxidant in the reaction mixture. L-Ascorbic acid was used as a standard and the results were expressed as micromole ascorbic acid equivalents per gram ( $\mu\text{M AAE/g}$ ) of extract.

ORAC was determined according to the method of Cao and Prior (1999) which measures antioxidant's scavenging activities against the peroxy radical of 2,2'-azobis (2-aminopropane) dihydrochloride (AAPH) at 37 °C. The test samples were added to fluorescein and loss of florescence corresponded to the extent of decomposition of the radical which is a measure of the antioxidant power of the sample. ORAC values were expressed as micromoles Trolox equivalents ( $\mu\text{M TE}$ ) per gram of extract.

#### *Determination of tyrosinase inhibiting activity*

The tyrosinase enzyme assay was performed according to the methods described by Chompo *et al.* (2012) and Vardhan *et al.* (2014). Extract samples were constituted to a stock concentration of 1 mg/ml by dissolution with DMSO, and further dilutions were then done. Kojic acid as control drug, tyrosinase (500 Units/mL in sodium phosphate

buffer) and substrate (2mM L-Tyrosine) were used for the assay as modified in earlier study by Popoola et al., 2015. Enzyme activity was determined by reading the absorbance at 490 nm and percentage tyrosinase inhibition calculated using the formula:

$$\text{Tyrosinase inhibition (\%)} = [(A - B) - (C - D)] / (A - B) \times 100$$

Where, A = absorbance of the control with the enzyme, B = absorbance of the control without the enzyme, C = absorbance of the test sample with the enzyme and D = absorbance of the test sample without the enzyme.

### *Data analysis*

All data from the assays of antioxidant parameters were analysed and the final results calculated using Microsoft Excel (Version 2010). Results were expressed as mean  $\pm$  standard deviation of triplicate values per sample. Graphpad Prism version 5.0 was used for the analysis of tyrosinase activities and calculation of IC<sub>50</sub>. ANOVA was used for comparison of mean values and  $p < 0.05$  considered to be statistically significant.

## **Results**

### *Flavonoids concentration and antioxidant capacities of extracts*

The flavonoids content and antioxidant capacities of each of the extract types are presented in Table 1. The ethylacetate extract of *Basella alba* leaves contains much higher concentrations of flavanol ( $13.44 \pm 0.03 \mu\text{mol/g}$ ) and flavonol ( $175.54 \pm 0.01 \mu\text{mol/g}$ ) when compared to both methanolic and aqueous extracts which contain negligible amounts of the two flavonoids. Incidentally, FRAP value was also highest in the ethylacetate fraction ( $117.99 \pm 0.01 \mu\text{mol AAE/g}$ ) as compared to methanolic ( $46.64 \pm 0.00 \mu\text{mol AAE/g}$ ) and aqueous ( $48.17 \pm 0.00 \mu\text{mol AAE/g}$ ) leave extracts of the plant. However, the highest values for TEAC ( $439.90 \pm 0.07 \mu\text{mol TE/g}$ ) and ORAC ( $1384.40 \pm 90.65 \mu\text{mol TE/g}$ ) were recorded in the aqueous extract when compared to values for methanolic (TEAC;  $44.20 \pm 0.03 \mu\text{mol TE/g}$ , ORAC;  $1286.25 \pm 9.00 \mu\text{mol TE/g}$ ) and ethylacetate (TEAC;  $61.30 \pm 0.07 \mu\text{mol TE/g}$ , ORAC;  $483.09 \pm 35.00 \mu\text{mol TE/g}$ ) extracts.

### *Tyrosinase inhibiting activity of extracts*

The effects of *Basella alba* extracts on mushroom tyrosinase activity using L-Tyrosine as substrate in an in-vitro system is reported in Table 2. The results indicated that all extracts have a direct inhibitory activity against mushroom tyrosinase at effective concentrations of  $193.58 \pm 0.02$ ;  $203.33 \pm 0.02$  &  $222.83 \pm 0.03$   $\mu\text{g/mL}$  for aqueous, methanol and ethylacetate extracts respectively. These antityrosinase activities of the three extracts were significantly ( $p < 0.01$ ) weaker when compared to that of standard substance, kojic acid at  $\text{IC}_{50}$  of  $7.33 \pm 1.78$   $\mu\text{g/mL}$ .

### **Discussion**

The findings in this study suggest that the aqueous extract of *Basella alba* leaves exhibit stronger antioxidant properties when compared to methanolic and ethylacetate fractions, despite containing less flavonol and flavanol than the ethylacetate fraction. A significant correlation is said to exist between the total phenolic content and antioxidant activities of *Basella alba* (Roshan et al., 2012), which suggests that there may be some phenolic compounds other than flavonol and flavanol that are extractable in aqueous, but not in ethylacetate or methanolic fractions. This may be responsible for the much higher TEAC and ORAC values for the aqueous fraction and further studies to investigate this should involve isolation of active compounds in each extract type for more specific assays. On the other hand, the FRAP reading was observed to be much lower in aqueous compared the ethylacetate fractions, which further confirms the inconsistency that is known to characterize the measurement of total antioxidant capacities (Jansen and Ruskovska, 2013; Janaszewska and Bartosz, 2002) and is partly because each assay measures different components of total antioxidant capacity (Winter et al., 2009). Furthermore, TEAC and ORAC readings are believed to be more representative of the antioxidant capacities of the various extract types since these assays directly measure the ability of the extracts to inhibit oxidation, while FRAP is an indirect assay that measures the ability of the extracts to reduce iron complexes (Rubio et al., 2016).

The results further show that all of the extracts from the different solvents displayed similar anti-tyrosinase activity within the range of  $193.58 - 222.83$   $\mu\text{g/mL}$ . Therefore, the

plant *Basella alba* can be a potential source of further investigation for new anti-tyrosinase agents. The expected active constituent from the quantitative determination of the chemical composition (Table 1) is primarily made up of flavanols in ethylacetate while flavonols are abundant in all the three types of extract. This indicates that the compounds responsible for the observed biological activities are highly extractable in both moderately polar (ethylacetate) and polar (methanol and water) solvents.

Although the outcome of this investigation gave moderate antityrosinase activities, this study suggested that *Basella alba* could play an important role in the regulation of tyrosinase activity and could possibly act as a natural inhibitor of browning reaction and formation of freckles in the skin.

## **Conclusion**

The findings from this study led to the conclusion that aqueous extraction of *Basella alba* leaves produced the best yield with regards to antioxidant as well as tyrosinase inhibiting effects of the plant. This also suggests a direct relationship between antioxidant and tyrosinase inhibiting activities of *Basella alba*. Additionally, a significant part of the antioxidant activities of *Basella alba* can be attributed to components other than flavonol and flavanol and the plant can be a healthy source of natural antityrosinase compounds.

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## **Conflict of interest**

The authors declare that there is no conflict of interest with regard to publishing any aspect of this study

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## TABLES

Table 1: Flavonoids content and antioxidant capacities of three different extracts of *Basella alba* leaves

| Extraction solvent | Flavanol ( $\mu\text{mol/g}$ ) | Flavonol ( $\mu\text{mol/g}$ ) | TEAC ( $\mu\text{mol TE/g}$ ) | FRAP ( $\mu\text{mol AAE/g}$ ) | ORAC ( $\mu\text{mol TE/g}$ ) |
|--------------------|--------------------------------|--------------------------------|-------------------------------|--------------------------------|-------------------------------|
| EtOAC              | $13.44 \pm 0.03$               | $175.54 \pm 0.01$              | $61.30 \pm 0.07$              | $117.99 \pm 0.01$              | $483.09 \pm 35.00$            |
| MeOH               | $0.28 \pm 0.00$                | $89.70 \pm 0.02$               | $44.20 \pm 0.03$              | $46.64 \pm 0.00$               | $1286.25 \pm 9.00$            |
| Aq.                | $0.19 \pm 0.00$                | $79.67 \pm 0.01$               | $439.90 \pm 0.07$             | $48.17 \pm 0.00$               | $1384.40 \pm 90.65$           |

$\mu\text{mol/g}$  = Micromole per gram;  $\mu\text{mol TE/g}$  = Micromole trolox equivalent per gram;  $\mu\text{mol AAE/g}$  = Micromole amino acid equivalent per gram; EtOAC = Ethylacetate; MeOH = Methanol; Aq. =Aqueous

Table 2: Tyrosinase inhibiting activity of extracts

| Fraction   | Antityrosinase $\text{IC}_{50} \pm \text{SD}$ |
|------------|---|
| EtOAC      | $222.83 \pm 0.03^*$                           |
| MeOH       | $203.33 \pm 0.02^*$                           |
| Aq.        | $193.58 \pm 0.02^*$                           |
| Kojic acid | $7.33 \pm 1.78$                               |

\* $P < 0.01$  vs. Kojic acid ; EtOAC = Ethylacetate;

MeOH = Methanol; Aq. =Aqueous

## Chapter IV

### **Reproductive parameters in streptozotocin-Induced diabetic male Wistar rats: Beneficial role of *Basella alba* Aqueous leave extract**

**Short Title;** *Basella alba* ameliorates diabetes-induced reproductive dysfunction

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## Abstract

**Background/Aim:** Diabetes mellitus (DM) is widely reported to have adverse effect on most systems of the body. This study investigated the effects of DM on male reproductive parameters and possible role of aqueous leave extract from *Basella alba* in ameliorating such effects.

**Materials and Methods:** Male rats (n = 40) aged 8-10 weeks were randomly divided into 4 equal groups namely; Healthy Control (HC), Diabetic Control (DC), Healthy Treatment (HT) and Diabetic Treatment (DT). DC and DT animals were made diabetic via single intraperitoneal injection of STZ (55 mg/kg). Treatment was oral via gavage (HC + DC received 0.5 ml/100 g normal saline daily, HT + DT received 200 mg/kg plant extract daily) and lasted 4 weeks. Fasting blood sugar and body weights were recorded weekly, animals were sacrificed upon completion of treatments and tissues collected for further analysis.

**Results:** There was significant ( $P < 0.05$ ) decrease in body, testicular and epididymal weight in DC compared to HC rats. Relative testicular weight was significantly increased ( $P < 0.0001$ ) in both DC and DT compared to HC. Sperm concentration, viability and morphology, were all significantly reduced in DC versus HC rats ( $P < 0.0001$ ), but improved in DT versus DC ( $P < 0.05$ ). Histopathological examination of the testes and epididymis showed degenerative changes in DC specimens that were alleviated in DT rats. **Conclusion:** Aqueous extract of *Basella alba* play a major role in ameliorating male reproductive complications due to streptozotocin-induced diabetes mellitus.

**Key words:** Male Reproduction, Streptozotocin, Diabetes, *Basella alba*, Aqueous extract.

## Introduction

Diabetes mellitus (DM) have been described as a metabolic disease that is characterized by hyperglycaemia due to failure of insulin secretion, inappropriate insulin action or a combination of both anomalies. The two major types are Insulin dependent or type 1 diabetes mellitus (T1DM) and non-insulin dependent or type 2 diabetes mellitus (T2DM), both of which are associated with disturbances of carbohydrate, protein and fat metabolism [1]. The beta-islet cells of Langerhans in the endocrine pancreas are responsible for the production of insulin, a peptide hormone which plays a key role in the metabolism of carbohydrate and other food classes [2]. Diabetes is often a consequence of either destruction of beta-islet cells resulting in insulin deficiency ( T1DM) or a failure of the hormone to exert its effects on target organs due to insulin resistance (T2DM) [3]. This knowledge of the pathophysiological basis of the diseases has been employed to produce the clinical picture of DM in experimental animals for the purpose of research.

Streptozotocin (STZ) is one of the chemical agents commonly used to induce different types of DM in various animal models due to its toxic effect on the insulin producing beta-islet cells of the pancreas. The metabolic characteristics and severity of DM induced by STZ is dependent on a number of factors, including the STZ dosage regime, strain and gender of the animal [4].

DM was reported to affect 177 million people worldwide in the year 2000, with the figure projected to reach 300 million people by the year 2025 [5]. However, by 2011, the International Diabetic Federation reported that, 366 million people were already affected by DM globally and the incidence was expected to rise to 522 million by the year 2030 [6]. These alarming projections, in addition to the challenges associated with conventional drug treatments led to current research trends where various medicinal plants are studied for their potential in the treatment of DM. The focus is often directed towards the ability of these plants to lower blood sugar and possible improvement of the general well-being of diabetic patients, with little or no attention to whether or not specific systemic complications of DM are reversed by these plant agents.

*Basella alba* is one such plant that have been reported to lower blood sugar and improve general wellbeing in diabetic rats [7]. It is a green vegetable plant that originated from India [8], but is also grown throughout the tropics as a perennial plant and in warmer temperate regions as an annual crop. Its thick, semi-succulent heart-shaped leaves have a mild flavour and mucilaginous texture. The common names include; Ceylon spinach, Malabar spinach or Indian spinach, and it is a branched, climbing plant [9]. Among many other uses, the plant has been reportedly used to alleviate weak libido in men suffering from subfertility in some parts of Cameroon [10]. In the southern part of Nigeria, *Basella alba* leave is commonly prepared as vegetable sauce which serves as condiment to most of the common staple food. Other systemic effects of this plant that have been reported include its potential as hematinic, antidiarrheal, anticonvulsant, anti-inflammatory, antifungal and analgesic agent [9].

DM is reportedly complicated by a wide variety of male reproductive dysfunction ranging from reduced libido to infertility and impotence [11]. Reports on the impact of the disease on sperm parameters and semen quality are rather inconsistent, with discrepancies regarding the actual parameters affected and extent of the effects [12].

The mechanism by which *Basella alba* lowers blood sugar is not known and whether or not this anti-hyperglycemic effect translates to reversal of certain complications of DM is yet to be demonstrated in any study. This present work was targeted at investigating a possible role for the aqueous extract of *Basella alba* at reversing some reproductive complications of DM in male Wistar rats.

