

**THE ANTIDIABETIC PROPERTIES OF FOUR PLANTS
GROWN IN INDIA AND KWAZULU-NATAL, SOUTH
AFRICA, SUITABLE FOR DIABETIC MANAGEMENT**

RAYMOL GEORGEKUTTY

January 2015

UNIVERSITY OF ZULULAND

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RAYMOL GEORGEKUTTY

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Degree of Doctor of Philosophy in Biochemistry**

SUPERVISOR: PROF A.R. OPOKU



DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

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DECLARATION

The research described in this thesis was carried out in the Department of Biochemistry and Microbiology at the University of Zululand, KwaDlangezwa, under the supervision of Prof. A.R. Opoku. These studies have not otherwise been submitted in any form for any degree or diploma at any University. Where use has been made of the work of others, it is duly acknowledged in the text.

Raymol Georgekutty

I certify that the above statement is correct.

Professor A.R. Opoku

DEDICATION

**Call to me, and I will answer you; I will tell you wonderful
and marvelous things that you know nothing about**

Jeremiah 33:3

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ABSTRACT

Over the years, it has been suggested that various medicinal plants have anti-glycaemic properties which could help in the management of diabetes mellitus, a complex metabolic disorder. We aimed to evaluate the nutritional content (proximate and mineral) of four ayurvedic plants: *Abelmoschus esculentus* (L.) Moench, *Momordica charantia* L., *Moringa oleifera* Lam, *Solanum melongena* L. to identify nutritional differences between the Indian and South African varieties. An assessment of the anti-glycaemic activity of the aqueous and boiled extracts of the samples from South Africa was carried out by monitoring glucose, triglyceride and cholesterol levels as well as body weight in alloxan-induced diabetic rats and their control group over a 15 day period. Ninety-two Sprague-Dawley rats were separated into 7 groups (see table 3.3). Diabetes was induced in 48 rats by a single intraperitoneal injection of alloxan, 150mg/kg body weight. The apparent mechanism of anti-glycaemia was also investigated using the inverted intestine test, the glycogen content within the liver, the activity of Na/K ATPase and by assessing glucokinase and hexokinase activity by analysing liver and gastrocnemius muscle tissue.

Results revealed that all four plants were reported to have protein, fats, glucose and starch, with the South African varieties showing higher protein content than the Indian varieties. According to mineral analysis by the varian inductively coupled plasma (ICP), all the four plants are rich in minerals. The *in vivo* studies revealed that all the plant extracts showed hypoglycaemic effects within the two week study period except the *A.esculentus* boiled extract. Preliminary experiments demonstrated that aqueous

extracts of *M.charantia* and *A.esculentus* had maximum antidiabetic potential compared to the other plant extracts. The aqueous extract of *M.charantia* and *A.esculentus* fruits, at a dose of 300 mg/kg body weight, showed a statistically significant lowering of the glucose level of alloxan induced diabetic rats compared to the control group from 22.78 mmol/L to 8.1 mmol/L and 22.2 mmol/L to 13.3 mmol/L respectively, after 15 days of treatment. The enteral use of *M.charantia* and *A.esculentus* extract resulted in an increase in body weight in diabetic rats and a reduction in body weight in the diabetic control rats. *M.charantia* and *A.esculentus* extracts lowered the entry of glucose into the intestine. Treatment with *M.charantia* and *A.esculentus* increased the glycogen content by 47.3% (*M.charantia* aqueous) 59.2% (*M.charantia* boiled), 55% (*A.esculentus* aqueous) compared to the diabetic control group.

The evaluation of Na⁺/K⁺-ATPase activity revealed a more statistically significant inhibitory activity (25%) with the use of *A.esculentus* compared to *M.charantia* (12.5%). Hexokinase and glucokinase activity was reduced in the diabetic control rats as compared to the non-diabetic rats, with respective percentage decreases in activity of 66.7% and 83.3%. Treatment with *Momordica charantia* and *Abelmoschus esculentus* significantly increases the activity of these enzymes ($P < 0.001$ as compared to diabetic controls). On the other hand, there was no change in glucokinase activity in non-diabetic treated groups.

Histological analysis of diabetic control livers showed slight degenerative changes, which was within normal histological limits. Analysis of groups treated with extract showed mild diffuse sinusoidal congestion with mild hydropic degeneration which was within normal histological limits. Gastrocnemius muscle analysis of diabetic control, diabetic and non-diabetic treated groups appeared within normal histological limits. This revealed that the plant extracts of *M.charantia* and *A.esculentus* were non-toxic and thus advisable for safe consumption. The inclusion of these vegetables on a regular basis in the daily diet, together with medication and exercise would improve general health and blood glucose control in diabetic patients.

LIST OF ABBREVIATIONS USED

AOAC :	Association of Official Analytical Chemists
<i>A. esculentus</i> :	<i>Abelmoschus esculentus</i> (L.) Moench
ATP:	Adenosine triphosphate
DM:	Diabetes Mellitus
DTT:	Dithiothreitol
EDTA:	Ethylene diamine tetra-acetic acid
EGTA :	Ethylene glycol tetra-acetic acid
GK:	Glucokinase
GLUT:	Glucose transporter
GLUT-2:	Glutathione 2
GLUT-4:	Glutathione 4
HK:	Hexokinase
HEPES:	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
ICP:	Inductively Coupled Plasma
In	India
KZN:	Kwazulu-Natal
<i>M. charantia</i> :	<i>Momordica charantia</i> L
<i>M. oleifera</i> :	<i>Moringa oleifera</i> Lam
MTT:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaCl:	Sodium chloride
NAD:	Nicotinamide adenine dinucleotide
NADH:	Reduced nicotinamide adenine dinucleotide
NADP:	Nicotinamide adenine dinucleotide phosphate
SA	South Africa
<i>S. melongena</i> :	<i>Solanum melongena</i> L.
Tris-HCl:	Trizma hydrochloride
WHO:	World Health Organisation

RESEARCH OUTPUT

1. Presentations

Georgekutty R and Opoku A.R. *In vivo* anti-diabetic potentials of *Momordica charantia* L. in the management of type 2 diabetes. A research paper presented at the research seminars in the Faculty of Science and Agriculture, University of Zululand on 17th October, 2014.

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Georgekutty R and Opoku A.R. The Proximate Composition of two vegetables: Okra (*Abelmoschus esculentus* L.) and Brinjal (*Solanum melongena* L.) from India and South Africa. The SAAB (South African Association of Botanists), Venda University on 11-15th January 2015.

2. Manuscripts submitted for editorial consideration

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Georgekutty, R and Opoku, A.R. 2014. Anti-diabetic effects of freeze-dried overnight aqueous extract and boiled extract of *Abelmoschus esculentus* fruit on normal and diabetic rats.

TABLE OF CONTENTS

	page
DECLARATION	ii
DEDICATION	iii
ACKNOWLEDGEMENTS	iv
ABSTRACT	vi
LIST OF ABBREVIATIONS USED	ix
RESEARCH OUTPUT	x
TABLE OF CONTENTS	xii
LIST OF TABLES	xx
LIST OF FIGURES	xxi

CHAPTER 1

1.1 INTRODUCTION	1
1.2 Structure of the Thesis	2

CHAPTER 2

REVIEW OF LITERATURE	3
2.1 Diabetes Mellitus	3
2.2 Classification	4

2.2.1 Type 1 Diabetes Mellitus	4
2.2.2 Type 2 Diabetes Mellitus	6
2.2.3 Gestational Diabetes	8
2.2.4 Other causes	9
2.3. Epidemiology of diabetes mellitus	10
2.4. Risk factors	11
2.5 Diagnosis of Diabetes	11
2.6 Pathophysiology of diabetes mellitus	12
2.6.1 Mechanism of Insulin Mediated Glucose Uptake	12
2.7 Treatment of Diabetes Mellitus – Non-insulin agents	15
2.7.1 Insulin Secretagogues	15
2.7.1.1 <i>Sulphonylureas</i>	15
2.7.1.2 <i>Meglitinides</i>	16
2.7.2 Insulin Sensitisers	17
2.7.2.1 <i>Biguanides</i>	17
2.7.2.2 <i>Thiazolidinediones</i>	18
2.7.3 Other Agents	19
2.7.3.1 <i>Alpha glucosidase Inhibitors</i>	20
2.7.3.2 <i>Dipeptidyl Peptidase-IV Inhibitors</i>	20
2.7.3.3 <i>SGLT 2 Antagonists</i>	21
2.8 Plants used for the treatment of diabetes	23
2.8.1 <i>Abelmoschus esculentus</i> (L).	25

2.8.2. <i>Momordica charantia</i> L. BITTER GOURD, Karela, Family: Cucurbitace	27
2.8.3 <i>Moringa oleifera</i> Lam. DRUMSTICK; Muringa ; Family: Moringaceae	29
2.8.4 <i>Solanum melongena</i> L. EGGPLANT/BRINJAL; Baigon; Family: Solanaceae	31
2.9 Aim of the study	33
2.10 objectives	33

CHAPTER: 3

MATERIALS AND METHODS	34
3.1 Chemicals and reagents Sigma–Aldrich Chemical Co	34
3.2 Equipment	35
3.3 Collection and preparation of plant materials	35
3.4 Proximate analysis:	36
3.4.1 Determination of Moisture Content	36
3.4.2 Determination of Ash Content	36
3.4.3 Determination of Protein Content	37
3.4.4 Determination of fat content	37
3.4.5 Carbohydrate Analysis	37
3.5 Mineral analysis:	38
3.6 Preparation of freeze-dried Plant extract	38
3.6.1 Preparation of samples:	39

3.7 Animal experiments	39
3.7.1 To determine the hypoglycaemic effects of the four plant extracts on normal and diabetic rats	40
3.7.2 Harvesting of liver and muscle tissue	42
3.7.3 Histology of liver and gastrocnemius muscle	42
3.8. To determine the hypoglycaemic mechanism of the plant extracts in diabetic rats	42
3.8.1. Transport of glucose across a membrane of rat's intestine	42
3.8.2. The effect of extract on Na/K ATPase	43
3.8.3 Assessment of the glycogen content	44
3.8.4 Glucokinase Activity in Liver	44
3.8.5 Hexokinase Activity in Muscle	45
3.8.6 Statistical analysis	45

CHAPTER: 4

RESULTS

4.1 General	46
4.2 Evaluation and comparison of the nutritional values of selected vegetables from India and South Africa	46
4.2.1 Proximate composition:	47
4.2.2 Mineral content:	48

4.3	Effects of Plant extract on Body Weight of different groups of normal & alloxan induced diabetic rats.	51
4.3.1	Effect of <i>Momordica charantia</i> fruit extract on Body weight	51
4.3.2	Effect of <i>Abelmoschus esculentus</i> fruit extract on body weight	52
4.3.3	Effect of <i>Moringa oleifera</i> leaf extract on body weight	53
4.3.4	Effect of <i>Solanum melongena</i> fruit extract on body weight	54
4.3.5	Effect of all 4 plant extracts on body weight	55
4.4	Hypoglycemic effects of <i>Momordica charantia</i> fruit extract on normal and alloxan induced diabetic rats	56
4.4.1	Effect of <i>M.charantia</i> extracts on blood sugar:	56
4.4.2	Effect of <i>M.charantia</i> extracts on Triglyceride	57
4.4.3	Effect of <i>M.charantia</i> extracts on Cholesterol	58
4.5	Anti-diabetic effects of freeze-dried, overnight aqueous and boiled extracts of <i>Abelmoschus esculentus</i> (okra) fruit on normal and diabetic rats.	58
4.5.1	Anti diabetic effects of <i>A.esculentus</i> fruit extracts on blood glucose levels	59.
4.5.2	Anti diabetic effects of <i>A.esculentus</i> fruit extracts on Triglyceride levels	60
4.5.3	Anti diabetic effects of <i>A.esculentus</i> fruit extracts on Cholesterol levels	61
4.6	Hypoglycaemic effects of freeze-dried overnight aqueous and boiled extracts of <i>Moringa oleifera</i> leaf extracts on normal and diabetic rats.	61
4.6.1	Hypoglycaemic effects of <i>Moringa oleifera</i> leaf on Blood sugar	62
4.6.2	Hypoglycaemic effects of <i>Moringa oleifera</i> leaf on Triglyceride	63
4.6.3	Hypoglycaemic effects of <i>Moringa oleifera</i> leaf on Cholesterol	64

4.7 Hypoglycaemic effects of freeze-dried overnight aqueous and boiled extract of <i>Solanum melongena</i> (Brinjal) fruit on normal and diabetic rats.	65
4.7.1 Hypoglycaemic effects of <i>S. melongena</i> on Blood glucose level	65
4.7.2 Hypoglycaemic effects of <i>S. melongena</i> on Triglyceride	66
4.7.3 Hypoglycaemic effects of <i>S. melongena</i> on Cholesterol	67
4.8 Hypoglycaemic effects of freeze-dried overnight aqueous and boiled extracts of all 4 plant extracts together (1:1 ratio) on normal and diabetic rats.	68
4.8.1 Hypoglycaemic effects of all 4 plant extracts on Blood sugar.	68
4.8.2 Hypoglycaemic effects of all 4 plant extracts on Triglyceride	69
4.8.3 Hypoglycaemic effects of all 4 plant extracts on Cholesterol	70
4.9 Efficacy of all 4 selected plants extracts with metformin in blood glucose level	71
4.10 Determination of the apparent hypoglycaemic mechanism of the plant extracts (<i>M.charantia</i> and <i>A.esculentus</i>) in diabetic rats	73
4.10.1 <i>In vitro</i> transport of glucose across rat's intestine	73
4.10.2 The effect of extract on Na/K ATPase	74
4.10.3 Assessment of the glycogen content	75
4.10.4 Enzyme Glucokinase and Hexokinase	77
4.10.5. Histopathological analysis of liver and gastrocnemius muscle tissues of normal and experimental groups of rats	78.

