UNIVERSITY OF KWAZULU-NATAL

Effects of *Momordica balsamina* methanolic extract on cardiovascular and haematological function in streptozotocin-induced diabetic rats: effects on selected markers

Asiphaphola Ludidi

2018

EFFECTS OF *MOMORDICA BALSAMINA* METHANOLIC EXTRACT ON CARDIOVASCULAR AND HAEMATOLOGICAL FUNCTION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS: EFFECTS ON SELECTED MARKERS

by

Asiphaphola Ludidi

216017652

Supervisor: Dr P.S Ngubane

Co-Supervisor: Dr A Khathi

Discipline of Human Physiology

School of Laboratory Medicine and Medical Sciences

College of Health Sciences

Date of submission: 16 January 2018

Submitted in fulfilment for the degree of Master of Medical Science in the School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal

Preface

Chronic hyperglycaemia observed in diabetic patients has been associated with the development of cardiovascular complications, which increases the risk of heart failure. In addition, hyperglycaemia induces haematological changes that compromise red blood cell function thus decreasing the oxygen-carrying capacity of the blood to the cardiovascular system, further aggravating cardiac disorders. The use of anti-diabetic agents have been associated with the progression of the pathology of haematological and cardiac function disorders. The World Health Organization however, has proposed the use of medicinal plants as an alternative as some of these plants possess anti-hyperglycaemic and cardio-protective properties which is of benefit in alleviating hyperglycaemia-induced cardiovascular complications. The goal therefore, is to investigate the effects of *Momordica balsamina* methanolic extract on haematological and cardiovascular function which may bring to light, the mechanisms by which this plant may use to ameliorate hyperglycaemia-induced cardiovascular complications in a streptozotocin-induced diabetic rat model.

Declaration

I, **Asiphaphola Ludidi** (student number: **216017652**), hereby declare that the dissertation entitled (**Effects of *Momordica balsamina* methanolic extract on cardiovascular and haematological function in streptozotocin-induced diabetic rats: effects on selected markers**) is the result of my own investigation and research and that it has not been submitted in part or full for any other degree or to any other University or Tertiary Institution. Where use was made of others work, it has been duly acknowledged. The research done in this study was carried out under the supervision of Dr P.S. Ngubane and Dr A. Khathi.

_____

Student: Asiphaphola Ludidi (216017652)

_____

Supervisor: Dr P.S. Ngubane

_____

Co-Supervisor: Dr A. Khathi

Date: _____

Plagiarism declaration

School of Laboratory Medicine and Medical Sciences, College of Health Sciences

MASTER'S DEGREE IN MEDICAL SCIENCES 2018

1. I know that plagiarism is wrong. Plagiarism is to use another's work and pretend that it is one's own.
2. Each contribution to, and quotation in, this thesis from the works of other people has been attributed and has been cited and referenced.
3. This thesis is my own work.
4. I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his or her own work.

Signature_____

Acknowledgements

I would like to firstly thank my God who has carried me through the duration of my master's degree and keeping me safe at all times as I walked alone in the middle of the night to and from campus, in preparation to submit my thesis. The following people have greatly contributed to the success of my project:

- Dr Phikelelani Ngubane who has been a great mentor to me and his constant smile even after missing submission dates. I am truly blessed to have a mentor that is patient and constantly believing in me even when I did not believe in myself. You have honestly helped me find my true passion for research.
- Dr Andile Khathi who has always kept a smile on his face, even when he was correcting me. Your mentorship and constant strife to make me the best researcher has truly made you more than a mentor to me, but an older brother. I will forever be grateful for your advice, jokes and warmth in your ways of imparting knowledge.
- Dr Ntethelelo Sibiya, Ms Anelisiwe Siboto, Mixo Charirty Baloyi, Lindokuhle Mabuza, Mr Mlindeli Gamede, Mluleki Luvuno and the rest of the UKZN endocrinology group, I appreciate your inputs and constant encouragement to do better. I would not have survived the unbearable long hours during experiments if you all had not been there to support me. May you continue giving and availing yourselves, selflessly to others as you did for me. In Xhosa we say "Umntu ngumntu ngabantu" meaning that nobody is an island, we all need someone. May God bless you all.
- My family and friends who have been praying for me and constantly encouraging me to not give up. Your love, consistent support and patience during all these academic years has motivated me to follow my true passion.
- Mr Dennis Makhubela, Bab'Dennis (as we call you), has been my father figure here in Durban. I know that seeing us mature and soar to greater heights brings you joy. I now understand why you have constantly availed yourself to assist me in any way you could. I hope to always make you proud. You are truly God sent to us.
- The academic and technical staff of the Human Physiology Department for their willingness to assist me in every way possible. Thank you.
- The UKZN Biomedical Resource Centre staff for the exceptional training, assistance and support during the time of conducting my animal studies. Thank you.
- The National Research Foundation and UKZN College of Health Science for the financial support towards my research project. Thank you.

Table of Contents

List of Figures

List of Tables

List of Appendices

Abbreviations list

AMPK	activated protein kinase
AGE	advanced glycation end product
ATP	adenosine triphosphate
ANOVA	one-way analysis of variance
AR	aldose reductase
α	alpha
APLT	aminophospholipid translocases
Ang-II	angiotensin-II
AREC	animal research ethics committee
β	beta
BRU	biomedical research unit
1, 3 BPG	1, 3 bisphosphoglycerate
BHT	butylated hydroxytoluene
Ca^{2+}	calcium ion
CML	carboxymethyllysine
CT-I	cardiotrophin-I
^{o}C	celcius
CLIA	chemiluminescence immunoassay
CCK	cholecystokinin
CoBNeST	Conference of Biomedical and Natural Sciences and Therapeutics
CHS	College of Health Sciences
CRP	C-reactive protein
3-DG	3-deoxyglucosone
DNA	deoxyribonucleic acid
DC	diabetic control
DM	diabetes mellitus
DAG	diacylglycerol
DMSO	dimethyl sulphoxide
DPX	distyrene plasticizer xylene
eNOS	endothelial cell NO synthase
ET-I	endothelin-I
EDHF	endothelium-derived hyperpolarizing factor

ELISA	enzyme-linked immunosorbent assay
EGCG	epigallocatechin-gallate
EPO	erythropoietin
FS	forward scatter
FITC	fluorescein isothiocyanate
FACS	Fluorescence-activated cell sorter
FFA	free fatty acids
GPx	glutathione peroxidase
G3P	glyceraldehyde 3-phosphate
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
Hb	haemoglobin
H & E	haematoxylin and eosin
H/B	heart to body weight ratio
HRP	horseradish peroxidase
h	hour
HCl	hydrochloric acid
H_2O_2	hydrogen peroxide
OH^{-2}	hydroperoxyl
OH^{-}	hydroxyl radical
INS	insulin
i.p.	intraperitoneally
JAK	Janus kinase
JNK	c-Jun-N terminal kinase pathway
Kg	kilogram
λ	lambda
L	litre
LDL	low-density lipoprotein
Ltd	limited
MA	masilinic acid
MDA	malondialdehyde
MAP	mean arterial pressure
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume

MTF	metformin
µ	micro
µg	micrograms
µl	microlitre
MV	microvesicle
m	milli
mg	milligram
ml	millilitre
mmHg	millimeters of mercury
MAPK	mitogen activated protein kinase
MB	*Momordica balsamina* methanolic extract
NAD^+	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NO_2	nitrogen dioxide
NO	nitric oxide
NC	non-diabetic control
NF-κβ	nuclear factor kappa-beta
PYY	peptide YY
p.o.	per os (orally)
PPAR	peroxisome proliferator-activated receptor
PBS	Phosphate buffered saline
PI3K	phosphatidylinositol-4, 5-bisphosphate
PS	phosphatidylserine
3-PK	3-phosphokinase
PLSCR	phospholipid scramblases
PUFA	polyunsaturated fatty acids
PKC	protein kinase C
ROS	reactive oxygen species
RAGE	receptor for advanced glycation end product
RBC	red blood cell
RLU	relative light unit
SS	Side scatter
STAT	signal transducer and activator of transcription
SD	Sprague-Dawley

SEM	standard error of means
SOD	superoxide dismutase
STZ	streptozocin
s.c.	subcutaneously
TBA	thiobarbituric acid
TBARs	thiobarbituric acid reactive substances
TNF α	tumuor necrosis factor α
T1D	type one diabetes
USA	United States of America
UKZN	University of KwaZulu-Natal

Study outline

The current dissertation is presented in manuscript format. It consists of 7 sections viz. dissertation abstract, chapter 1: introduction/literature review, chapter 2: manuscript 1, chapter 3: manuscript 2, chapter 4: synthesis, conclusions, and appendices. The dissertation abstract states the purpose of the study and summarizes the findings of the study. Chapter 1 is a brief background and a germane literature review to highlight the gaps that exist in literature and how the current study aims to fill these gaps. Chapter 2 is the first novel research paper that seeks to evaluate the effects of a medicinal plant, *Momordica balsamina* methanolic extract on cardiovascular dysfunction. A.Ludidi, A Khathi, N.H Sibiya and P.S Ngubane are authors to this paper. The manuscript is currently under review in the journal of Chemico-Biological Interaction. Chapter 3 entails the second research study manuscript, which sought to investigate the effects of *Momordica balsamina* methanolic extract on haematological function, which may unveil some of the mechanisms by which this plant may exert its cardio-protective effects. A Ludidi, M.C Baloyi, A Khathi, N.H Sibiya and P.S Ngubane are authors to this paper. The manuscript is prepared for submission to the journal of Biomedicine and pharmacotherapy. Chapter 4 is the synthesis, which discusses the link between the two studies and highlights the main findings for the specific aims of the current project. Appendices include the letter of ethical clearance, abstract and certificate of presentations to various conferences and journal's guideline to authors for both research papers.

Abstract

Background

The hyperglycaemia-induced haemanetic changes reduces the oxygen-carrying capacity of erythrocytes, thus aggravating cardiovascular disorders in diabetic patients. The conventional therapies have been shown to be associated with the progression of haematological and cardiovascular dysfunction, which may not be favorable for patients with congestive heart failure. We have previously shown the anti-hyperglycaemic and antioxidant properties of *Momordica balsamina* (MB) methanolic extract which may be of benefit in alleviating cardiovascular disorders, thus providing an effective alternative therapy. The current study therefore, investigated the short-term effects of MB methanolic extract on cardiovascular and haematological function in streptozotocin-induced diabetic rats.

Methods

Briefly, air-dried MB leaves were extracted with methanol to yield a methanolic extract. STZ-induced diabetic rats were divided into untreated and treated groups with insulin (170 μg kg^{-1} s.c.) and metformin (500 mg kg^{-1} p.o.) as standard drugs. MB (250 mg kg^{-1} p.o.) was administered twice daily for 5 weeks. Blood glucose concentration, body weight and blood pressure were monitored weekly for 5 weeks. Terminally, animals were sacrificed after which blood, heart and kidneys were collected for haematological and biochemical analysis. Histological analysis was also performed on the hearts.

Results

MB significantly decreased blood glucose concentration from week 3-5 by comparison with diabetic untreated animals. Treatment with MB reduced oxidative stress in the plasma, kidney and heart while improving their antioxidant status compared with untreated diabetic animals. This was associated with increased EPO secretion by the kidneys thus improving RBC production and haemoglobin concentrations. MB moderately increased erythrocyte indices: mean cell volume (MCV), mean cell haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) by comparison with untreated diabetic animals. MB ameliorated heart hypertrophy and decreased CRP, CT-I and Ang-II concentrations by comparison with untreated diabetic animals. MB also decreased MAP by comparison with untreated diabetic animals.

Conclusion

MB administration protects against hyperglycaemia-induced cardiovascular and haematological changes by attenuating hyperglycaemia, oxidative stress in both the kidney and heart tissues of STZ-induced diabetic rats, which may reduce the risks of cardiac myopathology complications in diabetes mellitus.

Chapter 1: Literature review

1. Background

Sustained hyperglycaemia is the pathogenic feature of DM which has been shown to result in microvascular and macrovascular complications (1, 2). Macrovascular complications have been found to be the most common cause of deaths amongst diabetic patients with cardiovascular disorders accounting for 52% of deaths in type 2 diabetes mellitus (T2DM) and 44% in type 1 diabetes mellitus (T1DM) (3, 4). Studies indicate that hyperglycaemia-induced haematological changes such as altered red blood cell function play a significant role in the progression of cardiac dysfunction (5). Although conventional treatment decreases hyperglycaemia, their use is associated with undesirable side effects which amongst many include increased blood viscosity which is of concern in patients with congestive heart failure (6). As a result, the progression of macrovascular complications intensifies even during treatment (7, 8). There is therefore, a need for alternative treatment, which will lower blood glucose concentration and alleviate complications associated with diabetes. Medicinal plants have been shown to possess, with minimal side effects, anti-hyperglycaemic and cardio-protective effects in STZ-induced diabetic animal models and therefore could potentially serve as an effective alternative (9-11). The mechanisms by which these medicinal plants exert their hypoglycaemic, anti-hyperglycaemic effects and ameliorate hyperglycaemia-induced cardiovascular disorders, are still to be fully understood. We have previously reported on the anti-hyperglycaemic and reno-protective properties of *Mormodica balsamina*, which may be beneficial in alleviating hyperglycaemia-induced cardiac dysfunction. This study therefore, investigated whether *Mormodica balsamina* can avert the decline of cardiac function often seen in diabetic humans and animal models. The following section discusses the effects of hyperglycaemia on cardiac function.

1.1 Effects of hyperglycaemia on cardiovascular function

Hyperglycaemia has been shown to increase reactive oxygen species (ROS) including superoxide resulting in oxidative stress, which induces mitochondrial dysfunction through caspase 3 activation, which induces apoptosis in cardiomyocyte (12, 13). Furthermore, ROS activates scramblases in red blood cell (RBC) membrane to expose phosphatidylserine (PS) to the extracellular space, where annexin-V, a marker for RBC undergoing apoptosis, which targets it for engulfment by macrophages (14, 15). The latter decreases RBC count which decreases the oxygen carrying capacity of blood, further aggravating cardiac dysfunction due to decreased oxygen supply to the cardiomyocytes. Studies however, have shown that some medicinal plants have the ability to decrease caspase 3 activation which may delay apoptosis in cardiomyocyte (16).

Other mechanisms that induce cardiac cell death include the increased activation of the polyol and protein kinase C (PKC) pathways and advanced glycation end products (AGEs) formation which are further discussed below (17-21).

1.1.1 The role of the polyol pathway in cardiac dysfunction

The polyol pathway is a metabolic pathway where aldose reductase (AR) uses nicotinamide adenine dinucleotide phosphate (NADPH) as its cofactor to reduce excess glucose to sorbitol. In addition, NADPH is required as a cofactor during the catalytic regeneration of glutathione by glutathione reductase which is an antioxidant enzyme (22). The accumulation of sorbitol as seen in cardiac cells induces osmotic stress which has been proposed to promote apoptosis, however the mechanism is still unknown (23). Sorbitol is oxidized by sorbitol dehydrogenase to fructose with nicotinamide adenine dinucleotide (NAD^+) being reduced to NADH (24). Hyperglycaemia therefore decreases the NAD^+/NADH ratio (25). However medicinal plants have been shown to possess phytochemicals that inhibit AR which may protect the heart from ischemic injury (26, 27). Furthermore, the up regulation of the polyol pathway has been shown to promote ROS production thus activating other cellular damaging mechanisms such as the protein kinase C (PKC) pathway which has been shown to induce haematological changes that shorten the life span of erythrocytes , further promoting cardiovascular dysfunction (28).

1.1.2 The role of the protein kinase C (PKC) pathway in cardiac dysfunction

As mentioned previously, the up regulation of the polyol pathway decreases the NAD^+/NADH ratio, which blocks NAD^+-dependant glyceraldehyde 3-phosphate dehydrogenase (GAPDH) from converting glyceraldehyde 3-phosphate (G3P) to 1,3 bisphosphoglycerate (1,3 BPG) (29). As a result, there is an increased amount of G3P, which

is a substrate for the synthesis of α-glycerol phosphate (29, 30). Furthermore, α-glycerol phosphate is a precursor of diacylglycerol (DAG) and DAG is known to activate some protein kinase C (PKC) isoforms (31). In the hearts of diabetic patients, both PKC-α and PKC-β isoforms are both upregulated which is associated with an irregular contractility progressing towards heart failure (32). The activation of the PKC has also been linked with the decreased RBC deformability thus inducing eryptosis (33). Medicinal plants such as *Centaurium erythraea* however, have been shown to decrease the activation of PKC in erythrocytes thereby restoring RBC membrane integrity and reducing the rate of agglutination (34, 35).

1.1.3 The role of advanced glycation end products (AGEs) in cardiac dysfunction

Hyperglycaemia has been shown to be associated with increased advanced glycation end products (AGEs) (36). AGEs develop when a reducing sugar such as glucose reacts non-enzymatically with an amino group of a protein to form Amadori products (37, 38). Then after days to weeks, the Amadori products undergo reactions that further rearrange to form irreversibly cross-linked senescent macroprotein derivatives which are called AGEs (37). When an AGE interacts with its receptor (RAGE), it generates oxidative stress in various cell types including cardiomyocytes and evoke inflammatory and thrombogenic reactions (39). AGEs thus play a significant role in the development and progression of cardiovascular complications in diabetic patients thus their formation and accumulation progress at accelerated rates in diabetic patients (40). Furthermore, AGEs have also been shown to induce the cross-linking of integral membrane proteins such as the sodium-potassium pump (Na^+/K^+ ATPase) of erythrocytes, resulting in the reduced activity of this pump (41, 42). Medicinal plants however, have been shown to possess flavonoids which decrease the AGEs-induced ROS production, this way restoring the function of glycated enzymes (43). The increased formation of AGEs, enhanced production of reactive oxygen species (ROS) and stimulation of protein kinase C (PKC), have been proposed to contribute to the endothelial dysfunction in diabetic patients which is further discussed below (44, 45).

1.2. Endothelial dysfunction

Free radicals also target and modify major extra-cellular and long lived proteins such as elastin, laminin and collagen to form glycoproteins (46). The presence of these glycoproteins in the vascular wall is associated with the development of endothelial dysfunction and cardiovascular complications including atherosclerosis (47). In addition, hyperglycaemia contributes to the progression of cardiovascular dysfunction, however, dislipidaemia, is another factor that contributes to endothelial dysfunction resulting in the development of cardiac dysfunction (48).

Endothelial dysfunction is defined as the inability of the endothelium to maintain vascular homeostasis, which is also associated with the decreased bioavailability of nitric oxide (NO) (49). This decrease is caused by the decreased expression of endothelial cell NO synthase (eNOS), inefficient eNOS activation, insufficient substrate or cofactors for eNOS and lastly, the accelerated degradation of NO_2 by ROS (50, 51). As a result, endothelial dysfunction is accompanied by increased vasoconstrictors including endothelin-1 (ET-1) and angiotensin II (49, 52). Additionally, there is increased thrombosis, cell growth in the vascular walls and poor regulation of inflammation (52). The endothelium orchestrates the production of vasodilators including NO, prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF) in order to maintain arterial tone and blood flow (53). Both vasoconstrictors and vasodilators work in synergy to maintain a homeostatic arterial patency and compliance (52). The pathology of endothelial dysfunction therefore results in increased arterial tone, which promotes vasospasm and increased arterial stiffness (54). Shear stress is the frictional force between blood flow and the endothelial wall (55). An increase in blood flow, increases shear stress however the diameter of the vessel is inversely proportional to shear stress (56). In healthy arteries, an increase in arterial flow which alters the diameter stimulates a "flow-mediated dilation", therefore decreasing shear stress, thus maintaining homeostasis (57). Additionally, the endothelium plays a role in maintaining the chronic changes in the structure of the arterial lumen because of chronic changes in blood flow (58). Endothelial dysfunction therefore contributes to the clinical expression of atherosclerosis leading to cardiovascular dysfunction (59). Atherosclerosis is the narrowing of the arteries due to endothelial injury and inflammation (60). Additionally, the accumulation of the oxidized low-density lipoprotein (LDL) particles in the arterial walls may be promoted by a vasoconstrictor known as angiotensin II (Ang- II),resulting in vascular blockage (61). The inhibition of vascular blood flow eventually leads to the bursting of the vascular wall (62). Furthermore, oxidized LDL accumulation, results in monocyctes infiltrating the arterial wall and differentiate into macrophages in order to accumulate oxidized lipids to form foam cells (63). These foam cells stimulate macrophages proliferation and attract T-lymphocytes. T-lymphocytes induce smooth muscle proliferation in the arterial walls and collagen accumulation resulting in the formation of a lipid-rich atherosclerotic lesion with a fibrous cap. Vascular occlusion is increased thereby increasing the risk of rupture, which further contributes to cardiovascular dysfunction. ROS has also been shown to also induce the nuclear factor-kappa B (NF-κB) to transcriptional activation of many transcriptional genes relevant to inflammation, immunity and atherosclerosis (64). As a result, there is elevated tumour necrosis factor α (TNF α), plasma

cardiotropin-I and C-reactive protein concentration in the circulation (65). These pro-inflammatory cytokines serve as experimental markers for the progression of cardiovascular diseases (65).

In addition, hyperglycaemia increases the accumulation of sorbitol in the cells, thus, osmotic stress has been postulated to underly the mechanism involved in the development of diabetic microvascular complications (66). Medicinal plants such as *Portulca oleracea* L. have been shown to improve endothelial dysfunction by promoting the activity of eNOS thereby restoring vasodilation and vasoconstriction which may improve the increased blood pressure of diabetic patients which may further improve cardiac dysfunction (67, 68).

Hyperglycaemia has also been shown to induce haemanetic changes including RBC dysfunction associated with an increased risk of developing cardiac dysfunction and diabetic anaemia if left untreated. These haemanetic changes are further discussed below.

1.3. Effects of hyperglycaemia on red blood cell function

1.3.1 Effects of hyperglycaemia-induced ROS on red blood cell function

Amongst the several factors that contribute to the haematological changes in diabetic patients, hyperglycaemia increases free radical accumulation resulting in oxidative stress, which target and damage cellular molecules namely: protein and lipids eventually altering cellular function (69-71). The up regulation of the polyol pathway as mentioned in the above section, increases the accumulation of sorbitol which results in osmotic stress (72). Osmotic stress has been shown to induce red blood cell (RBC) shrinkage as observed by a decrease in mean corpuscular volume (MCV) in diabetic patients (73, 74). The shrinkage of RBCs disturbs membrane proteins such calcium (Ca^{2+}) channels resulting in RBC death, a process known as eryptosis (75). Activated Ca^{2+}channels increase intracellular influx of Ca^{2+}, which activate aminophospholipid translocases (APLT) that activate phospholipid scramblases (PLSCR) thus inducing the exposure of phosphatidilne serine (PS) to the extracellular fluid (76). In addition, conversion of sorbitol to fructose, which is further metabolized to fructose-3-phosphate by 3-phosphokinase (3-PK), results in the generation of 3-deoxyglucosone (3-DG) (77). 3-DG is the central precursor of an array of AGEs, particularly carboxymethyllysine (CML)–protein adducts in erythrocytes (78). CML adducts have been shown to increase to thiobarbituric acid reactive substances (TBARs) including malondialdehyde (MDA) which increases oxidative stress thus increasing RBC membrane rigidity, decreasing the life-span of RBCs and promoting eryptosis (79). Cardiovascular function is therefore compromised; however, there are

scavenging systems or antioxidants such as superoxide dismutase (SOD), which neutralizes ROS. Furthermore, the imbalance of these protective antioxidant mechanisms due to hyperglycaemia as seen in DM, is associated with other complications such as increased lipid peroxidation (80). Medicinal plants such as *Ferulga angulata* have been shown to contain bioactive compounds that improve the antioxidant status including decreasing lipid peroxidation levels of experimental diabetic animals (81, 82). This could be of benefit in red blood cell function since lipid peroxidation induces hemolysis of RBC, thus increasing the risk of cardiovascular complications (83).

1.3.2 Effects of lipid peroxidation on RBC function

Lipid peroxidation is a result of increased ROS, with the hydroxyl radical (OH^-) and hydroperoxyl (OH^{-2}) profoundly affecting lipids. The OH^- radical is the most chemically reactive species of the ROS and produced from O_2 in cell metabolism. Lipid peroxidation is described as the process of oxidants such as free radicals or non-radical species attack lipids containing carbon-carbon double bond (s), that involve hydrogen abstraction from a carbon, with oxygen insertion resulting in lipid peroxyl radicals and hydroperoxides. Glycolipids, phospholipids and cholesterol and polyunsaturated fatty acids (PUFA) are targets for damaging and peroxidative modification (84). PUFA arachidonic acid, which is a component of cell membrane phospholipids, is peroxidized to finally form malondialdehyde (MDA) which is used as a biomarker for lipid peroxidation. The accumulation of MDA increases intracellular fluid viscosity, which disturbs haemoglobin (Hb) function, ultimately altering the function of RBC indices as observed by the decreased mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). As a result of prolonged decreased Hb concentrations, anaemia ensues due to the decreased Hb transporting oxygen to cells. The changes in RBC indices also result in decreased RBC fragility which is associated with a disrupting in the asymmetry of membrane phospholipids where cell membrane scrambling occurs accompanied with phosphatidylserine (PS) translocation to the external cell surface (85). PS exposure signals for eryptosis through phagocytosing cells engulfing targeted RBCs resulting in the rapid clearance of the affected erythrocytes (86). The increased rate of eryptosis, which invariably leads to the development of anaemia thus aggravating cardiovascular dysfunction (87, 88). However, the ability of some medicinal plants such as *Nigella sativa,* to stabilise the fragility of RBC thus decrease the rate of rupturing, which prolongs the life span of erythrocytes therefore, may improve diabetic myopathy (89, 90).