## **Materials and Methods**

### *Preparation of Plant extract*

Fresh leaves of *Basella alba* was collected from various humid locations in south western Nigeria, The leaves were separated from the stem, washed and then air-dried at room temperature for a period of three to four weeks. The plant was earlier identified and authenticated at the Department of Botany, University of Ibadan, Nigeria with voucher number (UIH-22391).

Aqueous extract was prepared as described by Iloki-Assanga et al., 2015 [13] with slight modification. Briefly, the dried leaves were grinded into fine powder and 100 g of this was added to 1000 ml of distilled water at 100°C and the mixture was maintained at that temperature in water bath for a further 5 minutes. It was subsequently removed from the heat and allowed to infuse for a further 30 minutes before being filtered. The filtrate was freeze-dried to form a powdery extract which was dissolved in normal saline and administered to the rats by oral gavage.

#### *Animals and study design*

Ethical approval for the study, (reference number; CPUT/HWS-REC 2015/A04) was granted by the Health and Wellness Sciences Research Ethics Committee (HWS-REC) of the Cape Peninsula University of Technology, Cape Town, South Africa. Forty (40) male Wistar rats, aged 8-10 weeks were given free access to food (standard rat chow) and water unless when fasted prior to fasting blood sugar (FBS) sample collection. They were subjected to standard atmospheric conditions with a twelve hours light/dark cycle. Animals were randomly divided into four groups (n = 10) and treated as follows; Healthy control (HC) and Diabetic control (DC) animals received normal saline at 0.5 ml/100g body weight daily, and Healthy Treatment (HT) and Diabetic Treatment (DT) rats were given the plant extract at an oral dose of 200 mg/kg body weight daily. Treatment in all four groups was administered by gavage via a metal endotracheal tube. The maintenance and care of experimental animals throughout the study complied with National Institutes of Health guidelines for the humane use of laboratory animals.

#### *Induction of diabetes mellitus*

Diabetes was induced in rats from the diabetic control and diabetic treatment groups through a single intraperitoneal injection of STZ, 55 mg/kg after an overnight fast. STZ was dissolved in ice cold citrate buffer (0.1 M) at a pH of 4.5 and prepared fresh, immediately before administration. Animals were allowed access to food and water afterwards and fasting blood sugar was recorded seventy-two hours later to confirm diabetes. Rats were considered diabetic and included in the study only when FBS was above 11.1 mmol/L (200mg/dL) [14].

### *Measurement of FBS and weight*

FBS was recorded weekly in all four groups from tail capillary blood, by pricking the tip of the tail with a sterile lancet to express one or two drops of blood. This was applied to the glucometer (ONETOUCH® Ultra2) strip and FBS determined. Weight was recorded weekly using a portable electronic balance.

### *Sample and organ collection*

All animals were euthanized after four weeks of treatment. Blood was collected via cardiac puncture into serum clot activator tubes (VACUETTE®), centrifuged at 4000rpm for 10min at 4°C and serum stored at -80°C for hormonal assays. The right testes and epididymis were removed, weighed and caudal epididymal spermatozoa were isolated for immediate analysis, while left testes and epididymis were preserved for histological studies. Relative organ weight was calculated by dividing the organ weight by the animal's total body weight.

### *Sperm isolation*

The caudal epididymis was dissected out and freed of all fat and excess tissues. 0.5 cm of the distal end was then cut off, placed in 1ml of HAMS solution in a petri dish, chopped into four smaller pieces and incubated at 37°C for 1minute to allow the spermatozoa to swim out before evaluation.

### *Sperm concentration and motility*

Both the concentration and motility of the spermatozoa were determined by computer-aided sperm analysis (CASA), using the Sperm Class Analyzer, version 5.0 (SCA®, Microptic, Barcelona, Spain).

One minute after the onset of incubation of the epididymis in the medium (HAMS solution), 2 µl of the medium was removed with a pipette close to the edge of the hazy portion (i.e. the cloud of sperm swimming out of the epididymis). Subsequently, one 2 µl chamber of a standard count eight chamber slide (Leja® Netherland) was filled and placed on the heated (37°C) microscope stage and analysed under X40 magnification.

Nikon E200 microscope was used with phase '2' setting and spermatozoa fields visualized on camera (Basler® A312fc) at a frame rate of 25 fps (Frames per second). Minimum of 1000 spermatozoa from at least five different fields were evaluated per sample.

### *Sperm viability*

Sperm viability was determined by the Eosin/Nigrosin (E/N) dye exclusion staining technique. To the sperm suspension, we added eosin and nigrosin in a 1:2:3 ratio and mixed in 1.5 µl eppendorf tube. A drop (10 µl) of this mixture was placed on a microscope slide and a thin smear prepared and allowed to air dry. After twenty four hours of air-drying, a cover slip was mounted with DPX and allowed to further dry prior to evaluating it with a light microscope using X100 magnification. The percentage viability was determined by evaluating 100 spermatozoa per slide and recording the number of live cells. Live spermatozoa were unstained (white), while dead spermatozoa took up the eosin stain and appeared pink/red.

### *Sperm morphology*

The percentage of morphologically normal spermatozoa was determined after staining the spermatozoa with Sperm Blue® according to the technique described by Van Der Horst and Maree [15]. A thin smear was made from the sperm suspension (10 µl) and the slide was left to air dry for twenty-four hours. The slide was subsequently immersed slowly in the sperm blue staining solution for sixty seconds after which it was placed at an angle of 60° - 80° for ten seconds to allow for draining of the excess stain. Finally the slide was gently immersed in distilled water (3-6 seconds), drained and left to air dry. Dry slides were cover slipped using DPX and evaluated on the Sperm Class Analyser®. Nikon E200 microscope was used with a blue filter and phase 'A' setting under X60 magnification. Only spermatozoa which do not overlap with each other or with background staining were considered for evaluation (fifty spermatozoa per slide).

### *Reproductive Hormones*

Testosterone levels in all the serum samples was determined by radioimmunoassay technique using testosterone rat/mouse enzyme-linked immunosorbent assay (ELISA) kit (DEV9911) according to the manufacturer's specifications (Demeditec<sup>®</sup>).

Both serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels were determined using the MILLIPLEX<sup>®</sup> MAP Rat Pituitary Magnetic Bead Panel (RPTMAG-86K), employing Luminex<sup>®</sup> xMAP<sup>®</sup> technology.

### *Histopathological Analysis*

Histological study of the testes and epididymis was conducted by fixing samples from each rat in 10% buffered formalin immediately after excision. These were washed, trimmed and processed by embedding in paraffin. The specimens were sectioned at 5  $\mu$ m thickness and stained with Hematoxylin and Eosin (H&E stain). Slides were examined microscopically according to the method of Luna [16] under the guidance of a histopathologist who was blinded to the study.

### *Statistical analysis*

Data was analysed using Graphpad Prism version 5, and values were expressed as mean  $\pm$  SEM. Differences between group means were determined by one-way analysis of variance (ANOVA). Bonferroni post-test used to compare data from all four groups. A value for  $P < 0.05$  was considered to be statistically significant.

## **Results**

### *Fasting blood sugar*

The FBS of diabetic animals (DC =  $18.69 \pm 1.37$ mmol/L, DT =  $17.74 \pm 1.34$ mmol/L) were significantly elevated ( $p < 0.0001$ ) compared to that of the healthy animals (HC =  $4.48 \pm 0.15$ mmol/L, HT =  $4.51 \pm 0.15$ mmol/L) at the onset of the study. The FBS of DC animals significantly increased over the four weeks treatment period ( $18.69 \pm 1.37$ mmol/L vs.  $24.71 \pm 1.14$ mmol/L,  $p < 0.001$ ). After 4 weeks of treatment, the FBS of DT animals receiving *Basella alba* was significantly reduced compared to their initial

values ( $10.71 \pm 0.41\text{mmol/L}$  vs.  $17.74 \pm 1.34\text{mmol/L}$ ,  $p < 0.0001$ ), as well as significantly reduced compared to FBS levels in diabetic (DC) animals that only received normal saline ( $10.71 \pm 0.41\text{mmol/L}$  vs.  $24.71 \pm 1.14\text{mmol/L}$ ,  $p < 0.001$ ). FBS remained unchanged in both control and treated healthy rats (Fig. 1a).

### *Body weight*

The body weight of both HC ( $291.9 \pm 11.26\text{g}$  vs.  $333.6 \pm 13.36\text{g}$ ) and HT ( $272.4 \pm 4.43\text{g}$  vs.  $306.1 \pm 4.58\text{g}$ ) animals increased significantly ( $p < 0.05$ ) during the 4 weeks experimental period. The percentage weight gain was 14% for HC and 12% for HT animals.

A significant loss ( $p < 0.001$ ) in body weight was observed in DC ( $268.0 \pm 16.79\text{g}$  vs.  $182.8 \pm 11.75\text{g}$ ) and DT ( $251.3 \pm 10.42\text{g}$  vs.  $183.5 \pm 5.50\text{g}$ ) animals during the same period of time. DC and DT animals lost 32% and 27% weight respectively (Fig. 1b).

### *Organ and relative organ weights*

The testicular weights (right) of DC and DT animals were significantly lower ( $p < 0.001$ ) when compared to that of both HC and HT animals. *Basella alba* treatment had no effect on the testicular weight of healthy animals (HC vs. HT, not significant) or diabetic animals (DC vs. DT, not significant) as depicted in Fig. 2a. Upon calculating the relative testicular weights (i.e. Right testis weight/Body weight), it was observed that these ratios were significantly higher ( $p < 0.001$ ) in DC and DT animals compared to HC and HT animals. Yet again, no differences were observed in ratios between *Basella alba* treated animals and their respective controls (i.e. HC vs. HT and DC vs. DT) (Fig. 2b).

Similarly, epididymal weights (right) of DC and DT animals were significantly lower ( $p < 0.0001$ ) when compared to both HC and HT animals. However, the epididymal weight of *Basella alba* treated diabetic animals (DT) was significantly higher ( $p < 0.005$ ) compared to their control (DC) animals (Fig. 2c). The relative epididymal weight (Right epididymis weight/Body weight) was also significantly higher ( $p < 0.05$ ) in DT animals when compared to DC. No differences were observed among the relative epididymal weights of DT, HT and HC animals (Fig. 2d).

### *Sperm parameters*

Sperm concentration was significantly lower ( $p < 0.05$ ) in the DC rats when compared to the HC, but significantly higher ( $p < 0.05$ ) in DT animals compared to DC. Four weeks of *Basella alba* treatment did not affect the sperm concentration of healthy animals (i.e. HC vs. HT, not significant). No significant differences were observed in the total motility of spermatozoa among all 4 groups. The percentage of viable spermatozoa was significantly lower in the diabetic control ( $p < 0.001$ ) and diabetic treatment ( $p < 0.05$ ) groups when compared to the healthy control. However, the viability values were significantly higher ( $p < 0.001$  and  $p < 0.05$ ) in the healthy and diabetic treatment groups respectively when compared to the diabetic control group. The percentage normal morphology was also significantly lower ( $p < 0.001$ ) in the diabetic control group compared to healthy controls and significantly higher ( $p < 0.001$  and  $p < 0.05$ ) in healthy treatment and diabetic treatment groups respectively in comparison to diabetic control (See Table 1).

### *Serum testosterone, LH and FSH*

Serum testosterone levels were significantly increased in the diabetic control group when compared to the remaining three groups ( $p < 0.001$ ) as shown Fig. 3a. A similar trend was also observed for serum LH with significantly higher levels in DC animals compared to all three of the other groups ( $p < 0.0001$ ) (Fig. 3b). There was no significant difference in the levels of FSH among all four experimental groups as illustrated in Fig. 3c.

### *Histopathology of the testes and epididymis*

Histopathological examination of the testes of HC and HT rats showed no visible lesions (Fig. 4a and c) when compared to DC specimens which displays patchy arrangement of severe germinal tissue necrosis of the seminiferous tubules and moderate edema of the testicular interstitium. The affected tubule sections are interspersed between normal non-necrotic sections (Fig. 4b). The testes of diabetic rats treated with aqueous extract of *Basella alba* (DT) showed nearly normal histology, with mild to moderate interstitial congestion (Fig. 4d).

Similarly, the histological picture of the epididymis in DC rats revealed evidences of fragmentation and reduced density of intraluminal spermatozoa (Fig. 5b), while intraluminal spermatozoa structure and population appear normal in HC, HT and DT rats (Fig. 5a, c and d respectively).

## **Discussion**

Diabetes is a metabolic disorder of epidemic proportions and when left untreated the disease affects the organ systems of humans as well as animals in various ways. DM has been shown in previous studies to impact male reproductive fertility in addition to many other systemic effects [17]. DM was induced in this study via a single intraperitoneal injection of STZ which resulted in marked elevation of FBS three days afterwards in the DC and DT animals at the onset of the experiment. The results of our study showed a deterioration in most of the reproductive parameters measured (sperm concentration, viability and normal morphology), as well as in testicular and epididymal histology in the untreated diabetic (DC) rats. Incidentally, rats in this group expressed abnormally high levels of testosterone and LH in their serum. This finding may appear to differ from the commonly reported trend of low serum testosterone in male diabetic subjects that exhibit poor reproductive parameters [18, 19]. It is therefore important to note that the level of serum testosterone in male diabetic subjects is dependent on a number of factors.

One of such determinants of the level of serum testosterone is the type of diabetes mellitus induced. Low levels of serum testosterone is commonly found in T2DM, a picture described as hypogonadotrophic hypogonadism, whereas, serum testosterone is usually significantly raised in T1DM especially in adolescents and middle aged men [20, 21]. Another factor relevant to this finding is the pattern of change in body weight, since body mass index have been reported to relate inversely with serum testosterone concentration [21, 22]. This is partly because testosterone is aromatized and converted to estradiol by aromatase enzyme in adipocytes thereby reducing the concentration of testosterone in blood. In T1DM, tissue uptake of glucose (the primary source of energy) is compromised due to insulin deficiency caused by the destruction of insulin producing beta-islet cells of the pancreas [23]. During conditions of insulin deprivation, there is

marked metabolic alterations in the body that culminate in increased basal energy expenditure and profound protein catabolic state [24]. This partly explains the weight loss commonly seen in T1DM and the high percentages of weight loss observed in DC and DT animals in this study. The catabolism of body fat mass implies less adipocyte will be available to convert testosterone to estradiol by aromatization, leading to a higher serum concentration of testosterone [25, 26]. Significant loss of body fat may account for the severe weight loss observed in the diabetic control rats which suggest additional reason for the elevated serum testosterone in the animals.