4.10.5.1 Histopathological analysis of liver in normal and experimental groups of rats	78
4. 10.5.2 Histopathological analysis of Muscle tissues in normal and experimental group of rats	81
CHAPTER 5	
DISCUSSION	84
CHAPTER 6	
CONCLUSION	97
Suggestions for further studies	100
REFERENCES	101
Appendix A	127
Details of preparation of some reagents	127
Appendix B	129
Details of Methodology	
B.1. Proximate Analysis	129
B.1.1. Determination of Moisture Content	129
B.1.2 Determination of Ash Content	129
B.1.3. Determination of Protein Content	130

B.1.4. Determination of fat Content	131
B.1.5 Carbohydrate Analysis	132
B.2 Mineral analysis:	133
B.3 Determine the hypoglycaemic mechanism of the plant extract in Diabetic rats	135
B.3.1 Effect of extract on intestinal glucose absorption	135
B.3.2 The effect of extract on Na/K ATPase	135
B.3.3 Assessment of the glycogen content	136
B.3.3.1 Preparation of Standard glucose:	136
B.3.4 Glucokinase Activity in Liver	138
B.3.5 Hexokinase Activity in Muscle	138
Appendix C	141
Ethic Clearance	141
Appendix D	143
Research Output	143
International/National Conference	143

LIST OF TABLES

Table 2.1 Plants used for the treatment of diabetes mellitus in the Indian traditional System of medicine.	24
Table 3.1 Experimental set up of the animals	41
Table 4.1 Proximate composition of the four vegetables of India and South Africa	47
Table 4.2 Nutrient composition of the four vegetables of India (In) and South Africa	50
Table 4.3 Comparison of efficacy of all 4selected plants extracts with metformin in blood glucose level.	71
Table 4.4 Showing the amount of glycogen present in the liver of different study groups.	76
Table 4.5 Effect of administration of selected doses of <i>M.charantia</i> and <i>A. esculentus</i> on enzymes in carbohydrate metabolism in rats.	77
Table B.1 Protocol for the standard curve for Nitrogen	131
Table B.2 Protocol for standard glucose curve	133
Table B.3 Protocol for standard Starch curve	133
Table B.4 Preparation of working standard solutions	134
Table B.5 Parameters	134

LIST OF FIGURES

Figure 2.1 Type 1 Diabetes	5
Figure 2.2 Type 2 Diabetes	6
Figure 2.3 showing the mechanism of action of insulin	13
Figure 2.4 Insulin mediated glucose uptake	14
Figure 2.5 Mechanism of action of beta-cell functioning and effect of sulphonylureas on insulin release	16
Figure 2.6 Mode of action of SGLT1 and SGLT2	22
Figure 2.7 Leaves, flowers, fruits and seeds of <i>Abelmoschus esculentus</i>	26
Figure 2.8 Leaves, flowers, fruits and seeds of <i>Momordica charantia</i>	28
Figure 2.9 Leaves, flowers, fruits and seeds of <i>Moringa oleifera</i>	30
Figure 2.10 Leaves, flowers and fruits of <i>Solanum melongena</i>	32
Figure 3.1 Effect of Extract on glucose uptake	43
Figure 4.1 Effect of <i>M.charantia</i> fruit extracts on body weight (g) in normal and diabetic rats.	51
Figure 4.2 Effect of <i>A. esculentus</i> fruit extracts on body weight (g) levels in normal and diabetic rats.	52
Figure 4.3 Effect of <i>Moringa oleifera</i> leaf extracts on body weight (g) levels in normal and diabetic rats.	53
Figure 4.4 Effect of <i>S. melongena</i> fruit extracts on body weight (g) levels in normal and diabetic rats.	54
Figure 4.5 Effect of all 4 plant extracts together (1:1 ratio) on Body weight (g) levels in normal and diabetic rats.	55

Figure 4.6 Effect of <i>M.charantia</i> extracts on blood glucose (mmol/L) levels in normal and diabetic rats.	56
Figure 4.7 Effect of <i>M.charantia</i> extracts on triglyceride (mmol/L) levels in normal and diabetic rats.	57
Figure 4.8 Effect of <i>M.charantia</i> extracts on cholesterol (mmol/L) levels in normal and diabetic rats.	58
Figure 4.9 Effect of <i>A. esculentus</i> fruit extracts on blood glucose (mmol/L) levels in normal and diabetic rats.	59
Figure 4.10 Effect of <i>A. esculentus</i> fruit extracts triglyceride (mmol/L) levels in normal and diabetic rats.	60
Figure 4.11 Effect of <i>A. esculentus</i> fruit extracts on cholesterol (mmol/L) levels in normal and diabetic rats.	61
Figure 4.12 Effect of <i>Moringa oleifera</i> leaf extracts on blood glucose (mmol/L) levels in normal and diabetic rats.	62
Figure 4.13 Effect of <i>Moringa oleifera</i> leaf extracts on triglyceride (mmol/L) levels in normal and diabetic rats.	63
Figure 4.14 Effect of <i>Moringa oleifera</i> leaf extracts on cholesterol (mmol/L) levels in normal and diabetic rats.	64
Figure 4.15: Effect of <i>S. melongena</i> fruit extracts on blood glucose (mmol/L) levels in normal and diabetic rats.	65

Figure 4.16 Effect of <i>S. melongena</i> fruit extracts on triglyceride (mmol/L) levels in normal and diabetic rats.	66
Figure 4.17 Effect of <i>S. melongena</i> fruit extracts on cholesterol (mmol/L) levels in normal and diabetic rats.	67
Figure 4.18 Effect of all 4 plant extracts together (1:1 ratio) on blood glucose (mmol/L) levels in normal and diabetic rats.	68
Figure 4.19 Effect of all 4 plant extracts together (1:1 ratio) on triglyceride (mmol/L) levels in normal and diabetic rats.	69
Figure 4.20 Effect of all 4 plant extracts together (1:1 ratio) on cholesterol (mmol/L) levels in normal and diabetic rats.	70
Figure 4. 21 Effect of Extract on glucose uptake in rat's intestine.	73
Figure 4.22 Inhibitory activity of <i>Momordica charantia</i> and <i>Abelmoschus esculentus</i> on Na ⁺ /K ⁺ -ATPase	74
Figures 4.23 A -- I Histology of Liver	79
Figures 4.23 J – R Histology of Muscles	82
Figure 5.1 Functioning of the Na ⁺ /K ⁺ ATPase pump	94
Figure B.1 Standard graph calculated the amount of carbohydrate	137
Figure B.2 Gastrocnemius muscle of rat	140

CHAPTER 1

1.1 INTRODUCTION

Diabetes mellitus is a complex metabolic disorder that results from an absolute or relative deficiency of insulin with or without insulin resistance (Erasto *et al.*, 2005; Abo *et al.*, 2008; Bhattacharyya 2009). The burden of diabetes and its complications are great and continue to grow in both developing and developed countries. It is estimated that 25% of the world population is affected by this disease (Erasto *et al.*, 2005). Estimates from the World Health Organisation (WHO) have projected an increase in the incidence of diabetes from 173 million in the year 2000 to 300 million in 2025, of which 228 million are estimated to be in developing countries (Venkat Narayan *et al.*, 2000; Mckinlay and Marceau, 2000). This rapid increase in developing countries is attributed to industrialisation and a change in lifestyle, particularly diet (Mashige *et al.*, 2008).

Dietary management is a fundamental part of the management of Diabetes (Gorman, 2003; Love, 2003; Liu *et al.*, 2004). Medicinal plants are also widely utilised by African traditional healers in the treatment of Diabetes (Hutchings, 1996; Erasto *et al.*, 2005; Abo *et al.*, 2008). Ayurveda is a science that uses herbal medicines to treat various human ailments including diabetes, and has identified several plant species in India that possess medicinal value (Grover *et al.*, 2002; Saxena and Vikram, 2004; Shekelle *et al.*, 2005).

Among the plants listed as possessing anti-diabetic properties and are thus being used as Ayurveda are: *Abelmoschus esculentus* (L.) Moench, *Momordica charantia* L., *Moringa oleifera* Lam, *Solanum melongena* L.

The aim of this study was to evaluate the nutritional content of four ayurvedic plants: *Abelmoschus esculentus* (L.) Moench, *Momordica charantia* L, *Moringa oleifera* Lam, *Solanum melongena* L. grown in India and KwaZulu-Natal, South Africa. This study also examines the anti-diabetic activities of the aqueous and boiled extracts of fruit and leaf samples of the South African varieties and the apparent mechanism in the reduction of glucose levels in alloxan-induced diabetic rats.

1.2 Structure of the Thesis

Chapter One gives a brief background introduction for the study.

Chapter Two gives the literature review of the study. It also describes the aim and objective of the study conducted.

Chapter Three describes the materials and methods used in the study.

Chapter Four gives the overall results of all the findings of the study.

Chapter Five comprises the discussion drawn from the study.

Chapter Six comprises the conclusions drawn from the study.

Recommendations are also suggested in this chapter

References

Appendix

CHAPTER 2

REVIEW OF LITERATURE

2.1 Diabetes Mellitus

“**Diabetes**” originates from the word “siphon”, a Greek word meaning a tube, which allows the flow of liquid. This was due to the fact that patients with diabetes passed an excessive amount of urine. “Mellitus” means “sweet” which refers to the sweet urine that is produced by a patient with diabetes due to excessive glucose in the urine (Bhattacharyya, 2009).

Diabetes mellitus is a complex metabolic disorder that results from an absolute or relative deficiency of insulin with or without insulin resistance (Erasto *et al.*, 2005; Abo *et al.*, 2008; Bhattacharyya, 2009). Insulin is a hormone secreted by the pancreas for the metabolism of carbohydrates, proteins and fats (Mckinlay and Marceau, 2000; Viridi *et al.*, 2003; Mitra, 2007; Catsicas, 2009).

Insulin is the principal hormone secreted by the beta cells from the Islet of Langerhans. It plays a key role in the uptake of glucose into cells, especially the liver, muscle and adipose tissue. Therefore, a total or relative insulin production deficiency together with peripheral insulin resistance plays a central and crucial role in the development of diabetes mellitus (American Diabetes Association, 2014).

Insulin plays an important role in maintaining glucose homeostasis. Main sources of glucose include intestinal absorption of glucose, glycogenolysis (breakdown of glycogen) and gluconeogenesis (generation of glucose from non-carbohydrate substances). Insulin inhibits the process of glycogenolysis and gluconeogenesis and increases the uptake of glucose into the cells and the storage of glucose in the form of glycogen (glycogenesis). Insulin also delays the breakdown of fats to free fatty acids and ketone bodies and increases the storage of fat into adipose tissue (Gardner, 2011). The deficiency in insulin results in hyperglycaemia (Mashige *et al.*, 2008; Bhattacharyya, 2009). Weight loss, polyuria (frequent urination), polydipsia (increased thirst), and polyphagia (increased hunger) are the typical symptoms found in untreated diabetes. This may either develop rapidly (weeks or months) in type 1 diabetes, or have a more insidious onset or even be absent in Type 2 diabetes (Cooke and Plotnick 2008).

2.2 Classification

2.2.1 Type 1 Diabetes Mellitus

Type 1 Diabetes Mellitus, also known as insulin dependent diabetes mellitus (**IDDM**) or juvenile onset diabetes, accounts for approximately 10% of cases. In this type of diabetes, there is a loss of beta cell function in the Islets of Langerhans, thus there is either **little** or **no insulin** production from the pancreas (Figure 2.1).