1.4 Effects of hyperglycaemia on erythropoietin secretion

Hyperglycaemia-induced microvascular complications including nephropathy, have been shown to be associated with a decrease in red blood cell formation since the kidney is the primary stimulus for erythropoiesis via erythropoietin (EPO) secretion (91, 92). Diabetic patients, as a result, develop anaemia if left untreated (93, 94). However, diabetic anaemia arises from a number of factors such as increased reactive oxygen (ROS) species and lipid peroxidation (95, 96). Some isolated derivatives of medicinal plants such as chlorigenic, caffeine and ferulic acid have been shown to improve EPO secretion thus erythropoiesis in experimental animals however (97, 98).

It is clear therefore that treatment strategies, should aim to lower blood glucose and alleviate hyperglycaemic-induced micro and macrovascular complications (99).

1.5. Management strategies

The management of hyperglycaemia and DM associated complications relies on various treatment strategies, which involves the use of anti-hyperglycaemic agents such as biguanides, sulphonylureas, alpha glucosidase inhibitors, thiozolidinediones and insulin injections (100). Metformin and insulin however, have been shown to affect cardiac and RBC function as highlighted below.

1.5.1 Metformin

Biguanides such as metformin, decreases hepatic glucose output and decreases fasting glycaemia (101). However, it may lead to renal dysfunction as it may increase lactic acidosis which is extremely rare (less than 1 case per 100,000 treated patients) but potentially fatal (102). Although metformin has been shown to have cardio-protective effects, it has been shown to contribute to the progression of anaemia through the malabsorption of vitamin B_{12} which is important for erythropoiesis and RBC maturation (103).

1.5.2 Insulin

Insulin is currently the primary means of treatment for DM however, the administration of insulin as a bolus is associated with hyperinsulinaemia which increases blood volume thus inducing cardiac failure (104). Increased blood volume/ venous return over prolonged periods, induces cardiac hypertrophy (105). Insulin has also been shown to promote fibrosis in cardiac muscle which disturbs cardiac contractility. Furthermore, increased insulin has also been shown to increase sodium retention and hence increases plasma volume and ultimately blood pressure (106). An increase in plasma volume is of concern in patients with congestive heart failure (107). Insulin has been shown to promote RBC agglutination which increases blood

viscosity and mean arterial pressure (MAP) which further aggravates cardiac dysfunction (107).

Studies have also indicated that conventional drugs are still not accessible in some parts of our rural communities (108-110). There is therefore a need for alternative treatment strategies that will lower blood glucose and alleviate hyperglycaemia-induced micro and macrovascular complications (111). Medicinal plants have been shown to possess, with minimal side effects, anti-hyperglycaemic, reno and cardio-protective effects in STZ-induced diabetic animal models and therefore could potentially serve as an effective alternative (112-116). The mechanisms by which these medicinal plants exert their hypoglycaemic, anti-hyperglycaemic and ameliorate hyperglycaemic induced complications such as cardiovascular disorders, are still to be fully understood. In this study, the focus is on *Momordica Balsamina'*s effect on selected markers for cardiovascular and haematological function.

1.5.3 Medicinal plants and their effects on haematological and cardiovascular function

Various medicinal plants such as *Parkia biglobosa, Cyclopia maculata* (honeybush) *Momordica charantia*, *Syzygium cordatum* (Hochst.) have been shown to possess anti-hyperglycaemic, reno-protective, cardiovascular effects and haemoprotective activities (117-120). These are amongst several thousands of medicinal plants used to manage diabetes in Africa (121, 122). *Parkia biglobosa* called by different local names such as 'afitin' in Benin City, Nigeria, has been shown to initiate insulin secretion from the pancreatic beta cells, however the seeds are often fermented and consumed for nutritional condiment (123). In addition, *Parkia biglobosa* has been shown to improve red blood cell count and haemoglobin concentrations of streptozotocin-induced diabetic rats (124). *Cyclopia maculata* (honeybush) which is rich in hesperidin has been shown to possess cardio-protective effects via the PPAR-c pathway in an ischemic heart disease model in diabetic rats (125). In addition, pre-treatment with hesperidin was shown to significantly improve mean arterial pressure, reduce left ventricular end-diastolic pressure, and improve both inotropic and lusitropic function of the heart as compared to controls (125). A plant derived flavanol with antioxidant properties, epigallocatechin-gallate (EGCG), has been shown to protect ROS induced cellular DNA, with a much higher potency in reducing lipid peroxidation, as a result, RBC membrane integrity may be improved (108, 126, 127). *Momordica charantia;* Karela (Hindi) and Bitter Gourd (English) is a common folklore remedy for DM as it has demonstrated anti-hyperglycaemic effects in various animal models (128). Extracts from the leaves, fruit pulp, seeds and the whole plant are used to treat diabetes in various anima models (128). Shibib and

colleagues, have previously shown an ethanolic extract of *Momordica charantia* to be anti-hyperglycaemic and stimulate erythropoiesis in STZ-induced diabetic rats (129). Razif, et al. have also shown the cardio-protective effects of *Momordica charantia* through improving the disturbed metabolism of the vasodilator nitric oxide, thereby decreasing blood pressure (130). However, the study focused on the function of the aorta and vascular function and did not highlight the effects of *Momordica charantia* on the cardiac muscle. Furthermore, studies conducted on *Momordica charantia* have not fully explored the effects of this plant on RBC structure and function (131). In our laboratory, we have previously shown anti-hyperglycaemic effects of leaf extracts from *Syzygium cordatum* (Hochst.) [Myrtaceae] (132). This plant has been shown to possess bioactive compounds such as masilinic acid (MA) which possesses reno-protective and cardio-protective effects through a number of mechanisms including decreasing oxidative stress while improving the antioxidant status which may be of benefit in alleviating haematological changes induced by oxidative stress as a result of hyperglycaemia (133). However, the structure and markers for cardiac damage such as hypertrophy which is marked by an increase in cardiotropin-I, was not evaluated to further establish the cardio-protective effects of MA in these animals. Furthermore, cardiac damage is further aggravated by RBC dysfunction which has also not been assessed in studies that use *Syzygium cordatum* (Hochst.) [Myrtaceae] as treatment. The effects of *Syzygium cordatum* on haematological parameters are therefore not fully understood.

Medicinal plants evidently improve cardiac function and erythropoiesis; however, further investigations are warranted on their effects on red blood cell structure and function. Compromising RBC structure increases the risk of RBC haemolysis and clearance, which exacerbates cardiac dysfunction in diabetic patients. Studying RBC function provides a tool to understand the mechanism/s by which these medicinal plants exert their cardio-protective effects.

1.6 Basis of the study

Of interest to our study is *Momordica balsamina,* a plant commonly known as “Intshungu” in isiZulu, South Africa, which is in the same family as *Momordica charantia*. *Momordica balsamina* is an African pumpkin that belongs to the cucurbitaceae family of plants widespread in Namibia, Botswana, Swaziland and all provinces of Southern Africa (134). The seeds, fruits, leaves and stems have been reported to contain nutritional and medicinal compounds that render them anti-hyperglycaemic (135). However, the effects of this plant on hyperglycaemic induced haematological changes and cardiovascular dysfunction have not been shown. In our

laboratory however, we have recently shown that MB possesses anti-hyperglycaemic and reno-protective effects and ameliorates kidney dysfunction in STZ-induced diabetic rats (136). Against this background, we investigated the effects of MB on cardiac dysfunction since renoprotection has been shown to be associated with improved EPO secretion, erythropoiesis and cardiovascular dysfunction in diabetic animal models (136). In addition, since we have previously shown the anti-hyperglycaemic effects of MB in STZ-induced diabetic rats, we sought to also investigate the effects of MB on hyperglycaemic induced red blood cell dysfunction in STZ-induced diabetic rats. This is in an effort to establish the mechanisms by which MB may improve cardiac dysfunction.

1.7 Aim

The aim of the study therefore is to evaluate the effects of *Momordica balsamina* methanolic extract on cardiovascular and haematological function in STZ-induced diabetic rats.

1.8 Objectives

The objectives of the study were to investigate the effects of *Momordica balsamina* methanolic extract on:

- blood glucose concentration of STZ-induced diabetic rats
- oxidative stress in the plasma, heart and kidney of STZ-induced diabetic rats
- mean arterial blood pressure in STZ-induced diabetic rats
- histological changes caused by hyperglycaemia on the cardiac muscle of STZ-induced diabetic rats
- endothelial function (plasma angiotensin-II concentration) in STZ-induced diabetic rats
- cardiac inflammation (heart cardiotrophin-I, heart and plasma CRP concentration) in STZ-induced diabetic rats
- red blood cell structure (annexin-V) and function (RBC profile) in STZ-induced diabetic rats

1.9 References

1. Chawla A, Chawla R, Jaggi S. Microvasular and macrovascular complications in diabetes mellitus: distinct or continuum? Indian journal of endocrinology and metabolism. 2016;20:546.

2. Domingueti CP, Dusse LMSA, das Graças Carvalho M, de Sousa LP, Gomes KB, Fernandes AP. Diabetes mellitus: the linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. Journal of diabetes and its complications. 2016;30:738-45.

3. Morrish N, Wang S-L, Stevens L, Fuller J, Keen H, Group WMS. Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. Diabetologia. 2001;44:14.

4. Tarquini R, Lazzeri C, Pala L, Rotella CM, Gensini GF. The diabetic cardiomyopathy. Acta diabetologica. 2011;48:173-81.

5. Salvagno GL, Sanchis-Gomar F, Picanza A, Lippi G. Red blood cell distribution width: A simple parameter with multiple clinical applications. Critical reviews in clinical laboratory sciences. 2015;52:86-105.

6. Varghese JF, Patel R, Yadav U. Metabolic syndrome: A forerunner of cardiovascular diseases. Edorium journal of biochemistry. 2017;2:4-6.

7. Mesa J. New insulin types in type 1 diabetes mellitus. Medicina clínica (english edition). 2015;145:70-5.

8. Chang H-Y, Li X, Karniadakis GE. Modeling of Biomechanics and Biorheology of Red Blood Cells in Type 2 Diabetes Mellitus. Biophysical journal. 2017;113:481-90.

9. Zeng Y, Guan M, Li C, Xu L, Zheng Z, Li J, Xue Y. Bitter melon (Momordica charantia) attenuates atherosclerosis in apo-E knock-out mice possibly through reducing triglyceride and anti-inflammation. Lipids in health and disease. 2018;17:251.

10. Kamboj A. Phytochemical characterization and antidiabetic potential of standardized total methanolic extract and phytosomes of Momordica dioica roxb. ex Willd. fruit. International journal of green pharmacy 2017;11:157-67.

11. Bahar E, Akter K, Lee G, Lee H, Rashid H, Choi M, Bhattarai K, Hossain M, Ara J, Mazumder K, Raihan O. β-Cell protection and antidiabetic activities of Crassocephalum crepidioides (Asteraceae) Benth. S. Moore extract against alloxan-induced oxidative stress via regulation of apoptosis and reactive oxygen species (ROS). BMC complementary and alternative medicine. 2017;17:179.

12. Barlaka E, Görbe A, Gáspár R, Pálóczi J, Ferdinandy P, Lazou A. Activation of PPARβ/δ protects cardiac myocytes from oxidative stress-induced apoptosis by suppressing generation of reactive oxygen/nitrogen species and expression of matrix metalloproteinases. Pharmacological research. 2015;95:102-10.

13. Nickel AG, Von Hardenberg A, Hohl M, Löffler JR, Kohlhaas M, Becker J, Reil JC, Kazakov A, Bonnekoh J, Stadelmaier M, Puhl SL. Reversal of mitochondrial transhydrogenase causes oxidative stress in heart failure. Cell metabolism. 2015;22:472-84.

14. Jagadish S, Hemshekhar M, NaveenKumar SK, Kumar KSS, Sundaram MS, Girish KS, Rangappa KS. Novel oxolane derivative DMTD mitigates high glucose-induced erythrocyte apoptosis by regulating oxidative stress. Toxicology and applied pharmacology. 2017;334:167-79.

15. Yu M, Xie R, Zhang Y, Liang H, Hou L, Yu C, Zhang J, Dong Z, Tian Y, Bi Y, Kou J. Phosphatidylserine on microparticles and associated cells contributes to the hypercoagulable state in diabetic kidney disease. Nephrology dialysis transplantation. 2018;33:115-2127.

16. Atawodi SE, Adepoju OA, Nzelibe HC. Antihyperglycaemic and hypolipidemic effect of methanol extracts of Ageratum conyzoides L (Asteraceae) in normal and diabetic rats. Tropical journal of pharmaceutical research. 2017;16:989-96.

17. Forbes JM, Cooper ME. Mechanisms of Diabetic Complications. Physiological Reviews. 2013;93:137-88.

18. Fowler MJ. Microvascular and macrovascular complications of diabetes. Clinical diabetes. 2011;29:116-22.

19. Seferović PM, Paulus WJ. Clinical diabetic cardiomyopathy: a two-faced disease with restrictive and dilated phenotypes. European heart journal. 2015;36:1718-27.

20. Ojha S, Kurdi A, Sadek B, Kaleem M, Cai L, A Kamal M, Rajesh M. Phytochemicals as prototypes for pharmaceutical leads towards drug development against diabetic cardiomyopathy. Current pharmaceutical design. 2016;22:3058-70.

21. Hansen SS, Aasum E, Hafstad AD. The role of NADPH oxidases in diabetic cardiomyopathy. Biochimica et biophysica Acta (BBA)-molecular basis of disease. 2018;1864:1908-13.

22. Kurian GA, Rajagopal R, Vedantham S, Rajesh M. The role of oxidative stress in myocardial ischemia and reperfusion injury and remodeling: revisited. Oxidative medicine and cellular longevity. 2016;2016:1-14.

23. Sasso FC, Rinaldi L, Lascar N, Marrone A, Pafundi PC, Adinolfi LE, Marfella R. Role of Tight Glycemic Control during Acute Coronary Syndrome on CV Outcome in Type 2 Diabetes. Journal of diabetes research. 2018;2018:8.

24. Anupama N, Rani MP, Shyni G, Raghu K. Glucotoxicity results in apoptosis in H9c2 cells via alteration in redox homeostasis linked mitochondrial dynamics and polyol pathway and possible reversal with cinnamic acid. Toxicology in Vitro. 2018;53:178-92.

25. Jagdale AD, Bavkar LN, More TA, Joglekar MM, Arvindekar AU. Strong inhibition of the polyol pathway diverts glucose flux to protein glycation leading to rapid establishment of secondary complications in diabetes mellitus. Journal of diabetes and its complications. 2016;30:398-405.

26. Tang J, Zuo M. Assessment of renal function and oxidative stress after alprostadil combined with valsartan treatment of early diabetic nephropathy. Journal of Hainan medical university. 2017;23:157-60.

27. Bonini SA, Premoli M, Tambaro S, Kumar A, Maccarinelli G, Memo M, Mastinu A. Cannabis sativa: A comprehensive ethnopharmacological review of a medicinal plant with a long history. Journal of ethnopharmacology. 2018;227:300-315.

28. Mortuza R, Chakrabarti S. Glucose-induced cell signaling in the pathogenesis of diabetic cardiomyopathy. Heart failure reviews. 2014;19:75-86.

29. Park YJ, Choe SS, Sohn JH, Kim JB. The role of glucose-6-phosphate dehydrogenase in adipose tissue inflammation in obesity. Adipocyte. 2017;6:147-53.

30. Shamsaldeen YA, Alsugoor MH, Lione LA, Benham CD. Dysfunction in nitric oxide synthesis in streptozotocin treated rat aorta and role of methylglyoxal. European journal of pharmacology. 2019;842:321-8.

31. Yang Q, Vijayakumar A, Kahn BB. Metabolites as regulators of insulin sensitivity and metabolism. Nature Reviews Molecular Cell Biology. 2018;19:654-72.

32. Tham YK, Bernardo BC, Ooi JY, Weeks KL, McMullen JR. Pathophysiology of cardiac hypertrophy and heart failure: signaling pathways and novel therapeutic targets. Archives of toxicology. 2015;89:1401-38.

33. Nguyen DB, Ly TBT, Wesseling MC, Hittinger M, Torge A, Devitt A, Perrie Y, Bernhardt I. Characterization of microvesicles released from human red blood cells. Cellular physiology and biochemistry. 2016;38:1085-99.

34. Zhirnov VV, Iakovenko IN. The osmotic resistance, and zeta potential responses of human erythrocytes to transmembrane modification of Ca2+ fluxes in the presence of the imposed low rate radiation field of 90Sr. International journal of radiation biology. 2015;91:117-26.

35. Đorđević M, Mihailović M, Jovanović JA, Grdović N, Uskoković A, Tolić A, Sinadinović M, Rajić J, Mišić D, Šiler B, Poznanović G. Centaurium erythraea methanol extract protects red blood cells from oxidative damage in streptozotocin-induced diabetic rats. Journal of ethnopharmacology. 2017;202:172-83.

36. Simm A, Müller B, Nass N, Hofmann B, Bushnaq H, Silber R, Bartling B. Protein glycation—Between tissue aging and protection. Experimental gerontology. 2015;68:71-5.

37. Chilelli NC, Burlina S, Lapolla A. AGEs, rather than hyperglycemia, are responsible for microvascular complications in diabetes: A “glycoxidation-centric” point of view. Nutrition, metabolism and cardiovascular diseases. 2013;23:913-9.

38. Yamagishi S, Matsui T, Ishibashi Y, Isami F, Abe Y, Sakaguchi T, Higashimoto Y. Phytochemicals against advanced glycation end products (AGEs) and the receptor system. Current pharmaceutical design. 2017;23:1135-41.

39. Yamagishi S-i, Fukami K, Matsui T. Crosstalk between advanced glycation end products (AGEs)-receptor RAGE axis and dipeptidyl peptidase-4-incretin system in diabetic vascular complications. Cardiovascular diabetology. 2015;14:2.

40. Abdallah HM, El-Bassossy H, Mohamed GA, El-Halawany AM, Alshali KZ, Banjar ZM. Phenolics from garcinia mangostana inhibit advanced glycation endproducts formation: effect on amadori products, cross-linked structures and protein thiols. Molecules. 2016;21:251.

41. Humayoun M, Khalid A, Mahmood M, Hussain S. Evaluation of Sodium Pumps Activity in Patients of Lahore City Suffering from Diabetes Mellitus Type. Pakistan journal of medical & health sciences. 2016;10:643-5.

42. Nowotny K, Jung T, Höhn A, Weber D, Grune T. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. Biomolecules. 2015;5:194-222.

43. Ramlagan P, Rondeau P, Planesse C, Neergheen-Bhujun V, Bourdon E, Bahorun T. Comparative suppressing effects of black and green teas on the formation of advanced glycation end products (AGEs) and AGE-induced oxidative stress. Food & function. 2017;8:4194-209.

44. Behl T, Kotwani A. Potential of angiotensin II receptor blockers in the treatment of diabetic retinopathy. Life sciences. 2017;176:1-9.

45. Russell N, O'Brien RC. Diabetes and hypertension: is treating hyperglycemia useful? Diabetes and hypertension. 2016:136.

46. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TPA. Recent advances in Indian herbal drug research guest editor: Thomas Paul Asir Devasagayam Indian herbs and herbal drugs used for the treatment of diabetes. Journal of clinical biochemistry and nutrition. 2007;40:163-73.

47. Adamopoulos C, Piperi C, Korkolopoulou P, Dalagiorgou G, Spyropoulou A, Gargalionis AN, Kandaraki EA, Papavassiliou A. Interference of Advanced Glycation

End-products signaling with collagen cross-linking in human endothelium. Endocrine. 2016;15:18.

48. Karunanayake E, Welihinda J, Sirimanne S, Adorai GS. Oral hypoglycaemic activity of some medicinal plants of Sri Lanka. Journal of ethnopharmacology. 1984;11:223-31.

49. Grover J, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. Journal of ethnopharmacology. 2002;81:81-100.

50. Matsuda H, Yuhao L, Murakami T, Matsumura N, Yamahara J, Yoshikawa M. Antidiabetic principles of natural medicines. III. Structure-related inhibitory activity and action mode of oleanolic acid glycosides on hypoglycemic activity. Chemical and pharmaceutical bulletin. 1998;46:1399-403.

51. Förstermann U, Xia N, Li H. Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. Circulation research. 2017;120:713-35.

52. Singh K, Chandra V, Barthwal K. Letter to the editor: Hypoglycaemic activity of Acacia arabica, Acacia benthami and Acacia modesta leguminous seed diets in normal young albino rats. Indian journal of physiology and pharmacology. 1975;19:167.

53. Barthelmes J, Nägele MP, Ludovici V, Ruschitzka F, Sudano I, Flammer AJ. Endothelial dysfunction in cardiovascular disease and Flammer syndrome—similarities and differences. EPMA Journal. 2017;8:99-109.

54. Liu J, Yao J, Li X, Song Y, Wang X, Li Y, Yan B, Jiang Q. Pathogenic role of lncRNA-MALAT1 in endothelial cell dysfunction in diabetes mellitus. Cell death & disease. 2014;5:1506.

55. White S, Satta S, Hazell G, Teasdale J, Peachey A, Sala-Newby G, McKay T, Johnson JL, Alexander Y. P3487Progress towards a tissue culture model to investigate endothelial erosion of plaques. European heart journal. 2017;38:3487.

56. Ahmed I, Adeghate E, Sharma A, Pallot D, Singh J. Effects of Momordica charantia fruit juice on islet morphology in the pancreas of the streptozotocin-diabetic rat. Diabetes research and clinical practice. 1998;40:145-51.

57. Beyer AM, Zinkevich N, Miller B, Liu Y, Wittenburg AL, Mitchell M, Galdieri R, Sorokin A, Gutterman DD. Transition in the mechanism of flow-mediated dilation with aging and development of coronary artery disease. Basic research in cardiology. 2017;112:5.

58. Canović EP, Zollinger AJ, Tam SN, Smith ML, Stamenović D. Tensional homeostasis in endothelial cells is a multicellular phenomenon. American journal of physiology-cell physiology. 2016;311:528-35.

59. Gimbrone Jr MA, García-Cardeña G. Endothelial cell dysfunction and the pathobiology of atherosclerosis. Circulation research. 2016;118:620-36.

60. Singh R, Devi S, Gollen R. Role of free radical in atherosclerosis, diabetes and dyslipidaemia: larger-than-life. Diabetes/metabolism research and reviews. 2015;31:113-26.

61. Cahill PA, Redmond EM. Vascular endothelium–gatekeeper of vessel health. Atherosclerosis. 2016;248:97-109.

62. Kattoor AJ, Pothineni NVK, Palagiri D, Mehta JL. Oxidative stress in atherosclerosis. Current atherosclerosis reports. 2017;19:42.

63. Groh L, Keating ST, Joosten LA, Netea MG, Riksen NP, editors. Monocyte and macrophage immunometabolism in atherosclerosis. 2018;40:203214.

64. Basta G, Lazzerini G, Massaro M, Simoncini T, Tanganelli P, Fu C, Kislinger T, Stern DM, Schmidt AM, De Caterina R. Advanced glycation end products activate endothelium through signal-transduction receptor RAGE. Circulation. 2002;105:816-22.

65. Gullestad L, Ueland T, Vinge LE, Finsen A, Yndestad A, Aukrust P. Inflammatory cytokines in heart failure: mediators and markers. Cardiology. 2012;122:23-35.

66. Bjornstad P, Lanaspa MA, Ishimoto T, Kosugi T, Kume S, Jalal D, Maahs DM, Snell-Bergeon JK, Johnson RJ, Nakagawa T. Fructose and uric acid in diabetic nephropathy. Diabetologia. 2015;58:1993-2002.

67. Afolabi Clement Akinmoladun M, Olaleye T, Farombi EO. Cardiotoxicity and Cardioprotective Effects of African Medicinal Plants. Toxicological survey of African medicinal plants. 2014;395.

68. Zhou Y, Xin H, Rahman K, Wang S-J, Peng C, Zhang H. Portulaca oleracea L.: a review of phytochemistry and pharmacological effects. BioMed research international. 2015;2015.

69. Nwose E, Jelinek H, Richards R, Kerr P. Erythrocyte oxidative stress in clinical management of diabetes and its cardiovascular complications. British journal of biomedical science. 2007;64:35-43.

70. Mahmood R, Qasim NM. Protective effect of creatine against hyperglycemia induced oxidative damage in human erythrocytes. Free radical biology and medicine. 2017;112:32.