The dosage and schedule of STZ administration in rats play a key role in determining the features of the DM induced. A single intraperitoneal injection of STZ at dosages between 40 – 60mg/kg is said to induce symptoms that mimic T1DM [27]. This assertion is well corroborated in the results of this study with the finding of features like hyperglycaemia, severe weight loss and elevated serum testosterone and LH in the diabetic control group following single intraperitoneal injection of STZ at 55mg/kg. The concurrent elevation of both testosterone and LH suggests target organs' resistance to testosterone and failure of its negative feedback regulation of LH secretion from the anterior pituitary. DM has been previously reported to have adverse effects on the functionality of the hypothalamus-pituitary-gonadal axis, resulting in an abnormal sexual steroid feedback and this is attributed to abnormal steroid transport and pituitary insensitivity [28]. This may result as a consequence of the effect of diabetic autonomic neuropathy on the hypothalamus and the pituitary gland [19].

Furthermore, the sustained hyperglycaemia ultimately leads to the generation of high levels of advanced glycation end-products (AGEs) which are injurious to spermatozoa and the process of spermatogenesis [12]. The metabolic challenges induced by STZ in the diabetic animals will also contribute an increased production of reactive oxygen species (ROS) [18], which result in oxidative stress status when the level outnumber antioxidants, thereby culminating in deterioration of reproductive parameters [29].

The findings from this study clearly indicated that oral administration of the aqueous extract of *Basella alba* significantly reversed the observed deterioration in reproductive parameters (either partially or completely) in the diabetic treatment group when

compared to diabetic control. This can be attributed to a number of beneficial effects of the extract that led to the reduction in percentage weight loss (reduced fat catabolism and increased testosterone aromatization) which might have contributed to lowering of serum testosterone back to within normal range in the DT group. Additionally, the restoration of normal testosterone and LH levels in DT rats may suggest that the plant extract is capable of stimulating enhanced target organ and pituitary sensitivity to testosterone, which restores proper negative feedback regulation along the hypothalamus-pituitary-testis axis. *Basella alba* has also been reported to contain numerous phytochemicals like flavonoids and phenolic compounds which are capable of neutralizing the oxidative potential of ROS by the donation of hydroxyl groups [30]. The antihyperglycemic effect of the extract was also very evident in the result of this study, and this suggests a reduction in the generation of AGEs, thereby ameliorating the deleterious effect on the gonads and spermatozoa. However, the mechanism by which *Basella alba* exerts the antihyperglycemia cannot be explained within the scope of this study. Stimulation of pancreatic islet regeneration, up regulation of insulin receptors and possible insulin-like action by the plant extract itself are some of the possibilities that will be explored in future studies to unravel this observation.

## **Conclusion**

The findings from this study confirmed a possible role for *Basella alba* in the management of male factor infertility secondary to T1DM. Regulation of the release and actions of reproductive hormones are important aspects of this beneficial effect of the plant. However, further studies involving an in-depth analysis of the phytochemical components and the in vivo antioxidant activities of the plant extract may be necessary to fully explain how this is achieved. Additionally, the administration of *Basella alba* leave extract for a longer duration and post-treatment cohabitation with female animals will give further information about the actual effect on fecundity of diabetic rats.

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## **Conflict of interest**

The authors declare no conflict of interest concerning any part of this study.

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None

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## TABLE

**Table 1. Sperm parameters in control and treatment groups after four weeks of experiment**

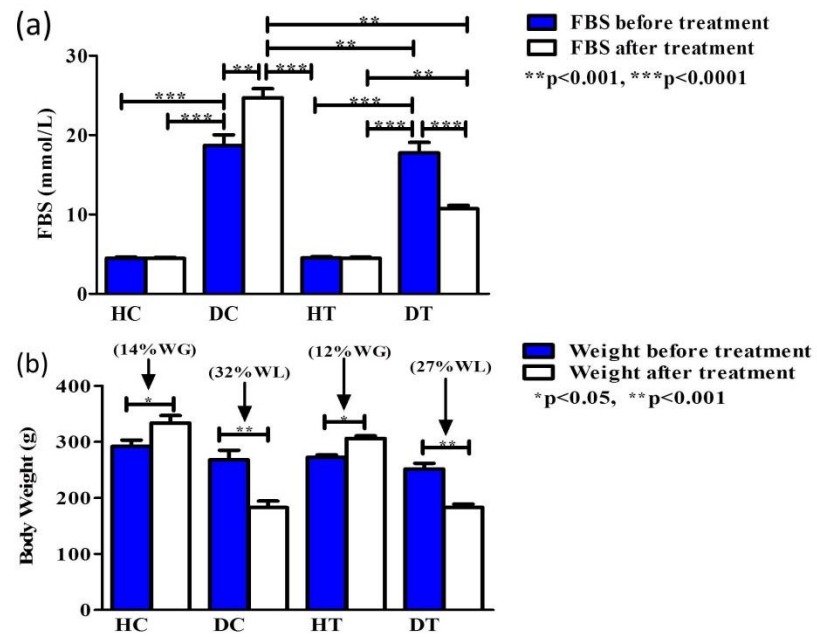
| Parameters                 | Healthy control | Diabetic control | Healthy treatment         | Diabetic treatment         |
|----------------------------|-----------------|------------------|---------------------------|----------------------------|
| Concentration (million/ml) | 4.50 ± 0.49     | 2.23 ± 0.57 *    | 3.88 ± 0.88               | 4.52 ± 0.63 <sup>a</sup>   |
| Motility (%)               | 74.30 ± 7.23    | 53.30 ± 10.37    | 70.90 ± 6.48              | 69.50 ± 6.88               |
| Viability (%)              | 70.13 ± 2.0     | 31.67 ± 1.63**   | 70.43 ± 1.73 <sup>b</sup> | 50.50 ± 1.73 <sup>*a</sup> |
| Normal Morphology (%)      | 62.96 ± 2.71    | 41.67 ± 2.33**   | 68.00 ± 2.80 <sup>b</sup> | 53.67 ± 2.39 <sup>a</sup>  |

Values expressed as mean ± SEM for 10 rats per group. Statistical significance were represented as;

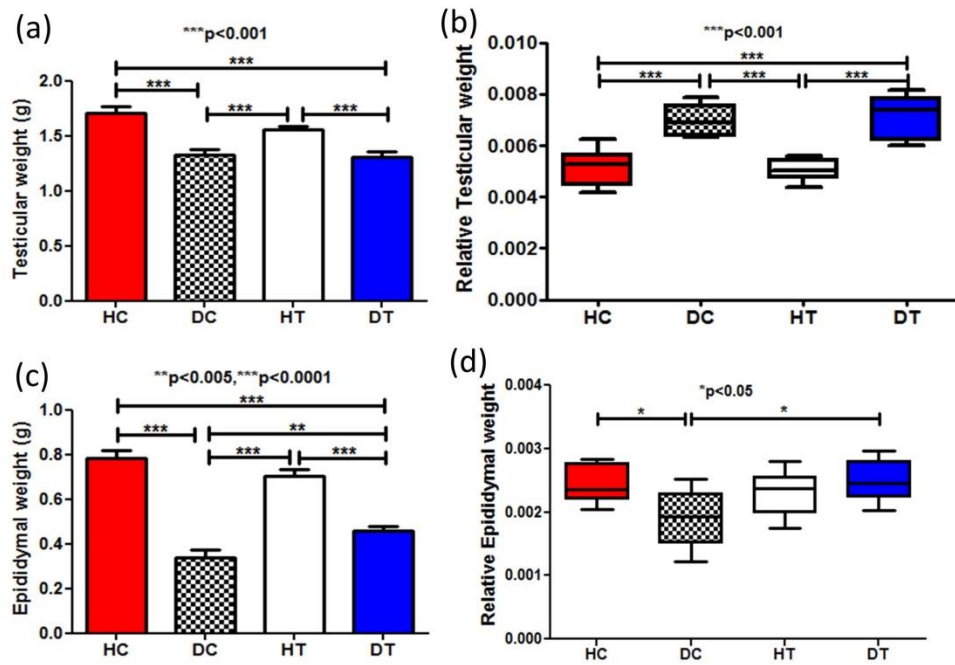
\*P < 0.05, \*\*P < 0.001 vs. Healthy control

<sup>a</sup>P < 0.05, <sup>b</sup>P < 0.001 vs. Diabetic control

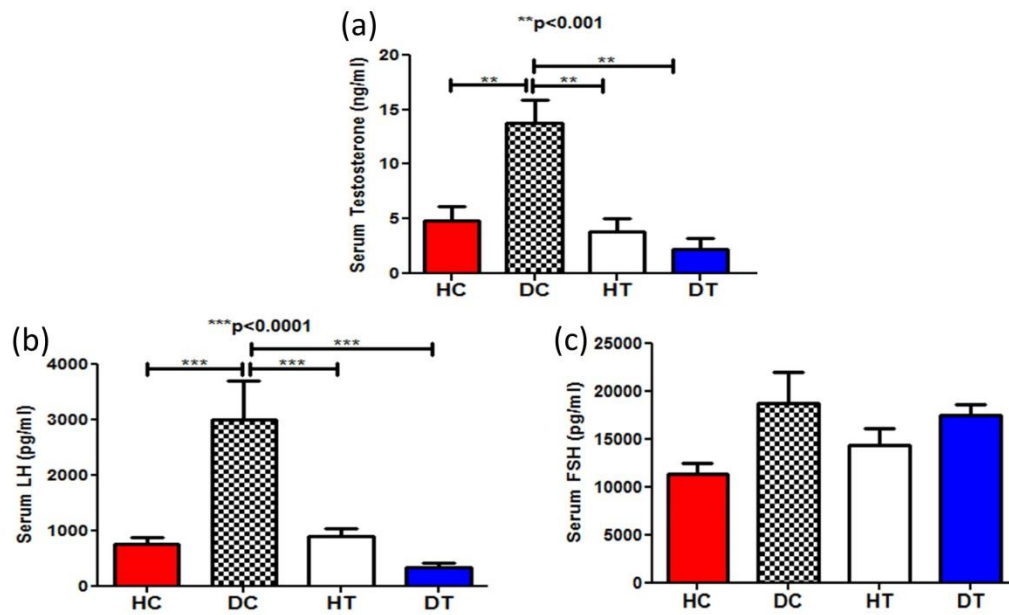
## Figures



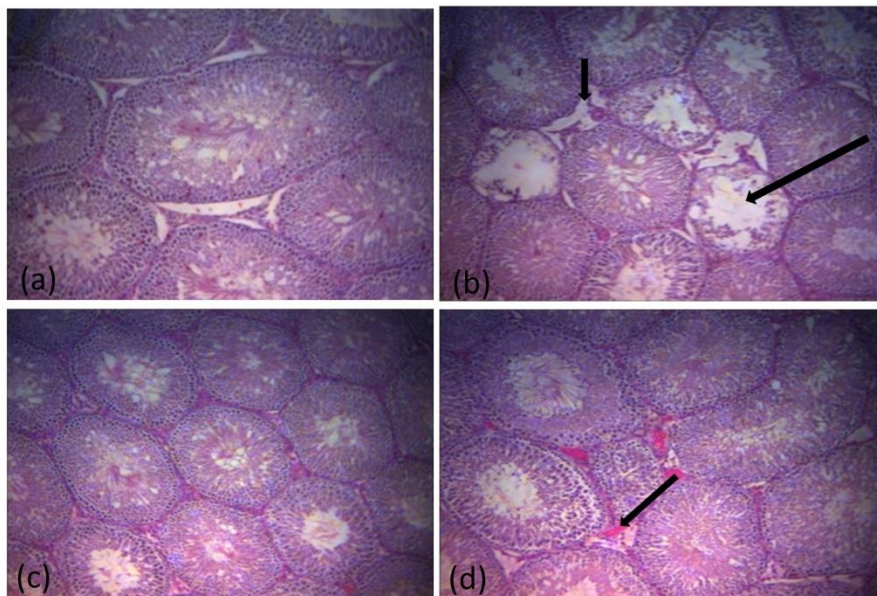
**Fig. 1** (a) Fasting blood sugar (FBS) before commencement of treatment and after four weeks of treatment. (b) Body weight changes before and after treatment [WG=Weight gain, WL=Weight loss]



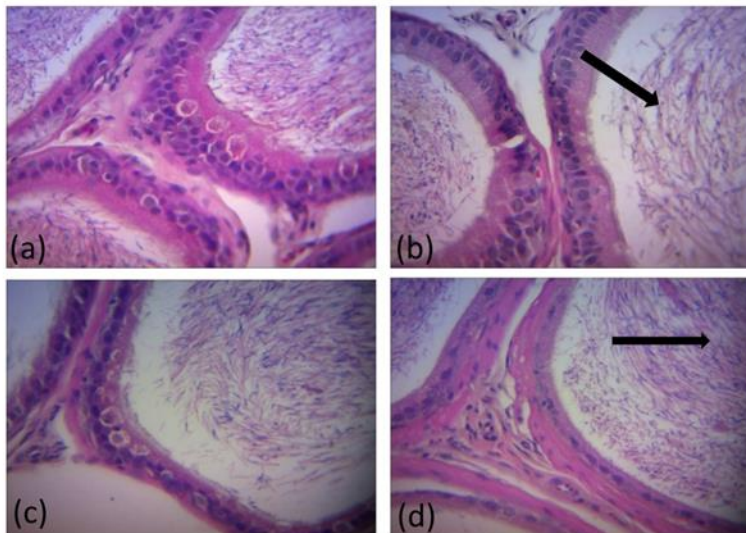
**Fig. 2** (a) Average weights of right testis after treatment. (b) Average relative testicular weight after treatment. (c) Average weights of right epididymis after treatment. (d) Average relative testicular weight after treatment.