Figure 2.1 Type 1 Diabetes

(<http://www.yalescientific.org/2013/12/targeting-diabetes-at-its-source-preserving-insulin-production-in-type-1-diabetes/>.)

The destruction of the insulin secreting beta cells in the pancreas is caused by cellular mediated autoimmunity which includes immunological, genetic and environmental factors (Gorman, 2003; Chen Chou, 2004). In (1986), George Eisenbarth proposed the first pathogenesis for the autoimmune destruction of the beta cells. He suggested a chronic autoimmune process caused by unknown causes. Subsequently, various autoantibodies to insulin, glutamic acid decarboxylase were implicated (Rother, 2007). The most important genetic risk factor for the development of the disease is associated with the HLA (human leukocyte antigen) locus as well as forty other non-HLA polymorphisms. Environmental factors include infections that mediate the direct infection of the beta cells (Rother, 2007). Type 1 diabetes usually develops in young people. They depend on insulin injections daily (Mashige *et al.*, 2008). Due to a shortage of insulin and decreased glucose utilisation of the cells, the body switches to

fatty acid metabolism, thus producing an accumulation of ketone bodies. Diabetic ketoacidosis (DKA) is one of the life threatening complications of Type 1 diabetes; however, it can occur in Type 2 diabetes in certain circumstances. DKA requires prompt treatment. Delays in treatment can lead to death, as it is a medical emergency (Kitabchi, 2009).

2.2.2 Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus is also known as adult onset diabetes or non-insulin dependent diabetes mellitus (NIDDM). It is a complex metabolic disorder and there is an inability of the body to respond appropriately to the insulin produced (Figure 2.2), a concept known as insulin resistance, followed by a relative deficiency in insulin production (beta cell dysfunction).

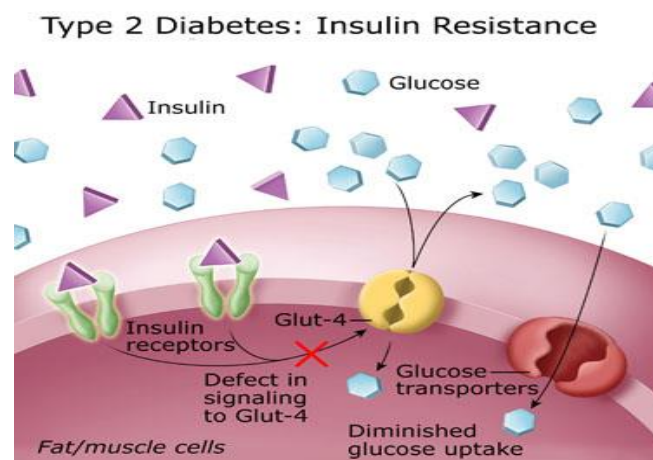


Figure 2.2 Type 2 Diabetes

(<http://dtk.ucsf.edu/images/charts/1.e.jpg>)

The insulin receptor plays the central role with regard to defective responsiveness of body tissues to insulin. Type 2 diabetes usually develops after the age of 45 years (Gorman, 2003; Chen Chou, 2004). It results from an intricate interaction between genetics and lifestyle factors. Genetic factors include insulin resistance and beta cell dysfunction. Lifestyle factors are important in the development of type 2 diabetes including obesity (body mass index greater than 30), sedentary lifestyle involving little physical exercise, poor diet and stress. It can be controlled by medication, diet and exercise. Prolonged medication may require administration of insulin. Approximately 90 to 95% of diabetes is type 2 (Venkat Narayan *et al.*, 2000; Mashige *et al.*, 2008).

Pancreatic beta cell dysfunction involves a gradual decline in insulin secretion together with the failure of beta cells to compensate for this insulin resistance. Several different hypotheses have been proposed. The hypotheses of beta cell toxicity suggest that glucose has a direct toxic effect on the beta cells. Hyperglycaemia impairs functions of genes, major enzymes and metabolic pathways in the beta cells, thus eventually leading to a decline in insulin secretion (Leahy, 2005).

The hypotheses of beta cell exhaustion suggest that chronic hyperglycaemia has an exhaustive effect on insulin secretion due to the progressive depletion of cellular components required for insulin production (Leahy, 2005).

The hypotheses of lipid induced beta cell toxicity suggest that due to increased adipocyte lipolysis there is increased free fatty acid concentration. Hyperglycaemia inhibits beta oxidation of free fatty acids which leads to accumulation of fatty acyl CoA,

which in turn decreases the production of ATP, thus decreasing insulin secretion (Robertson *et al.*, 2004).

Complications can be separated into microvascular and macrovascular complications.

Microvascular complications include diabetic nephropathy, neuropathy and retinopathy.

Macrovascular complications include cerebrovascular accidents, coronary artery disease and peripheral arterial disease (Robertson *et al.*, 2004).

2.2.3 Gestational Diabetes

Gestational Diabetes is defined as glucose intolerance that is first diagnosed with the onset of pregnancy and usually resolves once the pregnancy is over. However, this increases the risk of developing Type 2 diabetes later in life (Chen Chou, 2004). About 5-10% of women with gestational diabetes have been found to develop Type 2 diabetes at a later stage. Gestational diabetes resembles Type 2 diabetes in that there is a combination of inadequate insulin secretion and peripheral sensitivity. It occurs in 2-10% of all pregnancies (National diabetes statistics, 2011). Management includes intense medical supervision throughout the pregnancy, together with dietary changes, and in some cases, insulin may be used. If left untreated, gestational diabetes can cause macrosomia (high birth weight), congenital cardiac, nervous system and skeletal muscle anomalies, respiratory distress syndrome and in severe cases, perinatal death can occur (National diabetes statistics, 2011).

2.2.4 Other causes

These include any disease that causes extensive damage to the pancreas, which include chronic pancreatitis, pancreatectomy, pancreatic neoplasia, cystic fibrosis, haemochromatosis etc.

This classification of diabetes is used by the American Diabetes Association Committee Report (2014). This is not the only one used in the medical community. The American Diabetes Association publications are directed mainly towards diabetic patients and the general public for diabetic awareness. The new classification based on aetiology and recommended by the Current Medical Diagnosis and Treatment (CMDT) (Lange Medical Book, 2003) includes Type 1 Diabetes (with two subtypes - immune-mediated and idiopathic); Type 2 Diabetes with two subgroups – non-obese and obese. The acronyms, IDDM and NIDDM are not eliminated. They are still in use but IDDM stands for immune-mediated diabetes mellitus or type 1 and NIDDM stands for non-immune mediated type or type 2 (Tierney *et al.*, 2003).

Other specific type of diabetes mellitus are classified as follows: MODY (maturity-onset diabetes of the young); diabetes due to mutant insulin; diabetes due to mutant insulin receptors and diabetes associated with a mutation of the mitochondrial DNA. Gestational diabetes is not a part of the above-mentioned classification. According to CMDT, pregnancy is associated with changes due to placental lactogen and increased circulating levels of oestrogens and progesterone. This leads to insulin resistance, thus resulting in elevated blood levels of insulin, glucose and triglycerides.

2.3 Epidemiology of diabetes mellitus

The burden of diabetes and its complications are great and continue to grow in both developing and developed countries. It is estimated that 25% of the world population is living with this disease (Erasto *et al.*, 2005). In 1995 there were approximately 135 million people diagnosed with diabetes worldwide (Venkat Narayan *et al.*, 2000). In 2000, the World Health Organization (WHO) publication estimated diabetes in adults to be approximately 173 million, of which two thirds were estimated to live in developing countries (Abo *et al.*, 2008). In 2010, the estimated figure rose to 221 million in the world (WHO, 2010). By 2025, the worldwide prevalence of diabetes is expected to be 300 million (Venkat Narayan *et al.*, 2000; Mckinlay and Marceau, 2000) of which 228 million are estimated to be in developing countries and 72 million in developed countries (Mckinlay and Marceau, 2000).

This rapid increase in developing countries is attributed to industrialisation and a change in lifestyle, particularly diet (Mashige *et al.*, 2008). The countries with the largest prevalence of diabetes mellitus are India, China and The United States of America (Venkat Narayan *et al.*, 2000; Mckinlay and Marceau, 2000).

In South Africa the prevalence of type 2 diabetes is steadily increasing. In 2003 it was estimated that 1.5 million South Africans had diabetes (Love, 2003). In 2009 this figure increased to 6.5 million (*Netcare-Diabetes, 2009*). It was estimated that the prevalence of diabetes among South African Indians was 13%, 5-8% for Blacks and 4% in Whites (Ramaiya *et al.*, 1991; Health 24.com, 2010).

2.4 Risk factors

The exact cause of diabetes is uncertain; however, both genetic and environmental factors contribute to the onset of the disease. Genetic factors have been suggested as a cause of Type 2 diabetes because of a large proportion of individuals with diabetes having a positive family history (Ramaiya *et al.*, 1991; Bhattacharyya, 2009). There are several environmental factors that contribute to the onset of type 2 diabetes: unhealthy diets, sedentary lifestyles, obesity especially abdominal adiposity and an increase in psychological stress are risk factors in the development of type 2 diabetes. (Abo *et al.*, 2008; Ramaiya *et al.*, 1991; Venkat Narayan *et al.*, 2000; Gorman, 2003; Piji *et al.*, 2009). Other risk factors include excessive alcohol intake and smoking (Bhattacharyya, 2009). Diabetes may also be drug induced, particularly from steroids (Ferner, 1992).

2.5 Diagnosis of Diabetes

Diagnosis of Diabetes is based on both symptoms experienced by patients, together with abnormalities in blood glucose levels. Abnormalities of blood glucose levels include a fasting plasma glucose $\geq 7\text{mmol/L}$ or a 2 hour plasma glucose $\geq 11.1\text{mmol/L}$ during an oral glucose tolerance test (SEMDSA Guidelines, 2009).

At an individual level, management of diabetes includes resources for home glucose monitoring, regular medical care, oral medications, dietary management; regular exercise (Ramaiya *et al.*, 1991; Venkat Narayan *et al.*, 2000; Boule *et al.*, 2001; Gorman, 2003; Bhattacharyya, 2009; Piji *et al.*, 2009) and in more advanced cases,

insulin injections (Alberti and Zimmet, 1998; Mashige *et al.*, 2008). Dietary management is a fundamental part of the management of diabetes (Gorman, 2003; Love, 2003; Liu *et al.*, 2004).

2.6 Pathophysiology of diabetes mellitus

The pancreas plays an important role in glucose metabolism through the synthesis of the hormones insulin and glucagon. Low blood glucose stimulates the α - cells of the pancreas to release glucagon. Glucagon binds specific cell surface receptors in the liver, which in turn causes the activation of adenylate cyclase for cAMP production. cAMP then activates protein kinase A, which in turn activates glycogen phosphorylase via phosphorylation by an activated phosphorylase kinase. Active glycogen phosphorylase breaks down glycogen through phosphorylytic cleavage into glucose-6-phosphate that is released into the blood (Mathews *et al.*, 2000).

2.6.1. Mechanism of Insulin Mediated Glucose Uptake

High blood glucose levels stimulate beta cell secretion of insulin, which acts on the insulin receptor, stimulating the glucose transporter (GLUT4) to move from the vesicles to the plasma membrane. The binding of insulin onto the receptors on muscle and adipose tissue, markedly increases the uptake of glucose. Insulin receptors are present on most cells of the body but the highest concentrations are found in the liver, muscles and in adipose tissue (Czech and Corvera, 1999).