71. Khan AN, Khan RA, Ahmad M, Mushtaq N. Role of antioxidant in oxidative stress and diabetes mellitus. Journal of pharmacognosy and phytochemistry. 2015;3:217-20.

72. Jamwal S, Sharma S. Vascular endothelium dysfunction: a conservative target in metabolic disorders. Inflammation research. 2018:1-15.

73. Pinzón-Díaz CE, Calderón-Salinas JV, Rosas-Flores MM, Hernández G, López-Betancourt A, Quintanar-Escorza MA. Eryptosis and oxidative damage in hypertensive and dyslipidemic patients. Molecular and cellular biochemistry. 2018;440:105-13.

74. Lang E, Lang F. Triggers, inhibitors, mechanisms, and significance of eryptosis: the suicidal erythrocyte death. Biomed research international. 2015;2015:1-6.

75. Qadri SM, Bissinger R, Solh Z, Oldenborg P. Eryptosis in health and disease: A paradigm shift towards understanding the (patho) physiological implications of programmed cell death of erythrocytes. Blood reviews. 2017;31:349-61.

76. Bevers EM, Williamson PL. Getting to the outer leaflet: physiology of phosphatidylserine exposure at the plasma membrane. Physiological reviews. 2016;96:605-45.

77. Henning C, Glomb MA. Pathways of the Maillard reaction under physiological conditions. Glycoconjugate journal. 2016;33:499-512.

78. Maessen DE, Stehouwer CD, Schalkwijk CG. The role of methylglyoxal and the glyoxalase system in diabetes and other age-related diseases. Clinical science. 2015;128:839-61.

79. Gwozdzinski K, Pieniazek A, Tabaczar S, Jegier A, Brzeszczynska J. Investigation of oxidative stress parameters in different lifespan erythrocyte fractions in young untrained men after acute exercise. Experimental physiology. 2017;102:190-201.

80. de Souza Bastos A, Graves DT, de Melo Loureiro AP, Júnior CR, Corbi SCT, Frizzera F, Scarel-Caminaga RM, Câmara NO, Andriankaja OM, Hiyane MI, Orrico SR. Diabetes and increased lipid peroxidation are associated with systemic inflammation even in well-controlled patients. Journal of diabetes and its complications. 2016;30:1593-9.

81. Gamede M, Mabuza L, Ngubane P, Khathi A. The Effects of Plant-Derived Oleanolic Acid on Selected Parameters of Glucose Homeostasis in a Diet-Induced Pre-Diabetic Rat Model. Molecules. 2018;23:794.

82. Ngubane PS, Masola B, Musabayane CT. The effects of Syzygium aromaticum-derived oleanolic acid on glycogenic enzymes in streptozotocin-induced diabetic rats. Renal failure. 2011;33:434-9.

83. Sheweita S, Mashaly S, Newairy A, Abdou H, Eweda S. Changes in oxidative stress and antioxidant enzyme activities in streptozotocin-induced Diabetes mellitus in rats: Role of Alhagi Maurorum extracts. Oxidative medicine and cellular longevity. 2016;2016:1-8.

84. Gallo G, Bruno R, Taranto A, Martino G. Are polyunsaturated fatty acid metabolites, the protective effect of 4-hydroxytyrosol on human red blood cell membranes and oxidative damage (4-hydroxyalkenals) compatible in hypertriglyceridemic patients? Pharmacognosy magazine. 2017;13:561.

85. Waibel S, Bissinger R, Bouguerra G, Abbès S, Lang F. Saquinavir Induced Suicidal Death of Human Erythrocytes. Cellular physiology and biochemistry. 2015;37:1973-82.

86. Almasry M, Jemaà M, Mischitelli M, Faggio C, Lang F. Stimulation of Suicidal Erythrocyte Death by Phosphatase Inhibitor Calyculin A. Cellular physiology and biochemistry. 2016;40:163-71.

87. Calderón-Salinas JV, Muñoz-Reyes EG, Guerrero-Romero JF, Rodríguez-Morán M, Bracho-Riquelme RL, Carrera-Gracia MA, Quintanar-Escorza MA. Eryptosis and oxidative damage in type 2 diabetic mellitus patients with chronic kidney disease. Molecular and cellular biochemistry. 2011;357:171-9.

88. Prestes AdS, dos Santos MM, Ecker A, Zanini D, Schetinger MRC, Rosemberg DB, da Rocha JB, Barbosa NV. Evaluation of methylglyoxal toxicity in human erythrocytes, leukocytes and platelets. Toxicology mechanisms and methods. 2017;27:307-17.

89. Barros FJ, Costa RJO, Cesário FRAS, Rodrigues LB, da Costa JGM, Coutinho HDM, Galvao HB, de Menezes IR. Activity of essential oils of Piper aduncum anf and Cinnamomum zeylanicum by evaluating osmotic and morphologic fragility of erythrocytes. European journal of integrative medicine. 2016;8:505-12.

90. Amin HAM, Arihan O, Ragbetli MC. Effect of thymoquinone administration on erythrocyte fragility in diethylnitrosamine administered rats. Journal of cellular biotechnology. 2017;3:1-7.

91. Carlsson P-O, Korsgren O, Le Blanc K. Mesenchymal stromal cells to halt the progression of type 1 diabetes? Current diabetes reports. 2015;15:46.

92. Droguett A, Marchant V, Sanchez Y, Valderrama G, Burgos ME, Carpio D, Kerr B, Ruiz-Ortega M, Egido J, Mezzano S. FP448 Tubular overexpression of gremlin in transgenic mice aggravates renal damage in diabetic nephropathy. Nephrology dialysis transplanation. 2015;30:221.

93. Niu H, Chang C, Niu C, Cheng J, Lee K. Erythropoietin ameliorates hyperglycemia in type 1-like diabetic rats. Drug design, development and therapy. 2016;10:1877.

94. Oh K, Lee D, Kim W, Han B, Lee S, Bae K. Metabolic adaptation in obesity and type II diabetes: myokines, adipokines and hepatokines. International journal of molecular sciences. 2016;18:8.

95. Gradinaru D, Margina D, Ilie M, Borsa C, Ionescu C, Prada G. Correlation between erythropoietin serum levels and erythrocyte susceptibility to lipid peroxidation in elderly with type 2 diabetes. Acta physiologica hungarica. 2015;102:400-8.

96. Brown DI, Griendling KK. Regulation of signal transduction by reactive oxygen species in the cardiovascular system. Circulation research. 2015;116:531-49.

97. Ekweogu C, Nwankpa P, Egwurugwu J, Etteh C, Ugwuezumba P, Chukwuemeka O. Effects of Ethanol Extract of Cola lepidota Seed on Lipid Profile and Haematological Parameters of Albino Wistar Rats. International journal of microbiology and applied sciences. 2018;7:3178-86.

98. Li W, Tang Y, Qian Y, Shang E, Wang L, Zhang L, Su S, Duan JA. Comparative analysis of main aromatic acids and phthalides in Angelicae Sinensis Radix, Chuanxiong Rhizoma, and Fo-Shou-San by a validated UHPLC–TQ-MS/MS. Journal of pharmaceutical and biomedical analysis. 2014;99:45-50.

99. Donaghue KC, Wadwa RP, Dimeglio LA, Wong TY, Chiarelli F, Marcovecchio ML, Salem M, Raza J, Hofman PL, Craig ME. Microvascular and macrovascular complications in children and adolescents. Pediatric diabetes. 2014;15:257-69.

100. Chakrabarti R, Rajagopalan R. Diabetes and insulin resistance associated disorders: disease and the therapy. Current science-Bangalore-. 2002;83:1533-8.

101. Foretz M, Guigas B, Bertrand L, Pollak M, Viollet B. Metformin: from mechanisms of action to therapies. Cell metabolism. 2014;20:953-66.

102. Tahrani A, Varughese G, Scarpello J, Hanna F. Metformin, heart failure, and lactic acidosis: is metformin absolutely contraindicated? BMJ: British medical journal. 2007;335:508.

103. Liu KW, Dai LK, Jean W. Metformin-related vitamin B12 deficiency. Age and ageing. 2006;35:200-1.

104. Sankaralingam S, Alrob OA, Zhang L, Jaswal JS, Wagg CS, Fukushima A, Padwal RS, Johnstone DE, Sharma AM, Lopaschuk GD. Lowering body weight in obese mice with diastolic heart failure improves cardiac insulin sensitivity and function: implications for the obesity paradox. Diabetes. 2015:141050.

105. Riehle C, Abel ED. Insulin signaling and heart failure. Circulation research. 2016;118:1151-69.

106. Herman ME, O'Keefe JH, Bell DS, Schwartz SS. Insulin therapy increases cardiovascular risk in type 2 diabetes. Progress in cardiovascular diseases. 2017;60:422-34.

107. Tahrani AA, Bailey CJ, Del Prato S, Barnett AH. Management of type 2 diabetes: new and future developments in treatment. The Lancet. 2011;378:182-97.

108. McEwen LN, Casagrande SS, Kuo S, Herman WH. Why Are Diabetes Medications So Expensive and What Can Be Done to Control Their Cost? Current diabetes reports. 2017;17:71.

109. Chow CK, Ramasundarahettige C, Hu W, AlHabib KF, Avezum Jr A, Cheng X, Chifamba J, Dagenais G, Dans A, Egbujie BA, Gupta R. Availability and affordability of essential medicines for diabetes across high-income, middle-income, and low-income countries: a prospective epidemiological study. The lancet Diabetes & endocrinology. 2018;6:798-808.

110. Boutayeb A, Lamlili M, Boutayeb W. Health promotion and diabetes care in developing countries. Diabetes & metabolism. 2016;42:302.

111. Mutyambizi C, Pavlova M, Chola L, Hongoro C, Groot W. Cost of diabetes mellitus in Africa: a systematic review of existing literature. Globalization and Health. 2018;14:3.

112. Mahmoud MF, El Ashry FEZZ, El Maraghy NN, Fahmy A. Studies on the antidiabetic activities of Momordica charantia fruit juice in streptozotocin-induced diabetic rats. Pharmaceutical biology. 2017;55:758-65.

113. Al-Shaqha WM, Khan M, Salam N, Azzi A, Chaudhary AA. Anti-diabetic potential of Catharanthus roseus Linn. and its effect on the glucose transport gene (GLUT-2 and GLUT-4) in streptozotocin induced diabetic wistar rats. BMC complementary and alternative medicine. 2015;15:379.

114. Zangeneh MM, Goodarzi N, Zangeneh A, Tahvilian R, Najafi F. Amelioration of renal structural changes in STZ-induced diabetic mice with ethanolic extract of Allium saralicum RM Fritsch. Comparative clinical pathology. 2018:1-7.

115. Dludla PV, Muller CJ, Joubert E, Louw J, Essop MF, Gabuza KB, Ghoor S, Huisamen B, Johnson R. Aspalathin protects the heart against hyperglycemia-induced oxidative damage by up-regulating Nrf2 expression. Molecules. 2017;22:129.

116. Toma A, Makonnen E, Mekonnen Y, Debella A, Adisakwattana S. Antidiabetic activities of aqueous ethanol and n-butanol fraction of Moringa stenopetala leaves in streptozotocin-induced diabetic rats. BMC complementary and alternative medicine. 2015;15:242.

117. Ogunyinka BI, Oyinloye BE, Osunsanmi FO, Opoku AR, Kappo AP. Protective effects of Parkia biglobosa protein isolate on streptozotocin-induced hepatic damage and oxidative stress in diabetic male rats. Molecules. 2017;22:1654.

118. Ajuwon O, Ayeleso A, Adefolaju G. The Potential of South African Herbal Tisanes, Rooibos and Honeybush in the Management of Type 2 Diabetes Mellitus. Molecules. 2018;23:3207.

119. Czompa A, Gyongyosi A, Szoke K, Bak I, Csepanyi E, Haines DD, Tosaki A, Lekli I. Effects of Momordica charantia (Bitter Melon) on ischemic diabetic myocardium. Molecules. 2017;22:488.

120. Maroyi A. Syzygium Cordatum Hochst. ex Krauss: An Overview of Its ethnobotany, phytochemistry and pharmacological properties. Molecules. 2018;23:1084.

121. Cemek M, Kağa S, Şimşek N, Büyükokuroğlu ME, Konuk M. Antihyperglycemic and antioxidative potential of Matricaria chamomilla L. in streptozotocin-induced diabetic rats. Journal of natural medicines. 2008;62:284-93.

122. Erukainure OL, Narainpersad N, Singh M, Olakunle S, Islam MS. Clerodendrum volubile inhibits key enzymes linked to type 2 diabetes but induces cytotoxicity in human embryonic kidney (HEK293) cells via exacerbated oxidative stress and proinflammation. Biomedicine & pharmacotherapy. 2018;106:1144-52.

123. Ibrahim MA, Habila JD, Koorbanally NA, Islam MS. Butanol fraction of Parkia biglobosa (Jacq.) G. Don leaves enhance pancreatic β-cell functions, stimulates insulin secretion and ameliorates other type 2 diabetes-associated complications in rats. Journal of ethnopharmacology. 2016;183:103-11.

124. Ekperikpe US, Owolabi OJ, Olapeju BI. Effects of Parkia biglobosa aqueous seed extract on some biochemical, haematological and histopathological parameters in streptozotocin induced diabetic rats. Journal of ethnopharmacology. 2019;228:1-10.

125. Bhardwaj P, Khanna D, Balakumar P. Catechin averts experimental diabetes mellitus-induced vascular endothelial structural and functional abnormalities. Cardiovascular toxicology. 2014;14:41-51.

126. Eng QY, Thanikachalam PV, Ramamurthy S. Molecular understanding of Epigallocatechin gallate (EGCG) in cardiovascular and metabolic diseases. Journal of ethnopharmacology. 2017;2010:296-310.

127. Eng QY, Thanikachalam PV, Ramamurthy S. Molecular understanding of Epigallocatechin gallate (EGCG) in cardiovascular and metabolic diseases. Journal of ethnopharmacology. 2018;210:296-310.

128. Ali L, Khan AKA, Hassan Z, Mosihuzzaman M, Nahar N, Nasreen T, Nur-e-Alam M, Rokeya B. Characterization of the hypoglycemic effects of Trigonella foenum graecum seed. Planta medica. 1995;61:358-60.

129. Shibib BA, Khan LA, Rahman R. Hypoglycaemic activity of Coccinia indica and Momordica charantia in diabetic rats: depression of the hepatic gluconeogenic enzymes glucose-6-phosphatase and fructose-1, 6-bisphosphatase and elevation of both liver and red-cell shunt enzyme glucose-6-phosphate dehydrogenase. Biochemical journal. 1993;292:267-70.

130. Abas R, Othman F, Thent ZC. Effect of Momordica charantia fruit extract on vascular complication in type 1 diabetic rats. Experimental and clinical sciences journal. 2015;14:179-89.

131. Cortez-Navarrete M, Martínez-Abundis E, Pérez-Rubio KG, González-Ortiz M, Villar MM-d. Momordica charantia Administration Improves Insulin Secretion in Type 2 Diabetes Mellitus. Journal of medicinal food. 2018;21:672-677.

132. Musabayane C, Mahlalela N, Shode F, Ojewole J. Effects of Syzygium cordatum (Hochst.)[Myrtaceae] leaf extract on plasma glucose and hepatic glycogen in streptozotocin-induced diabetic rats. Journal of ethnopharmacology. 2005;97:485-90.

133. Mkhwanazi BN, Serumula MR, Myburg RB, Van Heerden FR, Musabayane CT. Antioxidant effects of maslinic acid in livers, hearts and kidneys of streptozotocin-induced diabetic rats: effects on kidney function. Renal failure. 2014;36:419-31.

134. Thakur GS, Bag M, Sanodiya BS, Bhadauriya P, Debnath M, Prasad G, Bisen PS. Momordica balsamina: a medicinal and neutraceutical plant for health care management. Current pharmaceutical biotechnology. 2009;10:667-82.

135. Aworh OC. From lesser-known to super vegetables: the growing profile of African traditional leafy vegetables in promoting food security and wellness. Journal of the Science of Food and Agriculture. 2018;98:3609-13.

136. Siboto A, Sibiya N, Khathi A, Ngubane P. The Effects of Momordica balsamina Methanolic Extract on Kidney Function in STZ-Induced Diabetic Rats: Effects on Selected Metabolic Markers. Journal of Diabetes Research. 2018;2018:1-8.

Chapter 2

Prologue

Manuscript 1

Hyperglycaemia has been shown to induce cardiovascular complications observed in diabetic patients, which have been shown to progress cardiac failure. Furthermore, current anti-diabetic agents have been associated with the advancement of cardiac disorders. Alternative treatment strategies are therefore needed. We have previously shown the anti-hyperglycaemic and reno-protective effects of *Momordica balsamina*. Since improved renal function has been shown to be associated with improved cardiac function in diabetic animals, the current study, evaluated the effects of *Momordica balsamina* methanolic extract on selected cardiovascular function markers in a streptozotocin-induced diabetic rat model that has not been shown.

The effects of *Momordica balsamina* methanolic extract on cardiovascular function in STZ-induced diabetic rats: **effects on selected markers.**

The present manuscript was prepared for publication according to the **Chemico-Biological Interaction** journal's guidelines to authors. see (Appendix 2).

The effects of *Momordica balsamina* methanolic extract on cardiovascular function in STZ-induced diabetic rats: effects on selected markers

A. Ludidi[1], A. Khathi[1], N. H. Sibiya[2], P. S. Ngubane[1]

[1]School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Private Bag X54001, Durban, 4000, South Africa

[2]Faculty of Pharmacy, P O Box 94, Rhodes University, Grahamstown, 6140, South Africa

Correspondence should be addressed to Asiphaphola Ludidi: asaludidi6@gmail.com

Abstract

Background: Renal dysfunction and cardiovascular disorders are amongst the leading causes of deaths in Diabetes Mellitus (DM). Insulin administration has been associated with the progression of cardiac disorders due to hyperinsulinemia however, medicinal plants have been shown to have antidiabetic effects. We have previously shown the ameliorative effects of *Momordica balsamina* methanolic extract (MB) on renal dysfunction in streptozotocin (STZ)-induced diabetic rats however, the cardiovascular effects have not yet been established. The study therefore, investigated the effects of MB on cardiovascular function in STZ-induced diabetic rats.

Methods: Air-dried *Momordica balsamina* leaves were sequentially extracted with methanol to yield a methanolic extract. Short-term (5 weeks) effects of MB on cardiovascular function were assessed in STZ-induced diabetic rats treated twice daily with MB (250 mg kg^{-1} p.o.). Insulin (170 μg kg^{-1} s.c.) and metformin (500 mg kg^{-1} p.o.). Blood glucose concentration, body weight and blood pressure were monitored weekly for 5 weeks. Thereafter, the animals were sacrificed terminally, collecting blood and hearts for biochemical and histological analysis.

Results: MB significantly decreased blood glucose concentration from week 3-5 by comparison with diabetic untreated animals. Treatment with MB reduced oxidative stress while improving the antioxidant status compared with untreated diabetic animals. MB treatment decreased CRP, CT-I and Ang-II concentrations by comparison with untreated diabetic animals. MB decreased MAP by comparison with untreated diabetic animals. Histopathological hypertrophy was observed in untreated diabetic rats that was ameliorated by the administration of MB.

Conclusions: The administration of MB protects against hyperglycemia-induced cardiovascular changes, which may reduce the risks of cardiovascular complications in diabetes mellitus.

Key words: Cardiovascular disorder, C-reactive protein, oxidative stress, *Momordica balsamina*, cardiomyocyte, mean arterial pressure

1 Introduction

Diabetes mellitus (DM) is the leading non-communicable disease, which is associated with a significantly high morbidity and mortality rate and estimated to affect approximately 693 million people by 2045 (1-3). Cardiovascular complications have been shown to contribute significantly to the total number of deaths caused by DM (4, 5). Metabolic syndromes, including DM, doubles the risk of developing cardiovascular complications, therefore diabetic patients have a two to five-fold greater mortality rate due to cardiovascular disease (6). Chronic hyperglycemia contributes to the progression of cardiovascular dysfunction via increased oxidative stress and endothelial dysfunction (7, 8). Oxidative stress due to the overproduction of superoxide induces an alteration in the morphology and function of the cardiomyocyte as shown by cardiac hypertrophy which is marked by an increase in secretion of cardiotrophin-I (9). Furthermore, chronic inflammation is promoted and thus the increase in proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), C-reactive protein (CRP) and cardiotrophin-I (CT-I) which indicate cardiac damage (10, 11). In addition, hyperglycemia induced reactive oxygen species (ROS) production results in the reduction in the bioavailability of nitric oxide (NO) (12). NO decline is the result of the reduced expression of endothelial cell NO synthase (eNOS), inefficient eNOS activation, insufficient substrate or cofactors for eNOS and accelerated degradation of NO_2 by ROS (13, 14). Furthermore, endothelial dysfunction is accompanied by increased vasoconstrictors including angiotensin-II (15, 16). The pathology of endothelial dysfunction therefore, results in increased arterial stiffness and increased arterial pressure (17). In addition, dyslipidemia has been associated with an increase in atherosclerotic plaque formation, which narrows the vascular lumen, therefore raising blood pressure (18). The long-term increase in blood pressure is associated with cardiac myopathy, cardiac hypertrophy and eventually cardiac failure (19, 20).

There are however, conventional treatments to manage diabetes and associated complications with insulin being the mainstay treatment (21). The administration of insulin as a bolus however, has been shown to be associated with hyperinsulinemia, increased sodium retention

and therefore increased blood pressure (22). Moreover, insulin and oral hypoglycemic agents have been shown to deteriorate liver function and increase plasma volume which is of concern in patients with congestive heart failure (22, 23). These challenges have led to the investigation of alternative therapeutic interventions with less undesirable effects.

Traditionally, various plants have been shown to alleviate diabetes-associated complications. Of interest to our study is *Momordica balsamina*, commonly known as "Intshungu", a plant widespread in Namibia, Botswana, Swaziland and all provinces of South Africa (24). The plant has been reported to possess hypoglycemic effects and improves renal function in streptozotocin (STZ)-induced diabetic rats (25, 26). Despite these developments, the cardiovascular effects of this plant remain elusive hence the interest in investigating the effects of MB on cardiovascular function in STZ-induced diabetic rat model. We envisaged that monitoring blood glucose concentration, mean arterial pressure (MAP), oxidative stress and inflammatory status may provide a holistic insight on the effects of cardiovascular function as a result of MB administration (25).

2 Materials and methods

2.1 Drugs and chemical reagents:

Streptozotocin (Sigma Aldrich Chemical Company, Missouri, St Louis, USA); glucose ($C_6H_{12}O_6$),calcium chloride ($CaCl_2$), citric acid, monosodium citrate (Merck chemicals (Pty) Ltd Wadeville, Johannesburg, South Africa); metformin, dimethyl sulphoxide (Sigma-Aldrich, St Louis, Missouri, United States of America); insulin (NovoRapid pen refill, Novordisk Pty Ltd, Sandton, South Africa).

2.2 Plant extraction

The leaves of *Momordica balsamina* were identified and authenticated by Professor H Baijnath, the former chief taxonomist/curator of the University of KwaZulu-Natal department of botany. The extraction of *Momordica balsamina* leaves were performed in the School of laboratory Medicine and Medical Sciences, at the University of KwaZulu-Natal, Westville Campus, using a previously validated protocol formerly reported by our laboratory (27). Briefly, the air-dried *Momordica balsamina* leaves (1.15kg) were sequentially extracted by cold percolation with methanol (95%, 6.9L) for 24 h. The methanolic extract was recovered from the mixture and methanol was added to the pulp for further extraction. To maximize the extraction process to increase the yield (609g), the process was repeated three times. The three extracts were

combined to yield a concentrated methanolic extract at a reduced pressure (22-26mmHg) and temperature of 45-60 ºC.

2.3 Animals

Male Sprague-Dawley (SD) rats (250 -300 g, n=30) bred and housed in the Biomedical Research Unit (BRU) of University of KwaZulu-Natal were used in the present study. The animals were maintained under standard laboratory conditions of constant temperature (22±2 ºC), CO_2 content of <5000 p.m., relative humidity of 55±5% and illumination (12 h light/dark cycles) and the noise levels of less than 65 decibels and ad libitum access to food and water. Procedures involving animals and their care were conducted in conformity with the institutional guidelines of the University of KwaZulu-Natal (AREC/023/017M). Individual rats were housed in Makrolon polycarbonate metabolic cages (Techniplast, Labotec, South Africa) and were acclimatized in metabolic cages for 5 days before commencement of the study.

2.4 Induction of diabetes mellitus

The induction of type 1 diabetes mellitus by a single intraperitoneal injection of 60 mg kg^{-1} streptozotocin, which was freshly prepared in 0.1 M citrate, buffer (pH 4.5). Control group received the vehicle, citrate buffer through the same route. Animals that presented glucosuria after 24 h, when tested by urine strips (Rapidmed Diagnostics, Sandton, South Africa) were considered diabetic. Seven days after the induction of diabetes, animals that had a blood glucose concentration greater than 20 mmol L^{-1} were considered as having stable diabetes.