**Fig. 3** (a) Serum testosterone levels after treatment. (b) Serum Luteinizing hormone (LH) levels after treatment. (c) Serum Follicle stimulating hormone (FSH) levels after treatment.



**Fig. 4** Histopathological Photomicrographs (H & E, x100) showing; (a) Testes of a healthy control (HC) rat with normal seminiferous tubules. (b) Testes of a diabetic control (DC) rat with severe germinal tissue necrosis of the seminiferous tubules (Long arrow) and moderate edema of the testicular interstitium (Short arrow). (c) Testis of a healthy treatment rat (HT) with normal seminiferous tubules. And (d) Testis of a diabetic treatment rat (DT) with mild to moderate interstitial congestion (See arrow).



**Fig. 5** Histopathological Photomicrographs (H & E, x400) showing; (a) Epididymis of a healthy control (HC) rat with no visible lesion. (b) Epididymis of a diabetic control (DC) rat with normal epithelium, but evidence of fragmentation and reduced density of intraluminal spermatozoa (See arrow). (c) Epididymis of a healthy treatment rat (HT) with no visible lesion. And (d) Epididymis of a diabetic treatment rat (DT) with improved spermatozoa population when compared to DC specimen (See arrow).

## Chapter V

### **Modulation of inflammatory cytokines and islet morphology as therapeutic mechanisms of *Basella alba* in streptozotocin induced diabetic rats**

**Short title:** *Basella alba* modulate cytokines and islet morphology

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#### **Abbreviations**

Ba – *Basella alba*

STZ - Streptozotocin

DM – Diabetes mellitus

T1DM – Type I Diabetes mellitus

FBS – Fasting blood sugar

IL - Interleukin

TNF – Tumor necrosis factor

IFN - Interferon

MCP-1 – Monocyte chemotactic protein

SEM – Standard error of means

ANOVA – Analysis of variance

## **Abstract**

The mechanism of antidiabetic effect of *Basella alba* as previously reported is unknown. This study investigated the role of *Basella alba* aqueous leave extract in modulating inflammatory cytokines and islet morphology in streptozotocin (STZ) induced diabetic rats. Forty (40) male Wistar rats, aged 8-10 weeks were randomly divided into four groups (n=10) and treated as follows: Healthy control (H-c) and Diabetic control (D-c) animals received normal saline 0.5ml/100g body weight daily, while Healthy Treatment (H-Ba) and Diabetic Treatment (D-Ba) rats received the plant extract 200mg/kg body weight daily, all by oral gavage. Diabetes was induced in D-c and D-Ba rats by a single intraperitoneal injection of STZ (55mg/kg). Weight and fasting blood sugar (FBS) were recorded weekly for four weeks, after which rats were euthanized and samples collected for further analysis. FBS was significantly reduced ( $p < 0.0001$ ) in D-Ba, but further increased ( $p < 0.001$ ) in D-c rats after the experiment. The absolute (H-c and H-Ba vs. D-c,  $p < 0.05$ ) and relative (D-Ba vs. H-c,  $p < 0.05$  and D-Ba vs. H-Ba,  $p < 0.005$ ) weight of the pancreas were significantly higher. Rats in D-c had significantly higher serum interleukin-1b ( $p < 0.001$  vs. H-c,  $p < 0.05$  vs. H-Ba and D-Ba) and monocyte chemotactic protein-1 ( $p < 0.0001$ ), but lower interleukin-10 ( $p < 0.05$ ) when compared to the other groups. Histopathological examination showed severe interstitial congestion, reduced islet area ( $p < 0.0001$ ) and increased islet cell density in D-c vs. D-Ba. From these findings, it was concluded that the aqueous extract of *Basella alba* stimulates recovery of Beta-islet morphology in STZ induced diabetic rats by modulating peripheral production of inflammatory cytokines.

**Key words;** Inflammation, cytokines, islet morphology, *Basella alba*, diabetes

## Introduction

Cytokines are members of a large group of polypeptides which mediate inflammatory and immune responses in tissues and play a central role in the pathophysiology of most chronic diseases, including diabetes mellitus (DM) (1). A rather simplistic classification of this family of proteins divides them into pro- and anti-inflammatory cytokines, and the process of inflammation is believed to be as a result of interplay between these two groups (2). However, more recent views on the role of cytokines in inflammation suggests that such classification may not be all-encompassing as certain cytokines have been found capable of both pro- and anti-inflammatory activities depending on the prevailing cytochemical condition (2). Conventionally, cytokines such as interleukin-1 (IL-1), interleukin-12 (IL-12), interleukin-18 (IL-18), gamma-interferon (IFN-gamma), tumor necrosis factor (TNF), and granulocyte-macrophage colony stimulating factor are considered to be pro-inflammatory, while interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-13 (IL-13), transforming growth factor-beta and interferon alpha (IFN- $\alpha$ ) belong to the anti-inflammatory category (2). Chemokines, like monocyte chemoattractant protein-1 (MCP-1), are closely associated with cytokines and equally play key roles in inflammatory processes. During the progression of type 1 diabetes mellitus (T1DM), pro-inflammatory cytokines have been found to invade islet cells of the pancreas and exert cytotoxic effects on the beta cells (3). Therefore, T1DM is essentially an inflammatory disease of the endocrine pancreas where there is preferential destruction of insulin producing beta islet cells via a cytokine mediated autoimmune reaction (4). Understanding the specific role played by inflammatory cytokines in the pathophysiology of DM is usually a daunting task due to the complex physiological functions of the cytokines and their inhibitors. However, an ever-changing dynamics exist between the pro- and anti-inflammatory components of the immune system, and in immune mediated diseases like T1DM, the anti-inflammatory mediators appear to provide insufficient protection over the pro-inflammatory activities of the system (5).

The destruction of insulin producing islet beta-cells of the pancreas result in insulin deficiency and consequently hyperglycemia, which is the primary anomaly in insulin dependent DM. The circulating levels of most pro-inflammatory cytokines are usually elevated in DM largely due to hyperglycemia. This is said to stimulate increased blood

levels of cytokines via an oxidative mechanism (6, 7). Hyperglycemia also results in hyperosmolality of the extracellular fluid, a physiological state which enhance the production of cytokines by mononuclear cells in the peripheral circulation (8). In the conclusion of the study by Otto and colleagues, it was suggested that hyperglycemia causes tissue inflammation by stimulating cytokine production via osmotic stress.

Streptozotocin (STZ) and alloxan are two diabetogenic agents commonly used to induce experimental DM in laboratory animals through their ability to generate reactive oxygen species via different routes and thereby induce cytotoxic effects on beta-cells of the pancreas (9). STZ is a methylating agent that causes extensive beta-cell necrosis when injected as a single high dose in rodents. Multiple low doses induce partial beta-cell apoptosis, which triggers an autoimmune reaction that eventually eliminate the remaining cells (10). It was originally used as an alkylating agent for the treatment of metastatic tumors of islet cells, but was later discovered to be diabetogenic by Rakieten et al in 1963 (11) and has since then become a chemical agent of choice for the induction of experimental DM (12).

Diabetes continues to pose a serious threat to human survival and constitute a major health challenge worldwide despite all the treatment options currently available. Recent studies reported 8.3% prevalence rate with 387 million people affected by the disease worldwide and 46.3% cases still remaining undiagnosed (13). The majority of these cases are said to live in underdeveloped and developing countries where resources for disease management are scarcely available. These underscore the need for continuous investigation of new treatment modalities for this dreadful disease.

*Basella alba* (Ba) is a type of green leafy vegetable which belongs to the family *Basellaceae* and said to be native to South Asia, but also widely cultivated in tropical Africa. Some common names of this perennial vine includes; Ceylon spinach, Malabar spinach, Saan choy and many more depending on the geographical location. The plant has a deep green stem with thick and fleshy oval shaped leaves arranged all through the length of the creeping stem. It is often mistaken for the counterpart in the basellaceae family, *Basella rubra*, which has a pinkish or purple stem with pink colored veins running through the leaves (14). Ba have been reported for its ability to lower

blood sugar and improve health in diabetic rats when administered orally as an aqueous extract (15). Little is however known in terms of the mechanism by which Ba exerts its antidiabetic actions and thus forms the main focus of this present study. The research was aimed at investigating the modulation of cytokine levels in serum and stimulation of Beta-islet cell regeneration as possible antidiabetic mechanisms of Ba.

## **Materials and Methods**

### *Preparation of Plant extract*

The research plant, Ba was sourced fresh from Osun state in south western Nigeria, identified and authenticated by the Department of Botany, University of Ibadan, Nigeria with voucher number (UIH-22391). The leave was then washed and dried at room temperature (to preserve heat labile components). The dried leaves were subsequently grounded to a powder and 100 g was extracted in 1000 ml of distilled water according to the method described by Iloki-Assanga et al., 2015 (16). The liquid extract was filtered using muslin cloth and the filtrate was freeze-dried over twenty-four hours to get a powdery extract that was subsequently dissolved in normal saline. This formed the stock solution that was administered to the rats by oral gavage during the course of this study.

### *Animal care and ethical considerations*

Male Wistar rats were obtained from Stellenbosch University animal house and acclimatized in the animal experimental laboratory of Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa. Animals had free access to food (standard rat chow) and water with the exception of periods prior to fasting blood sugar (FBS) sample collection when they were fasted overnight. Housing was under standard atmospheric conditions and animals were exposed to a light/dark cycle of 12hours. Compliance with the National Institutes of Health Guidelines (National Institutes of Health Publication No. 80-23, revised 1978) for the handling of Laboratory animals was ensured. The Health and Wellness Sciences Research Ethics Committee (HWS-REC) of Cape Peninsula University of Technology, Cape Town, South Africa,

granted ethical approval for this study with reference number; CPUT/HWS-REC 2015/A04.

### *Study design*

Forty (40) male Wistar rats, aged 8-10 weeks were randomly divided into four groups (n=10) and treated as follows: Healthy control (H-c) and Diabetic control (D-c) animals received oral normal saline at 0.5ml/100g body weight daily, while Healthy Treatment (H-Ba) and Diabetic Treatment (D-Ba) rats were given the plant extract daily at a dose of 200mg/kg body weight by oral gavage.

### *Induction of diabetes mellitus*

Rats from the diabetic control and diabetic treatment groups were given a single intraperitoneal injection of STZ (55 mg/kg) after an overnight fast. Prior to administration, STZ was prepared fresh by dissolving in ice cold citrate buffer (0.1M) at a pH of 4.5. Animals were given food and water afterwards and FBS was recorded 72hours later to confirm diabetes. Rats with FBS reading greater than 11.1 mmol/L (200 mg/dl) were considered diabetic (17) and included in the study.

### *Measurement of FBS and weight*

FBS and weight were recorded weekly in all animals from the four experimental groups. Blood for FBS was collected from the tail capillaries, by pricking the tip of the tail with a sterile lancet and expressing one or two drops of blood. This was applied appropriately to the glucometer (ONETOUCH<sup>®</sup> Ultra2) strip and FBS determined. Weight was recorded using a portable electronic balance.

### *Pancreas and blood sample collection*

All animals were euthanized via exsanguination under high dose (100mg/kg) intraperitoneal sodium pentobarbital at the completion of four weeks of treatment. Blood was collected via cardiac puncture into serum clot activator tubes (VACUETTE<sup>®</sup>), centrifuged at 4000rpm for 10min at 4°C, serum removed and stored at -80°C for the assessment of serum cytokine levels. The pancreas was carefully removed, washed

and weighed and then preserved in formalin for histological studies. Relative weight of the pancreas was calculated by dividing the pancreas weight by the animal's total body weight.

#### *Assessment of serum cytokines*

Evaluation of the various cytokine levels was done in triplicate serum samples using multiplex technology. Samples were removed from -80°C and kept at room temperature to thaw prior to assay. Analysis of cytokine levels was done using the Bio-Plex Pro™ Reagent kit with 1 x 96-well flat bottom plate, Cat. #171-304070M (USA). Assays were performed according to the product protocol manual (Merck Millipore, Billerica, MA, USA) and the Bio-Plex platform (Bio-Rad Laboratories, Hercules, CA, USA) was used for plate reading.

#### *Histopathological analysis of the pancreas*

Pancreatic tissue samples from each rat were fixed in 10% buffered formalin immediately after removal and weighing. The tissues were processed by embedding in paraffin wax and sectioned at 4-6µm thickness before staining with Hematoxylin and Eosin (H&E stain). The prepared slides were examined microscopically at x40, x100 and x400 magnifications with a Digital Microscope, VJ-2005 DN model Bio-microscope® (China).

#### *Determination of islet cell area and density*

The morphometric analysis was done using TS View CX Image® Software, file version 6.2.4.3 and Motic Image 2000 (China). For each slide, the islet parenchyma were observed and several pictures taken at x400 magnification. Discreet random islet areas were determined using Motic Plus 2000 (China) photomicrography software. Manual counting of viable cells in each area was performed. Summation of all islet areas per slide was done to estimate the total area and likewise summation of cell count values yielded the total cell count per slide. Total islet density was determined in each slide by the formula: Total cell count/ Total area of count.

### *Statistical analysis*

Graphpad Prism version 5.0 was used to analyze all data. Values were expressed as mean  $\pm$  SEM and differences between group means determined by one-way analysis of variance (ANOVA). Bonferroni post-test was applied and statistical significance was considered at values for  $P < 0.05$ .

## **Results**

### *Effect of treatments on fasting blood sugar*

Following a single intraperitoneal injection of STZ in D-c and D-Ba rats, FBS in both groups before the commencement of treatments was significantly ( $p < 0.0001$ ) higher than FBS in H-c and H-Ba rats. After four weeks of treatment, the FBS levels did not change significantly in rats from the H-c and H-Ba groups. D-c rats showed a significant increase ( $p < 0.001$ ), while there was a significant ( $p < 0.0001$ ) reduction of FBS in D-Ba rats when compared to readings recorded in the respective groups before treatment commenced (See Table 1).