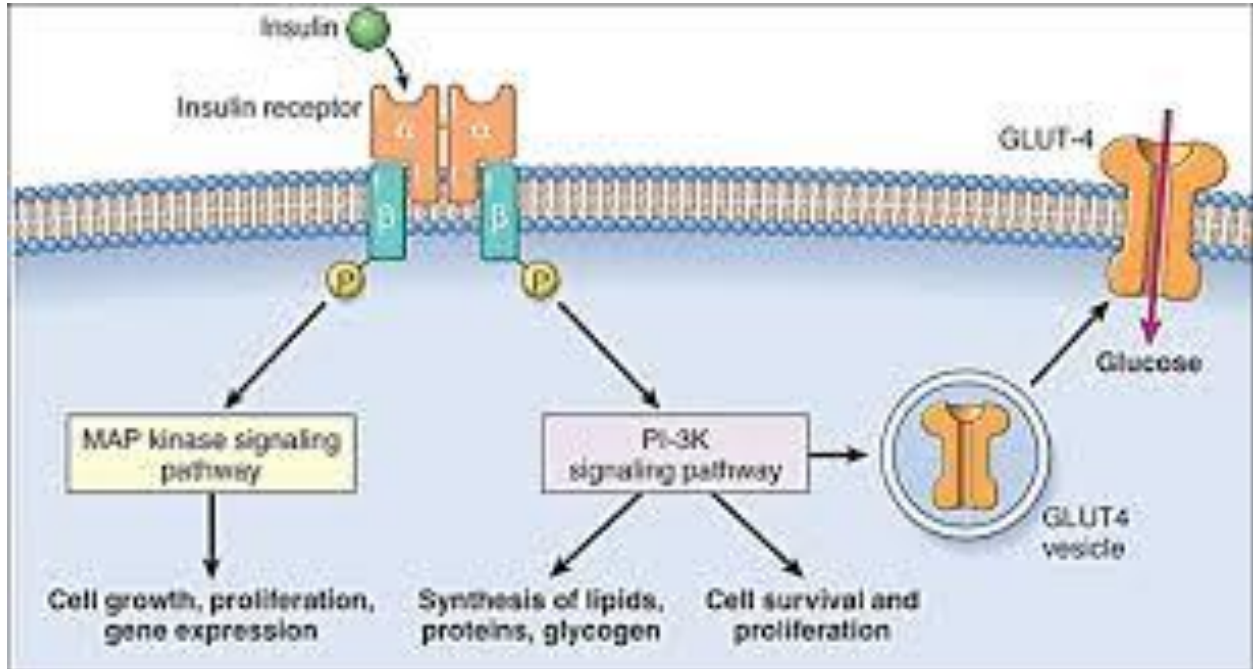


Fig. 2.3 showing the mechanism of action of insulin (usmle.bichemistryformedics.com)

Insulin receptors belong to the tyrosine kinase class. Figure 2.3 shows the insulin receptor, cytoplasm of the cell and the plasma membrane. Insulin receptors have two α subunits and two β subunits. α subunits include the binding site for insulin. The end of the β subunits project into the cytoplasm as tyrosine kinase domains (Czech and Corvera, 1999).

Once insulin is bound to the α subunit, the β subunits automatically phosphorylate activating the tyrosine kinase enzyme. The activation of β subunits by phosphorylation results in the activation of two pathways inside the cell (Bryant *et al.*, 2002).

1. Phosphatidylinositol-3kinase (PI-3K) signalling pathway results in the entry of glucose through GLUT4 molecules from the blood into the cytoplasm. PI-3K signalling pathway are also involved in the synthesis of lipids, proteins and glycogen.

2. The 2nd pathway activated by β subunits phosphorylation is a MAP kinase signalling pathway.

This is involved in cell growth, proliferation and gene expression. (Bryant *et al.*, 2002). When insulin is no longer available to bind to the α subunits, the GLUT4 in the cell membrane of the muscle cell is translocated back in to the cytoplasm so glucose can no longer enter the cell. Exercise can cause GLUT4 translocation to the muscle cell surface by activating the mitogen activated protein kinase (MAPK) pathway (Lemieux *et al.*, 2003). Carbohydrates in excess are stored as glycogen in the liver and muscle with the rest being stored in adipocytes. Both these conversions are facilitated by insulin (Figure 2.4).

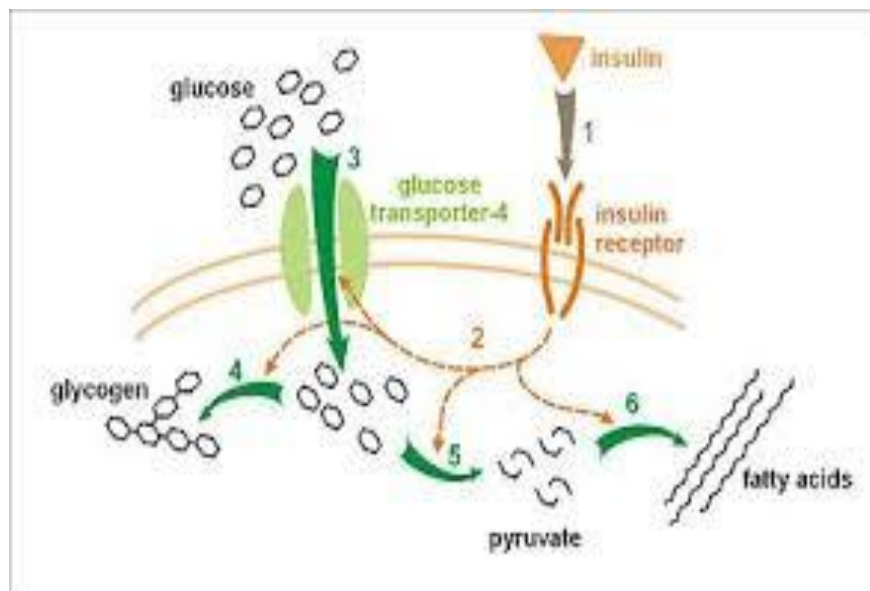


Figure 2.4: Insulin mediated glucose uptake
(<http://faculty.alverno.edu/bowneps/MSN621/2009%20tutorials/DiabetesMellitus.ppt>)

2.7 Treatment of Diabetes Mellitus – Non-insulin agents

The treatment of Type 2 Diabetes Mellitus can be divided into various broad categories: insulin secretagogues, insulin sensitizers and those affecting glucose pharmacokinetics. The focus of treatment is on achieving tight glycaemic control in order to prevent or delay the complications of diabetes. Diabetes is an evolving field of study and the management thereof is always undergoing change and optimisation.

2.7.1 Insulin Secretagogues

2.7.1.1 *Sulphonylureas*

Sulphonylureas are one of the first available oral hypoglycaemic agents. They act by stimulating functioning beta-cells to increase production of insulin, thus lowering blood glucose levels (Koski, 2004). As seen in Figure 2.5, they function by binding to their high-affinity receptors (SUR1) on the pancreatic beta-cell surface, which are associated with K_{atp} channels and inhibit the efflux of potassium through the channel and in so doing cause cell membrane depolarisation. Depolarisation results in the opening of voltage-gated calcium channels that lead to calcium entry and the subsequent release of insulin from secretory granules within the beta cell (Ashcroft,1996). Sulphonylureas thus function as insulin secretagogues.

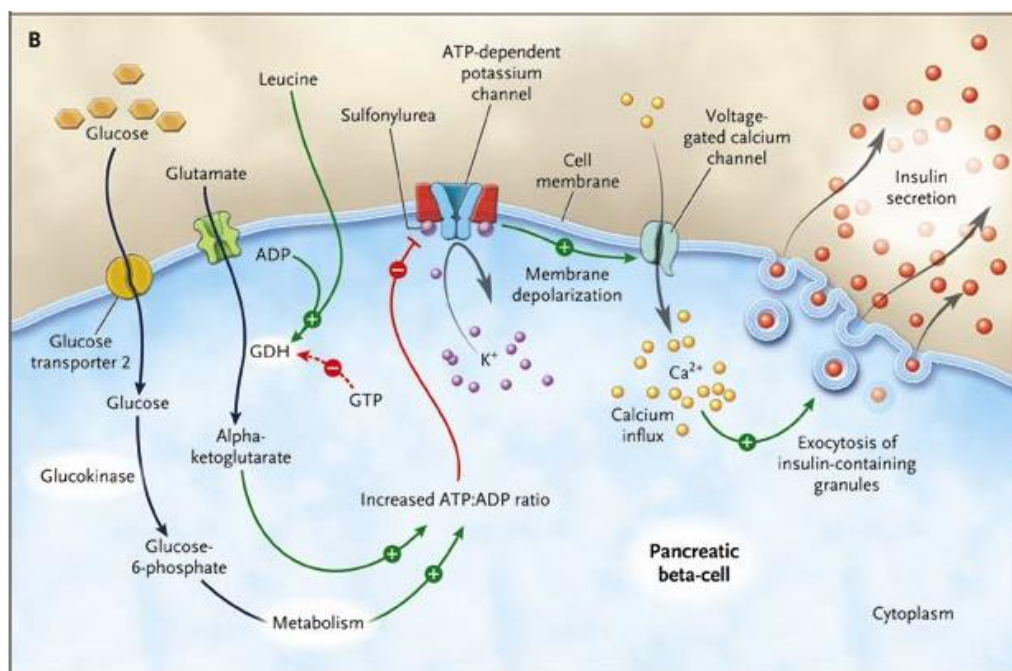


Figure 2.5 Mechanism of action of beta-cell functioning and effect of sulphonylureas on insulin release (Big Tomato, 2014)

Sulphonylureas are divided into first and second generation sulphonylureas. The second generation agents, although more effective and having a better side-effect profile, still may cause multiple side effects, some of which may be life threatening (Inzucchi, 2002). The major side-effects of the sulphonylureas include hypoglycaemia and weight gain (Smith *et al.*, 2010).

2.7.1.2 Meglitinides

Meglitinides are shorter acting non-sulphonylurea insulin secretagogues. They act on the same ATP-dependent K^+ channels as sulphonylureas, but exert their action via a different binding site. Similar to the sulphonylureas, by closing the potassium channels

of the pancreatic beta cells, they open voltage-gated calcium channels, enhancing insulin release (Rendell, 2004). Meglitinides are shorter-acting than sulphonylureas. They are thus taken at the beginning of a meal and induce insulin surges which are short-lived. This decreases the risk of delayed hypoglycaemia and also has a potential benefit in those where hypoglycaemia is a significant risk, including the elderly, patients with renal and hepatic failure (Goldberg *et al.*, 1998). The short action of these agents decreases risk of hypoglycaemia and weight gain, but consequently requires multiple daily dosing. The lower protein binding as compared to sulphonylureas ensures fewer drug interactions.

2.7.2 Insulin Sensitisers

2.7.2.1 Biguanides

Biguanides function by lowering serum glucose by increasing insulin induced suppression of hepatic glucose production and increasing insulin induced utilisation of glucose in peripheral tissue (Wiernsperger, 1999). It is suggested that the effects are brought about by increased tyrosine kinase activity of the insulin receptors, thus increasing both glycogen synthesis (in the liver) and the recruitment and transport of GLUT4 transporters to the plasma membrane (in peripheral tissue) (Koski, 2004). Biguanides thus act as insulin sensitisers. Unlike the sulphonylureas, biguanides do not increase weight gain and thus are preferred in obese patients and younger type 2 diabetic patients (Golay, 2008). Biguanides also decrease the release of free fatty acids

from adipose tissue and subsequently lower cholesterol and triglyceride levels (Cabezas *et al.*, 2012).

A biguanide in itself has no effect on insulin release from the pancreas and thus the risk of hypoglycaemia is decreased. The major site of action of biguanides is the liver and is contraindicated in patients with liver dysfunction (King, 2014). The most serious and life threatening complication of biguanide use is lactic acidosis. The risk of lactic acidosis is increased in patients with concurrent renal disease; however, the overall incidence is low (1 case per 33 000 patient years) (Stang *et al.*, 1999). The more common side-effects of biguanide therapy include gastrointestinal side-effects including abdominal pain, nausea and vomiting, heartburn, diarrhoea and bloating – although patients become more tolerant to use with time (Bailey *et al.*, 1996).

2.7.2.2 Thiazolidinediones

Thiazolidinediones, similar to Metformin, work by increasing insulin sensitivity. They, however, have different mechanisms of action as evidenced by the synergistic improvement of glycaemic control when used concurrently (Inzucchi, 1998). They activate peroxisome proliferator-activated receptor (PPAR)-gamma, a nuclear regulatory protein found mainly in adipocytes and myocytes that is involved in the transcription of glucose and fat metabolism genes (Vidal-Piug *et al.*, 1997). These PPARs act on peroxisome proliferator responsive elements which enhance the mRNA production of insulin-dependent enzymes, via insulin sensitive genes (Vidal-Piug *et al.*, 1997).

Compared to the other agents, thiazolidinediones have a slower onset of action. Effects begin within 2 weeks, with the maximal benefit only being seen after about three months (Kumar *et al.*, 1996).

As these drugs are not insulin secretagogues, the risk of hypoglycaemia is minimized when taken as monotherapy. The use of these drugs have been associated with weight gain, and is thought to be secondary to fluid retention with a 1% decrease in haematocrit over time. In some patients, fluid retention may trigger congestive cardiac failure (Del Prato *et al.*, 2007). There was initial concern about an increase in cardiac events related to the use of these agents. However, multiple studies including the ADOPT (Haffner, 2007) study and the DREAM (Gagnon, 2007) trial showed that therapy with these drugs may prevent progression of the disease. All thiazolidinediones cause only a small increase in low-density lipoprotein (LDL) levels and a more considerable increase in high-density lipoprotein (HDL) levels and therefore a decrease in LDL to HDL ratio inferring a benefit in terms of cardiac events (Sutton *et al.*, 2002). There has also numerous reports suggesting idiosyncratic reactions to the drugs particularly troglitazone. Numerous case reports also report hepatotoxicity associated with the use of thiazolidinediones with cases of severe hepatotoxicity within 2 weeks of starting rosiglitazone (Forman, 2000; Al-Salman, 2000). The FDA thus recommends the regular monitoring of transaminase levels every 2 months once patients have been started on thiazolidinediones.