2.5 Experimental design

To study the short-term effects *of Momordica balsamina* methanolic extract (MB) on the cardiovascular system over 5 weeks of treatment, experimental rats were divided into separate groups of non-diabetic (group 1) and STZ-induced diabetic (group 2-5) male Sprague-Dawley rats, with six rats per group. Group 1 was treated with the drug vehicle DMSO to serve as a negative control. Group 2 was treated with *Momordica balsamina* methanolic extract (250 mg kg^{-1}, p.o.). Positive controls in Group 3 and 4 were treated with insulin (175 µg kg^{-1}, s.c.) and metformin (500 mg kg^{-1}, p.o.), respectively. Non-diabetic animals in Group 5 served as absolute control. MB was administered twice daily at 09h00 and 15h00. Food and water intake, body weight, mean arterial blood pressure (MAP) and blood glucose concentration were monitored over the 5-week period. MAP was measured using the non-invasive tail cuff method with photoelectric sensors (IITC Model 31 Computerised Blood Pressure Monitor, Life Sciences, Woodland Hills, California, USA) as previously described (28), while blood glucose

concentrations were measured via the tail pricking method. All parameters were assessed every 3rd day at 09:00 am for the duration of experimental period.

2.6 Tissue sample harvesting

At the end of the 5-week experimental period, all animals were sacrificed by exposing to halothane via a gas anesthetic chamber (100 mg kg^{-1}) for 3 minutes (Biomedical Resource Unit, UKZN, Durban, South Africa). Thereafter, blood was collected by cardiac puncture (RBCP) into individual pre-cooled heparinized container and centrifuged (Eppendorf centrifuge 5403, Germany) at 4 °C, 503 g for 15 minutes and separated plasma were stored at -80 in a Bio Ultra freezer (Snijers Scientific, Holland) for hormonal analysis. The heart tissues were removed and weighed before snap freezing in liquid nitrogen and then stored in a BioUltra freezer (Snijers scientific, Tilburg, Netherlands) -80 °C until use.

2.7 Biochemical analysis

2.7.1 C-reactive protein and angiotensin-II measurement

Heart and plasma C-reactive protein (CRP) and plasma angiotensin-II (Ang-II) were analysed using separate specific ELISA kits (Elabscience and Biotechnology, WuHan) that use the Sandwich-ELISA method. Kits included micro ELISA plates, which were coated with antibody specific to CRP and angiotensin-II, respectively. Standards and samples were pipetted into the appropriate wells of the micro ELISA plate and incubated for 90 minutes. The plate relevant biotinylated detection antibody (100 µL) was then added and incubated for 60 minutes. Avidin-Horseradish Peroxidase (HRP) conjugate (100 µL) was added to each micro-plate well and incubated for 30 minutes. Unbound components were washed out. Substrate solution (100 µL) was added to each micro-plate well. After incubating for a further 15 minutes, the stop solution (50 µL) was added. The optical density was measured using a Nano spectrophotometer (BMG Labtech, Ortenburg, Baden-Wurttemberg, Germany) at the wavelength of 450 nm. The concentration of the samples was extrapolated from the respective standard curves.

2.7.2 Cardiotrophin-I measurement

Heart cardiotrophin-I (CT-I) was analysed using a separate specific CLIA kit (Elabscience and Biotechnology, WuHan) that uses the Sandwich- CLIA method. The kit included a micro CLIA plate, which was coated with an antibody specific to CT-I. Standards and samples were pipetted into the appropriate wells of the micro CLIA plate and incubated for 90 minutes. The plate relevant biotinylated detection antibody (100 µL) was then added and incubated for 60 minutes.

Avidin-Horseradish Peroxidase (HRP) conjugate (100 µL) was added to each micro-plate well and incubated for 30 minutes. Unbound substances were washed out. Substrate mixture solution (100 µL) was added to each micro-plate well. After incubating for not more than 5 minutes protecting from the light the relative light unit (RLU) was measured using the GloMax® 96 Microplate Luminometer (BMG Labtech, Ortenburg, Baden-Wurttemberg, Germany). The concentration of the samples were extrapolated from the respective standard curves.

2.7.3 Oxidative stress

To establish the effects of treatment on oxidative stress in the plasma and heart muscle, levels of malondialdehyde (MDA), a commonly known marker of oxidative stress, were measured in untreated and treated experimental animals using a biochemical assay as shown below. The antioxidant defense enzymes: superoxide dismutase (SOD) and glutathione peroxidase (GPx) were also measured in untreated and treated experimental animals (29).

2.7.3.1 Malondialdehyde measurement

Heart tissues (50 mg) were homogenized in 500 µL of 0.2% phosphoric acid, respectively. The homogenate was centrifuged at 400 x g for 10 min. Thereafter, 400 µL of the homogenate and 100 µL plasma were supplemented with 400 µL 2% phosphoric acid and then separated into three glass tubes, each receiving equal volumes of the solution. Subsequently, 200 µL of 7% phosphoric acid was added into all glass tubes followed by the addition of 400 µL of thiobarbituric acid (TBA)/butylated hydroxytoluene (BHT) into two glass tube (sample tests) and 400 µL of 3 mM hydrochloric acid (HCl) into the third glass tube (blank). To ensure an acidic pH of 1.5, 200 µL of 1 M, HCl was added to sample and blank test tubes. All solutions were heated at 100℃ for 15 min, and allowed to cool to room temperature. Butanol (1.5 mL) was added to the cooled solutions; the sample tests were vortexed for 1 min to ensure rigorous mixing and allowed to settle until two phases are distinguished. The butanol phase (top layer) was transferred to Eppendorf tubes and centrifuged at 13,200 x g for 6 min. The samples were aliquoted into a 96-well microtiter plate in triplicate and the absorbance was read at 532 nm (reference 600 nm) on a BioTek µQuant spectrophotometer (Biotek, Johannesburg, South Africa). The absorbance from these wavelengths were used to calculate the concentration of MDA using Beer's Law.

Concentration of MDA (mM)

$$= \frac{\text{Average Absorbance}}{\text{Absorption coefficient } (156\ \text{mmol}^{-1})}$$

2.7.3.2 Superoxide dismutase and glutathione peroxidase measurement

Superoxide dismutase (SOD) and glutathione peroxide (GPx) in both the plasma and heart tissues were analysed using separate specific ELISA kits (Elabscience and Biotechnology, WuHan) that use the Sandwich-ELISA method. Kits included micro ELISA plates that were coated with antibody specific to SOD and GPx, respectively. Standards and samples were pipetted into the relevant wells of the micro ELISA plate and incubated for 90 minutes. The plate relevant biotinylated detection antibody (100 µL) was then added and incubated for 60 minutes. Avidin-Horseradish Peroxidase (HRP) conjugate (100 µL) was added to each micro-plate well and incubated for 30 minutes. Unbound components were washed out. Substrate solution (100 µL) was added to each micro-plate well. After incubating for a further 15 minutes, the stop solution (50 µL) was added. The optical density was measured using a Nano spectrophotometer (BMG Labtech, Ortenburg, Baden-Wurttemberg, Germany) at the wavelength of 450 nm. The concentration of the samples was extrapolated from the respective standard curves.

2.8 Histology of the heart

Heart tissues were fixed in formalin overnight, paraffin embedded and processed for sectioning. 0,5 µM sections were made and stained with hematoxylin and eosin (H&E) to analyze the cardiomyocyte size and the arrangement of cardiomyocyte myofibres using Leica microsystems for analysis.

For the assessment of histological cardiovascular changes after treatment, the heart tissues of non-diabetic, untreated STZ-induced diabetic rats and treated STZ-induced diabetic rats were dissected out for fixation with 10% formaldehyde solution at the end of the 5-week experimental period. Excess formalin was removed from the heart tissues by rinsing with water. The heart tissues were then dehydrated in 70%, 80%, 90%, as well as 100% ethanol for 2, 2, 3 and 2 minutes respectively. To remove the alcohol, the tissues were then submerged in xylene rendering the tissue translucent. In preparation for sectioning, the tissues were embedded in wax blocks which were sectioned at a thickness of 3-5µm using a rotary microtome (Robert-Bosch-Straße, Walldorf, Baden-Württemberg, Germany). Sectioned tissues were then mounted onto clean slides and dried by placing them on a Ransom warming plate. 250 ml of xylene was then used to de-paraffinize the sections twice for 3 min each time. The rehydration of tissue sections in decreasing concentrations of 100%, 90%, 70% and 50% ethanol for 2 minutes each time in preparation for staining. The tissue slides were then flooded with hematoxylin, using a Pasteur pipette and allowed to stand for 5 minutes and the slide was tilted to remove the

hematoxylin stain. Using tap water, the slides were then gently flood with a Pasteur pipette three times or until the sections stained purple turned blue. Pasteur pipette was used over the tissue sections to drop the Eosin stain and left to stand for 3-5minutes. Tap water was used to rinse off the residual eosin on the tissue sections/slide. Tissue slides were then dehydrated by submerging the slides in increasing 90% and 100% ethanol. Sections were then cleared with then cleared with xylene in preparation for permanent mounting by dropping distyrene plasticizer xylene (DPX) mounting glue directly over the tissue section using an applicator, then covering with a cover slip. The sections were allowed to dry overnight. The processed tissue sections were then visualized and captured using a Leica Scanner, SCN400 and Slide Path Gateway LAN software for analysis (Leica Microsystems CMS, Wetzlar, Germany).

2.9 Statistical analysis

All data are expressed as means ± standard error of means (SEM). GraphPad Prism Instat Software (version 5.00, GraphPad Software, San Diego, California, USA) was used to perform statistical analysis. Blood glucose and MAP were analysed using analysis of variance (ANOVA) followed by Bonferroni post hoc test. ANOVA was used to analyze terminal parameters to compare the differences between control and experimental groups. Values of $p<0.05$ were regarded statistical significant between the compared groups.

Results

3.1 Blood glucose concentration

Figure 1 shows blood glucose concentration of non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}) metformin (MTF) and insulin (INS) over an experimental period of 5weeks. The untreated STZ-induced diabetic animals sustained a significantly high blood glucose concentration over the period of 5 weeks by comparison with non-diabetic control $^{\alpha}$ (DC vs NC, $p<0.05$, Figure 1). Treatment with MB, insulin and metformin showed a significant decrease in blood glucose from week 3,4 and 5 by comparison with the diabetic control animals *(MB vs DC, $p<0.05$, Figure 1). Blood glucose concentrations of animals treated with insulin significantly decreased at week 5 by comparison with animals treated with MB λ (INS vs MB, $p<0.05$, Figure 1).

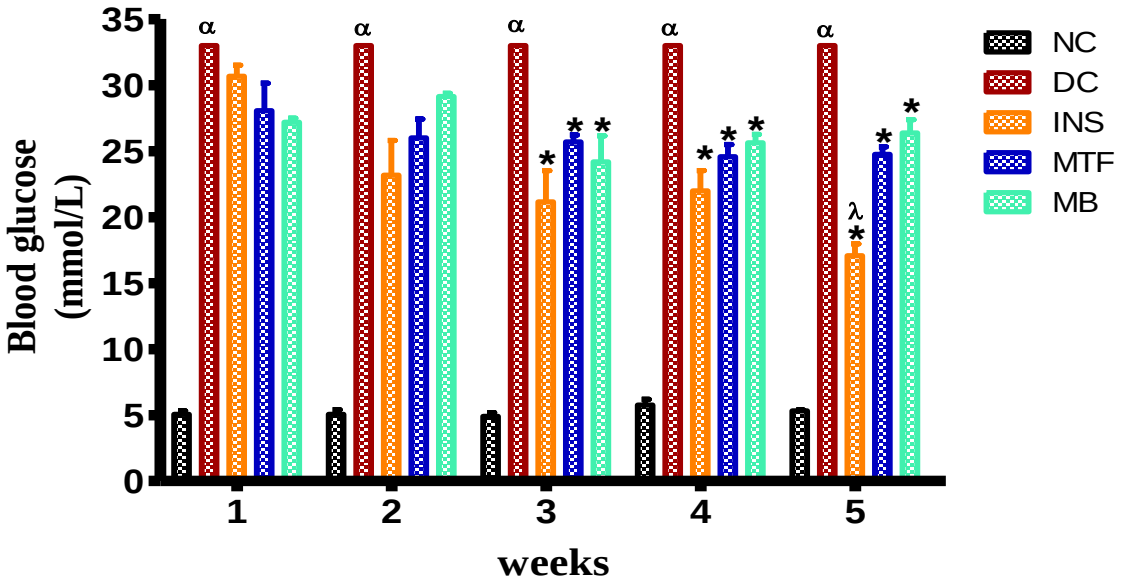

Figure 1: Comparison of blood glucose concentration in non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS) over the period of 5 weeks. Values are presented as means and vertical bars indicate SEM (n=6 in each group). α $p<0.05$ by comparison with non-diabetic control animals. * $p<0.05$ by comparison with diabetic control animals. λ $p<0.05$ by comparison with MB treated animals.

3.2 Mean arterial blood pressure

Figure 2 shows the mean arterial pressure (MAP) of non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS) over an experimental period of 5 weeks, measured 24 hours after treatment. The diabetic control group showed a significantly high MAP throughout the 5-week experimental period by comparison with non-diabetic control $^{\alpha}$ (DC vs NC, $p<0.05$, Figure 2). However, treatment with MB decreased MAP similarly to metformin and insulin from week 3 to 5 by comparison with the untreated STZ-induced diabetic rats *(MB vs DC, $p<0.05$, Figure 2), while insulin significantly increased MAP from week 3 to week 5 by comparison with metformin and MB λ (INS vs MTF and MB, $p<0.05$, Figure 2).

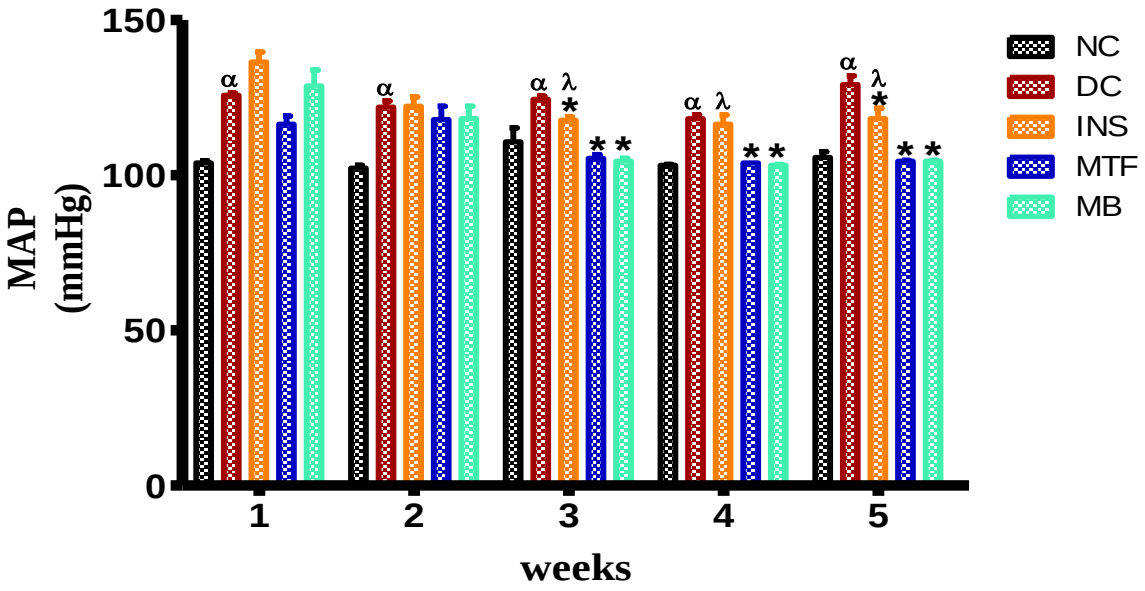

Figure 2: Comparison of MAP in non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS) over the period of 5 weeks. Values are presented as means and vertical bars indicate SEM (n=6 in each group). α $p<0.05$ by comparison with non-diabetic control animals. * $p<0.05$ by comparison with diabetic control animals. λ $p<0.05$ by comparison with MB treated animals.

3.3 Heart to body weight ratio

Table 1 compares the heart to body weight ratios of non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with MB (250 mg kg^{-1}), metformin (MTF) and insulin (INS) over a 5-week treatment period. Diabetic control rats exhibited an increase in heart to body weight ratio compared with non-diabetic control rats at the end of the 5-week experiment $^{\alpha}$ (DC vs NC, $p<0.05$). MB administration similarly to standard drugs, decreased heart to body weight ratio compared with STZ-induced diabetic control rats at the end of the 5-week experimental period *(MB vs DC, $p<0.05$).

Table 1: Comparisons of the (H/B) ratios of non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS). Values are expressed as mean ± SEM (n=6 in each group).

Experimental groups	**Final body weight (g)**	**Hearts weight (g)**	**H/B ratio (%)**
NC	307.00 ± 7.66	1.22 ± 0.08	0.39 ± 0.02
DC	169.17 ± 4.75 α	1.22 ± 0.06	0.72 ± 0.04 α
INS	281.92 ± 2.39 *	1.04 ± 0.05	0.37 ± 0.02 *
MTF	256.67 ± 10.17 *	1.12 ± 0.11	0.43 ± 0.04 *
MB	243.83 ± 5.39 *	1.08 ± 0.03	0.44 ± 0.02 *

α $p<0.05$ by comparison with non-diabetic control animals. * $p<0.05$ by comparison with diabetic control.

3.4 MDA, SOD and GPx concentrations

Table 2 Depicts the comparisons of MDA, SOD and GPx concentrations in both the heart and plasma of non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS) after 5 weeks of treatment. Untreated STZ-diabetic heart and plasma tissues presented with increased concentrations of MDA by comparison to untreated non-diabetic rats $^{\alpha}$ (DC vs NC, $p<0.05$). In addition, GPx concentrations in the plasma of untreated STZ-induced diabetic rats significantly decreased by comparison with non-diabetic animals $^{\alpha}$ (DC vs NC, $p<0.05$). The plasma MDA concentrations of MB treated animals similarly to standard drugs decreased by comparison to

untreated STZ-induced diabetic rats *(MB vs DC, p<0.05). Furthermore, GPx concentrations in the plasma of MB treated animals similarly to standard drugs increased by comparison to untreated non-diabetic rats *(MB vs DC, p<0.05). However, heart GPx concentrations of MB treated animals significantly decreased by comparison with untreated STZ-diabetic rats *(MB vs DC, p<0.05). Furthermore, heart and plasma SOD concentrations of MB treated animals, significantly decreased by comparison with untreated diabetic animals *(MB vs DC, p<0.05).

Table 2: Comparison of MDA, SOD and GPx concentrations of both plasma and heart tissues of non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS). Values are presented as means ± SEM (n=6 in each group).

	Plasma		**Heart**
MDA (µmol/g protein)	NC	0.68 ± 0.12	1.21 ± 0.04
	DC	9.37 ± 1.19 α	1.38 ± 0.09
	INS	0.54 ± 0.03 *	1.21 ± 0.02
	MTF	0.35 ± 0.06 *	1.26 ± 0.12
	MB	2.48 ± 0.05 *	1.15 ± 0.10 *
GPx Concentration (ng/mL)	NC	1.44 ± 0.03	1.14 ± 0.01
	DC	0.21 ± 0.04 α	1.37 ± 0.09
	INS	0.83 ± 0.01 *	1.39 ± 0.03
	MTF	1.67 ± 0.01 *	1.23 ± 0.06
	MB	1.06 ± 0.04 *	0.79 ± 0.05
SOD Concentration (ng/mL)	NC	35.39 ± 0.63	65.92 ± 0.98
	DC	33.55 ± 0.27	63.21 ± 1.92
	INS	31.68 ± 2.76	66.69 ± 1.11
	MTF	29.85 ± 2.44	65.17 ± 1.17
	MB	23.63 ± 0.87	33.55 ± 0.27

α p<0.05 by comparison with non-diabetic control animals. * p<0.05 by comparison with diabetic control.

3.5 CRP and CT-I concentrations

Table 3 shows the comparisons heart and plasma CRP and heart CT-I concentrations of non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS) after 5 weeks of treatment. Heart and plasma CRP with heart CT-I concentrations of untreated STZ-diabetic animals significantly increased by comparison with non-diabetic rats $^{\alpha}$ (DC vs NC, $p<0.05$). Furthermore, insulin sustained a high heart and plasma CRP and heart CT-I concentration similarly to untreated STZ-diabetic animals *(INS vs DC, $p<0.05$). However, MB similarly to metformin, significantly decreased heart and plasma CRP and heart CT-I concentrations of untreated STZ-diabetic animals by comparison to untreated STZ-induced diabetic rats (MB vs DC, $p<0.05$).

Table 3: Comparison of heart and plasma CRP and heart CT-I concentrations in non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS). Values are presented as means ± SEM (n=6 in each group).

Experimental groups	**CRP (ng/mL)**		**CT-I (pg/mL)**
	Plasma	**Heart**	**Heart**
ND	0.38 ± 0.01	5.61 ± 0.65	9.13 ± 0.85
DC	0.43 ± 0.01 α	14.31 ± 0.38 α	35.01 ± 0.26 α
INS	0.64 ± 0.03 *	15.66 ± 0.18 *	37.60 ± 1.62 *
MTF	0.42 ± 0.03 *	6.57 ± 0.21 *	11.14 ± 1.62 *
MB	0.32 ± 0.01 *	7.11 ± 0.72 *	9.73 ± 0.08 *

α $p<0.05$ by comparison with non-diabetic control animals.* $p<0.05$ by comparison with diabetic control.

3.6 Ang-II concentrations

Table 4 displays the comparisons of plasma angiotensin-II (Ang-II) of non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS) after 5 weeks of treatment. Plasma angiotensin-II concentrations of untreated STZ-diabetic rats significantly increased by comparison with

non-diabetic animals $^{\alpha}$ (DC vs NC, $p<0.05$). However, MB treated animals similarly to insulin and metformin treated animals exhibited a significantly decreased plasma Ang-II concentration*(MB vs DC, $p<0.05$).

Table 4: Comparison of plasma Ang-II concentrations of non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS). Values are presented as means ±SEM (n=6 in each group).

Experimental groups	**Plasma Ang-II (ng/mL)**
ND	3.98 ± 0.04
DC	4.34 ± 0.05 α
INS	4.01 ± 0.04 *
MTF	3.81 ± 0.06 *
MB	3.77 ± 0.09 *

α $p<0.05$ by comparison with non-diabetic control animals. * $p<0.05$ by comparison with diabetic control.

3.7 Histology of the heart

Figure 3 are the H & E photomicrographs illustrating the effects of treatment on the morphology of the heart tissue in (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}) metformin (MTF) and insulin (INS) after an experimental period of 5weeks. Photomicrograph of the non-diabetic untreated group showed single, oval and centrally located nuclei (N) of the cardiomyocytes. The cardiac myofibres (MF) in the non-diabetic control group were arrangement in a regular pattern (A). Nuclei of the cardiomyocytes in the diabetic control group (B) showed deformation in sizes and shapes. Moreover, the cardiac myofibres in the diabetic untreated group (B) showed disarrayed patterns compared to the non-diabetic untreated group (A). Nuclei of the cardiomyocytes in the insulin treated group (C) showed deformation in size and shape. Additionally, the insulin treated group (C) showed disarrayed cardiac myofibres similarly to the diabetic control group (B). The nuclei of the cardiomyocytes in the metformin treated group (D) showed an improvement in size and shape by comparison to the diabetic control group (B). In particular, the nuclei were single, oval and centrally located similarly to the cardiomycytes in the non-diabetic control group (A). The

arrangement of the cardiac myofibres in the metformin treated group improved to near normal compared to the non-diabetic control group (A). Nuclei of the cardiomyocytes in the MB treated group (E) showed an improvement in size and shape compared to the diabetic control group (B). Moreover, the nuclei were single, oval and centrally located similarly to the cardiomycytes in the non-diabetic control group. Arrangement of cardiac myofibres in MB treated group (E) improved to a near normal pattern compared to the non-diabetic control group (A). Magnification 20X-100 µm.