### *Effect of treatments on body weight, weight of pancreas and relative weight of pancreas*

After the four weeks period of treatment, the body weight and weight of the pancreas was significantly ( $p < 0.05$ ) lower in the two diabetic groups (D-c + D-Ba) compared to the healthy control rats. There was no significant difference in both body and pancreas weight between the two diabetic groups and the two healthy groups of rats. Even though body weight was significantly ( $p < 0.05$ ) lower in the diabetic treatment group when compared to healthy treatment animals, weight of pancreas did not differ significantly between the two groups. The relative weight of the pancreas was significantly higher in rats from the diabetic treatment group when compared to both healthy control ( $p < 0.05$ ) and healthy treatment ( $p < 0.005$ ) rats. There was no significant difference among relative pancreas weight of diabetic control rats and those of rats in the other three groups (Figure 1).

### *Effect of treatments on serum cytokines*

The serum levels of IL-1b was significantly higher in samples from the D-c group when compared to all of the other groups ( $p < 0.001$  vs. H-c and  $p < 0.05$  vs. H-Ba and D-Ba). Similarly, D-c samples had significantly ( $p < 0.0001$ ) higher levels of MCP-1 when compared to the other three groups (Figure 2). Serum levels of IL-4 and IL-13 did not differ significantly among any of the four experimental groups. However, IL-10 was significantly lower ( $p < 0.05$ ) in D-c samples when compared to the other groups. Furthermore, serum IL-10 levels in H-c samples was significantly higher ( $p < 0.05$ ) than levels in D-Ba samples (Figure 3).

### *Effect of treatments on pancreas histology*

Histopathological analysis of the pancreas showed normal interstitial architecture and islet sections in slides from the healthy control, healthy treatment and diabetic treatment groups. The islet sections however appears to be slightly larger in slides from the healthy treatment group when compared to the other groups. There was severe interstitial congestion and reduced islet sections in slides from the diabetic control group where rats did not receive the plant extract (Figure 4).

### *Effect of treatments on area and density of islet cell*

As shown in table 1, the Islet cell area was significantly ( $p < 0.0001$ ) reduced in D-c group when compared to the other three groups, while it was increased ( $p < 0.0001$ ) in the H-Ba group compared to all three of the other groups. On the other hand, Islet cell density was significantly ( $p < 0.0001$ ) lower in H-c, H-Ba and D-Ba rats when compared to D-c group. There was no significant difference in islet cell density among H-c, H-Ba and D-Ba rats.

## **Discussion**

The reduction in FBS observed in the diabetic rats treated with Ba aqueous extract (D-Ba) is a confirmation of the previously reported antihyperglycemic effect of the plant (15). Furthermore, potent mediators of inflammation like IL-1b (18) and MCP-1 were considerably lower in the serum samples of the treated diabetic rats compared to their

untreated counterparts, alluding to the possibility of a correlation between the antihyperglycemic (15) and anti-inflammatory (19, 20) effects of Ba. As mentioned in the introductory part of this study, hyperglycemia stimulates peripheral production of inflammatory cytokines via oxidative (6, 7) and osmotic stress (8) mechanisms. These mediators of inflammation play a major role in suppressing  $\beta$ -islet cell functions and ultimately inducing apoptosis of the cells by initiating and intensifying immune assaults on them (21). It implies that an unpleasant interplay exists between high serum levels of inflammatory mediators and hyperglycemia in a vicious cycle that culminates in deterioration of symptoms in T1DM. Prevention of one or all of hyperglycemia, production of pro-inflammatory cytokines/chemokines, or depletion of anti-inflammatory cytokines is therefore expected to have a positive impact on Beta-islet cell function and survival in an ongoing T1DM. In the present study, T1DM was induced by single intraperitoneal injection with a high dose of STZ (55mg/kg) to sufficiently ablate the insulin producing Beta-islet cells of the pancreas (22). The observation of lower blood sugar and pro-inflammatory cytokines, but higher IL-10 (the main anti-inflammatory cytokine assessed) in diabetic animals treated with the aqueous extract of Ba (i.e D-Ba group) is quite informative. These clearly highlight the actions underlying the anti-inflammatory role of the plant extract. Gomphrenin I (15S-betanidin 6-O- $\beta$ -glucoside) was identified as the principal coloring agent in Ba fruit and found to be a potent antioxidant and inhibitor of inflammation (23). It was observed to inhibit the transcription and expression of genes encoding certain inflammatory cytokines, including IL-1 $\beta$  (23). Although Gomphrenin I was not isolated during the phytochemical analysis of aqueous leave extract of Ba in a previous study (24), the possibility of its presence in the leave cannot be discarded in light of observations from this study. Furthermore, established chemical constituents of the leave extract like Tannins, Flavonoids, Mucilage and Saponin (19), have strong anti-oxidant properties (25, 26) which may also explain the ability of the extract to suppress the production of inflammatory cytokines. This is because generation of oxygen free radicals is known to contribute significantly to the initiation and progression of systemic as well as localized inflammatory responses via the activation of nuclear factors that stimulate release of cytokines (27).

The histopathologic findings in this study are also quite worthy of note. There appear to be a recovery of previously lost islet regions in the pancreas from diabetic rats that were

treated with the plant extract as evidenced by the significantly larger islet area when compared to pancreas sections from their untreated counterparts. Additionally, even though body weight was significantly lost in both diabetic groups, relative pancreatic weight loss was less in the diabetic rats treated with Ba extract suggesting a protective role for the plant as regards degenerative damage of the pancreas. Whether the larger islet area in the treated rats is as a result of regeneration of new Beta-islet cells or inhibition of progress of STZ-induced apoptosis cannot be ascertained within the limits of information accruable from the present data, but it opens a veritable topic for further research. It is also not exactly clear why a huge increase in islet area was observed in the healthy treatment group (H-Ba). This may be partly due to an unchallenged action of Ba in stimulating islet cell proliferation. Presently, curative therapies of T1DM are based on restoration of endogenous insulin production either by transplantation of Beta-islet cells or whole pancreas with concurrent immunosuppression (28, 29). This has its own demerits especially stemming from the continuous need to suppress the immune system and the increased risk of vascular and metabolic complications (29). It will therefore be a most welcomed innovation to be able to reinstitute insulin synthesis by using a natural medicinal agent that is capable of reenacting islet function well enough to achieve better glucose homeostasis in T1DM. Interestingly, islet cell density was observed to be highest in the diabetic control (D-c) rats that were not treated with the extract. This can be explained by increases in Alpha-islet and Delta-islet cell mass that usually occur following a selective STZ-induced destruction of the insulin producing Beta-islet cells (30). The inflammatory milieu in the STZ model of T1DM actually allows for the Alpha- and Delta-cells expanding and proliferating at a faster rate than usual (30) to compensate for the lost Beta-cells. There is also a marked increase in Beta-islet cell proliferation during the early stages of T1DM in humans (31), which may also be a contributory factor to the extremely high islet cell density observed in the diabetic control rats. Further studies in this respect may include differential staining of the various islet cell types to specifically account for the composition of the islet population and quantitative analysis of serum insulin and the other hormones produced by each cell type.

It was therefore concluded that the antidiabetic effect of Ba aqueous leave extract is largely dependent on the plant's ability to modulate the production of inflammatory

cytokines and influence pancreatic islet proliferation and possibly their function. The apparent recovery of islet sections observed in this study offers possible future hope of an alternative to pancreas/islet transplantation.

### **Acknowledgement**

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### **Conflicts of interest**

The authors declare no conflict of interest regarding the publication of any part of this study.

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## TABLE

Table 1: Fasting blood sugar before commencement and after completion of four weeks of *Basella alba* treatment, Islet cell area and Islet cell density of control and treatment rats

| Groups                    | Fasting blood sugar (mmol/L) |                           | Islet cell area (x10 <sup>5</sup> Sqµm) | Islet cell density (x10 <sup>-2</sup> Cells/Sqµm) |
|---------------------------|------------------------------|---------------------------|---|---|
|                           | Before Treatment             | After Treatment           |   |   |
| Healthy Control (H-c)     | 4.48 ± 0.15                  | 4.46 ± 0.14 <sup>ns</sup> | 2.54 ± 0.36 <sup>c</sup>                | 0.009 ± 0.00 <sup>#</sup>                         |
| Diabetic Control (D-c)    | 18.69 ± 1.36 <sup>***</sup>  | 24.71 ± 1.14 <sup>a</sup> | 0.03 ± 0.00 <sup>***</sup>              | 1.468 ± 0.23 <sup>***</sup>                       |
| Healthy Treatment (H-Ba)  | 4.51 ± 0.15                  | 4.48 ± 0.15 <sup>ns</sup> | 6.20 ± 0.31 <sup>***#</sup>             | 0.009 ± 0.00 <sup>#</sup>                         |
| Diabetic Treatment (D-Ba) | 17.74 ± 1.34 <sup>***</sup>  | 10.71 ± 0.41 <sup>b</sup> | 2.68 ± 0.45 <sup>#</sup>                | 0.012 ± 0.00 <sup>#</sup>                         |

Values are expressed as mean ± SEM, FBS readings before commencement of treatment in all groups were compared with that of H-c and readings before and after four weeks of treatment were compared in each group. Islet cell area and density in all experimental groups were compared with control groups. P values were denoted as;

<sup>ns</sup> Not significant, <sup>a</sup> p<0.001, <sup>b</sup> p<0.0001 vs. values before treatment.

<sup>\*\*\*</sup> p<0.0001 vs. H-c, <sup>#</sup> p<0.0001 vs. D-c

mmol/L = Millimoles per litre, Sqµm = Square micrometres

## LEGENDS FOR FIGURES

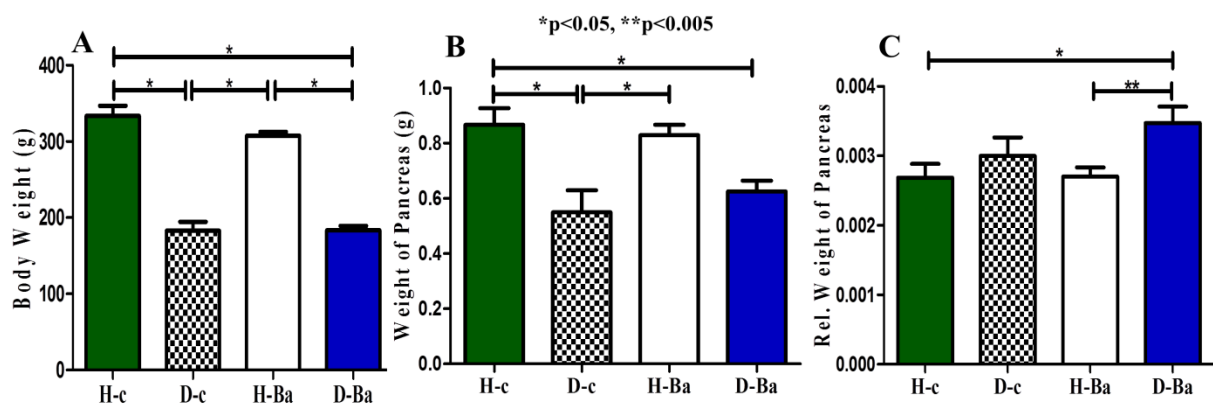
**FIGURE 1:** Body weight (A), weight of pancreas (B) and relative weight of pancreas (C) of rats after four weeks of *Basella alba* treatment.

**FIGURE 2:** Levels of pro-inflammatory cytokine, IL-1b (A) and chemokine, MCP-1 (B), in the serum of rats after four weeks of *Basella alba* treatment.

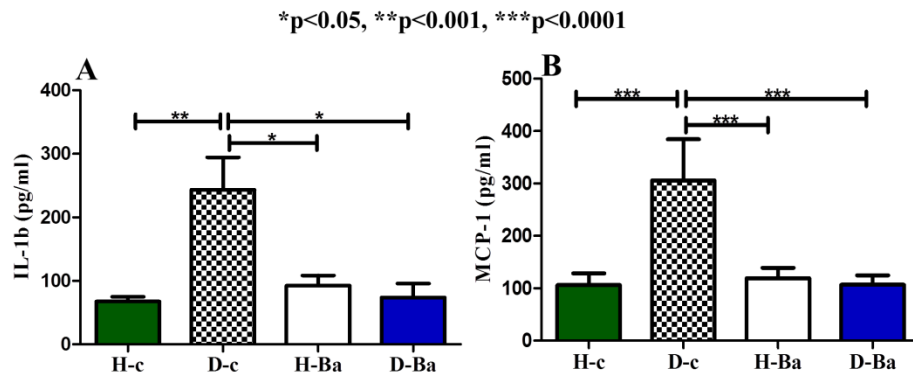
**FIGURE 3:** Levels of anti-inflammatory cytokines, IL-4 (A), IL-10 (B) and IL-13 (C) in the serum of rats after four weeks of *Basella alba* treatment [ns = Not significantly different]

**FIGURE 4:** Histopathological photomicrograph of the pancreas (H & E) showing slides from: (A) Healthy control rat with normal interstitium and islet section; (B) Diabetic control rat with reduced islet section (Long arrow) and severe interstitial congestion (Short arrow); (C) Healthy treatment rat with normal interstitium and islet section; And (D) Diabetic treatment rat with normal interstitium and islet section.