2.7.3 Other Agents

2.7.3.1 Alpha-Glucosidase Inhibitors

Alpha-glucosidase inhibitors are competitive inhibitors of membrane-bound intestinal α -glucosidases. Glucosidases hydrolyze oligosaccharides, trisaccharides and disaccharides to glucose and other monosaccharides in the small intestine and in so doing exert its anti-diabetic effect by delaying postprandial glucose absorption (van de Laar, 2008). The benefit to using α -glucosidase inhibitors is that their function is primarily within the intestine and thus only have minor systemic action (Rodger, 1995). A meta-analysis of studies on the use of acarbose showed a substantial decrease in oxidative stress from postprandial hyperglycaemia, thus resulting in a 35% risk reduction of cardiovascular disease and complications (Delorme *et al.*, 2005).

Despite their good safety record, inadequate gastrointestinal tolerability due to the large amounts of non-absorbed disaccharides in the intestinal tract, has substantially restricted their use. This results in abdominal discomfort, bloating, flatulence and diarrhoea, although these are reversible with discontinuation (Luna and Feingloss, 2001). The use of alpha glucosidase inhibitors have also been linked to elevated serum transaminase levels and thus, their use is contraindicated in patients with liver cirrhosis (Luna and Feingloss, 2001).

2.7.3.2 Dipeptidyl Peptidase-IV Inhibitors

Dipeptidyl peptidase-IV (DPP-IV) inhibitors are one of the newer agents, which were first approved for use by the FDA in 2006 (Pathak and Bridgeman, 2010). DPP-IV is involved in the degradation of various peptides including gastric inhibitory peptide (GIP)

and glucagon like peptide 1 (GLP 1). The endogenous incretins (GIP and GLP -1) are associated with a glucose dependent increase in insulin secretion (via insulin biosynthesis) and inhibition of glucagon release. The suppression of degradation of these peptides ensures better baseline glucose control (White, 2008). As the release of insulin by GIP and GLP 1, the risks of hypoglycaemia are decreased compared to other anti-diabetic agents (White, 2008).

The most common adverse reactions described with the use of DPP-IV inhibitors were upper respiratory tract infections, nasopharyngitis and headaches (Pathak and Bridgeman, 2010). Other adverse reactions that have been reported are serious allergic reactions including anaphylaxis, angioedema, and Stevens–Johnson syndrome (Princeton, 2009). There have also been reported cases of acute pancreatitis including fatal and non-fatal necrotizing pancreatitis, with use of certain DPP-IV inhibitors and thus patients should be monitored for signs and symptoms of pancreatitis and discontinue use if suspected (FDA, 2009).

2.7.3.3 SGLT 2 Antagonists

A new orally administered class of compounds are currently being tested for the treatment of diabetes mellitus type 2. This group targets renal glucose transport and plays a role in the induction of glycosuria. Glucose is freely filtered at the glomerulus and is then reabsorbed via active transport mechanisms in the proximal convoluted tubule. This occurs via 2 sodium-glucose co-transporters (SGLT 1 and SGLT2) as seen in figure 2.6. SGLT 1 is also found in the gut and other tissue and accounts for about

10% of glucose reabsorption whereas SGLT 2 is expressed exclusively in the proximal tubule and accounts for 90% of glucose reabsorption (Wilding, 2008). It is thus postulated that by exerting an inhibitory effect on renal SGLT activity, glucose is released in the urine instead of being reabsorbed.

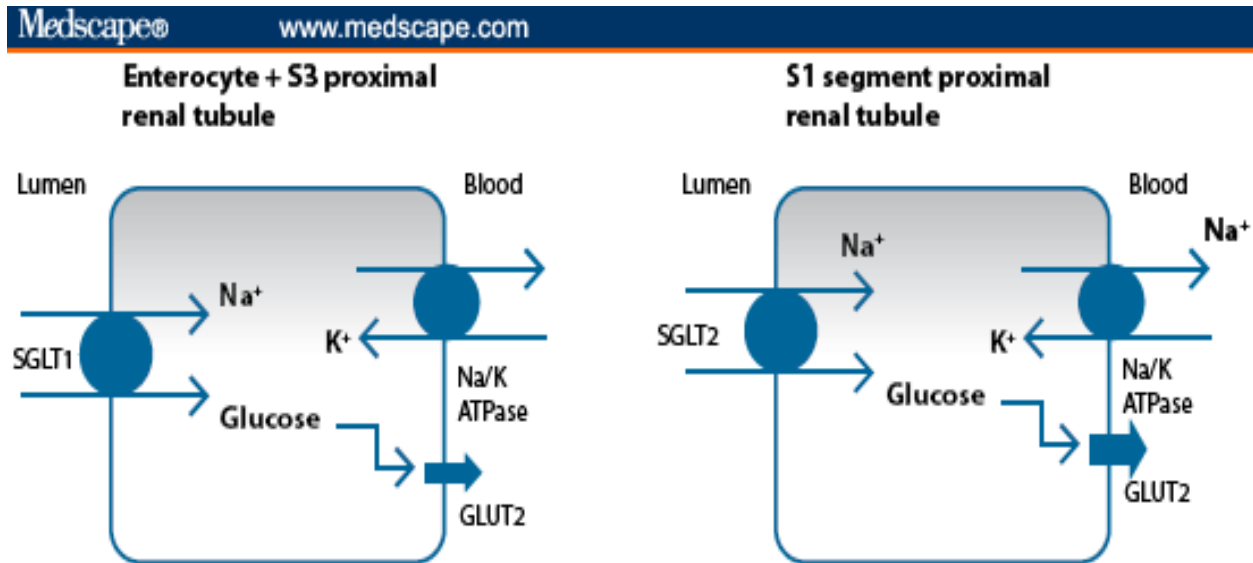


Figure 2.6: Mode of action of SGLT1 and SGLT2 (Wilding, 2008)

It is also suggested that the blocking the SGLT 2 pump causes natriuresis, therefore having a dual benefit of decreasing hypertension and fluid overload (Gilbert *et al.*, 2013).

SGLT 2 inhibitors have been associated with an increased risk of both urinary tract and genital infections (usually candida infections); possibly related to the increased amount of glucose in the urine (Bailey and Day, 2010). Other described side-effects include gastrointestinal discomfort, nausea, diarrhoea and constipation. Long term safety data is lacking regarding the use of SGLT 2 inhibitors (Kenkre *et al.*, 2013).

In light of the above discussion regarding the potential side-effects of the available diabetic drugs, it was deemed relevant to look for other alternatives that may assist with better glucose control.

2.8 Plants used for the treatment of diabetes

Plants have always been an exemplary source of medicine. Numerous drugs in clinical use have been derived from plants. An example of this is the hypoglycemic drug metformin, which is used in the treatment of diabetes. This drug was derived from *Galega officinalis*, L. Fabaceae family commonly known as French lilac (Grover *et al.*, 2002; Bailey & Day, 2004; Hadden, 2005). Medicinal plants are also widely utilized by African traditional healers in the treatment of diabetes (Hutchings, 1996; Erasto *et al.*, 2005; Abo *et al.*, 2008). The chemical constituents present in herbal drugs are considered to have better compatibility with the human body as they are part of the physiological functions of living flora. The herbal products denote safety in contrast to the synthetic drugs that are regarded as unsafe to both human health and the environment.

Ayurveda is a science that uses herbal medicines to treat various human ailments including diabetes and has identified several plant species in India that possess medicinal value (Grover *et al.*, 2002; Saxena and Vikram, 2004; Shekelle *et al.*, 2005).

A summary compiled from literature on some plants used for the treatment of diabetes is shown in Table 2.1.

Table 2.1: Plants used for the treatment of diabetes mellitus in the Indian traditional system of medicine. Indian Medicinal Plants with Antidiabetic Potential

Plant	Parts used	Reference
<i>Allium sativum</i>	rhizome	Pathirage and Yunman, 2012
<i>Cajanus cajan</i>	seeds	Grover <i>et al.</i> , 2002.
<i>Coccinia indica</i>	fruit	Grover <i>et al.</i> , 2002.
<i>Momordica charantia</i>	fruit	Grover <i>et al.</i> , 2002 ; Srivasta <i>et al.</i> , 1993; Viridi <i>et al.</i> , 2003.
<i>Ipomoea batatas</i>	fruit	Grover <i>et al.</i> , 2002.
<i>Musa sapientus</i>	flowers	Grover <i>et al.</i> , 2002.
<i>Murraya koeingii</i>	leaves	Grover <i>et al.</i> , 2002.
<i>Punica granatum</i>	fruit	Grover <i>et al.</i> , 2002.
<i>Trigonella foenum graecum</i>	seeds - methi	Kumar <i>et al.</i> , 2012
<i>Abelmoschus esculentus</i>	fruit	Hassan, 2009; Takano, 2007
<i>Moringa oleifera</i>	leaves	Fahey, 2005; Jaiswal <i>et al.</i> , 2007
<i>Solanum melongena</i>	fruit	Mutalik <i>et al.</i> , 2003; Kwon <i>et al.</i> , 2007
<i>Zingiber officinale</i>	rhizome	Bandari <i>et al.</i> , 2005
<i>Ocimum sanctum</i>	leaves	Pattanayak <i>et al.</i> , 2010
<i>Allium cepa</i> Linn.	rhizome	Gunjan <i>et al.</i> , 2011
<i>Brassica juncea</i> (Linn.)	seeds	Gunjan <i>et al.</i> , 2011
<i>Coccinia indica</i>	fruit	Gunjan <i>et al.</i> , 2011
<i>Cuminum cyminum</i> Linn.	fruit	Gunjan <i>et al.</i> , 2011

Among the plants listed as possessing anti-diabetic properties and thus being used as *Ayurveda* are: *Abelmoschus esculentus* (L.) Moench, *Momordica charantia* L, *Moringa oleifera* Lam and *Solanum melongena* L. These vegetables are often consumed by Indian mothers, but not in South Africa. Therefore, it has become necessary to evaluate these plants for their use as potential agents in the treatment of diabetes. The plants chosen for this study are known for their hypoglycemic effect (Table 2.1), but the comparison of the two types of fruit extracts (aqueous and boiled) has not yet been investigated.

2.8.1 *Abelmoschus esculentus* (L.) Moench, OKRA/LADY'S FINGER, Bhindi, Family: Malvaceae

Abelmoschus esculentus (L.) Moench, (Okra) is an annual herb 3-7 feet high, with hairy, semi-woody stems and palmately lobed leaves (Fig 2.7). The plant is considered to be African or Asian in origin. The flowers are large and yellow in colour with purple or red centers. The edible tender fruit is a capsule which contains numerous seeds (Mujeera and Balasubramanian, 2006; Adenipekun *et al.*, 2009). Okra contains protein, fat, carbohydrate, vitamin A, B, C, minerals and fibre (Tiwari *et al.*, 1998; Effiong *et al.*, 2009). Okra pods contain a mucilaginous substance, which stabilizes blood sugar and cholesterol levels (Hassan, 2009). The dietary fibre found in okra helps to stabilize blood sugar and prevents constipation (Takano, 2007).



Figure 2.7 Leaves, flowers, fruits and seeds of *Abelmoschus esculentus*
(<http://www.google.co.za/images?hl=img&q=okra&gbv=2&aq=f&aqi=g>)

2.8.2. *Momordica charantia* L. BITTER GOURD, Karela, Family: Cucurbitace

Momordica charantia L. (Bitter Gourd) is an annual herbaceous climber either allowed to trail on the ground or to climb on supports, with the aid of tendrils. It grows to 5m.

Leaves are simple, alternate lobed. Each plant bears separate yellow male and female flowers. The fruit are oblong, warty and have flat seeds (Fig 2.8). The plant is cultivated for its unripe fruit and has a bitter taste. The native country is uncertain, but the plant is cultivated widely in India, China and Africa (Bitter melon – Wikipedia, http://en.wikipedia.org/wiki/Bitter_melon).

The fruit is reported by various authors to be used in India and also in China as an appetite stimulant, blood purifier and to treat rheumatism, gout, and to lower blood sugar in diabetic patients (Baldwa *et al.*, 1977; Kedar and Chakrabarti, 1982; Zhang, 1992; Patel *et al.*, 1993; Patnaik, 1993; Raman and Lau, 1996 and Viridi *et al.*, 2003).