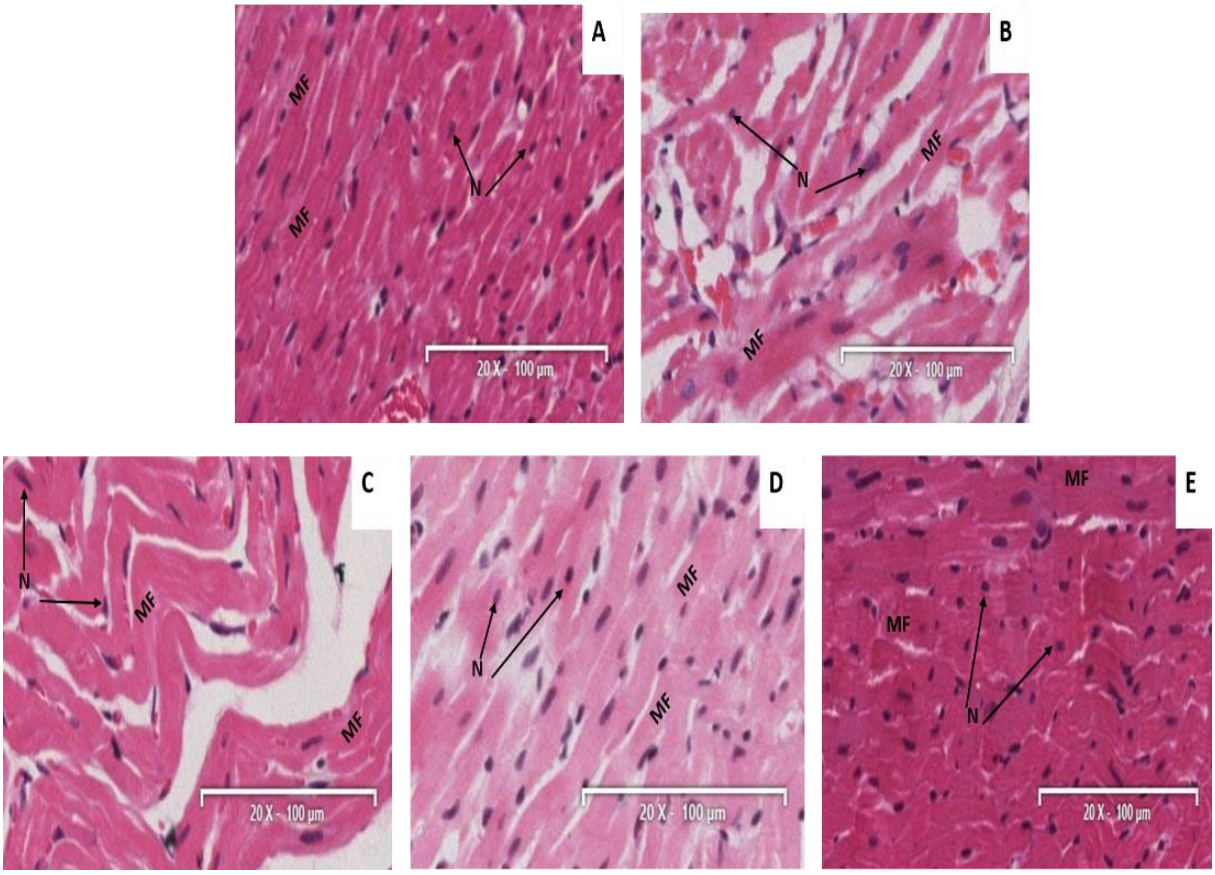

Figure 3: H & E photomicrographs illustrating cardiac tissue morphology in non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS) after 5 weeks of treatment. Magnification 20X-100 µm.

4 Discussion

The present study investigated whether treatment with a methanolic extract of *Momordica balsamina* ameliorates cardiovascular dysfunction commonly observed in diabetic patients and modelled in streptzotocin (STZ)-induced diabetic rats. We have previously shown that *Momordica balsamina* possess antihyperglycemic properties and improves kidney function.

Here in this study, *Momordica balsamina* improves oxidative stress, cardiovascular structure and function in STZ-induced diabetic rats.

Chronic hyperglycemia which is a characteristic in DM alters cardiovascular function and indeed STZ administration resulted in sustained hyperglycemia throughout the study due to the obliteration of pancreatic β-cells as shown in previous studies (30, 31). Administration of MB however, decreased glycemia in STZ-diabetic rats. Medicinal plants such as *Momordica charantia Linn* which is in the same family as *Momordica balsamina* have been shown to possess antihyperglycemic phytochemicals such as flavonoids, cucurbitane triterpenoids and phytosterols (32). Flavonoids have been shown to inhibit the activity of α-amylase and α-glucosidase *in vitro,* delaying postprandial glucose absorption (33). In addition, flavonoids have been shown to inhibit glucose transport through the inhibition of intestinal sodium-glucose co-transporters (34, 35). Interestingly, *Momordica balsamina* has been reported in our laboratory to decrease food intake of STZ-induced diabetic rats (26). *Momordica balsamina* therefore may maintain glycemic control through its antihyperglycemic effects similarly to other medicinal plants such as *Tripterygium wilfordii* possibly via delaying postprandial glucose absorption, thus activating the release of appetite modulation hormones such as cholecystokinin (CCK) and peptide YY(PYY) to inhibit feeding thus decreasing ghrelin secretion (36, 37).

An improvement in glycemic control has been shown to attenuate oxidative stress-induced debilitating cardiovascular complications in diabetic patients and experimental animals (38). Evidently, STZ-diabetic rats in the present study maintained increased oxidative stress, which was associated with the dysregulation of the antioxidant defense system, inflammation and endothelial function. The antioxidant defense system is adapted to scavenge ROS to minimize alteration in cell structure and function (39-41). It has been documented that hyperglycemia-induced glycation of proteins including superoxide dismutase and glutathione peroxide alters their structure reducing their efficiency (42). In agreement with previous studies, the suppression of GPx which detoxifies a ROS product- hydrogen peroxide- to water, was observed in our STZ-induced diabetic rats (43). This may be correlated with a rise in hyperglycemia-induced enzyme glycation, increasing oxidative stress (44, 45). Interestingly, MB administration improved the antioxidant status possibly through increasing GPx that scavenges ROS although SOD was not improved possibly due to glycation of this enzyme augmenting loss of enzyme activity. In addition, an increase in oxidative stress has been shown to dysregulate inflammation evidenced in the increase in CRP, which is a marker for increased

cardiovascular dysfunction in the STZ-induced diabetic animal. Moreover, *Momordica balsamina* attenuated the rise in CRP associated with the morphological and functional alteration of cardiomyocytes.

The progression of cardiac dysfunction was further evidenced in the present STZ-induced rats which may be attributed to the increase in the polyol pathway, resulting in the accumulation of sorbitol which induces osmotic stress in cardiomyocytes (46, 47). Osmotic stress activates the extracellular signal-regulated protein kinase and protein kinase C which are associated with cell growth, hence hypertrophy which may account for alteration in cardiomyocyte structure (48). Interestingly, MB attenuated the metabolic injury specifically to the cardiac muscle as observed by the restoration in the histological architecture of cardiomyocyte that may be associated with the decrease in oxidative stress, marked by MDA (49-51). This is in harmony with previous findings showing that an improvement in oxidative stress improves cardiac function (52, 53).

Hyperglycemia has been associated with an increase in MAP, which may be attributed to kidney dysfunction due to the increase in renal sodium reabsorption increasing blood volume in synergy with increased vasoconstrictors including angiotensin-II that may have stimulated hyperglycemia-induced collagen synthesis in the vasculature, consequently narrowing and stiffening the blood vessels (54). Resistance of blood flow therefore may have ensued hence an alleviated MAP as observed in the present STZ-induced diabetic animals (55). The increase in MAP forces the cardiac muscle to stretch, activating the release of CT-I by cardiac fibroblasts via the stimulation of the JAK/STAT pathway (56). JAK/STAT pathway activation through CT-I signals the expression and transcription of genes involved in immunity, proliferation and differentiation in the cardiomyocytes nucleus as observed in our STZ-induced diabetic animals (57, 58). An elevation in CT-I of STZ-induced diabetic animals therefore exacerbates cardiac dysfunction. Additionally, the increase in collagen synthesis in the cardiac muscle dysregulates the synchronous contractility of the cardiomyocytes hence cardiac myopathy (54, 59). In the present study, elevated MAP of STZ-induced rats coincides with the increase in heart to body weight ratio, suggesting cardiac hypertrophy. However, MB attenuated the rise in MAP possibly through decreasing Ang-II decreasing cardiac overload and mechanical stretch of the cardiac muscle, hence a decrease in CT-I. Additionally, the ability of MB to improve heart to body weight ratio similarly to metformin, may have improved cardiac hypertrophy. In addition, we have previously reported that MB improves kidney function and a decrease in MAP may be attributed to the restoration of electrolyte handling (26). Our results are in line with literature as medicinal plants such as *Prosopis gradulosa* have been shown to possess hypotensive, anti-

ischemic effects in addition to an increased cardiomyocyte insulin sensitivity. Furthermore, plants such as *Hibiscus subdariffa* have been shown to possess lipid lowering activity and reverse cardiac hypertrophy (60). As predicted, treatment with insulin in this study also showed an increase in MAP as seen in STZ-induced diabetic rats, as insulin is mitogenic and promotes proliferation of cells, which was associated with the observed cardiac hypertrophy although the heart to body weight ratio was decreased. In addition, supraphysiological administration of insulin to diabetic patients has been shown to increase sodium reabsorption associated with hyperinsulinemia and edema consequently contributing to the alleviated MAP which is associated with cardiac hypertrophy, progressing cardiovascular complications (61).

The present study is in agreement with the medicinal use of MB and provides evidence that it may delay the onset of the progression of cardiovascular complications in diabetic patients. In addition, since *Momordica balsamina* is widely spread in developing countries such as South Africa, this study may provide evidence that MB may indeed be a beneficial and easily accessible management strategy for diabetic patients.

5 Conclusion

Momordica balsamina administration attenuates hyperglycemia accompanied by an improvement in the antioxidant status. Furthermore, the administration of the extract ameliorated an increase in MAP, cardiac hypertrophy and inflammatory markers in STZ-induced diabetic rats. The observations further highlight the cardiovascular risk associated with insulin administration. In conclusion, the observations from this study support the use of medicinal plants as an alternative for the management of diabetes related complications.

6 Conflicts of Interest

The authors declare no conflict of interest, and the work has not been published elsewhere.

7 Acknowledgments

The authors appreciate assistance and support from the Biomedical Research Unit, University of KwaZulu-Natal. The current study was partly funded by NRF South Africa and the University of KwaZulu-Natal, Research Division.

8 References

1. Wang H, Naghavi M, Allen C, Barber RM, Carter A, Casey DC, Charlson FJ, Chen AZ, Coates, MM, Coggeshall M. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet. 2016;388:1459-544.

2. Bahendeka SK. Diabetes in sub-Saharan Africa: let us not forget type 1. The Lancet diabetes & endocrinology. 2017;5:575-7.

3. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes research and clinical practice. 2018;138:271-81.

4. Tarquini R, Lazzeri C, Pala L, Rotella CM, Gensini GF. The diabetic cardiomyopathy. Acta diabetologica. 2011;48:173-81.

5. Marzona I, Avanzini F, Lucisano G, Tettamanti M, Baviera M, Nicolucci A, Carla M. Are all people with diabetes and cardiovascular risk factors or microvascular complications at very high risk? Findings from the Risk and Prevention Study. Acta diabetologica. 2017;54:123-31.

6. Simi Joju K, Thangamani S, Radhakrishnan A. Prevalence of diabetic complications and other comorbidities among type 2 diabetes mellitus patients. 2017;6:1552-60.

7. Tinsley LJ, Kupelian V, D'Eon SA, Pober D, Sun JK, King GL. Association of glycemic control with reduced risk for large-vessel disease after more than 50 years of type 1 diabetes. The journal of clinical endocrinology & metabolism. 2017;102:3704-11.

8. Forbes JM, Cooper ME. Mechanisms of Diabetic Complications. Physiological reviews. 2013;93:137-88.

9. Lorenzo-Almoros A, Tunon J, Orejas M, Cortés M, Egido J, Lorenzo Ó. Diagnostic approaches for diabetic cardiomyopathy. Cardiovascular diabetology. 2017;16:28.

10. Schöttker B, Herder C, Rothenbacher D, Roden M, Kolb H, Müller H, Hermann B. Proinflammatory Cytokines, Adiponectin, and Increased Risk of Primary

Cardiovascular Events in Diabetic Patients With or Without Renal Dysfunction. Diabetes care. 2013;36:1703-11.

11. Cutando A, Montero J, Gómez-de Diego R, Ferrera M-J, Lopez-Valverde A. Effect of topical application of melatonin on serum levels of C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in patients with type 1 or type 2 diabetes and periodontal disease. Journal of clinical and experimental dentistry. 2015;7:628.

12. Kiani S, Aasen JG, Holbrook M, Khemka A, Sharmeen F, LeLeiko RM, Tabit CE, Farber A, Eberhardt RT, Gokce N, Vita JA, Hamburg NM. Peripheral artery disease is associated with severe impairment of vascular function. Vascular medicine. 2013;18:72-8.

13. Takahashi T, Harris RC. Role of endothelial nitric oxide synthase in diabetic nephropathy: lessons from diabetic eNOS knockout mice. Journal of diabetes research. 2014;2014:1-17.

14. Dellamea BS, Pinto LCF, Leitão CB, Santos KG, Canani LHS. Endothelial nitric oxide synthase gene polymorphisms and risk of diabetic nephropathy: a systematic review and meta-analysis. BMC medical genetics. 2014;15:9.

15. Loscalzo J. The identification of nitric oxide as endothelium-derived relaxing factor. Circulation research. 2013;113:100-3.

16. Ferreira I, Hovind P, Schalkwijk CG, Parving H-H, Stehouwer CD, Rossing P. Biomarkers of inflammation and endothelial dysfunction as predictors of pulse pressure and incident hypertension in type 1 diabetes: a 20 year life-course study in an inception cohort. Diabetologia. 2018;61:231-41.

17. Prenner SB, Chirinos JA. Arterial stiffness in diabetes mellitus. Atherosclerosis. 2015;238:370-9.

18. Hurtubise J, McLellan K, Durr K, Onasanya O, Nwabuko D, Ndisang JF. The different facets of dyslipidemia and hypertension in atherosclerosis. Current atherosclerosis reports. 2016;18:82.

19. Varghese JF, Patel R, Yadav U. Metabolic syndrome: A forerunner of cardiovascular diseases. Edorium journal of biochemistry. 2017;2:4-6.

20. Laurent S, Boutouyrie P. The structural factor of hypertension: large and small artery alterations. Circulation research. 2015;116:1007-21.

21. Vincent M, Nobécourt E. Treatment of diabetic ketoacidosis with subcutaneous insulin lispro: a review of the current evidence from clinical studies. Diabetes & metabolism. 2013;39:299-305.

22. Tahrani AA, Bailey CJ, Del Prato S, Barnett AH. Management of type 2 diabetes: new and future developments in treatment. The Lancet. 2011;378:182-97.

23. Chakrabarti R, Rajagopalan R. Diabetes and insulin resistance associated disorders: disease and the therapy. Current science-Bangalore-. 2002;83:1533-8.

24. Thakur GS, Bag M, Sanodiya BS, Bhadauriya P, Debnath M, Prasad G, Bisen PS. Momordica balsamina: a medicinal and neutraceutical plant for health care management. Current pharmaceutical biotechnology. 2009;10:667-82.

25. Joseph B, Jini D. Antidiabetic effects of Momordica charantia (bitter melon) and its medicinal potency. Asian Pacific journal of tropical disease. 2013;3:93-102.

26. Siboto A, Sibiya N, Khathi A, Ngubane P. The Effects of Momordica balsamina Methanolic Extract on Kidney Function in STZ-Induced Diabetic Rats: Effects on Selected Metabolic Markers. Journal of diabetes research. 2018;2018:1-8.

27. Mkhwanazi B, Serumula M, Myburg R, Heerden F, Musabayane C. antioxidant effects of maslinic acid in liver, hearts and kidneys of streptozotocin-induces diabetic rats: effects on kidney function. Renal failure. 2014;36:419-31.

28. Madlala HP, Van Heerden FR, Mubagwa K, Musabayane CT. Changes in renal function and oxidative status associated with the hypotensive effects of oleanolic acid and related synthetic derivatives in experimental animals. PloS one. 2015;10:128192.

29. Kasapoglu M, Özben T. Alterations of antioxidant enzymes and oxidative stress markers in aging. Experimental gerontology. 2001;36:209-20.

30. Wu J, Yan L-J. Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity. Diabetes, metabolic syndrome and obesity: targets and therapy. 2015;8:181.

31. Varsha MS, Thiagarajan R, Manikandan R, Dhanasekaran G. Vitamin K1 alleviates streptozotocin-induced type 1 diabetes by mitigating free radical stress, as well as inhibiting NF-κB activation and iNOS expression in rat pancreas. Nutrition. 2015;31:214-22.

32. Tan SP, Kha TC, Parks SE, Roach PD. Bitter melon (Momordica charantia L.) bioactive composition and health benefits: a review. Food reviews international. 2016;32:181-202.

33. Priscilla DH, Roy D, Suresh A, Kumar V, Thirumurugan K. Naringenin inhibits α-glucosidase activity: A promising strategy for the regulation of postprandial hyperglycemia in high fat diet fed streptozotocin induced diabetic rats. Chemico-biological interactions. 2014;210:77-85.

34. Kato CG, Gonçalves GdA, Peralta RA, Seixas FAV, de Sá-Nakanishi AB, Bracht L, Comar JF, Bracht A, Peralta RM. Inhibition of α-amylases by condensed and hydrolysable tannins: focus on kinetics and hypoglycemic actions. Enzyme research. 2017;2017:1-12.

35. Mudaliar S, Polidori D, Zambrowicz B, Henry RR. Sodium–Glucose Cotransporter Inhibitors: Effects on Renal and Intestinal Glucose Transport. From bench to bedside. 2015;38:2344-53.

36. Suh JH, Wang Y, Ho C-T. Natural dietary products and their effects on appetite control. Journal of agricultural and food chemistry. 2017;66:36-9.

37. Odeyemi S, Bradley G. Medicinal Plants Used for the Traditional Management of Diabetes in the Eastern Cape, South Africa: Pharmacology and Toxicology. Molecules. 2018;23:2759.

38. Faria A, Persaud SJ. Cardiac oxidative stress in diabetes: mechanisms and therapeutic potential. Pharmacology & therapeutics. 2017;172:50-62.

39. Umbarkar P, Singh S, Arkat S, Bodhankar S, Lohidasan S, Sitasawad SL. Monoamine oxidase-A is an important source of oxidative stress and promotes cardiac dysfunction, apoptosis, and fibrosis in diabetic cardiomyopathy. Free radical biology and medicine. 2015;87:263-73.

40. Jia G, DeMarco VG, Sowers JR. Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. Nature reviews endocrinology. 2016;12:144.

41. Ziegler D, Buchholz S, Sohr C, Nourooz-Zadeh J, Roden M. Oxidative stress predicts progression of peripheral and cardiac autonomic nerve dysfunction over 6 years in diabetic patients. Acta diabetologica. 2015;52:65-72.

42. Kayama Y, Raaz U, Jagger A, Adam M, Schellinger IN, Sakamoto M, Suzuki H, Toyama H, Spin JM, Tsao PS. Diabetic cardiovascular disease induced by oxidative stress. International journal of molecular sciences. 2015;16:25234-63.

43. Roslan J, Giribabu N, Karim K, Salleh N. Quercetin ameliorates oxidative stress, inflammation and apoptosis in the heart of streptozotocin-nicotinamide-induced adult male diabetic rats. Biomedicine & pharmacotherapy. 2017;86:570-82.

44. Münzel T, Gori T, Keaney Jr JF, Maack C, Daiber A. Pathophysiological role of oxidative stress in systolic and diastolic heart failure and its therapeutic implications. European heart journal. 2015;36:2555-64.

45. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. The Journal of clinical investigation. 2017;114:1752-61.

46. Anupama N, Rani MP, Shyni G, Raghu K. Glucotoxicity results in apoptosis in H9c2 cells via alteration in redox homeostasis linked mitochondrial dynamics and polyol pathway and possible reversal with cinnamic acid. Toxicology in Vitro. 2018;53:178-92.

47. Blake R, Trounce IA. Mitochondrial dysfunction and complications associated with diabetes. Biochimica et biophysica acta (BBA)-general subjects. 2014;1840:1404-12.

48. Mapanga RF, Essop MF. Damaging effects of hyperglycemia on cardiovascular function: spotlight on glucose metabolic pathways. American journal of physiology-heart and circulatory physiology. 2015;310:153-73.

49. Jemai H, Sayadi S. Heart histopathology and oxidative features in diabetic rats and protective effects of oleuropein. Advances in bioscience and biotechnology. 2015;6(06):383.

50. dos Santos KC, Cury SS, Ferraz APCR, Corrente JE, Gonçalves BM, de Araújo Machado LH, Carvalho RF, de Melo Stevenato Nakamune AC, Fabro AT, Freire PP, Corrêa CR. Recovery of Cardiac Remodeling and Dysmetabolism by Pancreatic Islet Injury Improvement in Diabetic Rats after Yacon Leaf Extract Treatment. Oxidative Medicine and cellular longevity. 2018;2018:1-9.

51. Ahmad M, Gwarzo M, Anwar S. Antioxidative and anti-hyperglycaemic effect of calotropis procera in alloxan induced diabetic rats. Journal of medicinal plants research. 2016;10:54-8.

52. Nasri H, Shirzad H, Baradaran A, Rafieian-Kopaei M. Antioxidant plants and diabetes mellitus. Journal of research in medical sciences: the official journal of Isfahan University of medical sciences. 2015;20:491.

53. Gupta NK, Srivastva N, Bubber P, Puri S. The antioxidant potential of Azadirachta indica ameliorates cardioprotection following diabetic mellitus-induced microangiopathy. Pharmacognosy magazine. 2016;12:371-78.

54. Bugger H, Abel ED. Molecular mechanisms of diabetic cardiomyopathy. Diabetologia. 2014;57:660-71.

55. Laakso M, Kuusisto J. Insulin resistance and hyperglycaemia in cardiovascular disease development. Nature reviews endocrinology. 2014;10:293.

56. Pan J, Fukuda K, Saito M. Mechanical stretch activates the JAK/STAT pathway in rat cardiomyocytes. Circulation research. 1999;84:1127–36.

57. Stephanou A. Role of STAT-1 and STAT-3 in ischaemia/reperfusion injury. Journal of cellular and molecular medicine. 2004;8:519-25.

58. Takeda K, Akira S. STAT family of transcription factors in cytokine-mediated biological responses. Cytokine & growth factor reviews. 2000;11:199-207.

59. Jia G, Whaley-Connell A, Sowers JR. Diabetic cardiomyopathy: a hyperglycaemia-and insulin-resistance-induced heart disease. Diabetologia. 2018;61:21-8.

60. Carvajal-Zarrabal O, Waliszewski SM, Barradas-Dermitz DM, Orta-Flores Z, Hayward-Jones PM, Nolasco-Hipólito C, Angulo-Guerrero O, Sánchez-Ricaño R, InfanzónPatricia RM, Trujillo RL. The consumption of Hibiscus sabdariffa dried calyx ethanolic extract reduced lipid profile in rats. Plant Foods for human nutrition (formerly qualitas plantarum). 2005;60:153-9.

61. Sibiya N, Ngubane P, Mabandla M. Cardio-protective effects of pectin-insulin patch in streptozotocin-induced diabetic rats Beneficial effects of insulin patch. Journal of diabetes. 2017;9:1073-78.

Chapter 3

Prologue

Manuscript 2

The haematological changes associated with hyperglycaemia have been shown to aggravate cardiovascular complications in diabetic patients. In addition, conventional treatment has been shown to alter haematological function, which eventually, advances cardiovascular dysfunction in diabetic patients. Hence the need of alternative treatment strategies. We have previously shown the anti-hyperglycaemic and reno-protective effects of *Momordica balsamina* in STZ-induced diabetic rats which may benefit patients with hyperglycaemia-induced haematological changes associated with cardiovascular dysfunction. The effects of MB on haematological function in STZ-induced diabetic rats however, have not been established. The present study therefore investigated the effects of *Momordica balsamina* methanolic extract on haematological function in STZ-induced diabetic rats with specific interests on red blood cell function. This is in an effort to establish the mechanisms by which MB may alleviate cardiovascular dysfunction in diabetic animal models.

The effects of *Momordica balsamina* methanolic extract on haematological function in STZ-induced diabetic rats: Effects on selected markers.

The present manuscript was prepared for publication according to the **Biomedicine and pharmacotherapy** journal's guidelines to authors. see (Appendix 3).

Biomedicine and pharmacotherapy
The Effects of *Momordica balsamina* Methanolic Extract on Haematological Function in Streptozotocin-induced Diabetic Rats: Effects on Selected Markers.