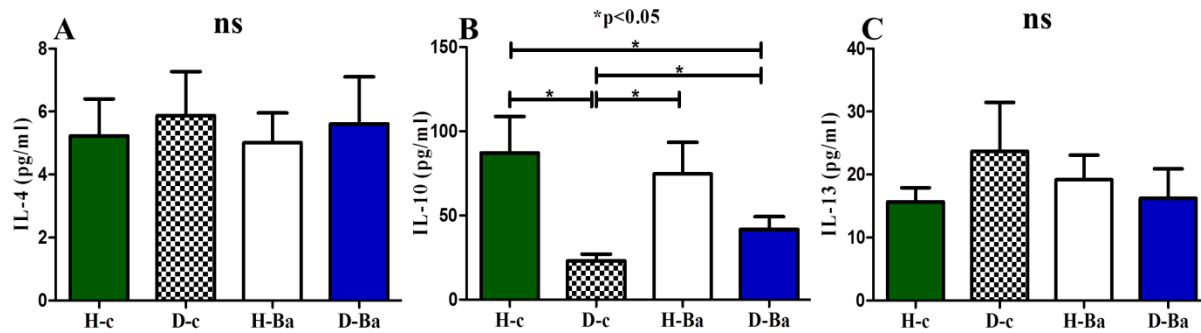
## FIGURES



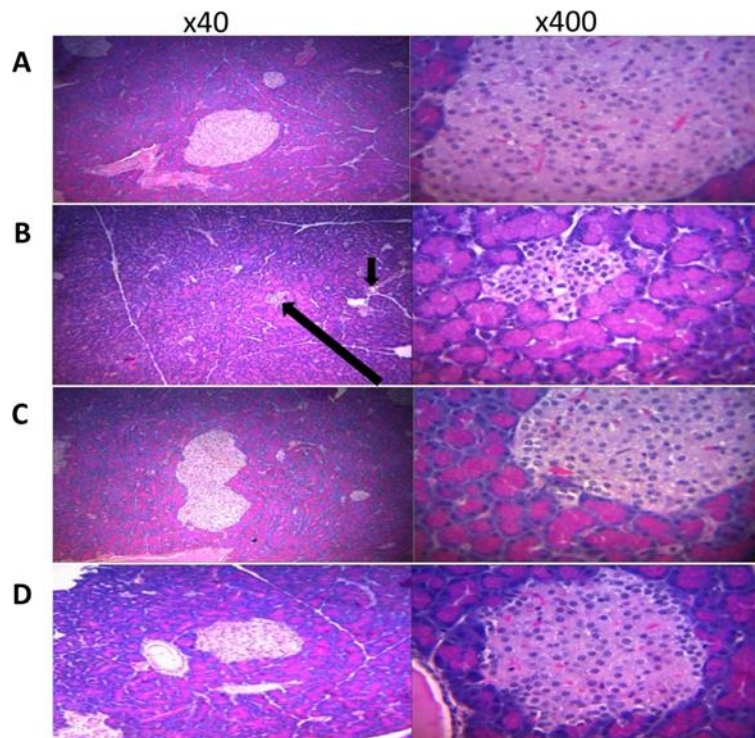
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## Chapter VI

### **Antioxidant activities of *Basella alba* aqueous leave extract in blood, pancreas and gonadal tissues of diabetic male Wistar rats**

**Short title:** *Basella alba* has systemic antioxidant effect

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#### **Authors' Contributions**

All four authors participated in conceptualizing, design, definition of intellectual content, literature search, experimental studies, data analysis, statistical analysis and in the preparation and editing of the final manuscript.

## **Abstract**

Oxidative stress is frequently identified as a key element in the pathophysiology of many complications of diabetes mellitus, including reproductive complications. The antioxidant potential of medicinal plants has been suggested for therapeutic focus in recent reports. This study investigated the effect of *Basella alba* aqueous leave extract on diabetes-induced oxidative stress. Forty male Wistar rats (8-10weeks) were randomly divided into four groups (n=10) and treated as follows; Control (C+N<sub>s</sub>) and Diabetic (D+N<sub>s</sub>) animals received oral normal saline 0.5ml/100g body weight daily, while Healthy Treatment (H+Ba) and Diabetic Treatment (D+Ba) rats were given *Basella alba* extract at an oral dose of 200mg/kg body weight daily. Treatment was by gavage and lasted four weeks in all groups. Diabetes was induced in D+N<sub>s</sub> and D+Ba rats by single intraperitoneal injection of streptozotocin (55mg/kg) and fasting blood sugar (FBS) recorded weekly in all rats afterwards. Animals were euthanized at the end of the experiment and blood samples, pancreas, testes and epididymis were preserved for analysis of oxidative stress biomarkers. Oral administration of aqueous leave extract of *Basella alba* significantly ( $p<0.0001$ ) lowered FBS in D+Ba rats. There was significantly higher blood superoxide dismutase (SOD) activity and serum FRAP (Ferric reducing antioxidant power), but lower serum concentration of conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS) in D+Ba compared to D+N<sub>s</sub> rats ( $p<0.05$ ). It was concluded from these findings that *Basella alba* exerts antioxidant effects in the gonads by enhancing antioxidant parameters in circulating blood, but not necessarily in the gonadal tissues.

**Key Words:** Antioxidant, *Basella alba*, Oxidative stress, Blood, Pancreas, Gonads

## Introduction

Oxidative stress as a result of increased production of free radicals is becoming more frequently identified as a key pathophysiological element in most complications of diabetes mellitus (DM) (Baynes and Thorpes, 1999; Ceriello et al., 2000; Telci et al., 2000; Matough et al., 2012). The body is equipped with a number of enzymatic and non-enzymatic antioxidant systems with the primary role of neutralizing free radicals like reactive oxygen species (ROS) and reactive nitrogen species (RNS), thereby rendering them non-toxic to body cells (Taheri et al., 2012). ROS are inevitable by-products of normal physiological processes in the body and are required to an extent in the immune system for antimicrobial actions and also function as signaling molecules in the maintenance of intracellular homeostasis (Halliwell et al., 1996 ). In conditions where the rate of generation of these free radicals is increased or the protective antioxidant mechanism is reduced, an imbalance occurs in favor of free radicals leading to increased oxidative stress and subsequent tissue damage (Tiwari et al., 2013). The resultant tissue damage is caused by the tendency for these unstable oxygen/nitrogen species to enter into reactions with cellular component by donating free electrons which results in DNA damage and peroxidative changes in cell membrane ( Matés et al., 1999). This is the basis of most health complications of diseases that trigger increased generation of systemic oxidative stress, including DM.

DM is a metabolic disorder that is characterized by chronic hyperglycemia due to relative or absolute insulin deficiency (American diabetes association, 2012). The two major classes of DM are insulin dependent or type 1 DM (insulin deficiency due to auto-immune  $\beta$ -islet cell damage) and non-insulin dependent or type 2 (target organ resistance to insulin) (Asmat et al., 2015). Both classes essentially encompass abnormalities in carbohydrate, fat and protein metabolism due to deficient insulin action (American diabetes association, 2009). The persistent hyperglycemia in DM stimulate increased generation of ROS, which coupled with the weakened body defense system, result in an imbalance between ROS and antioxidants that culminates in oxidative stress (Pandey et al., 2010). The generation of oxidative stress in DM occurs via a number of pathophysiological routes. Chronic hyperglycemia might result in auto-oxidation of glucose, a shift in redox balance, limited activities of antioxidant enzymes

like catalase (CAT) and superoxide dismutase (SOD) or depletion of low molecular weight antioxidants like vitamin E and reduced glutathione (GSH) (Haskins et al., 2003). Additionally, metabolic modification of proteins and/or lipids in DM leads to the formation of advanced glycation end-products (AGEs), which is said to increase generation of ROS as well as destroys the antioxidant enzyme system (Nowotny et al., 2015).

Dysfunctional free radical scavenging and increased production of ROS/RNS have been frequently implicated in the pathophysiology of male reproductive complications of DM (La Vignera et al., 2012; Jain and Jangir, 2014), as well as other systemic complications. The use of medicinal plants as alternative therapies in the management of DM and its complications is therefore largely hinged on both the free radical scavenging and anti-hyperglycemic potentials of the phytochemical components of such plants (Chan et al., 2002). *Basella alba*, also called Malabar or Indian spinach, is a creeping mucilaginous green vegetable that have been reported for its anti-hyperglycemic activity and successfully used in the treatment of experimental diabetes mellitus in rats (Bamidele et al., 2014). *Basella alba* is believed to originate from Asia but is widely cultivated and consumed as vegetable in the humid tropical regions of Africa and have been reportedly used to relieve sexual asthenia in Cameroonian men (Adhikari et al., 2012). Previous studies have also confirmed the androgenic properties of the plant (Nantia et al., 2009; Nantia et al., 2011) and unpublished experimental data by our research group demonstrated the value of *Basella alba* in alleviating some diabetes-induced abnormalities in sperm parameters. The antioxidant properties of some of the phytochemical components of the plant have been suggested as a possible reason for the antidiabetic and pro-fertility effects. This present study aimed at investigating the concise in vivo effect of oral administration of aqueous leaf extract of *Basella alba* on the various biomarkers of oxidative stress in the systemic circulation, pancreatic tissues as well as in the gonadal tissues of diabetic male rats.

## **Materials and methods**

### *Plant material and extract preparation*

Fresh *Basella alba* (Ba) was collected from multiple locations within Osun state, south western Nigeria and authenticated by the Department of Botany, University of Ibadan,

Nigeria under voucher number UIH-22391. The leaves were removed from the stem, washed and then dried at room temperature for about four weeks. This was macerated into fine powdery form and 100 g extracted in 1000 ml distilled water using the method of Nagappan (2012). The product of extraction was filtered through a muslin cloth and freeze-dried into powdery extract which was dissolved in normal saline to form the stock solution that was administered to rats by oral gavage during this study.

#### *Animal care and ethical considerations*

Male Wistar rats were obtained from the animal unit at Stellenbosch University and acclimatized in the animal experimental laboratory of the Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa. Animals had free access to food (standard rat chow) and water with the exception of periods prior to fasting blood sugar (FBS) sample collection when they were fasted overnight. Housing was under standard atmospheric conditions and animals were exposed to a light/dark cycle of 12hours. Compliance with the National Institutes of Health Guidelines (National Institutes of Health Publication No. 80-23, revised 1978) for the handling of Laboratory animals was ensured. The Health and Wellness Sciences Research Ethics Committee (HWS-REC) of Cape Peninsula University of Technology, Cape Town, South Africa, granted ethical approval for this study with reference number; CPUT/HWS-REC 2015/A04.

#### *Study design*

A total of forty (40) male Wistar rats, 8-10weeks of age were used for this study. These were randomly divided into four groups of ten rats each and treated as follows; Control (C+N<sub>s</sub>) and Diabetic (D+N<sub>s</sub>) animals received oral normal saline 0.5ml/100g body weight daily, and Healthy Treatment (H+Ba) and Diabetic Treatment (D+Ba) rats were given *Basella alba* extract at an oral dose of 200mg/kg body weight daily. Administration of the extract and normal saline was done by gavage using a metal endotracheal tube.

### *Induction of DM and measurement of fasting blood sugar (FBS)*

DM was induced in rats from diabetic control and diabetic treatment groups by single intraperitoneal injection of STZ, 55mg/kg subsequent to an overnight fast. STZ was prepared fresh just before each administration by dissolving in ice cold citrate buffer (0.1M) at a pH of 4.5. Animals were allowed access to food and water afterwards and FBS was recorded after 72hours to confirm diabetes. Rats with FBS above 11.1mmol/L (200mg/dL) were considered diabetic and included in this study (Pournaghi et al., 2012). FBS was subsequently recorded weekly in all animals from the four experimental groups after the commencement of treatment. Blood sample was taken from tail capillaries to determine FBS using a standard glucometer (ONETOUCH<sup>®</sup> Ultra2).

### *Preparation of serum and whole blood samples*

At the completion of four weeks of treatment, all animals were euthanized via exsanguination under high dose (100mg/kg) intraperitoneal sodium pentobarbital and blood was collected via cardiac puncture. For serum, blood was collected into serum clot activator tubes (VACUETTE<sup>®</sup>) and centrifuged at 4000 rpm in 4°C for 10 minutes to get the supernatant. Whole blood samples were dispensed into EDTA tubes and all samples were stored at -80°C until further biochemical analysis.

### *Preparation of tissue homogenates (Pancreas, Testes and epididymis)*

All animals in the four experimental groups were euthanized and dissected after blood sample collection. The pancreas, testes and epididymis were excised, washed and preserved at -80°C. Frozen samples of these tissues were weighed and homogenized in glass tubes on ice, centrifuged at 15000 rpm for 10 minutes at 4°C and the supernatant kept at -80°C for further analysis.

### *Analysis of reduced glutathione and antioxidant enzymes*

The concentration of reduced glutathione (GSH) was determined in whole blood samples and tissue homogenates according to the method of Asensi et al., (1999).

Superoxide dismutase (SOD) and catalase (CAT) activities were determined in whole blood and tissue homogenates using the method described by Ellerby and Bredesen (2000).

#### *Determination of antioxidant capacities*

Antioxidant capacity was measured in all serum samples by three different methods; Ferric reducing antioxidant power (FRAP) was determined according to the method of Benzie and Strain (1999), trolox equivalent antioxidant capacity (TEAC) was determined using the method described by Re et al., (1999) and oxygen radical antioxidant capacity (ORAC) according to the method of Cao and Prior (1999).

#### *Estimation of lipid peroxidation*

Lipid peroxidation was quantified via two methods; Initiation of peroxidation was assessed by measuring Conjugated dienes (CDs) concentration in serum samples according to the method of Recknagel and Glende (1984). Termination of lipid peroxidation was assessed by determining the concentration of thiobarbituric acid reactive substances (TBARS) in serum samples according to the method of Esterbauer and Cheeseman (1990).

#### *Statistical analysis*

Data for all parameters are expressed as mean  $\pm$  SEM. Graphpad Prism version 5.0 was used for the data analysis employing analysis of variance (ANOVA) for multiple comparisons with Bonferroni's post-test. Confidence interval was placed at 95% ( $p < 0.05$ ).

## **Results**

#### *Effect of Basella alba on FBS*

*Basella alba* significantly lowered FBS in rats from the diabetic treatment group after four weeks of treatment as opposed to the diabetic control group where FBS was

significantly higher after the experimental period ( $p < 0.0001$  and  $p < 0.001$  respectively versus FBS before treatment). This is shown in table 1.