Srivasta *et al.* (1993) reported that the aqueous extract of unripe fruit was more effective in diabetes than the powder of the dried fruit. Bitter gourd has reported anti-cancer effects (Raman and Lau, 1996), antioxidant properties (Krawinkel *et al.*, 2006) and is high in vitamin C (Patnaik, 1993).

Close indigenous relatives from South Africa, *Momordica balsamina* L. and *Momordica foetida* Schumacher are locally well-known and the leaves are eaten as vegetables and taken for diabetes in Kwazulu-Natal and the Eastern Cape (Hutchings, 1996) and used by Vhavenda in the Venda region more often with other vegetables to give a plaque

taste to the side dishes (Maanda and Bhat, 2010). Several studies have been carried out with *Momordica charantia* due to its popular medicinal value. The most widely studied being its anti-diabetic properties (Sharma *et al.*, 1960; Grover and Yadav 2004; Chaturvedi, 2005).



Figure 2.8 Leaves, flowers, fruits and seeds of *Momordica charantia*

<http://www.google.co.za/images?hl=en&gbv=2&tbs=isch:1&ei=EMjTTNqdAYmFswaMpu3sBQ&q=momordica+charantia&start=>

2.8.3 *Moringa oleifera* Lam. DRUMSTICK; Muringa ; Family: Moringaceae

Moringa oleifera Lam (Drumstick) is a perennial softwood tree (Foidl *et al.*, 2001). The tree is slender, with drooping branches that grow to approximately 10m in height. It is often cut back annually to 1 meter and allowed to regrow so that pods and leaves stay within arm's reach. It grows best in dry sandy soil, but does not tolerate freeze or frost. The leaves are alternate, tripinnate, leaflets, 12-18 mm long. Flowers are white or cream coloured (fig 2.9). The fruits are elongated and contain winged seeds (Ramachandran *et al.*, 1980). The tree is native to India, but these days it is cultivated in Africa, Central and South America, Sri Lanka, India, Mexico, Malaysia, Indonesia and the Philippines.

Moringa preparations have been reported to have anticancer, antibiotic, antiulcer, antispasmodic, anti-inflammatory, hypoglycaemic and antibacterial activity (Fahey, 2005). Moringa leaves have high vitamin A (Patnaik, 1993) and vitamin E contents (Brendler *et al.*, 2010). The moringa leaves are reported to contain more beta-carotene than carrots, more protein than peas, more vitamin C than oranges, more calcium than milk, more potassium than bananas and more iron than spinach (Palada and Chang 2003; Fahey 2005, Wikipedia, 2010). Moringa seeds may be used for water purification and softening hard water (Olsen, 1987 and Fahey, 2005).

A study conducted on normal and streptozotocin induced sub, mild and severely diabetic rats showed a significant hypoglycemic effect when administered an aqueous extract of *Moringa Oleifera* leaves. In the severely diabetic rats the fasting blood

glucose and postprandial glucose levels were reduced by 69.2% and 51.3% respectively (Jaiswal *et al.*, 2007).



Figure 2.9 Leaves, flowers, fruits and seeds of *Moringa oleifera*

http://www.google.co.za/images?hl=en&source=imghp&q=Moringa+oleifera&btnG=Search+Images&gbv=2&aq=f&aqi=&aql=&oq=&gs_rfai=

2.8.4 *Solanum melongena* L. EGGPLANT/BRINJAL; Baigon; Family: Solanaceae

Solanum melongena L. (Brinjal) is a bushy, annual plant which usually grows 2-4 feet tall with many large leaves. The flowers are violet-coloured and star shaped. The fruit is a fleshy berry containing many seeds, varying in size, shape and colour (Jilani *et al.*, 2008, Agro Atlas-Crops-*Solanum melongena* L.-Garden Eggplant). See figure 2.10. The plant is indigenous to India and cultivated widely in China, India, Bangladesh, Nepal and Sri Lanka (Hanson *et al.*, 2006).

The plant is indicated for the treatment of several diseases including diabetes, asthma and bronchitis (Mutalik *et al.*, 2003). Extracts from the fruit are reported to have significant effect in reducing blood cholesterol in humans (Patnaik, 1993; Guimarães *et al.*, 2000; Magioli and Mansur, 2005). It was reported that the eggplant is a wonderful source of potassium, manganese, copper, dietary fibre, magnesium, vitamin B1, B3, B6 and K. These dietary fibres help to lower blood sugar and blood cholesterol levels (Johnson, 2010). The fruit is reported to boost the body's natural healing powers due to its high antioxidant content (Mutalik *et al.*, 2003; Whitaker and Stommel, 2003; Sadilova *et al.*, 2006; Smith, 2010).

Numerous laboratory experiments and designs have been used to establish the hypoglycaemic effects of medicinal plants in the form of extracts prepared from leaves, seeds, stem barks, fruits or by ingesting the fruits themselves. The mechanisms contributing to the antidiabetic effects are mediated by various mechanisms mainly due to the high content of antioxidants, enhanced insulin sensitivity and stimulated insulin

response. Plant extracts from *Trigonella foenumgraecum* Linn have shown glucose reducing effects by enhancing the synthesis of glycogen in the liver (Vat *et al.*, 2003).



Figure 2.10 Leaves, flowers and fruits of *Solanum melongena*

<http://www.google.co.za/images?hl=en&gbv=2&tbs=isch:1&ei=1crTTliJlImztAbT-MDeBQ&q=Solanum+melongena&start=20&sa=N>

2.9. Aim of the study

This study will evaluate and compare the nutritional content of four plants grown in India and KwaZulu-Natal, South Africa, and also evaluate and compare the anti-diabetic (hypoglycaemic) properties of four plants of KwaZulu-Natal, South Africa (SA) suitable for diabetic management.

2.10 Objectives

1. Evaluate the nutritional properties of the four plant species of India and SA.
2. Investigate the hypoglycaemic effect of the plant extracts of four plant species of SA on rats.
3. Determine the apparent mechanism of the plant extracts in lowering the sugar level in rats:
 - Effect on the absorption of glucose (rat intestine)
 - Effect on the activity of Na⁺/K⁺-ATPase
 - The mobilisation of glycogen in the liver (glycogen storage, the activity of hepatic glucose kinase (GK) and gastrocnemius muscle hexose kinase (HK); histology of the tissues.

CHAPTER: 3

3. MATERIALS AND METHODS

This chapter briefly describes the major chemicals, reagents, and equipment that were used in the study. A brief description is also given. Details of the preparation of reagents and the details of the methodology are given in Appendix A and B respectively.

All the reagents and solvents used in the experiments were of analytical grade. Where animals were used, the guidelines for proper caring and conduct of animal experiments were followed. Approval (UZREC—NUMBER: 171110-030 PGD 2012/14 see Appendix C) for use of animals and experimental procedures was obtained from the University of Zululand Research Ethics Committee.

3.1 Chemicals and reagents Sigma–Aldrich Chemical Co. (St Louis, MO, U.S.

A, Steinheim, Germany): Alloxan monohydrate, Creatine phosphate, Creatine phosphokinase, DTT (Dithiothreitol), D-glucose, 1,1-dimethylbiguanide hydrochloride (metformin), ethylene glycol tetra-acetic acid (EGTA), Adenosine triphosphate (ATP), phosphoenolpyruvate, NAD, NADH, lactic dehydrogenase, Ouabain, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT), Glucose (GO) Assay kit, glucose 6 phosphate dehydrogenase, HEPES, Nicotinamide adeninedinucleotide phosphate (NADP), pancreatin, Pancreatic lipase, trizma hydrochloride (Tris-HCl), pyruvate kinase.

Merck, South Africa: Ethylenediaminetetraacetic acid (EDTA), ethanol, hexane, NaCl, dipotassium phosphate (K_2HPO_4), potassium diphosphate (KH_2PO_4), di-sodium hydrogen orthophosphate (Na_2HPO_4), sodium dihydrogen orthophosphate (NaH_2PO_4), anthrone, sulphuric acid, Kjeldahl tablets, halothane, $MgCl_2$, KOH, KCl, ICP Multi Element Standard Solution.

3.2 Equipment

Accutrend instrument, glucose, cholesterol and triglyceride strips (Roche, South Africa), pH meter(720-WTW series Inolab), Eppendorf centrifuge (5804 R- Merck) electric juicer (Philips, India), freeze-dryer (Virtis-SP, 6KSTES) homogenizer (IKA T10 basic, Polychem), incubator (Scientific, Polychem), oven (Gallenhamp, England), Rotary Evaporator (Heidolph instruments, Germany), Soxhlet extractor (JR selecta), Varian Inductively Coupled Plasma (ICP) (Agilent-735 ICP-OES).

3.3 Collection and preparation of plant materials

The seeds of *Momordica charantia* L, (bitter gourd), *Abelmoschus esculentus* (L). Moench, (okra), *Solanum melongena* L. (brinjal) and *Moringa oleifera* Lam, (Muringa) were collected from India and from Empangeni, KZN, South Africa. The seeds from India were supplied by a licensed importer of seeds from Verulam. The plants were cultivated from the collected seeds in the home garden of the researcher in Empangeni, KwaZulu-Natal. The relevant parts of the plants used were subjected to proximate analysis in

which moisture, ash, carbohydrate, proteins, fat and mineral values (Na, K, Fe, Mn, Mg, Cu, Ca, Co, Zn) were analysed according to prescribed methods (AOAC, 2003).

3.4 Proximate analysis:

3.4.1 Determination of Moisture Content

Moisture analysis was carried out using the drying oven method (AOAC, 2003). Two grams of sample was transferred to a previously dried and weighed crucible and placed in the oven thermostatically controlled at 105°C for 24 hours. The percentage loss in weight was expressed as percentage moisture content.

3.4.2 Determination of Ash Content

Ash content was analysed as per the method outlined in the (AOAC, 2003). Two grams of sample was transferred into a previously dried and weighed crucible and ignited for 4 hours in a furnace pre-heated to 600°C. The percentage residue weighed was expressed as ash content.

3.4.3 Determination of Protein Content

Total protein was determined by the Kjeldahl method (AOAC, 2003). The sample was digested with concentrated sulphuric acid and Kjeldahl catalyst. Ammonium sulphate solution was used as standard. The digest was then reacted with Nessler's reagent for colour development. The absorbance was read at 530 nm. Such values were multiplied by 6.25 to obtain the total protein concentration.

3.4.4 Determination of fat content

This estimation was performed using the soxhlet extraction method (AOAC, 2003). The dried sample was transferred to a paper thimble which was then plugged with cotton wool and placed in the Soxhlet extractor for 16 hours. One hundred and eighty millilitres of n-hexane was used to extract the lipid. The flask and its contents were then dried in a rotary evaporator at 40°C. The flask was weighed. The difference in weight is expressed as percentage crude lipid content.

3.4.5 Carbohydrate Analysis

Carbohydrate content was determined using the Hansen and Moller (1975) method. The sample was percolated with 80% ethanol to get soluble carbohydrates. After the ethanol percolation, the residue was mixed with 35% perchloric acid to obtain starch. The percolates were reacted with anthrone and the absorbance read at 630nm, against

distilled water blank. The amount of starch and soluble carbohydrate was then estimated from standard solutions prepared to give the standard graphs. Maize starch and glucose were used as standards for insoluble and soluble carbohydrates.

3.5 Mineral analysis:

All metals (Na, K, Fe, Mn, Mg, Cu, Ca, Co, Zn) were analysed by the Varian Inductively Coupled Plasma (ICP) method. The ashed sample material was digested with 10% Nitric acid for the dissolution of samples. After dissolution, the samples were transferred into a 100ml volumetric flask and made up to the mark with de-ionized water. The samples were filtered thoroughly and only the clear aqueous solutions were used for analysis. All assays were carried out in triplicate. A certified Merck-ICP Multi Element Standard Solution IV was used for the quantification of metals in the samples. A 10% nitric acid blank solution was used for the analysis.

3.6 Preparation of freeze-dried Plant extract

Unripe fruit of *Momordica charantia* (bitter gourd), *Abelmoschus esculentus* (okra), *Solanum melongena* (brinjal) and the leaves of *Moringa oleifera* (muringa) were washed thoroughly with water and cut into small pieces. An electric juicer was used to extract the juice from the fruit and leaves with water (1:1w/v). The extracted juice from the fruit was

frozen and freeze-dried. The powder was kept in airtight containers at -70° C (Mohammady *et al.*, 2012).