A. Ludidi[1], M. C Baloyi[1], A. Khathi[1], N. H. Sibiya[2], P. S Ngubane[1]

[1]Department of Human Physiology, University of KwaZulu-Natal, 4000, South Africa
[2]Department of Pharmacy, Rhodes University, 6140, South Africa

Correspondence should be addressed to Asiphaphola Ludidi; asaludidi6@gmail.com

Abstract

Background: Chronic hyperglycaemia-induced haemanetic changes increases the risk of cardiovascular complications in diabetic patients. The administration of Insulin injection as a bolus is accompanied with increased blood viscosity, which is not recommended for patients with congestive heart failure. *Momordica balsamina* (MB) methanolic extract has previously been shown to possess anti-hyperglycaemic and renal dysfunction ameliorative effects; however, the haematological effects of MB have not been shown. The current study therefore, investigated the short-term effects MB on selected haematological parameters in streptozotocin (STZ)-induced diabetic rats. **Methods:** Briefly, the air-dried *Momordica balsamina* leaves were sequentially extracted with methanol to yield a methanolic extract. STZ-induced diabetic rats were divided into untreated and treated groups with insulin (170 μg kg^{-1} s.c.) and metformin (500 mg kg^{-1} p.o.) MB (250 mg kg^{-1} p.o.). MB was administered twice daily for the 5-week experimental period. Blood glucose concentration was monitored weekly. Animals were sacrificed terminally. Blood and kidneys were collected for haematological and biochemical analysis respectively. **Results:** Treatment with MB significantly decreased blood glucose concentration and improved erythropoietin secretion, thus significantly increasing red blood cell production in treated diabetic animals by comparison to untreated animals. MB also significantly improved haemoglobin concentrations and moderately increased erythrocyte indices specifically, mean cell volume (MCV), mean cell haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) to no significance by comparison to untreated diabetic animals. MB treatment decreased the oxidative stress evoked by the induction of diabetes while improving the antioxidant status of treated animals by comparison to untreated animals respectively. **Conclusions:** Administration of *Momordica balsamina* methanolic extract protects against the injurious haematological changes induced by hyperglycaemia, which may reduce the risks of cardiovascular complications. **Key words**: Erythropoiesis, oxidative stress, *Momordica balsamina*, streptozotocin, hyperglycaemia

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycaemia (1). Sustained hyperglycaemia has been shown to progress the pathology of cardiac damage through haemanetic changes which include haemolysis of erythrocytes, consequent decrease in red blood cell count and haemoglobin concentrations which is associated with the development of diabetic anaemia and increases the risk of cardiovascular complications if left untreated (2). Hyperglycaemia has been shown to increase the probability of the non-enzymatic glycosylation of red blood cell membrane proteins resulting in the non-specific aggregation of protein molecules and alters the protein-protein and protein-lipid interaction leading to the

modification and disrupts the integrity/and symmetry of the erythrocyte membrane (3, 4). A loss of membrane symmetry increases erythrocyte aggregation, decreases mobility of the red blood cells and increases blood viscosity, which consequently elevates arterial pressure, thus increasing cardiovascular complications (5-7). In diabetic patients, sustained hyperglycaemia results in an increase in reactive oxygen species (ROS) and lipid peroxidation (8). Furthermore, chronic hyperglycaemia induced ROS production such as H_2O_2, crosses the erythrocyte membrane and oxidizes heme proteins, which have been shown to lead to the progressive loss of deformability and increased osmotic fragility of red blood cells (9). Consequently, diabetic patients with cardiovascular complications present with a decrease in haemoglobin (Hb) concentrations, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) which are haematological parameters for erythrocyte function (10). In addition. these changes induce the rapid initiation of apoptosis in damaged erythrocytes, decreasing the oxygen-carrying capacity of erythrocytes due to the increased haemolysis rate thereby significantly decreasing their lifespan (11). Apoptosis is characterised by membrane morphological alteration such as the exposure of the phosphatidylserine (PS) to the outer erythrocyte membrane surface which can be detected by staining with fluorescein isothiocyanate (FITC)-conjugated annexin-V, consequent membrane blebbing and microvesicle (MV) formation is observed (12).

In addition, diabetic nephropathy arising from prolonged hyperglycaemia is associated with a decrease in erythropoietin (EPO) secretion which is the primary stimulus for erythropoiesis (13). Inadequate EPO secretion results in the decreased RBC production and thus diabetic anaemia (14). Insulin is currently the primary means of treatment for DM in addition to the hypoglycaemic and anti-hyperglycaemic drugs in the market (15). However, insulin has been shown to increase the rate of agglutination of erythrocyte, increasing the viscosity of blood and consequently increases arterial pressure, which augments cardiac myopathy particularly in high cardiovascular risk diabetic patients. Furthermore drugs such as metformin have been shown to contribute to the progression of anaemia through the malabsorption of vitamin B_{12} (16). Vitamin B_{12} is important for normal erythropoiesis and RBC maturation, from the digestive tract. There is therefore a need for economical, alternative treatment for DM patients who are at risk of developing cardiovascular complications (17).

Various medicinal plants such as *Momordica charantia* and *Syzygium cordatum* (Hochst.) have been shown to possess anti-hyperglycaemic and reno-protective effects (18, 19). *Hemidesmus indicus Linn* has been shown to possess antioxidant activity and inhibited lipid peroxidation which may improve erythrocyte fragility and improve RBC function (20). Furthermore, medicinal plants such as *Prosopis gradulosa* have previously demonstrated the ability to increase haemoglobin concentrations (21).

Of interest to our study is *Momordica balsamina* (MB), a plant widespread in Namibia, Botswana, Swaziland and all provinces of Southern Africa (22). In our laboratory, we have recently shown anti-hyperglycaemic and reno-protective effects of MB in STZ-induced diabetic rats. The reno-protective effects may have via its antioxidant properties, which may be of benefit to diabetic patients with hyperglycaemic-induced haematological changes. (23). Hence the need to evaluate the haematological effects of this plant which are not yet established (24). The aim of this study therefore, is to investigate the effects of *Momordica balsamina* on selected haematological parameters in experimental STZ-induced diabetic rats.

Materials and methods

Drugs and chemicals

Dimethyl sulphoxide (Sigma-Aldrich, St Louis, Missouri, United States of America); glucose ($C_6H_{12}O_6$), metformin, monosodium citrate, calcium chloride ($CaCl_2$), citric acid, (Merck chemicals (Pty) Ltd Wadeville, Johannesburg, South Africa); streptozotocin (Sigma Aldrich Chemical Company, Missouri, St Louis, USA); insulin (NovoRapid pen refill, Novordisk Pty Ltd, Sandton, South Africa); FITC annexin-V (clone 563), BD Falcon round-bottom tubes (BD Biosciences, San Jose, CA); phosphate buffered saline (PBS) (Sigma Aldrich Co., St. Louis, MO).

Plant extraction

Professor H Baijnath, the former chief taxonomist/curator of the University of KwaZulu-Natal department of botany identified and authenticated *Momordica balsamina* leaves. The extraction of *Momordica balsamina* leaves were executed in the School of laboratory Medicine and Medical Sciences, at the University of KwaZulu-Natal, Westville Campus, following a previously validated protocol which has been previously reported by our laboratory (25). Briefly, the air-dried *Momordica balsamina* leaves (1.15kg) were sequentially extracted for 24 hours by cold percolation with methanol (95%, 6.9L). The methanolic extract was recovered from the mixture and methanol was added to the pulp for further extraction. To maximise the extraction process to increase the yield (609g), the process was repeated three times. The three extracts were combined to yield a concentrated methanolic extract at a reduced pressure (22-26mmHg) and temperature of 45-60 ºC.

Animals

In the present study, 30 male Sprague-Dawley rats (250-300 g) bred and housed in the Biomedical Research Unit (BRU) of University of KwaZulu-Natal. The rats were maintained under standard laboratory conditions of constant temperature (22±2 ºC), CO_2 content of <5000 p.m., relative humidity of 55±5% and illumination (12 h light/dark cycles) with the noise levels of less than 65 decibels. The animals were given standard rat chow daily and free access to water. Procedures performed on animal and their care were conducted in conformity with the institutional guidelines of the University of KwaZulu-Natal (AREC/023/017M). Before the study commenced, the animals were allowed to acclimatize for 5 days in metabolic cages.

Induction of diabetes mellitus

Type 1 diabetes mellitus were induced by a single intraperitoneal injection of 60 mg kg^{-1} STZ in freshly prepared 0.1 M citrate buffer (pH 4.5). Control group received the vehicle, citrate buffer via the same route. Animals that exhibited glucosuria after 24 h, when tested by urine strips were considered diabetic. Seven days following the induction of diabetes, the blood glucose concentration of STZ-induced diabetic animals above 20 mmol L^{-1} were regarded as stable diabetes.

Experimental design

The short-term effects of *Momordica balsamina* methanolic (MB) extract and standard drugs (insulin and metformin) were monitored over 5 weeks for haematological parameters in separate groups of non-diabetic (group 1) and STZ-induced diabetic (group 2-5) male Sprague-Dawley (SD) rats. Rats were individually housed in Makrolon polycarbonate metabolic cages (Techniplast, Labotec, South Africa). Group 1 received the drug vehicle DMSO to serve as a negative control. *Momordica balsamina* methanolic extract (250 mg kg^{-1}, p.o.) were administered to Group 2. Group 3 and 4 served were treated with insulin (175 µg kg^{-1}, s.c.) and metformin (500 mg kg^{-1}, p.o.), respectively, serving as positive controls. Non-diabetic animals in Group 5 served as absolute. MB was administered twice daily at 09h00 and 15h00. Blood glucose concentrations were measured every 3rd day at 09:00 for the duration of experimental period via the tail pricking method using the Elite® glucometer (Elite (Pty) Ltd., Health Care Division, South Africa) assessed.). The weekly haematocrit was measured with a micro-haematocrit, with 75x 16 mm capillary tubes filled with blood also collected similarly and centrifuged for 5 min (Cheesbrough, 2004). The EDTA anti-coagulated blood was collected into the capillary tubes, which were then sealed at one end with plasticine, and centrifuged at 3000g for 5 min, after which red cell levels in the capillary tubes were read using the microhaematocrit reader.

Terminal studies

At the end of the 5-week experimental period, all animals were sacrificed by exposing to halothane via a gas anaesthetic chamber (100 mg kg^{-1}) for 3 minutes (Biomedical Resource Unit, UKZN, Durban, South Africa). Thereafter blood was collected by cardiac puncture (RBCP) into individual pre-cooled heparinized container and centrifuged (Eppendorf centrifuge 5403, Germany) at 4 °C, 503 g for 15 minutes and separated plasma was stored at -80 °C in a Bio Ultra freezer (Snijers Scientific, Holland) for hormonal analysis. In addition, the remaining RBCs were stored in separate Eppendorf tubes and stored at -80 °C in a Bio Ultra freezer until use for flow cytometry analysis. The kidneys were removed for biochemical analysis.

Haematological analysis

Whole blood was collected from groups of untreated and treated diabetic animals to measure red blood cell (RBC) count, Mean Cell Haemoglobin (MCH), mean cell heamoglobin concentration (MCHC) and mean cell volume (MCV) using an automated haematology analyser (Beckman Coulter, California, United States).

Oxidative stress and erythropoietin analysis

To establish the effects of treatment on oxidative stress, levels of MDA, a commonly known marker of lipid peroxidation, were measured in plasma and kidneys using a biochemical assay as described below compared (26).The antioxidant defence enzymes: Superoxide dismutase (SOD) and glutathione peroxide (GPx) in plasma and kidney tissues of experimental animals were also measured using ELISA kits. Erythropoietin (EPO) concentrations were also

measured in the plasma of experimental animals of untreated and treated animals using an ELISA kit.

Malondialdehyde measurement

Kidney tissues (50 mg) were homogenized. The homogenate was centrifuged at 400 x g for 10 min. 500 µL of 0.2% phosphoric acid was added to the kidney homogenate and 100 µL plasma, respectively. Thereafter, 400 µL of both the homogenate and plasma were each supplemented with 400 µL 2% phosphoric acid and then separated into three glass tubes, each receiving equal volumes of the solution. Subsequently, 200 µL of 7% phosphoric acid was added into all glass tubes followed by the addition of 400 µL of thiobarbituric acid (TBA)/butylated hydroxytoluene (BHT) into two glass tube (sample tests) and 400 µL of 3 mM hydrochloric acid (HCl) into the third glass tube (blank). To ensure an acidic pH of 1.5, 200 µL of 1 M, HCl was added to sample and blank test tubes. All solutions were heated at 100℃ for 15 min, and allowed to cool to room temperature. Butanol (1.5 mL) was added to the cooled solution; the sample was vortexed for 1 min to ensure rigorous mixing and allowed to settle until two phases are distinguished. The butanol phase (top layer) was transferred to Eppendorf tubes and centrifuged at 13,200 x g for 6 min. The samples were aliquoted into a 96-well microtiter plate in triplicate and the absorbance was read at 532 nm (reference 600 nm) on a BioTek µQuant spectrophotometer (Biotek, Johannesburg, South Africa). The absorbance from these wavelengths were used to calculate the concentration of MDA using Beer's Law.

Concentration of MDA (mM)

$$= \frac{\text{Average Absorbance}}{\text{Absorption coefficient } (156\ \text{mmo}l^{-1})}$$

Superoxide dismutase and glutathione peroxidase and erythropoietin concentration

SOD and GPx concentrations in plasma and kidney tissues, plasma EPO were analysed using a specific ELISA kit (Elabscience and Biotechnology, WuHan) that uses the Sandwich-ELISA method. Kits included micro ELISA plate which were coated with antibody specific to SOD, GPx and EPO. Standards and samples were pipetted into the appropriate wells of the micro ELISA plate and incubated for 90 minutes. The plate relevant biotinylated detection antibody (100 µL) was then added and incubated for 60 minutes. Avidin-Horseradish Peroxidase (HRP) conjugate (100 µL) was added to each micro-plate well and incubated for 30 minutes. Unbound components were washed out. Substrate solution (100 µL) was added to each micro-plate well. After incubating for a further 15 minutes, the stop solution (50 µL) was added. The optical density was measured using a Nano spectrophotometer (BMG Labtech, Ortenburg, Baden-Wurttemberg, Germany) at the wavelength of 450 nm. The concentration of the samples was extrapolated from the respective standard curves.

Flow cytometry

A comparison of the percentage of red blood cells (RBC) expressing annexin-V among untreated and treated STZ-induced diabetic rats.

Red blood cell membrane analysis

Instrument set up: BD FACS Canto-II flow cytometer and BD FACSdiva software (BD Biosciences, San Jose, CA) were used to acquire data. In order to report standardized results, BD cytometer setup and tracking beads (BD Biosciences, San Jose, CA) were used for flow check to verify the cytometer's optical path and laminar flow.

Detector settings: This In order to detect small particles, forward scatter (FS) and side scatter (SS) parameters, were set at a log-scale (Figure 1 A). Voltages for the FS/SS photomultiplier tubes were set using an unstained fresh blood sample (Figure 1B). Antibody titration assays were then performed to detect optimal antibody concentrations.

Measurement of apoptotic red blood cell levels: Annexin-V FITC was used (Figure 1 B) as this antibody binds to the translocated phosphatidylserine (PS) from the inner leaflet of the plasma membrane to the outer leaflet consequently exposing PS to the external environment. Briefly, 50 µL of heparinized RBCs were stained with annexin-V FITC (1:10) antibody and incubated in the dark for 20 minutes at room temperature; samples were then suspended in 500 µL of PBS and analysed immediately.

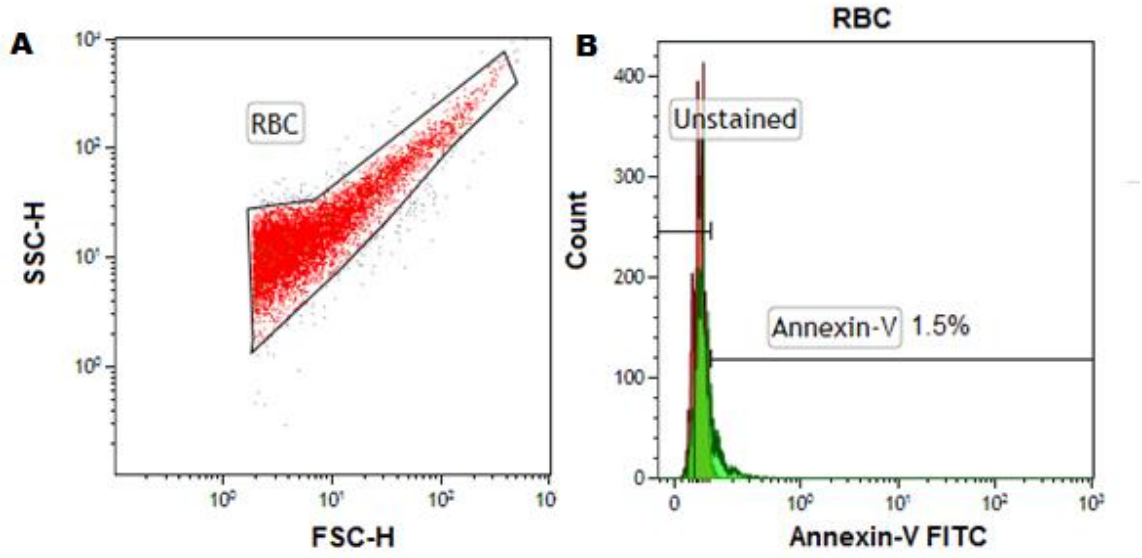

Figure 1: Gating strategy for annexin-V expression. The figure shows the gating strategy applied. (A) The colour dot plot depicts the red blood cells (RBCs) based on forward scatter (FSC) and side scatter (SS). (B) Demonstrates the expression of annexin-V.

Statistical analysis

All data are expressed as means ± standard error of means (SEM). To perform statistical analysis, GraphPad Prism Instat Software (version 5.00, GraphPad Software, San Diego, California, USA) was used. Blood glucose was analysed using analysis of variance (ANOVA) followed by Bonferroni post hoc test. ANOVA was used to analyse terminal parameters to analyse the differences between control and experimental groups. Values of $p<0.05$ were considered statistical significant between the compared groups.

Results

Blood glucose concentration

Figure 2 illustrates the blood glucose concentrations in non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS) over an experimental period of 5weeks. The untreated STZ-induced diabetic animals showed a significantly high blood glucose concentration throughout the period of 5 weeks by comparison with non-diabetic control $^{\alpha}$ (DC vs NC, $p<0.05$, Figure 2). Treatment with MB, insulin and metformin showed a significant decrease in blood glucose concentration from week three to week five by comparison with the diabetic control *(MB vs DC, $p<0.05$, Figure 2). At week 5, blood glucose concentrations of animals treated with insulin significantly decreased by comparison with animals treated with MB λ (INS vs MB, $p<0.05$, Figure 2).

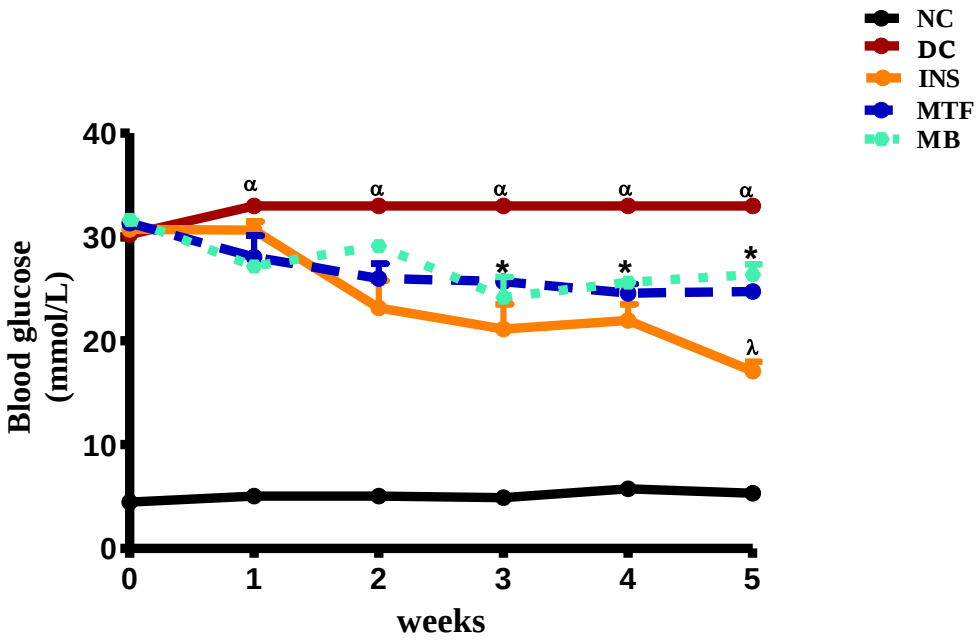

Figure 2: Blood glucose concentration in non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB), metformin (MTF) and insulin (INS) over the period of 5 weeks. Values are presented as means and vertical bars indicate SEM (n=6 in each group). α $p<0.05$ by comparison with non-diabetic control animals. * $p<0.05$ by comparison with diabetic control. λ $p<0.05$ by comparison with MB treated animals.

Haematological parameters

Table 1 shows the comparisons of haematological parameters in non-diabetic control (NC), diabetic control (DC) and STZ-induced diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS) at the end of the 5 weeks of treatment. Haematocrit and haemoglobin in untreated STZ-diabetic rats was significantly decreased in comparison to untreated non-diabetic rats. RBC in untreated STZ-diabetic rats were

significantly lower than untreated non-diabetic rats $^{\alpha}$ (DC vs NC, $p<0.05$). Interestingly, MB significantly increased the haematocrit, RBC and haemoglobin similarly to insulin and metformin treated rats by comparison with untreated STZ-diabetic rats *(MB vs DC, $p<0.05$). Red blood cell indices including MCV, MCHC, MCH in untreated animals slightly decreased by comparison non-diabetic animal. In addition, MB, insulin and metformin treatment moderately improved RBC indices in by comparison to untreated animals although no significance was reached.

Table 1: Shows the comparison of haematological parameters in non-diabetic control (NC), diabetic control (DC) and STZ-induced diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS). Values are presented as means ±SEM (n=6 per group).

	Treatment				
Parameters measured	NC	DC	INS	MTF	MB
RBC ($\times 10^6$cells/µL)	06.66 ± 0.41	05.39 ± 0.06 α	08.60 ± 0.31 *	08.56 ± 0.09 *	07.31 ± 0.44 *
Hct (%)	38.58 ± 0.05	31.38 ± 0.57 α	43.41 ± 2.34 *	44.15 ± 1.59 *	45.93 ± 0.43 *
Hb (g/dL)	13.07 ± 0.61	09.16 ± 0.18 α	15.23 ± 0.81 *	16.05 ± 0.47 *	15.82 ± 0.22 *
MCHC (g/dL)	37.18 ± 0.63	36.10 ± 0.20	35.60 ± 0.58	34.87 ± 0.81	36.15 ± 0.80
MCV (fL/cell)	51.83 ± 0.75	50.66 ± 0.21	52.83 ± 0.47	53.00 ± 0.58	51.33 ± 1.05
MCH (pg/cell)	19.21 ± 0.52	17.00 ± 0.29	19.33 ± 0.59	19.45 ± 0.67	18.98 ± 0.16

α $p<0.05$ by comparison with non-diabetic control animals. * $p<0.05$ by comparison with diabetic control.

MDA, SOD and GPx concentrations

Table 2 shows the comparisons of MDA, SOD and GPx concentrations in both plasma and kidney tissues in non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS) at the end of the 5 weeks of treatment. Plasma and kidney tissues of untreated STZ-diabetic animals presented with increased concentrations in MDA by comparison to untreated non-diabetic rats $^{\alpha}$ (DC vs NC, $p<0.05$). Furthermore, there was a significant decrease in both plasma and kidney

SOD and GPx concentrations in untreated STZ-diabetic animals, respectively, by comparison with untreated non-diabetic animals $^{\alpha}$ (DC vs NC, $p<0.05$). Both plasma and kidney tissues of MB treated STZ-induced diabetic rats, similarly to insulin and metformin treated rats, presented with a significant decrease in MDA concentrations by comparison to both plasma and kidney tissues in untreated diabetic rats *(MB vs DC, $p<0.05$). In addition, plasma and kidney GPx concentrations improved while plasma SOD concentrations deteriorated by comparison to untreated STZ-induced diabetic rats *(MB vs DC, $p<0.05$).

Table 2: Comparison of MDA, SOD and GPx concentrations of both the plasma and kidney tissues in non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS). Values are presented as means ±SEM (n=6).

		Plasma	**Kidney**
MDA (μmol/g protein)	NC	0.68 ± 0.12	0.99 ± 0.02
	DC	9.37 ± 1.19 α	4.21 ± 0.12 α
	INS	0.54 ± 0.03 *	1.10 ± 0.01 *
	MTF	0.35 ± 0.06 *	1.19 ± 0.01 *
	MB	2.48 ± 0.05 *	1.14 ± 0.01 *
GPx Concentration (ng/mL)	NC	1.44 ± 0.03	2137.31 ± 0.03
	DC	0.21 ± 0.04 α	1703.491 ± 0.03 α
	INS	0.83 ± 0.01 *	2062.21 ± 0.02 *
	MTF	1.67 ± 0.01 *	2125.75 ± 0.01 *
	MB	1.06 ± 0.04 *	21370.37 ± 0.03 *
SOD Concentration (ng/mL)	NC	35.39 ± 0.63	13.43 ± 0.76
	DC	33.55 ± 0.27	06.01 ± 0.09 α
	INS	31.68 ± 2.76	09.89 ± 0.10 *
	MTF	29.85 ± 2.44	10.11 ± 0.02 *
	MB	23.63 ± 0.87 *	10.08 ± 0.05 *

α $p<0.05$ by comparison with non-diabetic control animals. * $p<0.05$ by comparison with diabetic control.

Plasma erythropoietin concentrations

Figure 3 visualises the comparisons of plasma erythropoietin (EPO) concentration in non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS) after 5 weeks of the experimental period. Diabetic controls showed a significant decrease in plasma EPO

concentration by comparison to the non-diabetic control group $^{\alpha}$ (DC vs NC, $p<0.05$, Figure 3). Interestingly, the administration of MB, similarly to insulin, and metformin significantly increased plasma erythropoietin concentrations after the 5-week experimental period *(MB vs DC, $p<0.05$, Figure 3).