#### *Effect of Basella alba on enzymatic and non-enzymatic antioxidants*

As demonstrated in table 2, the blood concentration of non-enzymatic antioxidant GSH and antioxidant enzymes SOD and CAT were significantly lower in samples from rats in the D+N<sub>s</sub> group when compared to C+N<sub>s</sub> and H+N<sub>s</sub> rats ( $p < 0.001$  for GSH and SOD,  $p < 0.05$  for CAT). However, apart from the lower concentration of SOD in pancreas homogenates ( $p < 0.05$ ), the differences in concentration of these antioxidants in tissue homogenates were not statistically significant when the D+N<sub>s</sub> group was compared to C+N<sub>s</sub> group. SOD concentration in blood was also observed to be significantly higher ( $p < 0.05$ ) in diabetic rats treated with the plant extract (D+Ba) when compared with the untreated diabetic (D+N<sub>s</sub>) counterpart. However, all other differences observed in blood and tissue concentrations of the three antioxidant parameters between the D+Ba and D+N<sub>s</sub> groups were not statistically significant.

#### *Effect of Basella alba on the antioxidant capacities of serum*

Figure 1A demonstrates a significantly ( $p < 0.05$ ) lower FRAP in the serum samples from diabetic rats when compared to the remaining three groups. There was no significant difference in TEAC among the four experimental group (figure 1B), but ORAC was significantly higher in the control rat's serum compared to the other experimental groups and in H+Ba compared to D+Ba as shown in figure 1C ( $p < 0.0001$  and  $p < 0.05$  respectively).

#### *Effect of Basella alba on serum concentration of Conjugated dienes*

The level of CDs in the serum samples of rats in the experimental groups was demonstrated in figure 1D. CDs concentration was significantly ( $p < 0.001$ ) higher in the diabetic group of rats compared to the healthy rats (C+N<sub>s</sub> and H+Ba), but significantly ( $p < 0.05$ ) lower in the diabetic rats treated with the study extract (D+Ba) when compared with the untreated counterpart (D+N<sub>s</sub>).

### *Effect of Basella alba on concentration of thiobarbituric acid reactive substances*

As shown in figure 2A, serum concentration of TBARS was significantly higher in rats from D+N<sub>s</sub> group when compared to the other groups ( $p < 0.05$  vs C+N<sub>s</sub>, D+Ba, and  $p < 0.0001$  vs H+Ba). Similarly, as demonstrated in figure 2B, TBARS concentration was significantly higher in pancreas homogenates of rats from D+N<sub>s</sub> when compared to the other three groups ( $p < 0.0001$  vs C+N<sub>s</sub> and  $p < 0.001$  vs H+Ba, D+Ba). Despite equally higher concentrations of TBARS in both epididymis and testes homogenates of rats in the diabetic group, the observed differences were not statistically significant (figures 2C and 2D respectively).

### **Discussion**

In line with previous reports, findings in this study demonstrated the anti-hyperglycemic ability of *Basella alba* as confirmed by the lower FBS in D+Ba rats after four weeks of treatment (Table 1). This anti-hyperglycemic effect may be sufficient in bringing about significant correction in the oxidative stress status of the animals since prolonged hyperglycemia in itself have been said to induce oxidative stress (Haskins et al., 2003; Pandey et al., 2010). However, the possibility of the lowering of blood sugar being a direct consequence of antioxidant activity of some phytochemical components of the plant have also been suggested (Olajire and Azeez, 2011; Bamidele et al., 2014). The findings in this study confirmed both the antioxidant and antihyperglycemic effects of *Basella alba*, but it is not exactly certain if one occurred as a direct sequel of the other or both effects were achieved via separate mechanisms. Both events however appear to occur in a mutually synergistic cycle.

Changes in concentration and activities of enzymatic and non-enzymatic antioxidants in biological systems following an in vivo oxidative insult have been a subject of research controversy over the years. Whereas some studies have reported a decrease in the activity of endogenous antioxidants like GSH, SOD and CAT following exposure to oxidative stress (Pari and Umamaheswari, 2000; Bajaj and Khan, 2012; Taheri et al., 2012), others have reported increased activities of the antioxidant enzymes in the face of persistent exposure to oxidative stress (Shull et al., 1991; Michiels et al., 1994; Dias et al., 2010; Zelen et al., 2010). It is therefore imperative to acknowledge some of the

factors that may determine the level of antioxidant enzyme activities in biological tissues when subjected to oxidative stress. The duration and severity of oxidative stress is an important factor since antioxidant enzyme activity is presumed to increase initially in response to oxidative stressors but as the stress process become persistent, the enzyme activities may decrease due to damage to the proteins and genes responsible for the antioxidant enzyme expression. There is also a variation in the enzymatic activities in the various tissues of the body and for instance, pancreatic islets have been described as one of the tissues with the lowest intrinsic antioxidant defenses (Tiwari et al., 2013). In this present study, the result revealed a significant reduction in the concentration of GSH and activities of SOD and CAT in the blood samples of diabetic control rats, whereas, these animals showed no significant change in the expression of the antioxidants in the gonads and pancreas except for the reduced SOD activity observed in pancreas homogenate. This suggest worse antioxidant status in the systemic circulation compared to the tissues, and expectedly, the pancreas being the primary organ of insult in STZ diabetes, showed significantly reduced SOD activity. Furthermore, the dismutation of superoxide anions ( $O_2^-$ ) into molecular oxygen ( $O_2$ ) and hydrogen peroxide ( $H_2O_2$ ) by SOD is the first line of defense against ROS-induced tissue damage (Asmat et al., 2016), therefore generation of a greater volume of  $O_2^-$  in the pancreatic islet may explain why SOD activity is more significantly reduced in the pancreatic tissues of the diabetic rats. *Basella alba* appears to offer some form of protection against this imbalance in the treated diabetic group of rats as evidenced by the higher SOD activity when they were compared with rats from the untreated diabetic group. We hypothesized that this protective action of *Basella alba* leave extract may be due to it's ability to directly inactivate superoxide anions thereby sparing SOD, or due to a component of the extract that mimics SOD activity by enhancing dismutation of the anions into oxygen and hydrogen peroxide.

The more obvious confirmation of the antioxidant properties of *Basella alba* in our result was displayed in the effects it exerted on serum total antioxidant capacity and on lipid peroxidation in both serum and the tissues.

Total antioxidant capacity (TAC) is a term that is used in attempts to describe the cumulative effect of the various intrinsic antioxidants in body fluids (Sies, 2007) and is

said to increase in vivo after the consumption of foods rich in phenolic compounds (Cao et al., 1998) and flavonoids (Sies, 2007). In this study, TAC was assessed via three methods (FRAP, TEAC and ORAC), each of which employs three different mechanisms. TEAC and ORAC are direct assays that are based on the ability of the samples to inhibit substance oxidation while FRAP is indirect and based on evaluating the ability of the samples to reduce iron-complex from ferric to ferrous state (Rubio et al., 2016). FRAP was significantly increased in the group of diabetic rats treated with *Basella alba* when compared with the untreated counterpart, but TEAC and ORAC values were not significantly different between the two groups. This is a confirmation of the fact that TAC varies considerably depending on the assay method used since the various methods actually measure different components of TAC (Winter et al., 2009). In human serum, FRAP is said to measure mainly uric acid while TEAC measures mainly albumin but in addition, both assays also measure  $\alpha$ -tocopherol, bilirubin and ascorbic acid (Rubio et al., 2016).

The accumulation of products of lipid peroxidation, CDs and TBARS, was highly significant in the serum and pancreatic tissues of the untreated diabetic rats, but not in the testis and epididymis. This notwithstanding, data not published in the present study suggested gonadal complications of DM as evidenced by subnormal sperm parameters in the diabetic animals. This means systemic oxidative stress induced by DM can impact negatively on gonadal functions without necessarily causing significant local oxidative damage in the gonads. The lower concentration of TBARS observed in the diabetic rats that were treated with *Basella alba* for four weeks was equally significant in the serum and pancreas but not in the gonadal tissues. This is an indication that the antioxidant effect of *Basella alba* may be more of a systemic rather than localized tissue action and therefore, the beneficial effect in ameliorating diabetic complications is expected to be multisystemic.

## **Conclusion**

*Basella alba* ameliorate diabetes-induced oxidative stress and some of the complications associated with it. The antioxidant effect of the plant in diabetic rats is closely related to the anti-hyperglycemic effect and the two actions appear to enhance

each other mutually. This study also establishes the predominantly systemic nature of the antioxidant activities of *Basella alba* rather than localized tissue effects but further studies involving a wider variety of tissues will be needed to fully substantiate this observation.

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### **Conflict of interest**

The authors declare no conflict of interest with regards to the publication of any part of this study.

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## TABLES

**Table 1.** Fasting blood sugar before commencement and after the completion of treatment control and experimental groups.

| Groups                       | FBS (mmol/L)     |                           |
|------------------------------|------------------|---------------------------|
|                              | Before Treatment | After Treatment           |
| Control (C+N <sub>s</sub> )  | 4.48 ± 0.15      | 4.46 ± 0.14 <sup>ns</sup> |
| Diabetic (D+N <sub>s</sub> ) | 18.69 ± 1.36     | 24.71 ± 1.14 <sup>a</sup> |
| Healthy Treatment (H+Ba)     | 4.51 ± 0.15      | 4.48 ± 0.15 <sup>ns</sup> |
| Diabetic Treatment (D+Ba)    | 17.74 ± 1.34     | 10.71 ± 0.41 <sup>b</sup> |

Values expressed as mean ± SEM for 10 rats in each group. Values after treatment were compared with that before treatment in all groups. Statistical significance were denoted as; <sup>ns</sup> Not significant, <sup>a</sup> p < 0.001, <sup>b</sup> p < 0.0001 vs. values before treatment.

**Table 2.** Effect of treatments on enzymatic and non-enzymatic antioxidant activities in blood and tissue homogenates of rats from control and experimental groups

| Samples/Groups     | GSH<br>(U/mg protein) x 10 <sup>-3</sup> | SOD<br>(U/mg protein) x 10 <sup>-2</sup> | CAT<br>(U/mg protein) x 10 <sup>-3</sup> |
|--------------------|--|--|--|
| <b>Whole blood</b> |  |  |  |
| C+N <sub>s</sub>   | 0.005 ± 0.001                            | 0.020 ± 0.001                            | 0.10 ± 0.008                             |
| D+N <sub>s</sub>   | 0.002 ± 0.0003**                         | 0.006 ± 0.001**                          | 0.06 ± 0.007*                            |
| H+Ba               | 0.005 ± 0.001 <sup>b</sup>               | 0.014 ± 0.001* <sup>b</sup>              | 0.10 ± 0.008 <sup>a</sup>                |
| D+Ba               | 0.003 ± 0.001*                           | 0.012 ± 0.001* <sup>a</sup>              | 0.09 ± 0.012                             |
| <b>Pancreas</b>    |  |  |  |
| C+N <sub>s</sub>   | 1.63 ± 0.56                              | 110.4 ± 13.18                            | 3.76 ± 0.76                              |
| D+N <sub>s</sub>   | 3.94 ± 0.68                              | 56.22 ± 2.57*                            | 2.56 ± 0.32                              |
| H+Ba               | 2.02 ± 0.99                              | 129.70 ± 26.53                           | 4.36 ± 0.88                              |
| D+Ba               | 3.33 ± 0.01                              | 56.80 ± 5.03*                            | 3.43 ± 1.11                              |
| <b>Epididymis</b>  |  |  |  |
| C+N <sub>s</sub>   | 7.51 ± 2.24                              | 223.60 ± 40.47                           | 1.01 ± 0.23                              |
| D+N <sub>s</sub>   | 7.66 ± 2.69                              | 153.10 ± 13.36                           | 0.62 ± 0.21                              |
| H+Ba               | 12.89 ± 9.95                             | 176.80 ± 16.00                           | 1.09 ± 0.32                              |
| D+Ba               | 13.15 ± 9.30                             | 217.70 ± 30.07                           | 0.57 ± 0.12                              |
| <b>Testes</b>      |  |  |  |
| C+N <sub>s</sub>   | 74.72 ± 11.48                            | 166.30 ± 25.43                           | 3.10 ± 0.59                              |
| D+N <sub>s</sub>   | 81.77 ± 8.82                             | 94.23 ± 10.63                            | 1.78 ± 0.19                              |
| H+Ba               | 86.48 ± 17.80                            | 197.00 ± 32.25 <sup>a</sup>              | 4.03 ± 0.71 <sup>a</sup>                 |
| D+Ba               | 61.92 ± 7.07                             | 137.80 ± 10.11                           | 3.02 ± 0.47                              |

Values expressed as mean ± SEM for 10 rats in each group. Values from Diabetic group were compared with that from control group and treatment groups were compared with diabetic group.

U/mg protein = Enzyme Units per milligram protein.

Values statistically significant at;

\*p < 0.05 and \*\*p < 0.001 compared with control

<sup>a</sup>p < 0.05 and <sup>b</sup>p < 0.001 compared with diabetic

## FIGURES

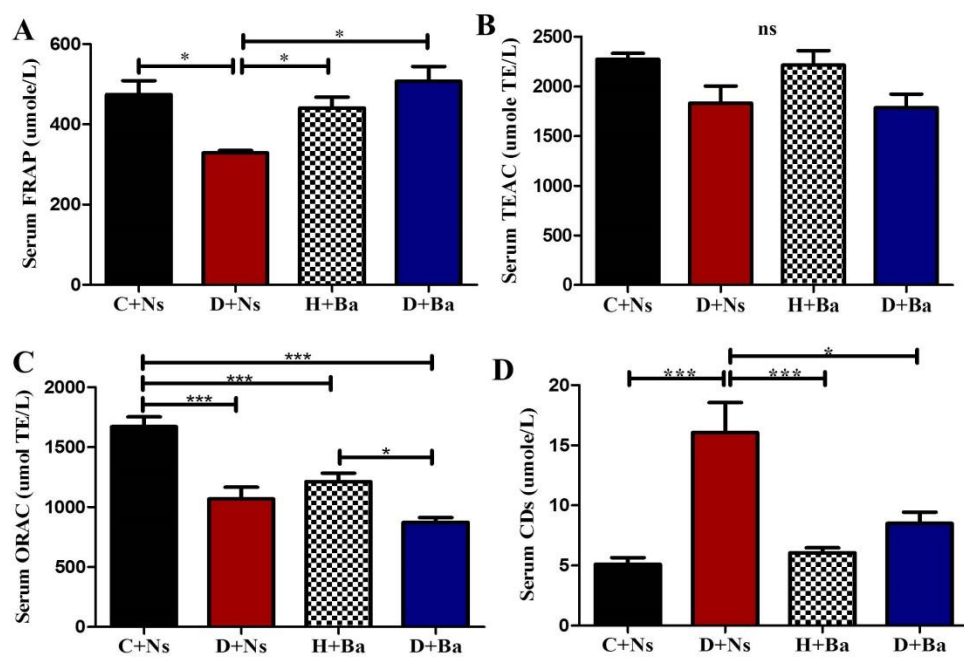


FIGURE 1. Antioxidant capacities and concentration of conjugated dienes in serum of rats from control and experimental groups.