3.6.1 Preparation of samples:

Two types of plant extracts were prepared from the freeze-dried samples of Kwa-Zulu Natal:

Extract 1: Placed fresh aqueous extract of the study vegetable part in cold water for 12 hours (Fasola *et al.*, 2010).

Extract 2: Boiled extract of the study vegetable part in water for 5 minutes, brought to room temperature and prepared the extract.

3.7 Animal experiments



Adult rats (*Sprague-Dawley*) of either sex (130-180g) were collected from the animal house in the Department of Biochemistry and Microbiology, University of Zululand. The animals were maintained under standard conditions (temperature $23 \pm 2^{\circ}$ C and 12h light/dark cycle). They had free access to standard pellet feed and enough drinking water.

3.7.1 To determine the hypoglycaemic effects of the four plant extracts on normal and diabetic rats

Ninety-two Sprague-Dawley rats were separated into 7 groups (see table 3.3). Groups 1, 2 and 3 were non-diabetic; groups 4, 5, 6 and 7 were diabetic. Groups 1 and 4 served as the control rats; groups 2 and 5 received the aqueous extracts, while groups 3 and 6 received boiled extracts. Group 7 received metformin. All animals had free access to food and water. The rats were weighed daily.

Diabetes was induced in 48 rats (groups 4, 5, 6 and 7) by a single intraperitoneal injection of alloxan, 150mg/kg body weight (Etuk, 2010 and Syiem *et al.*, 2009). The rats were allowed to rest for 3 days in order to stabilise the blood glucose. DM was confirmed in induced rats by testing blood glucose levels. A blood glucose level above 8 mmol/L was considered a diabetic state (Teoh *et al.*, 2010; Saha *et al.*, 2011). All rats, except the control groups, were fed with the extract 300 mg/kg BW per day for 15 days (Rao *et al.*, 1999; Vikrant *et al.*, 2001; Saha *et al.*, 2011 and Mohammady *et al.*, 2012) and the standard drug metformin (250mg/kg body weight) was administered to diabetic rats (group 7) for fifteen days (Maithili *et al.*, 2011, Yerima *et al.*, 2014). Mohammady *et al.* (2012) reported that the three doses of *M. charantia* (150, 300 and 600 mg/kg) produced varying significant hypoglycemic effects compared to the control group. But, the most effective dose was 300 mg/kg BW. The blood glucose, triglycerides and blood cholesterol were measured daily with the Accutrend instrument. Blood drops were collected by pinching the tail-end of the rats.

Table 3.1 Experimental set up of the animals

Plant Extract	Normal Rats 			Diabetic Rats 			
	Control	Experiment		Control	Experiment		
	Group1 (4rats)	Group2 (20 rats)	Group3 (20 rats)	Group4 (4 rats)	Group5 (20 rats)	Group6 (20 rats)	Group7 (4 rats)
<i>M. charantia</i>	Normal food No extract	Normal food + aqueous extract (300mg/kg bw)	Normal food + boiled extract (300mg/kg bw)	Normal food No extract	Normal food + aqueous extract (300mg/kg bw)	Normal food+ boiled extract (300mg/kg bw)	Normalfood+ Metformin (250mg/kg bw)
<i>A. esculentus</i>		Normal food + aqueous extract (300mg/kg bw)	Normal food + boiled extract (300mg/kg bw)		Normal food + aqueous extract (300mg/kg bw)	Normal food+ boiled extract (300mg/kg bw)	
<i>M. oleifera</i>		Normal food + aqueous extract (300mg/kg bw)	Normal food + boiled extract (300mg/kg bw)		Normal food + aqueous extract (300mg/kg bw)	Normal food+ boiled extract (300mg/kg bw)	
<i>S. melongena</i>		Normal food + aqueous extract (300mg/kg bw)	Normal food + boiled extract (300mg/kg bw)		Normal food + aqueous extract (300mg/kg bw)	Normal food+ boiled extract (300mg/kg bw)	
4 extracts (1:1:1:1)		Normal food + aqueous extract (300mg/kg bw)	Normal food + boiled extract (300mg/kg bw)		Normal food + aqueous extract (300mg/kg bw)	Normal food + boiled extract (300mg/kg bw)	

3.7.2 Harvesting of liver and muscle tissue

The rats were euthanized using halothane at day 15. Samples of liver and the gastrocnemius muscle were collected from each rat. Half of the rats' liver and muscle were stored in formalin at room temperature for histopathological analysis and the other half were rinsed with normal saline, wiped with filter paper, and kept at -80°C (Ugochukwu and Babady 2003; June *et al.*, 2012) until further analysis for assessment of the glycogen content and the activity of hepatic glucokinase (GK) and gastrocnemius muscle hexokinase (HK).

3.7.3 Histology of liver and gastrocnemius muscle

Histopathological studies were done at the Vet Diagnostics Laboratories at Pietermaritzburg.

3.8. To determine the hypoglycaemic mechanism of the plant extracts in diabetic rats

3.8.1. Transport of glucose across a membrane of rat's intestine

The upper part of the small intestine was obtained from Sprague Dawley rats, washed with saline solution. The inner surface of the gut segment was inverted with the help of a steel rod. Then the bottom part of the inverted segment was tied before filling it with

1ml of Potassium phosphate buffer (pH 6.9) which was immersed into a beaker containing 1% Pancreatin, 7 ml of 1% starch and plant extract (0.075 mg/ml). The glucose amount obtained outside the inverted intestine was taken to represent starch digestion, whereas the glucose amount inside the sac was taken to represent glucose absorption in the gut (Said *et al.*, 2007).

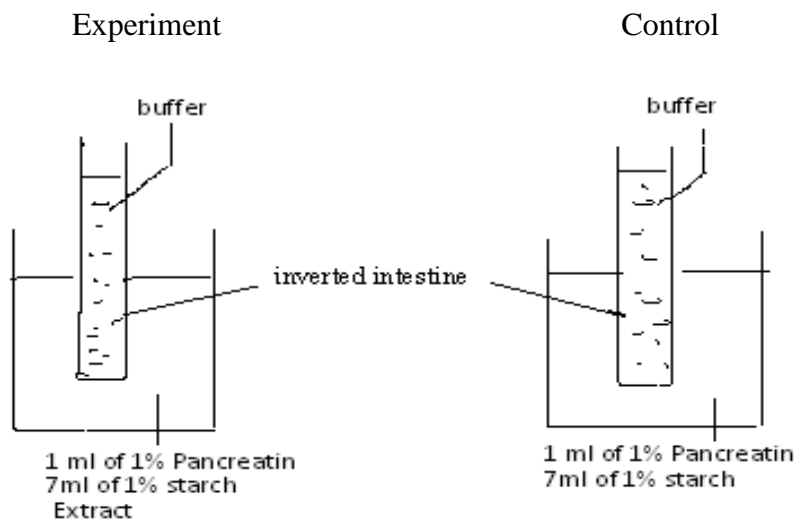


Figure 3.1 Effect of Extract on glucose uptake

3.8.2. The effect of extract on Na/K ATPase

A homogenized intestine was incubated with the extract and the activity of enzyme Na/K ATPase in the presence and absence of the extract was determined. Na⁺/K⁺-ATPase activity was determined through ouabain-sensitive ATP hydrolysis in intestinal samples (Vásárhelyi *et al.*, 1986). Na⁺/K⁺-ATPase activity was measured by the determination of the inorganic phosphate liberated through the hydrolysis of the substrate, adenosine

triphosphate, at 37° C. One unit of activity was defined as the amount of enzyme that catalyses the reaction of 1 μmol of ATP per minute.

3.8.3 Assessment of the glycogen content

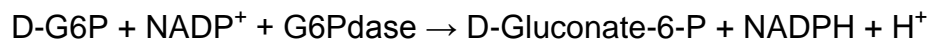
Glycogen content was measured as described by Ong and Khoo (2000). Liver tissues were homogenized (0.5:5w/v) of ice cold 30% KOH and boiled at 100°C for 30min. After cooling, the mixture was centrifuged at 5000 rpm for 15 minutes at 4°C. The supernatant was removed; glycogen was precipitated with 5 ml ethanol (95%), centrifuged, pelleted and washed with 5 ml ethanol (95%), and resolubilized in 1 ml distilled water. Glycogen content was estimated from standard glycogen treated with the anthrone reagent. (See Appendix B.3.3).

3.8.4 Glucokinase Activity in Liver

Weighed amounts of liver tissue were homogenized in nine volumes of buffer: 50mM Tris/HCl buffer (pH 7.4), 100 mM KCl, 10 mM DTT (dithiothreitol), and 1mM EDTA. After centrifugation at 12,000 g for 20 minutes at 4°C, the supernatants were collected in 2 ml micro tubes and preserved at -30°C until used to measure the enzyme activity (Newgard *et al.*, 1983 and Ugochukwu and Babady, 2003).

3.8.5 Hexokinase Activity in Muscle

The activity was measured by an assay in which the oxidation of glucose-6-phosphate produced is coupled to the reduction of NADP⁺ catalysed by G-6-P Dehydrogenase (G6Pdase) as shown:



Weighed amounts of muscle were homogenized in 10 volumes of buffer: 50mM Tris/HCl (pH7.5), 2 mM MgCl₂, 1mM EDTA, 30 mM DTT (Dithiothreitol). This was centrifuged at 20,000g for 15mins at 4⁰C and the supernatants collected in 2ml micro tubes and stored in at -30⁰C for the enzyme assay (Ugochukwu and Babady, 2003; Lwueke and Nwodo, 2008).

3.8.6 Statistical analysis

All experiments were repeated three times. Data were entered into Microsoft Excel and the means and standard error calculated. The statistical differences were determined by one-way analysis of variance, ANOVA (Graph Pad Prism) as well as Tukey test for multiple comparisons. P values <0.05 were considered significant.

CHAPTER 4: RESULTS

4.1 General

The study on antidiabetic properties of the plants were carried out to determine the antihyperglycemic activity of the four selected vegetables namely *Abelmoschus esculentus* (okra), *Momordica charantia* (bitter gourd), *Moringa oleifera* (drumstick), and *Solanum melongena* (brinjal), commonly used in India and South Africa. The four selected vegetables were analysed for the percentage of proximate composition of moisture content, ash, protein, fat, glucose and starch of both the Indian and South African varieties. The mineral composition of the four selected vegetables were analysed for the percentage of Ca, Co, Cu, Fe, K, Mg, Mn, Na and Zn of both Indian and South African Varieties. The nutritional values were determined by estimation of proximate analysis and mineral composition. The nutritional values of four selected vegetables of India and South Africa were compared. The *in vitro* effect of the plant extracts on blood glucose and some enzymes involved in glucose metabolism were determined. The results obtained from these analyses are presented in this chapter.

4.2 Evaluation and comparison of the nutritional values of selected vegetables from India and South Africa

4.2.1 Proximate composition:

The percentage of moisture, ash, protein, fat, glucose and starch contents of both the Indian and South African varieties of *Abelmoschus esculentus* (okra), *Momordica charantia* (bitter gourd), *Moringa oleifera* (drumstick), and *Solanum melongena* (brinjal) are given in Table 4.1.

Table 4.1 Proximate composition of the four vegetables of India and South Africa (n=3)

Plant Species	Country	Moisture%	Ash%	Fat%	Protein %	Glucose %	Starch %
<i>A. esculentus</i>	India	90.02 ± 0.22 a	7.20 ± 0.50 a	2.92 ± 0.15 b	1.14 ± 0.09 b	6.80 ± 0.35 a	9.17 ± 0.49 a
	SA	90.20 ± 1.16 a	6.47 ± 1.00 a	4.67 ± 0.23 a	1.64 ± 0.14 a	4.20 ± 0.06 b	5.62 ± 0.22 b
<i>M. charantia</i>	India	90.36 ± 2.32 a	10.97 ± 1.13 a	3.00 ± 0.58 b	1.97 ± 0.03 b	5.63 ± 0.20 a	10.87 ± 0.49 b
	SA	93.25 ± 0.42 a	8.20 ± 1.96 a	8.67 ± 1.36 a	6.33 ± 0.09 a	6.03 ± 0.15 a	16.87 ± 1.07 a
<i>M. oleifera</i>	India	76.74 ± 0.82 a	8.63 ± 0.12 a	16.30 ± 0.98 a	2.00 ± 0.29 b	6.55 ± 0.20 a	16.50 ± 0.29 b
	SA	72.50 ± 2.32 a	9.73 ± 0.74 a	12.10 ± 1.10 b	7.50 ± 0.58 a	4.95 ± 0.03 b	20.00 ± 1.04 a
<i>S. melongena</i>	India	91.80 ± 0.78 a	8.47 ± 0.44 a	2.84 ± 0.98 b	1.21 ± 0.05 b	6.00 ± 0.29 b	7.60 ± 0.23 b
	SA	92.37 ± 0.44 a	6.68 ± 0.53 a	7.87 ± 1.18 a	2.50 ± 0.25 a	6.97 ± 0.15 a	14.40 ± 0.81 a

Values are mean ± standard error. Values with the same letter along the column are not significantly different (P < 0.05).