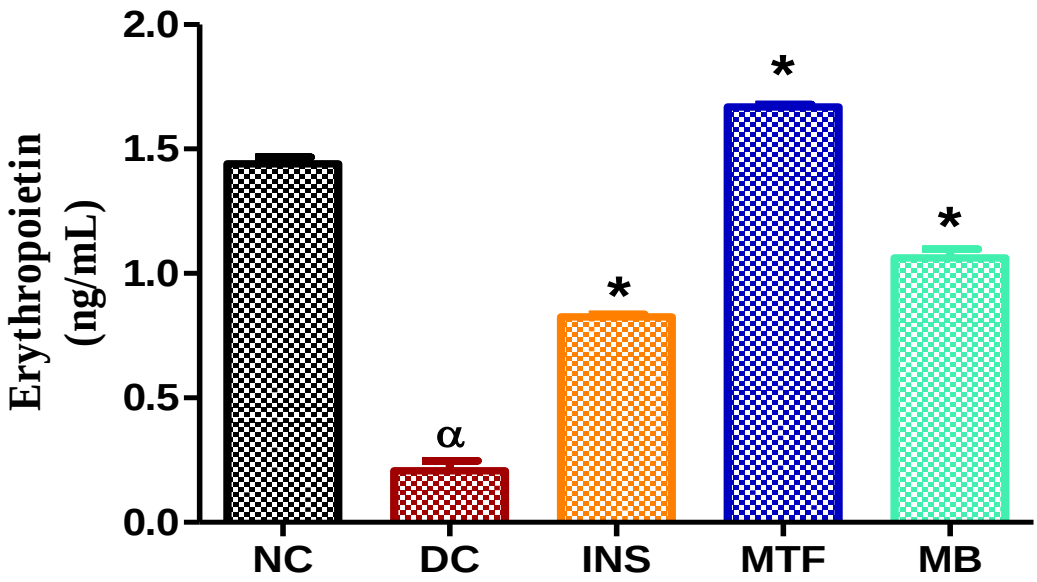

Figure 3: Plasma erythropoietin concentration in non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB), metformin (MTF) and insulin (INS) after the period of 5 weeks. Values are presented as means and vertical bars indicate SEM (n=6 in each group). α $p<0.05$ by comparison with non-diabetic control animals. * $p<0.05$ by comparison with diabetic control.

Percentage of annexin-V on red blood cell membrane

Figure 4 visualizes the comparisons percentage of annexin-V expressed on red blood cell membrane in non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB) (250 mg kg^{-1}), metformin (MTF) and insulin (INS) measured upon termination of the 5-week experimental period. Diabetic controls a significantly increased red blood cell annexin-V expression by comparison to the non-diabetic control group $^{\alpha}$ (DC vs NC, $p<0.05$, Figure 4). Insulin and metformin treated animals exhibited a significant decrease in the percentage of annexin-V expressed on red blood cell membrane by comparison with untreated STZ-induced diabetic animals *(INS vs DC, $p<0.05$, Figure 4),*(MTF vs DC, $p<0.05$, Figure 4). However, the administration of MB increased the percentage of annexin-V expressed on red blood cell membrane significantly by comparison to both untreated STZ-induced diabetic animals after the 5-week experimental period *(MB vs DC, $p<0.05$, Figure 4).

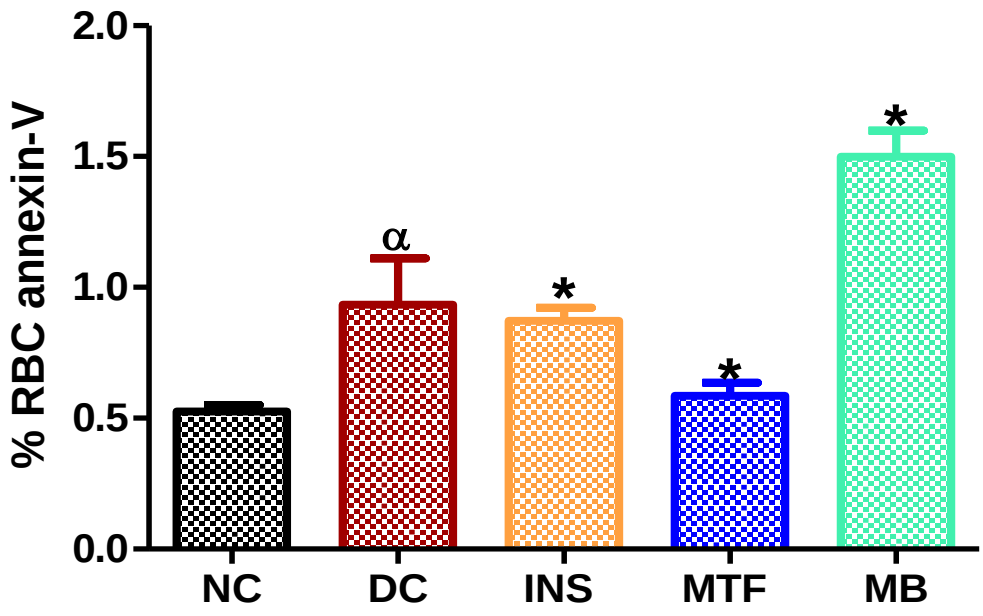

Figure 4: The percentage of red blood cell membranes expressing annexin-V in non-diabetic control (NC), diabetic control (DC) and diabetic animals treated with *Momordica balsamina* (MB), metformin (MTF) and insulin (INS) after the experimental period of 5 weeks. Values are presented as means and vertical bars indicate SEM (n=6 in each group). α $p<0.05$ by comparison with non-diabetic control animals. * $p<0.05$ by comparison with diabetic control.

Discussion

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycaemia which is associated with haematological changes that progress cardiovascular pathology (27, 28). The current study assessed the effects of treatment with *Momordica balsamina* methanolic extract on selected haematological parameters which have been shown to be altered under diabetic conditions, increasing cardiovascular dysfunction (29). In our laboratory, we have shown *Momordica balsamina* possesses anti-hyperglycaemic effects and ameliorates kidney dysfunction (23).

The red blood cells (RBC) are adapted to readily undergo cellular deformation without rupturing as it navigates through the vascular system for gaseous exchange between blood and tissues (30). Furthermore, hyperglycaemia has been shown to impair erythrocyte deformability through increasing lipid peroxidation and oxidative stress, which increases their risk of rupturing which may account for the decrease in red blood cell count in the STZ-induced diabetic animals. The decrease in the number of RBCs was accompanied by a significant decline in haemoglobin (Hb) among the STZ-induced diabetic animals (31). The severe deterioration in Hb observed in the STZ diabetic animals suggest that the animals were anaemic since the decline was well below that of normal expected hemoglobin range (32). The Hb molecule contains iron atoms which bind oxygen atoms to be transported throughout the system (33). The increased risk of ischemia would consequently correlate with the development of cardiac myopathy in STZ-induced diabetic animals (34). In addition, as hyperglcaemia increases the glycation rate of Hb, thereby raising extracorpuscular Hb that has been shown to

decrease RBC fragility thus shortening the life span of RBCs. However, treatment with insulin, metformin and MB improved Hb concentrations to within a normal expected range, possibly via an improvement of hyperglycaemic state which was also observed by Komolafe and et al. when treating STZ-induced diabetic rats with *Momordica charantia* (35). Notably, the homeostatic shift of a decline in Hb concentrations had no statistical significant impact on RBC indices such as MCV, MCH and MCHC. However, there was a physiological significance as RBCs can present with normal mean corpuscular volume, a condition known as normocytic anaemia which may be associated with kidney dysfunction (36). In harmony with a study conducted by Keskin and et al. , MCV, MCH and MCHC of untreated STZ-induced diabetic rats, showed no significant change at the end of the experimental period (37). It has also been extensively shown in literature that STZ-induced diabetic animals have an increase in mean arterial blood pressure, which may further increase the rupturing rate of erythrocytes (38). However, the antidiabetic properties of *Momordica balsamina* may have buffered the MDA concentrations, attenuating lipid peroxidation as the RBC membrane is rich in lipids to maintain its fluidity to facilitate deformability without rupturing which was in agreement with previous studies (39). In addition, antioxidant status was improved through increasing GPx, which detoxifies ROS product, hydrogen peroxide, to water thereby partially restoring membrane integrity (5, 40). However, hyperglycaemia results in disturbances in protein function due increased production of ROS and non-enzymatic glycation of many proteins including superoxide dismutase rendering it inefficient as observed in our experimental animals. A plant with antidiabetic properties such as *Allium sativum* has been shown to inhibit alanine production in ROS exposed erythrocytes thereby protecting erythrocytes from protein degradation, loss of deformability and increased osmotic fragility (8). Surprisingly, administration with *Momordica balsamina* did not improve the exposure of phosphatidylserine (PS) to the extracellular fluid, which may mark the erythrocytes for engulfment by local macrophages. A study conducted by Parminder K, et al showed that the seeds of *Momordica balsamina* are rich in balsamin, which is a 28 kDa protein that has been shown to promote apoptosis in breast cancer cells (41). The increase in the apoptotic activity of *Momordica balsamina* treated animals may be therefore attributed to some proapoptotic properties of this plant. Further studies on other antidiabetic compounds of *Momordica balsamina,* such as flavonoids warrant investigation to further understand the mechanism by which medicinal plants may restore RBC membrane integrity in STZ-induced diabetic rats. In addition, due to ethical constraints, the experimental period was five weeks; therefore increasing the treatment period may have shown an improvement on some haematological function markers.

The administration of STZ to mimic a type-1 diabetes rodent model has been shown to induce renal dysfunction which was previously observed in our laboratory (23). In addition, kidneys of STZ-induced diabetic rats in our study showed a decrease in the antioxidant status, which was associated with an insufficient erythropoietin secretion and RBC function. However, *Momordica balsamina* improved EPO secretion by the kidneys possibly through preventing the metabolic injury to peritubular interstitial fibroblast-like cells by advanced glycation end products (AGEs) formation. The increase in EPO, may account for the increase in RBC count and improvement in RBC indices in *Momordica balsamina* treated animals. Improving RBC function also restores the blood's oxygen-carrying capacity, which may improve cardiovascular dysfunction associated with diabetes.

Conclusion

As the foregoing discussion on the use of medicinal plants and their potential therapeutic effects on hyperglycaemia-induced haematological changes is underway, the current study enlightens some beneficial properties of *Momordica balsamina* administration. Results show improvement of the antioxidant status stemming from *Momordica balsamina*a's antidiabetic properties. This was associated with an improvement in RBC function possibly through increased EPO secretion although RBC membrane exposure of PS to the surface was not improved. Taken together, *Momordica balsamina* methanolic extract administration improves some haematological alterations associated with hyperglycaemia. Further investigations are envisaged to expand the indigenous knowledge in an effort to widen the spectrum range of easily accessible remedies for diabetes management.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

The authors appreciate assistance and support from the haematology group under the supervision of Dr B.B Nkambule and Biomedical Research Unit, University of KwaZulu-Natal. The current study was partly funded by NRF South Africa and the University of KwaZulu-Natal, Research Division.

References

1. Association AD. Diagnosis and classification of diabetes mellitus. Diabetes care. 2012;35:64-71.
2. Thomas MC, MacIsaac RJ, Tsalamandris C, Power D, Jerums G. Unrecognized anemia in patients with diabetes. Diabetes care. 2003;26:1164-9.
3. Awasthi S, Gayathiri S, Ramya R, Duraichelvan R, Dhason A, Saraswathi N. Advanced glycation-modified human serum albumin evokes alterations in membrane and eryptosis in erythrocytes. Applied biochemistry and biotechnology. 2015;177:1013-24.
4. Chen PM, Gregersen H, Zhao JB. Advanced glycation end-product expression is upregulated in the gastrointestinal tract of type 2 diabetic rats. World journal of diabetes. 2015;6:662.
5. Sila A, Kamoun Z, Ghlissi Z, Makni M, Nasri M, Sahnoun Z, et al. Ability of natural astaxanthin from shrimp by-products to attenuate liver oxidative stress in diabetic rats. Pharmacological reports. 2015;67:310-6.
6. Pamu P, Shanmugam M, Subramanian S. Beneficial Effects of Tephrosia purpurea Ethanolic Seed Extract on Lipids and Membrane Bound Enzymes in Experimental Diabetic Rats. Journal of young pharmacists. 2017;9:550.

7. Diederich L, Suvorava T, Sansone R, Keller IV TS, Barbarino F, Sutton TR, Kramer CM, Lückstädt W, Isakson BE, Gohlke H. On the effects of reactive oxygen species and nitric oxide on red blood cell deformability. Frontiers in physiology. 2018;9:332.
8. Suboh S, Bilto Y, Aburjai T. Protective effects of selected medicinal plants against protein degradation, lipid peroxidation and deformability loss of oxidatively stressed human erythrocytes. Phytotherapy research. 2004;18:280-4.
9. Shilov A, Avshalumov AS, Markovsky V, Sinitsyna E, Poleshchuk O. Changes of blood rheological properties in patients with metabolic syndrome. Russ med J. 2008;4:200-4.
10. Miikue-Yobe TFB. Effect of aqueous leaf extract of heinsia crinata on haematological and some biochemical indices of toxicity in streptozotocin induced diabetic rats. International journal of scientific research and innovative technology. 2015;2:116-126.
11. Wesseling MC, Wagner-Britz L, Huppert H, Hanf B, Hertz L, Nguyen DB, Berhardt I. Phosphatidylserine exposure in human red blood cells depending on cell age. Cellular physiology and biochemistry. 2016;38:1376-90.
12. Mindukshev IV, Krivoshlyk V, Dobrylko IA, Goncharov NV, Vivulanets EV, Kuznetsov SV, Krivchenko AI. Abnormalities of elastic and transporting properties of red blood cells under development of apoptosis. Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology. 2010;4:22-31.
13. Suresh S, Alvarez JC, Noguchi CT. Erythropoietin Eliminates Increased Bone Marrow Adiposity and Alters Bone Features in Obese Mice. Am Soc Hematology; 2017.
14. Association AD. Diagnosis and classification of diabetes mellitus. Diabetes care. 2014;37:81-90.
15. Leone A, Di Gennaro E, Bruzzese F, Avallone A, Budillon A. New perspective for an old antidiabetic drug: metformin as anticancer agent. Advances in Nutrition and Cancer. 2014;159:355-76.
16. Liu KW, Dai LK, Jean W. Metformin-related vitamin B12 deficiency. Age and ageing. 2006;35:200-1.
17. Hayanga JA, Ngubane SP, Murunga AN, Owira PM. Grapefruit juice improves glucose intolerance in streptozotocin-induced diabetes by suppressing hepatic gluconeogenesis. European journal of nutrition. 2016;55:631-8.
18. Cemek M, Kağa S, Şimşek N, Büyükokuroğlu ME, Konuk M. Antihyperglycemic and antioxidative potential of Matricaria chamomilla L. in streptozotocin-induced diabetic rats. Journal of natural medicines. 2008;62:284-93.
19. Kotnis MS, Patel P, Menon SN, Sane RT. Renoprotective effect of Hemidesmus indicus, a herbal drug used in gentamicin-induced renal toxicity. Nephrology. 2004;9:142-52.
20. Kainthla R, Kashyap R, Deopujari J, Purohit H, Taori G, Daginawala H. Effect of Hemidesmus indicus (Anantmool) extract on IgG production and adenosine deaminase activity of human lymphocytes in vitro. Indian journal of pharmacology. 2006;38:190.
21. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circulation research. 2000;87:840-4.
22. Thakur GS, Bag M, Sanodiya BS, Bhadauriya P, Debnath M, Prasad G, Bisen PS. Momordica balsamina: a medicinal and neutraceutical plant for health care management. Current pharmaceutical biotechnology. 2009;10:667-82.

23. Siboto A, Sibiya N, Khathi A, Ngubane P. The Effects of Momordica balsamina Methanolic Extract on Kidney Function in STZ-Induced Diabetic Rats: Effects on Selected Metabolic Markers. Journal of diabetes research. 2018;2018:1-9.
24. Joseph B, Jini D. Antidiabetic effects of Momordica charantia (bitter melon) and its medicinal potency. Asian Pacific journal of tropical disease. 2013;3:93-102.
25. Mkhwanazi B, Serumula M, Myburg R, Heerden F, Musabayane C. antioxidant effects of maslinic acid in liver, hearts and kidneys of streptozotocin-induces diabetic rats: effects on kidney function. Renal failure. 2014;36:419-31.
26. Kasapoglu M, Özben T. Alterations of antioxidant enzymes and oxidative stress markers in aging. Experimental gerontology. 2001;36:209-20.
27. Xanthopoulos A, Giamouzis G, Melidonis A, Kitai T, Paraskevopoulou E, Paraskevopoulou P, Patsilinakos S, Triposkiadis F, Skoularigis J. Red blood cell distribution width as a prognostic marker in patients with heart failure and diabetes mellitus. Cardiovascular diabetology. 2017;16:81.
28. Nuhu F, Bhandari S. Oxidative Stress and Cardiovascular Complications in Chronic Kidney Disease, the Impact of Anaemia. Pharmaceuticals. 2018;11:103.
29. Kawamura M, Paulsen MJ, Goldstone AB, Shudo Y, Wang H, Steele AN, Stapleton LM, Edwards BB, Eskandari A, Truong, VN. Tissue-engineered smooth muscle cell and endothelial progenitor cell bi-level cell sheets prevent progression of cardiac dysfunction, microvascular dysfunction, and interstitial fibrosis in a rodent model of type 1 diabetes-induced cardiomyopathy. Cardiovascular diabetology. 2017;16:142.
30. Muthinja JM, Ripp J, Krüger T, Imle A, Haraszti T, Fackler OT, Spatz JP, Engstler M, Frischknecht F. Tailored environments to study motile cells and pathogens. Cellular microbiology. 2018;20:12820.
31. Millot S, Delaby C, Moulouel B, Lefebvre T, Pilard N, Ducrot N, et al. Hemolytic anemia repressed hepcidin level without hepatocyte iron overload: lesson from Günther disease model. haematologica. 2017;102:260-70.
32. Jacob Filho W, Lima CC, Paunksnis MRR, Silva AA, Perilhão MS, Caldeira M, Bocalini D, de Souza RR. Reference database of hematological parameters for growing and aging rats. The Aging male. 2018;21:145-8.
33. Su Y, Xie Z, Kim GB, Dong C, Yang J. Design strategies and applications of circulating cell-mediated drug delivery systems. ACS biomaterials science & engineering. 2015;1:201-17.
34. Santos-Ribeiro D, Godinas L, Pilette C, Perros F. The integrated stress response system in cardiovascular disease. Drug discovery today. 2018;23:920-29.
35. Komolafe OA, Ofusori DA, Odukoya SA, Adewole OS. Momordica charantia restores compromised haematological parameters in streptozotocin-induced diabetic rats. Fiziologia-Physiology. 2016;26.
36. Buttarello M. Laboratory diagnosis of anemia: are the old and new red cell parameters useful in classification and treatment, how? International journal of laboratory hematology. 2016;38:123-32.
37. Yakhchalian N, Mohammadian N, Hatami K, Nosrati H, Yousofvand N. Hematological and Serum Biochemical Analysis of Streptozotocin-Induced Insulin Dependent Diabetes Mellitus in male adult Wistar Rats. bioRxiv. 2018:359844.

38. Sirdah MM. Protective and therapeutic effectiveness of taurine in diabetes mellitus: a rationale for antioxidant supplementation. Diabetes & Metabolic Syndrome: Clinical research & reviews. 2015;9:55-64.
39. Kalaycı D, Arpacı AH, Çomu FM, Güneş I, Beşkardeş E, Kurtipek Ö, Arslan M, Dikmen B. Investigation of the Effects of Sevoflurane and Desflurane on Erythrocyte Deformability in Transient Hyperglycemia. Gazi medical journal. 2017;29:2147.
40. Reczek CR, Chandel NS. ROS-dependent signal transduction. Current opinion in cell biology. 2015;33:8-13.
41. Ajji PK, Binder MJ, Walder K, Puri M. Balsamin induces apoptosis in breast cancer cells via DNA fragmentation and cell cycle arrest. Molecular and cellular biochemistry. 2017;432:189-98.

Chapter 4: Synthesis

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycaemia which has been associated with micro and macroangiopathies that contribute to the increased mortility and morbidity rate of diabetic patients (1). Chronic hyperglycaemia increases the risk of developing cardiovascular complications and induces haematological changes that may aggravate cardiac dysfunction (2). In this study, we investigated the short-term effects of *Momordica balsamina* (MB) methanolic extract administration on selected cardiovascular and haematological function markers in streptozotocin (STZ)-induced diabetic rats.

The induction of diabetes mellitus with STZ, an antibiotic that selectively destroys pancreatic β-cells, achieved a hyperglycaemic rat model as evidenced by the sustained hyperglycaemia of untreated animals. Studies have shown haematological and cardiovascular dysfunction in STZ-induced hyperglycaemic animal models. In addition, literature has shown a link between alleviating hyperglycaemia-induced haematological changes and improved cardiac myopathies. Medicinal plants have shown the ability to lower blood glucose and improve haematological and cardiovascular function although the mechanisms have not been fully established. In particular, the effects of these medicinal plants on RBC structure and function. Hence, the study investigated the effects of *Momordica balsamina* methanolic extract on haematological and cardiovascular function of STZ-induced diabetic rats. In this present study, the selective destruction of pancreatic β cell resulted in insufficient insulin secretion thereby resulting in sustained hyperglycaemia in untreated animals. Furthermore, there was a deterioration of cardiac function as observed by an increase in proinflammatory cytokines such as carditrophin-I and C-reactive protein. The deterioration of cardiac function may have been associated with the elevated oxidative stress as marked by an increase in malondialdehyde (MDA) of untreated STZ-induced diabetic animals in this study. The increased oxidative stress was also associated with endothelial dysfunction as evidenced by an increase in a vasoconstrictor Ang-II. Furthermore, the cardiac dysfunction may have also been due to hyperglycaemia-induced haematological changes via increased lipid peroxidation as shown by increased MDA concentrations, which have been shown to decrease RBC function as marked by decreased MCV, MCH and MCHC observed in untreated diabetic animals. Furthermore, an alteration in RBC indices shows a compromised red blood cell profile that was further shown by a decline in haemoglobin concentrations, may progress to anaemia if left untreated. Haemoglobin is a protein which binds oxygen in RBCs for transport to cells, therefore

assessing haemoglobin may enlighten researchers on drug-target for the management of DM related haematological complications (178). An improvement in hyperglycaemia, oxidative stress upon treatment with MB was observed, suggesting that MB may possess bioactive compounds, which improve the antioxidant status and promote RBC production thus improving both haematological and cardiac function in diabetic animals. Since there was an improvement in the RBC production, we further assessed the quality of the RBCs by investigating the integrity of the RBC membrane. The integrity of the membrane of RBCs must be maintained in order for it to deform with ease through the vasculature without rupturing. However, when the RBC membrane is compromised as marked by the exposure of phosphatidylserine (PS) to the exterior, it is marked for engulfment by macrophages. The administration of *Momordica balsamina* therefore improved some cardiovascular and haematological parameters.

References

1. Kawamura M, Paulsen MJ, Goldstone AB, Shudo Y, Wang H, Steele AN, et al. Tissue-engineered smooth muscle cell and endothelial progenitor cell bi-level cell sheets prevent progression of cardiac dysfunction, microvascular dysfunction, and interstitial fibrosis in a rodent model of type 1 diabetes-induced cardiomyopathy. Cardiovascular diabetology. 2017.

2. Berthiaume JM, Kurdys JG, Muntean DM, Rosca MG. Mitochondrial NAD+/NADH redox state and diabetic cardiomyopathy. Antioxidants & redox signaling. 2017.

3. Xanthopoulos A, Giamouzis G, Melidonis A, Kitai T, Paraskevopoulou E, Paraskevopoulou P, et al. Red blood cell distribution width as a prognostic marker in patients with heart failure and diabetes mellitus. Cardiovascular diabetology. 2017.

Conclusion

Our results taken together show that the administration of *Momordica balsamina* methanolic extract has the potential to alleviate the haematological changes induced by hyperglycaemia by attenuating hyperglycaemia, oxidative stress and promoting erythropoietin secretion thus improving red blood cell profile. Furthermore, *Momordica balsamina* methanolic extract also improved cardiac function as observed by a decrease in mean arterial pressure, inflammation and cardiac hypertrophy. The ability of medicinal plants such as *Momordica balsamina* to improve red blood cell function may therefore be a therapeutic target for alleviating cardiovascular dysfunction often observed in diabetic patients.

Recommendations

Further studies on the isolation of the bioactive compounds of *Momordica balsamina* need to beconducted in order to further understand the mechanisms by which this plant improves haematological and cardiac function. Furthermore, studying the effects of bioactive compounds on other haematological parameters such as profiling red blood cell development in the bone marrow, white blood cell activation and platelet regulation, which are impaired in hypergylcaemia-induced cardiovascular complications, of treated experimental animals will further unveil the mechanisms by which medicinal plants may improve cardiac function.