[\* $p < 0.05$ , \*\* $p < 0.001$ , \*\*\* $p < 0.0001$ , ns = Differences not statistically significant]

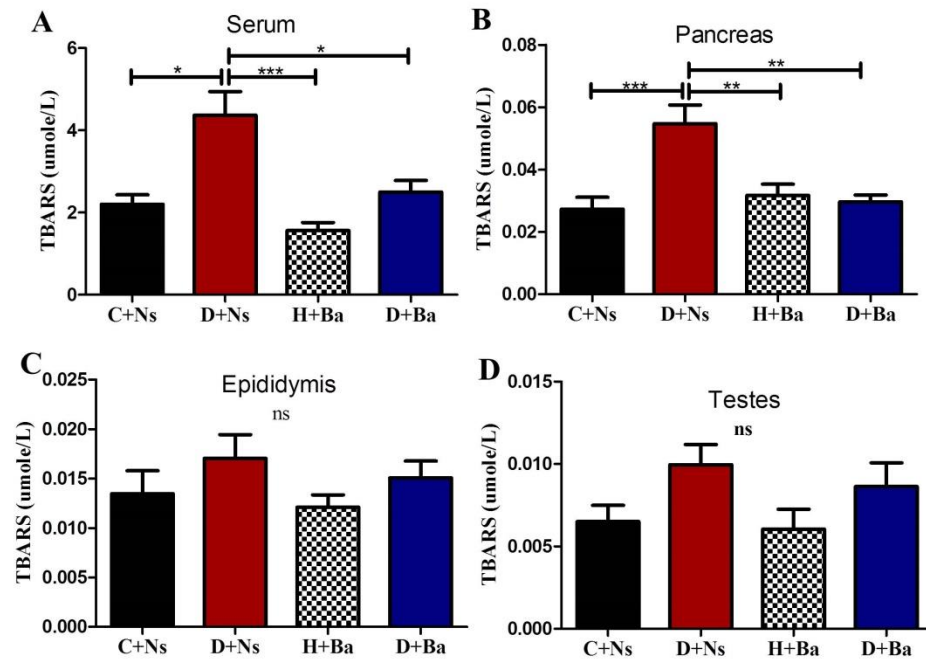


FIGURE 2. Concentration of thiobarbituric acid reactive substances (TBARS) in serum and tissue homogenates of rats from control and experimental groups.

[\*p<0.05, \*\*p<0.001, \*\*\*p<0.0001, ns = Differences not statistically significant]

## Chapter VII

### General Discussion and Conclusion

The evaluation of medicinal plants for the management of DM as reported in a number of previous studies have been largely based on the abilities of the plants to lower blood sugar either by mimicking insulin action or by stimulating increased insulin output (Patel *et al.*, 2012). The antihyperglycaemic activity of *Basella alba* was however not associated with any scientifically proven underlying mechanism prior to this study (Bamidele *et al.*, 2014; Azad *et al.*, 2016). The review of literature presented in Chapter II of this thesis emphasizes the key role played by oxidative stress in the occurrence of male reproductive complications of DM as well as many other complications of the disease (Moore and Wang, 2006; Hirata *et al.*, 2009; Mallidis *et al.*, 2011; Jain and Jangir, 2014). It is therefore not surprising that a large number of medicinal plants reported to alleviate reproductive dysfunctions in diabetic animals, achieve such effects by enhancing the *in vivo* antioxidant capacities of the animals (Mallick *et al.*, 2007; Abdolahnejad *et al.*, 2009; Shalaby and Hamowich, 2010; Thakur *et al.*, 2012; Wankeu-Nya *et al.*, 2013; Ghosh *et al.*, 2014; Khaki *et al.*, 2014). Expectedly, analysis of the antioxidant properties of *Basella alba* leaves formed the primary focus in the investigation of the mechanisms underlying its antidiabetic and profertility actions in this study.

#### 7.1 Determination of extraction solvent and dosage for *Basella alba* leaves

The conventional methods of treatment of DM presently available are frequently associated with challenges especially related to difficulty in maintaining normoglycaemia (Sena *et al.*, 2010), which underscores the importance of alternative therapies. Natural products such as vegetables in the form in which they are traditionally consumed are expected to exert little or no harmful effect on the body. The aqueous method of extraction was adopted for this study because it is believed to yield an extract with a composition that is similar to what the body absorbs from the vegetable following oral consumption as condiment with staple foods. Furthermore, from the preliminary comparative study reported in Chapter II, the aqueous extract of *Basella alba* had the

best antioxidant activity when compared to ethylacetate and methanol extracts of the plant. The study by Bamidele *et al* (2014) established the antidiabetic effect of *Basella alba* and reported a comparable degree of blood sugar reduction between diabetic rats treated with a standard drug (Metformin) and rats given *Basella alba* leave extract orally at a dose of 200 mg/kg body weight daily. This formed the basis for adopting 200 mg/kg body weight per day of the extract as the dosage for this study and the result thereof was consistent with the previous report.

## **7.2 Mechanisms of antidiabetic actions of *Basella alba* aqueous leave extract**

From the results of this study, a number of mechanistic explanations have been proffered with regards to the antidiabetic activities of *Basella alba* aqueous leave extract. The ability of the plant extract to significantly enhance the antioxidant capacity of blood and some biological tissues appear to be central to the prevention of diabetic complications and achievement of better glycaemic control. The antioxidant and antihyperglycaemic effects of the extract cannot be easily uncoupled into two unconnected processes. It is not exactly certain from this study which of the two is the primary actions, but both effects of the plant extract appear to potentiate each other in a mutual cycle. Prolonged elevation of blood glucose is a fundamental feature of DM that has been implicated in the pathophysiological explanation of most other features of the disease, including oxidative stress (Haskins *et al.*, 2003; Pandey *et al.*, 2010) and reproductive complications (Mallidis *et al.*, 2011). The antihyperglycaemic effect of *Basella alba* therefore played a major role in the correction of most diabetic induced reproductive complications as observed in the course of this study. Beyond simple stimulation of testosterone production by Leydig cells as reported in an earlier study (Nantia *et al.*, 2011), this study further demonstrated a rather regulatory effect of *Basella alba* on male gonadal hormones. This can be attributed to the removal or amelioration of the adverse effects of oxidative stress and AGEs on the hypothalamus-pituitary-testes axis (Jain and Jangir, 2014), via the antioxidant and antihyperglycaemic properties of the plant extract.

Modulation of the release of inflammatory cytokines is another key effect of *Basella alba* leave extract observed in this study to be a vital component of the overall antidiabetic

role of the plant. DM has been reportedly associated with chronic inflammation and systemic elaboration of many inflammatory biomarkers (Lontchi-Yimagou *et al.*, 2013). As a matter of fact, prolonged low-grade inflammation was described as critical to the natural course and complications of T2DM (Lontchi-Yimagou *et al.*, 2013), while the outcome of the immune attack on Beta-cells during the pathophysiology of T1DM is largely dependent on the Beta-cells' response to the inflammatory environment (Bending *et al.*, 2012). Generally, inflammatory processes are more frequently associated with T2DM where obesity-induced activation of adipocytes can result in increased circulating level of pro-inflammatory cytokines and initiation of insulin resistance (King, 2008). However, evidences from recent studies are beginning to lend credence to the importance of inflammatory processes in T1DM, especially in the progression of Beta-islet cell damage and worsening insulin deficiency (Devaraj *et al.*, 2007; King, 2008). Grunnet and Mandrup-Poulsen (2011), actually described T1DM as "an inflammatory disease of the pancreatic islet, in which insulin-producing Beta-cells are preferentially destroyed to varying degrees by the concerted action of auto-reactive T-cells and monocytic cells". This is corroborated in the findings of this present study by the higher serum levels of major mediators of inflammation like IL-1 $\beta$  and MCP-1 but lower anti-inflammatory cytokine, IL-10, observed in rats induced with T1DM when compared to control. Treatment with *Basella alba* leave extract in the diabetic treatment group of rats resulted in the reversal of this trend and elimination of the histological signs of local pancreatic inflammation that was observed in the untreated counterpart. It might be safe therefore, to assume the possibility of *Basella alba* scavenging for pro-inflammatory cytokines/chemokines and thereby limiting the progress of inflammatory damage to the Beta-islet cells of pancreas resulting in better insulin production. Further studies in this regard will involve quantification of the amount of insulin release with and without *Basella alba* treatment to fully substantiate this hypothesis.

Another striking observation in this study is the rather systemic nature of the antioxidant activities of *Basella alba*. The observed increases in the levels of enzymatic and non-enzymatic antioxidants like GSH, SOD and CAT following oral treatment with *Basella alba* aqueous leave extract was significant only in blood circulation but not in the pancreatic or the gonadal tissues. Similarly, the plant extract's protective effect against lipid peroxidation as evidenced by a lower concentration of TBARS in the diabetic

treatment group when compared to the diabetic control rats was also very significant in serum and pancreas but not in the gonadal tissues. Incidentally, diabetes induced oxidative stress is a system process with adverse effects on certain cellular processes particularly affecting tissues with lower intrinsic antioxidant defence mechanisms like the pancreatic islet cells (Yimam *et al.*, 2015). The fact that the antioxidant effect of *Basella alba* is more prominent at these sites of greater oxidative insult further support the suggestion that the extract may act by scavenging free radicals as well as mediators of inflammation.

### **7.3 Therapeutic potentials of *Basella alba* in the management of DM**

In a bid to circumvent the numerous challenges associated with conventional treatments of DM like insulin therapy and oral hypoglycaemic agents, attention in modern innovation is shifting towards curative therapy (Abdulazeez, 2015). The available curative treatment options currently being investigated are centred upon re-establishing endogenous production of insulin by transplantation of Beta-islet cells from human donors (Tatum *et al.*, 2017) or stem cell technology (Abdulazeez , 2015). These new innovations already come with their own limitations which include autoimmune rejection, availability/compatibility of donors, high cost among others. The histological observation of a recovery in the size of islet cell regions in diabetic rats treated with *Basella alba* extract offers a hope of future alternative in this regard. This suggest a possible natural means of achieving regeneration and replacement of lost Beta-islet cells devoid of the challenges associated with islet cell transplantation. Further studies are however required to fully characterize the events responsible for the observed increase in islet area before this can be ascertained.

The antioxidant and anti-inflammatory activities of *Basella alba* can also be exploited in the management of DM to achieve better scavenging for free radicals and proinflammatory cytokines/chemokines. This will significantly reduce the incidence of reproductive complications as well as other systemic complications of the disease and ensure better glycaemic control.

## 7.4 Conclusion

This thesis is a presentation of multiple congruent studies that highlighted new innovative insights into the mechanisms underlying the antidiabetic and profertility actions of orally administered aqueous leave extract of *Basella alba* in male Wistar rats. From the various reports, it can be concluded that the plant employs multiple mechanisms and the specific ones identified in this work include the following:

- Regulation of the release and actions of reproductive hormones; testosterone and luteinizing hormone probably through neutralization of the effect of diabetic induced oxidative stress on the hypothalamus-pituitary-testicular axis. This explains the improvement in reproductive parameters of diabetic rats following treatment with the plant extract as presented in Chapter IV.
- *Basella alba* modulates the serum levels of inflammatory cytokines and influence pancreatic islet proliferation and possibly function. The anti-inflammatory action is believed to contribute to the recovery of islet sections and better glycaemic control observed in treated diabetic rats as reported in Chapter V of this thesis.
- The antioxidant effect of the plant in diabetic rats appeared to be linked to the anti-hyperglycaemic effect and is predominantly a systemic, rather than localized tissue effect. Enhancement of reproductive parameters was achieved in the treated diabetic rats with significant differences only in the serum and pancreas antioxidant status but not in the gonadal tissues' antioxidant status. This is the observation in the study presented in Chapter VI.

Additionally, this work demonstrated in Chapter III that the aqueous extract of *Basella alba* leaves which is similar (in terms of composition) to the commonly consumed form of the vegetable, possesses better antioxidant activities when compared to extracts from common organic solvents like ethylacetate and methanol.

## 7.5 Further studies

This thesis has been able to explain a number of mechanisms underlying the antidiabetic effects of *Basella alba* aqueous leave extract based on the observations

from the component studies, however, many more aspects of this topic are yet unresolved:

- Further studies involving high-performance liquid chromatography (HPLC) to isolate active components of *Basella alba* leave extract is necessary. These specific components can then be used for similar experiments as described in this thesis with the aim of identifying the actual compound responsible for each effect. This will be highly instrumental to the possibility of eventually formulating a standard drug from the compounds inherent in the plant.
- There is need to design a study to specifically answer the question of 'whether or not insulin production is stimulated by the extract'. The design will include quantification of circulating insulin molecules in treatment and control groups.
- The observed increase in the size of islet cell sections following *Basella alba* extract administration raises interesting questions. Further studies involving differential staining of the islet cells and quantitative as well as qualitative analysis of the hormones produced by the various islet cell types will be very informative. It can then be ascertained if the observed enlargement is due to islet cell proliferation and which cell types are affected. Such a study will also involve insulin immune-staining to determine whether or not Beta-cells are functional in the experimental animals.
- Receptor studies via Western blotting and structural characterization of the bioactive components of the plant extract may also be helpful in determining the possibility of the plant extract having insulin like actions, ability to up-regulates insulin receptors or potentiates receptor-mediated insulin action.

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