Appendices

Appendix 1 - AREC ethics approval letter

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

12 June 2017

Ms Asiphaphola Ludidi (216017652)
School of Health Sciences
Westville Campus

Dear Ms Ludidi,

Protocol reference number: AREC/023/017M
Project title: The effects of *Momordica balsamina* on glucose metabolism in STZ diabetic rats: Effects on selected metabolic markers

Full Approval – Research Application

With regards to your revised application received on 07 June 2017. The documents submitted have been accepted by the Animal Research Ethics Committee and **FULL APPROVAL** for the protocol has been granted.

Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 12 June 2018.

Attached to the Approval letter is a template of the Progress Report that is required at the end of the study, or when applying for Renewal (whichever comes first). An Adverse Event Reporting form has also been attached in the event of any unanticipated event involving the animals' health / wellbeing.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

.....

Prof S Islam, PhD
Chair: Animal Research Ethics Committee

/ms

Cc Supervisor: Phikelelani S Ngubane
Cc Academic Leader Research: Professor J van Heerden
Cc Registrar: Mr Simon Mokoena
Cc NSPCA: Ms Stephanie Keulder
Cc BRU – Dr Sanil Singh

Animal Research Ethics Committee (AREC)
Ms Mariette Snyman (Administrator)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 8350 **Facsimile:** +27 (0) 31 260 4609 **Email:** animalethics@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Animal-Ethics.aspx

Appendix 2 – Manuscript 1 Journal guide

GUIDE FOR AUTHORS

Your Paper Your Way

We now differentiate between the requirements for new and revised submissions. You may choose to submit your manuscript as a single Word or PDF file to be used in the refereeing process. Only when your paper is at the revision stage, will you be requested to put your paper in to a 'correct format' for acceptance and provide the items required for the publication of your article. **To find out more, please visit the Preparation section below.**

INTRODUCTION

CHEMICO-BIOLOGICAL INTERACTIONS publishes research reports and review articles that examine the molecular, cellular, and/or biochemical basis of toxicologically relevant outcomes. Special emphasis is placed on toxicological mechanisms associated with interactions between chemicals and biological systems. Outcomes may include all traditional endpoints caused by synthetic or naturally occurring chemicals, both in vivo and in vitro. Endpoints of interest include, but are not limited to carcinogenesis, mutagenesis, respiratory toxicology, neurotoxicology, reproductive and developmental toxicology, and immunotoxicology.

CBI discourages papers that are descriptive in nature and that do not address toxicological mechanisms (e.g., reports of toxicological effects following chemical exposure in absence of mechanistic experiments). CBI also discourages papers reporting on toxicological effects from materials, such as plant extracts or herbal medicines, that have not been chemically characterized.

Types of paper

Chemico-Biological Interactions will publish, (1) Papers reporting results of original mechanistic research; (2) Review articles; (3) Letters to the Editor; (4) Announcements and advertisements. Review Articles - Outlines of papers for these sections should be submitted to the Editor in Chief. Announcements and Advertisements - Organisers of relevant meetings may submit announcements through the Editor-in-Chief for publication free of charge as space permits. Advertising rates can be obtained upon request.

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:

E-mail address

Full postal address

All necessary files have been uploaded:

Manuscript:

Include keywords

All figures (include relevant captions)

All tables (including titles, description, footnotes)

Ensure all figure and table citations in the text match the files provided

Indicate clearly if color should be used for any figures in print

Graphical Abstracts / Highlights files (where applicable)

Supplemental files (where applicable)

Further considerations

Manuscript has been 'spell checked' and 'grammar checked'

All references mentioned in the Reference List are cited in the text, and vice versa

Permission has been obtained for use of copyrighted material from other sources (including theInternet)

A competing interests statement is provided, even if the authors have no competing interests todeclare

Journal policies detailed in this guide have been reviewed

Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN

Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Studies in humans and animals

If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The manuscript should be in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations. The terms sex and gender should be used correctly.

Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

All animal experiments should comply with the ARRIVE guidelines and should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the authors should clearly indicate in the manuscript that such guidelines have been followed. The sex of animals must be indicated, and where appropriate, the influence (or association) of sex on the results of the study.

Conflict of interest

Chemico-Biological Interactions follows the ICMJE recommendations regarding conflict of interest disclosures. All authors are required to report the following information with each submission: All thirdparty financial support for the work in the submitted manuscript. All financial relationships with any entities that could be viewed as relevant to the general area of the submitted manuscript. All sources of revenue with relevance to the submitted work who made payments to you, or to your institution on your behalf, in the 36 months prior to submission. Any other interactions with the sponsor of outside of the submitted work should also be reported. Any relevant patents or copyrights (planned, pending, or issued). Any other relationships or affiliations that may be perceived by readers to have influenced, or give the appearance of potentially influencing, what you wrote in the submitted work.

As a general guideline, it is usually better to disclose a relationship than not. This information will be acknowledged at publication in a Transparency Document. Additional information on the ICMJE recommendations can be found at: http://www.icmje.org. The form for conflict of interest disclosure can be downloaded here, or at http://www.icmje.org/coi_disclosure.pdf (if this link does not display properly in your browser, please right-click the link and select "Save Target As..." or "Save Link as..." from the popup menu.)

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including

electronically without the written consent of the copyrightholder. To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

Preprints

Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Articles should make no assumptions about the beliefs or commitments of any reader, should contain nothing which might imply that one individual is superior to another on the grounds of race, sex, culture or any other characteristic, and should use inclusive language throughout. Authors should ensure that writing is free from bias, for instance by using 'he or she', 'his/her' instead of 'he' or 'his', and by making use of job titles that are free of stereotyping (e.g. 'chairperson' instead of 'chairman' and 'flight attendant' instead of 'stewardess').

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Article transfer service

This journal is part of our Article Transfer Service. This means that if the Editor feels your article is more suitable in one of our other participating journals, then you may be asked to consider transferring the article to one of those. If you agree, your article will be transferred automatically on your behalf with no need to reformat. Please note that your article will be reviewed again by the new journal. More information.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For gold open access articles: Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (more information). Permitted third party reuse of gold open access articles is determined by the author's choice of user license.

Author rights

As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing

Find out how you can share your research published in Elsevier journals.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Funding body agreements and policies

Elsevier has established a number of agreements with funding bodies which allow authors to comply with their funder's open access policies. Some funding bodies will reimburse the author for the gold open access publication fee. Details of existing agreements are available online.

Open access

This journal offers authors a choice in publishing their research:

Subscription

Articles are made available to subscribers as well as developing countries and patient groups throughour universal access programs.

No open access publication fee payable by authors.

The Author is entitled to post the accepted manuscript in their institution's repository and make this public after an embargo period (known as green Open Access). The published journal article cannot be shared publicly, for example on ResearchGate or Academia.edu, to ensure the sustainability of peerreviewed research in journal publications. The embargo period for this journal can be found below.

Gold open access

Articles are freely available to both subscribers and the wider public with permitted reuse.

A gold open access publication fee is payable by authors or on their behalf, e.g. by their researchfunder or institution.

Regardless of how you choose to publish your article, the journal will apply the same peer review criteria and acceptance standards.

For gold open access articles, permitted third party (re)use is defined by the following Creative Commons user licenses:

Creative Commons Attribution (CC BY)

Lets others distribute and copy the article, create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), include in a collective work (such as an anthology), text or data mine the article, even for commercial purposes, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, and do not modify the article in such a way as to damage the author's honor or reputation.

Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

For non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

The gold open access publication fee for this journal is **USD 2200**, excluding taxes. Learn more about Elsevier's pricing policy: https://www.elsevier.com/openaccesspricing.

Green open access

Authors can share their research in a variety of different ways and Elsevier has a number of green open access options available. We recommend authors see our open access page for further information. Authors can also self-archive their manuscripts immediately and enable public access from their institution's repository after an embargo period. This is the version that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and in editor-author communications. Embargo

period: For subscription articles, an appropriate amount of time is needed for journals to deliver value to subscribing customers before an article becomes freely available to the public. This is the embargo period and it begins from the date the article is formally published online in its final and fully citable form. Find out more.

This journal has an embargo period of 12 months.

Elsevier Researcher Academy

Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

Additional information

Manuscripts are accepted for review with the understanding that the same work has not been published, that it is not under consideration for publication elsewhere, and that its submission for publication has been approved by all of the authors and by the institution where the work was carried out. Further, it is understood that any person cited as a source of personal communications has approved such citation.

Chemico-Biological Interactions discourages papers that are descriptive in nature and that do not address toxicological mechanisms (e.g., reports of toxicological effects following chemical exposure in the absence mechanistic experiments). *Chemico-Biological Interactions* also discourages papers reporting on toxicological effects from materials, such as plant extracts or herbal medicines, which have not been chemically characterized.

It is a condition of publication that all manuscripts must be submitted in English to *ChemicoBiological Interactions* submission and review website http://ees.elsevier.com/chembioint. Authors are requested to transmit the text and art of the manuscript in electronic form to this address. Each manuscript must also be accompanied by a cover letter outlining the basic findings of the paper and their significance. Minimal exceptions will be exercised. The Editor welcomes submissions by the authors of the names and addresses of up to five individuals who could expertly review the paper, and who are not from the same institutions as the authors. The Editor reserves the right to use these or other reviewers. Should you be unable to provide an electronic version, please contact the editorial office prior to submission at toxcon@earthlink.net.

PREPARATION

NEW SUBMISSIONS

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.

As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or layout that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately.

References

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/ book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Formatting requirements

There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Artwork and Tables with Captions.

If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes. Divide the article into clearly defined sections.

Please ensure the text of your paper is double-spaced– this is an essential peer review requirement.

Figures and tables embedded in text

Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure or table.

Peer review

This journal operates a single blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. More information on types of peer review.

REVISED SUBMISSIONS

Use of word processing software

Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). See also the section on Electronic artwork.

To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Theory/calculation

A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

Title. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

Author names and affiliations. Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lowercase superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any

future queries about Methodology and Materials. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**

Present/permanent address. If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract of approx. 200-300 words is mandatory. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Graphical abstract

Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site.

Authors can make use of Elsevier's Illustration Services to ensure the best presentation of their images and in accordance with all technical requirements.

Highlights

Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). You can view example Highlights on our information site.

Keywords

A list of 3-6 key words, which convey the meaning of the paper as a whole, necessary for correct indexing and subsequent retrieval, must be submitted with the manuscript also. In the

event that key words are not supplied editorial discretion will be exercised in introducing appropriate words.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors build footnotes into the text, and this feature may be used. Should this not be

the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article.

Artwork

Electronic artwork General points

Make sure you use uniform lettering and sizing of your original artwork.

Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier.

Number the illustrations according to their sequence in the text.

Use a logical naming convention for your artwork files.

Indicate per figure if it is a single, 1.5 or 2-column fitting image.

For Word submissions only, you may still provide figures and their captions, and tables within asingle file at the revision stage.

Please note that individual figure files larger than 10 MB must be provided in separate source files.A detailed guide on electronic artwork is available.

You are urged to visit this site; some excerpts from the detailed information are given here. *Formats*

Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.

TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.

TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.

TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.

Please do not:

Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.

Supply files that are too low in resolution.

Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF) or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) in addition to color reproduction in print. Further information on the preparation of electronic artwork.

Figure captions

Ensure that each illustration has a caption. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884. Please note the format of such citations should be in the same style as all other references in the paper.

Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Data references

This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

References in a special issue

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software

Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley and Zotero, as well as EndNote. Using the word processor plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. More information on how to remove field codes.

Users of Mendeley Desktop can easily install the reference style for this journal by clicking the following link:

http://open.mendeley.com/use-citation-style/chemico-biological-interactions

When preparing your manuscript, you will then be able to select this style using the Mendeley plugins for Microsoft Word or LibreOffice.

Reference formatting

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume and issue/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference

style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style

Text: Indicate references by number(s) in square brackets in line with the text. The actual authors can be referred to, but the reference number(s) must always be given.

Example: '..... as demonstrated [3,6]. Barnaby and Jones [8] obtained a different result ....' *List:* Number the references (numbers in square brackets) in the list in the order in which they appear in the text.

Examples:

Reference to a journal publication:

[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, The art of writing a scientific article, J. Sci. Commun.

163 (2010) 51–59. https://doi.org/10.1016/j.Sc.2010.00372.

Reference to a journal publication with an article number:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon.

19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.

Reference to a book:

W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.

Reference to a chapter in an edited book:

G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z.Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.

Reference to a website:

Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/, 2003 (accessed 13 March 2003).

Reference to a dataset:

[dataset] [6] M. Oguro, S. Imahiro, S. Saito, T. Nakashizuka, Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1, 2015. https://doi.org/10.17632/ xwj98nb39r.1.

Journal abbreviations source

Journal names should be abbreviated according to the List of Title Word Abbreviations.

Video

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. . In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Data visualization

Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

Supplementary material

Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

Research data

This journal encourages and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To

facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. If you are sharing data in one of these ways, you are encouraged to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking

If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Mendeley Data

This journal supports Mendeley Data, enabling you to deposit any research data (including raw and processed data, video, code, software, algorithms, protocols, and methods) associated with your manuscript in a free-to-use, open access repository. During the submission process, after uploading your manuscript, you will have the opportunity to upload your relevant datasets directly to *Mendeley Data*. The datasets will be listed and directly accessible to readers next to your published article online.

For more information, visit the Mendeley Data for journals page.

Data in Brief

You have the option of converting any or all parts of your supplementary or additional raw data into one or multiple data articles, a new kind of article that houses and describes your data. Data articles ensure that your data is actively reviewed, curated, formatted, indexed, given a

DOI and publicly available to all upon publication. You are encouraged to submit your article for *Data in Brief* as an additional item directly alongside the revised version of your manuscript. If your research article is accepted, your data article will automatically be transferred over to *Data in Brief* where it will be editorially reviewed and published in the open access data journal, *Data in Brief*. Please note an open access fee of 500 USD is payable for publication in *Data in Brief*. Full details can be found on the Data in Brief website. Please use this template to write your Data in Brief.

MethodsX

You have the option of converting relevant protocols and methods into one or multiple MethodsX articles, a new kind of article that describes the details of customized research methods. Many researchers spend a significant amount of time on developing methods to fit their specific needs or setting, but often without getting credit for this part of their work. MethodsX, an open access journal, now publishes this information in order to make it searchable, peer reviewed, citable and reproducible. Authors are encouraged to submit their MethodsX article as an additional item directly alongside the revised version of their manuscript. If your research article is accepted, your methods article will automatically be transferred over to MethodsX where it will be editorially reviewed. Please note an open access fee is payable for publication in MethodsX. Full details can be found on the MethodsX website. Please use this template to prepare your MethodsX article.

Data statement

To foster transparency, we encourage you to state the availability of your data in your submission. This may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you will have the opportunity to indicate why during the submission process, for example by stating that the research data is confidential. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page.

Additional information

It is understood that with submission of this article the authors have complied with the institutional policies governing the humane and ethical treatment of the experimental subjects, and that they are willing to share the original data and materials if so requested.

AFTER ACCEPTANCE

Online proof correction

Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word:

in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors. If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.

We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's Webshop. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

AUTHOR INQUIRIES

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.

You can also check the status of your submitted article or find out when your accepted article will be published.

Appendix 3 – Manuscript 2 Journal guide

Journal Title

Concise and Informative Article Title

Firstname M. I. Lastname,[1] Firstname A. Lastname,[2] and Firstname B. Lastname[1,2]

[1] Department, Institute, City ZIP/Post code, Country.

[2] Department, Institute, City ZIP/Post code, Country.

Correspondence should be addressed to Firstname B. Lastname; lastname@institution.edu

Abstract

The abstract should be a single, self-contained paragraph which summarises the manuscript. Ideally, it will provide a brief context for the study, before describing the scientific approach and some key results in a qualitative manner. It should finish with a sentence to describe the implications for the field. The abstract must not include references, figures or tables.

Introduction

The introduction should be succinct, with no subheadings. Limited figures may be included only if they are truly introductory, and contain no new results.

Materials and Methods

The materials and methods section should contain sufficient detail so that all procedures can be repeated. It may be divided into headed subsections if several methods are described.

Results and Discussion

Subheadings

The results and discussion may be presented separately, or in one combined section, and may optionally be divided into headed subsections.

Advice on Equations

Equations should be provided in a text format, rather than as an image. Microsoft Word's equation tool is acceptable. Equations should be numbered consecutively, in round brackets, on the right-hand side of the page. They should be referred to as Equation 1, etc. in the main text.

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \qquad (1)$$

Advice on Figures

At the point of submission, authors may provide all figures embedded within the manuscript at a convenient break near to where they are first referenced or, alternatively, they may be provided as separate files. All figures should be cited in the paper in a consecutive order.

Where possible, figures should be displayed on a white background. When preparing figures, consider that they can occupy either a single column (half page width) or two columns (full page width), and should be sized accordingly. All figures must have an accompanying caption which includes a title and, preferably, a brief description (see Figure 1).

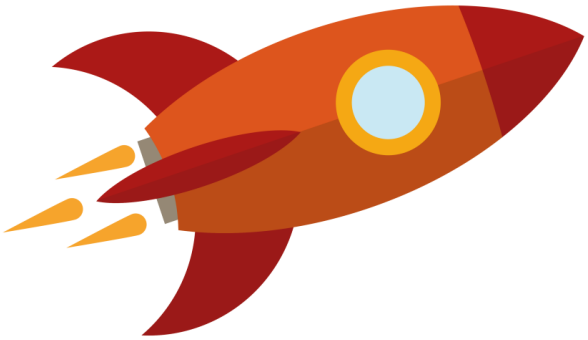

Figure 1: Basic rocket ship design. The rocket ship is propelled with three thrusters and features a single viewing window. The nose cone is detachable upon impact.

The caption can also be used to explain any acronyms used in the figure, as well as providing information on scale bar sizes or other information that cannot be included in the figure itself. Plots that show error bars should include in the caption a description of how the error was calculated and the sample size (see Figure 2).

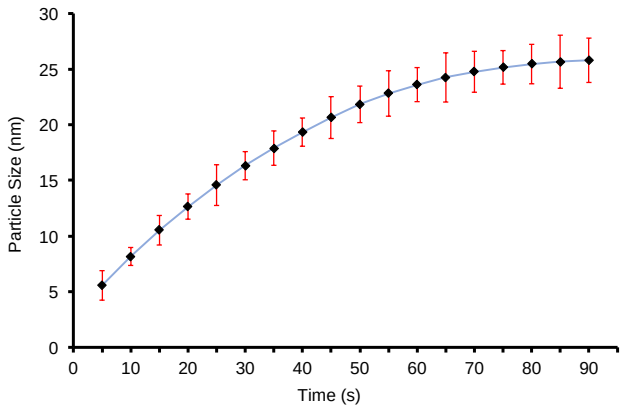

Figure 2: Plot of nanoparticle size with respect to time, recorded over a 90 s period. The error bars represent the standard deviation of measurements for 20 particles in five separate sample runs (n = 100).

If a figure consists of multiple panels, they should be ordered logically and labelled with lower case roman letters (i.e., a, b, c, etc.). If it is necessary to mark individual features within a panel (e.g., in Figure 3a), this may be done with lowercase Roman numerals, i, ii, iii, iv, etc. All labels should be explained in the caption. Panels should not be contained within boxes unless strictly necessary.

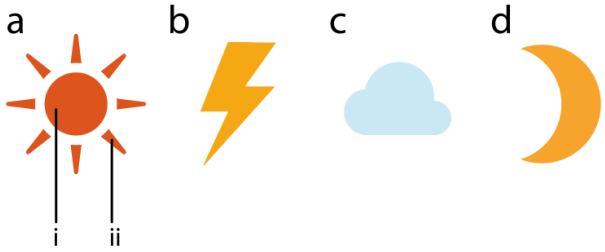

Figure 3: Representations of some common weather symbols. (a) The sun with (i) core, and (ii) rays. (b) Thunder bolt. (c) Cloud. (d) Moon.

Upon acceptance, authors will be asked to provide the figures as separate electronic files. At that stage, figures should be supplied in either vector art formats (Illustrator, EPS, WMF, FreeHand, CorelDraw, PowerPoint, Excel, etc.) or bitmap formats (Photoshop, TIFF, GIF, JPEG, etc.). Bitmap images should be of at least 300 dpi resolution, unless due to the limited resolution of a scientific instrument. If a bitmap image has labels, the image and labels should be embedded in separate layers.

Advice on Tables

Every table must have a descriptive title and, if numerical measurements are given, the units should be included in the column heading. Vertical rules should not be used (see Table 1). Tables should be cited consecutively in the text.

Table 1: Temperature and wildlife count in the three areas covered by the study.

Location	T [° C]	Turtles	Sharks	Octopuses	Starfish
Blue Lagoon	21.2	5	3	4	543
Regent's Canal	5.2	8	0	24	312
Shark Bay	12.8	4	7	9	122

Conclusions

The Conclusions section should clearly explain the main findings and implications of the work, highlighting its importance and relevance.

Data Availability

A data availability statement is compulsory for research articles and clinical trials. Here, authors must describe how readers can access the data underlying the findings of the study, giving links to online repositories and providing deposition codes where applicable. For more information on how to compose a data availability statement, including template examples, please visit: https://www.hindawi.com/research.data/#statement.

Conflicts of Interest

This section is compulsory. A competing interest exists when professional judgment concerning the validity of research is influenced by a secondary interest, such as financial

gain. We require that our authors reveal any possible conflict of interest in their submitted manuscripts. If there is no conflict of interest, authors should state that "The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper."

Some of the information you choose to provide here may constitute your "sensitive personal data". Please read our Privacy Policy for further information on how we handle your personal data.

Funding Statement

Authors should state how the research and publication of their article was funded, by naming financially supporting bodies followed by any associated grant numbers in square brackets.

Acknowledgments

An Acknowledgements section is optional and may recognise those individuals who provided help during the research and preparation of the manuscript.

Supplementary Materials

If Supplementary Materials are provided (e.g., audio files, video clips or datasets) they should be described here. Note that authors are responsible for providing the final Supplementary Materials files that will be published along with the article, which are not modified by our production team. You should remember to reference the Supplementary Materials' contents at appropriate points within the manuscript. We recommend citing specific items, rather than referring to the Supplementary Materials in general, for example: "See Figures S1-S10 in the Supplementary Material for comprehensive image analysis."

References

References will be reformatted in house, there is no need to adhere to a specific style at the point of submission. Authors are responsible for ensuring that the information in each reference is complete and accurate. All citations in the text must be numbered consecutively in square brackets, before any punctuation, for example, "as discussed by Smith [1]," and "as discussed elsewhere [2,3]." All uncited references will be automatically removed. The references should not contain footnotes. For your information, our citation style is:

[x] Author initials and surname, "Title in sentence style," Journal title, vol. (volume number), no. (issue number), pp. (page numbers separated by an en-dash), Year.

For example:

[1] J. D. Watson and F. H. C. Crick, "A structure for deoxyribose nucleic acid," *Nature*, vol. 171, no. 4356, pp. 737–738, 1953.

For articles with six or more authors, the first three authors are listed followed by ‘et al.’. When journals use only article numbers, no page numbers are necessary. For example:

[2] B. P. Abbott, R. Abbott, T. D. Abbott et al., “Observation of Gravitational Waves from a Binary Black Hole Merger,” *Physical Review Letters*, vol. 116, no. 6, Article ID 061102, 2016.

Appendix 4 – Certificate of CoBneST (PSSA) 2018 conference attendance

MYCPD

Discovery Health

Continuing Professional Development

Certificate of attendance

This is to certify that

Ms Asiphaphola Ludidi

LUDIDI

Attended the

CoBNeST 2018

on

07-Oct-2018 - 10-Oct-2018

at

Spier Wine Farm - Stellenbosch

Accredited by

University of Stellenbosch

Category	CPD Points	CPD Level	Accreditation #
Clinical	18	1,00	MDB006-MD024-0011-9-2018

M. R. Goodman

Dr Maurice Goodman
Head of Health Profession Strategy
Discovery health

- MYCPD CONNECT -

Appendix 5 – Abstract of CHS symposium 2018 poster presentation

Chronic hyperglycaemia as observed in Diabetes Mellitus (DM) has been shown to increase the probability of the non-enzymatic glycosylation of red blood cell membrane proteins resulting in the non-specific aggregation of protein molecules and alters the protein-protein and protein-lipid interaction leading to the modification and damage of the erythrocyte membrane (1). The modifications increase erythrocyte aggregation, decreased mobility of the red blood cells and increased blood viscosity which consequently elevates arterial pressure, this way inducing cardiac myopathy (2). However, the administration of insulin and other conventional drugs have been associated with the increased rate of agglutination of erythrocyte, increasing the viscosity of blood and consequently increases arterial pressure and disruption of erythrocyte function, which augments cardiac myopathy particularly in high cardiovascular risk diabetic patients (3). There is therefore a need for economical, alternative treatment such as medicinal plants for DM patients who are at risk of developing cardiovascular complications. Traditionally various plants have been shown to alleviate diabetes associated complications. Of interest to our study is *Momordica balsamina.* (MB) commonly known as "Intshungu" is a plant widespread in provinces of Southern Africa (4). In our laboratory, we have recently shown anti-hyperglycaemicic and reno-protective effects of MB. However, the haemantic effects of this plant are not yet known (5, 6).