The table 4.1 shows that the percentage of moisture, ash, fat, protein, glucose, and starch content of these vegetables ranged from 72.50 to 93.25, 6.47 to 10.97, 2.84 to 16.30, 1.14 to 7.50, 4.20 to 6.97 and 5.62 to 20.00 percent respectively. *A.esculentus*, *M.charantia*, *M.oleifera* and *S.melongena* show no significant difference in percentage of moisture and ash content irrespective of their country of origin. *A.esculentus*, *M. charantia* and *S. melongena* from South Africa show higher percentage of fat content compared to those from India, but significantly higher percentage of fat content in *M. oleifera* is observed in the Indian variety.

The percentage of protein content is significantly higher in all the South African varieties. The percentage of glucose content is significantly higher in the Indian varieties of *A.esculentus* and *M.oleifera*, whereas the South African variety of *S.melongena* shows higher glucose content. There is no marked significant difference in the percentage of glucose content in *M. charantia*. The South African variety of *M.charantia*, *M.oleifera*, and *S.melongena* has significantly higher percentage of starch content than those from India whereas the starch content is higher in the Indian variety of *A.esculentus*.

4.2.2 Mineral content:

The results of mineral analysis of *Abelmoschus esculentus*, *Momordica charantia*, *Moringa oleifera* and *Solanum melongena* are shown in Table 4.2. The percentage of mineral content in four selected plants of Indian and South African varieties, ranged from 1.42 to 5.64 (Calcium), 0.002 to 0.012 (Cobalt), 0.009 to 0.018 (Copper), 0.054 to 0.232 (Iron), 5.54 to 18.48 (Potassium), 2.01 to 6.14 (Magnesium), 0.014 to 0.033 (Manganese), 0.643 to 2.328 (Sodium) and 0.013 to 0.059 (Zinc) percent. *A.esculentus*, *M.charantia*, *M.oleifera* and *S.melongena* show no significant difference in their percentage of copper, sodium and zinc content in both India and South Africa. *A.esculentus*, *M.oleifera* and *S.melongena* from South Africa shows significantly higher percentage of cobalt content compared to those from India. An opposite scenario is

evident for *M.charantia* which shows higher percentage of cobalt content in the Indian counterpart. The calcium content is significantly higher in the Indian variety of *M.charantia* with no significant difference among the rest of the other vegetables. *S. melongena* shows a significantly higher percentage of iron concentration among the South African varieties compared to those from India, with no significant difference in percentage of iron content among the rest of the other vegetables. The percentage of potassium content of the Indian variety of *M. oleifera* is significantly higher than its South African counterpart with no significant difference among the rest of the vegetables. There is no noted significant difference in magnesium percentage in the vegetables except *A. esculentus*, which shows high concentration in the South African variety compared to those from India. The percentage of manganese is significantly higher in the Indian variety of *M.charantia* compared to the South African variety with no significant difference in manganese content among the other vegetables.

Table 4.2 Nutrient composition of the four vegetables of India (In) and South Africa (SA) (n=3)

Plant Species	Country	Ca %	Co %	Cu %	Fe %	K %	Mg %	Mn %	Na %	Zn %
<i>A. esculentus</i>	India	3.01 ± 0.35 a	0.004 ± 0.0004b	0.018 ± 0.002 a	0.054 ± 0.097a	12.22 ± 0.15a	3.19 ± 0.48b	0.033 ± .001a	2.328 ± 0.19a	0.038 ± 0.003a
	SA	5.64 ± 1.01 a	0.011 ± 0.0004a	0.014 ± 0.004 a	0.084 ± 0.099a	15.15 ± 3.67a	6.14 ± 0.17a	0.026 ± .007a	1.566 ± 0.34a	0.059 ± 0.01a
<i>M. charantia</i>	India	1.87 ± 0.08 a	0.011 ± 0.002a	0.016 ± 0.005 a	0.097 ± 0.01a	12.09 ± 1.45 a	2.32 ± 0.41 a	0.031±0.004a	0.643 ± 0.05a	0.023 ± 0.001a
	SA	1.42 ± 0.02 b	0.002 ± 0.0001b	0.013±0.0002 a	0.099 ± 0.01a	14.09 ± 0.21 a	2.45 ± 0.32 a	0.014±0.002b	0.850 ± 0.13a	0.027 ± 0.003a
<i>M. oleifera</i>	India	4.27 ± 1.11 a	0.003 ± 0.0005b	0.009±0.0002a	0.111 ± 0.02a	8.87 ± 0.47 a	2.01 ± 0.54a	0.031±0.003a	2.186 ± 0.56a	0.016 ± 0.002 a
	SA	3.06 ± 1.19 a	0.007 ± 0.002a	0.014 ± 0.004a	0.232 ± 0.07a	5.54 ± 0.57 b	3.37 ± 0.32a	0.025±0.01a	2.218 ± 0.21a	0.022 ± 0.002 a
<i>S. melongena</i>	India	2.23 ± 0.05 a	0.005 ± 0.0001b	0.018 ± 0.001a	0.053 ± .001b	18.48 ± 1.55a	2.25 ± 0.30 a	0.020±0.004a	1.43 ± 0.03 a	0.013 ± 0.001 a
	SA	2.40 ± 0.10 a	0.012 ± 0.0003a	0.018 ± 0.006a	0.109 ± 0.01 a	14.41 ± 0.30a	2.61 ± 0.67 a	0.027±0.005a	1.25 ± 0.28 a	0.023 ± 0.004 a

Values are mean ± standard error. Values with different letters within a column differ significantly (P < 0.05). 1%=10000ppm

4.3 Effects of Plant extract on Body Weight of different groups of normal & alloxan induced diabetic rats.

4.3.1 Effect of *Momordica charantia* fruit extract on body weight

Figure 4.1 shows the change in body weight in the control and experimental groups of rats. The oral feeding of *M.charantia* extracts resulted in an increase in body weight. Diabetic rats fed with the aqueous extract however showed less weight gain compared to their corresponding controls. A steady increase in body weight is observed, which is significant in all the groups except on day 5 and day10 of the diabetic rats fed with aqueous extract.

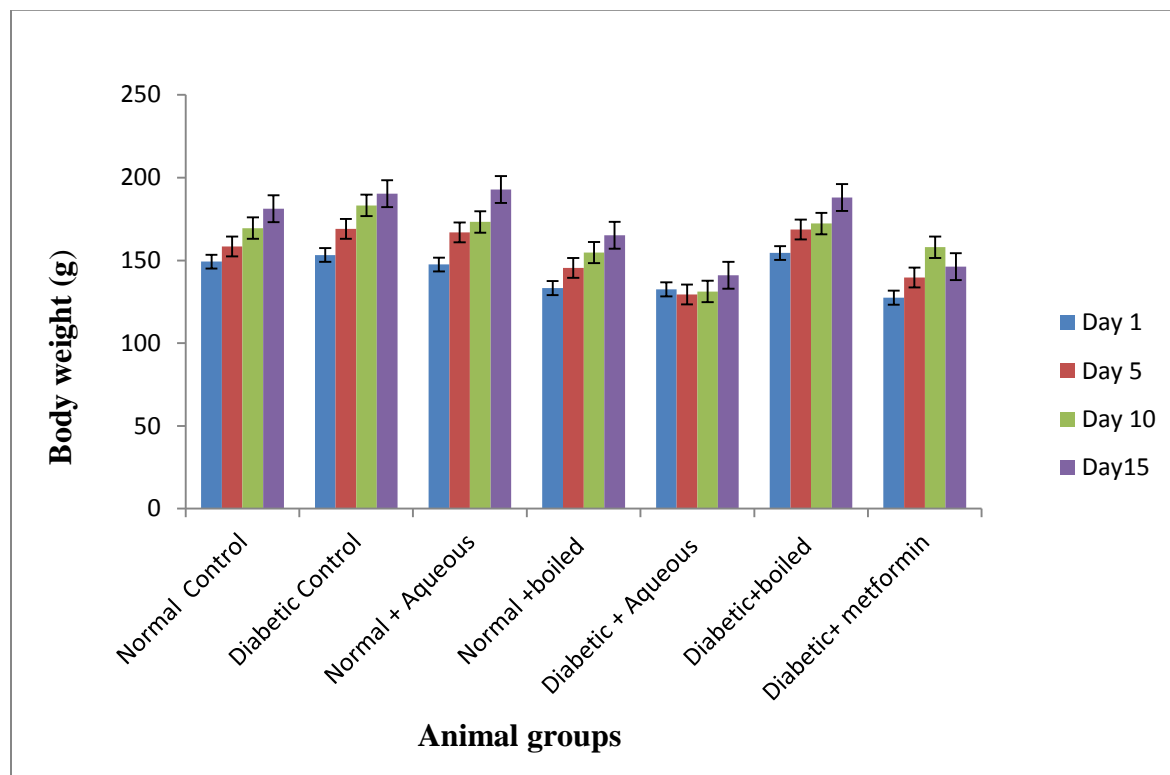


Figure 4.1 Effect of *M.charantia* fruit extracts on body weight (g) in normal and diabetic rats.

4.3.2 Effect of *Abelmoschus esculentus* fruit extract on body weight

The figure 4.2 shows the effect of *A. esculentus* fruit extract on body weight levels in normal and diabetic rates. The oral feeding of *A.esculentus* extracts resulted in an increase in body weight. Diabetic animals fed with the boiled *A.esculentus* extract shows less weight gain than their corresponding controls. A steady increase in the body weight is observed, which is significant in all the groups except on day 10 and day 15 of the diabetic rats treated with boiled extract.

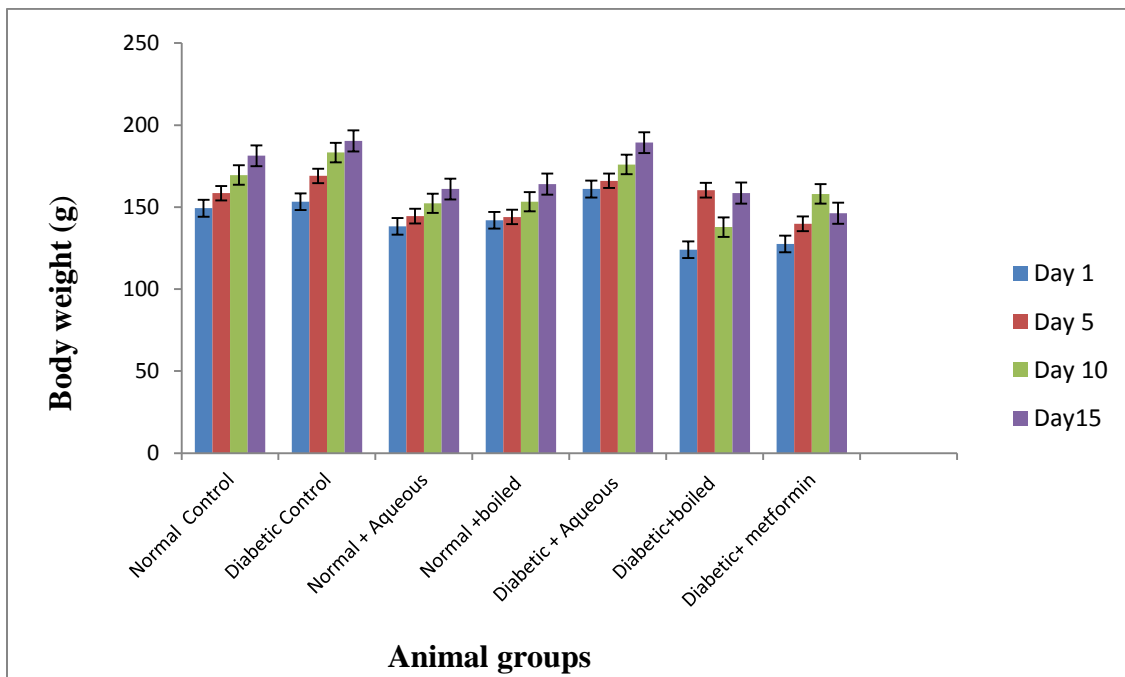


Figure 4.2 Effect of *A. esculentus* fruit extracts on body weight (g) levels in normal and diabetic rats.

4.3.3 Effect of *Moringa oleifera* leaf extract on body weight

The extracts of *M. oleifera* leaf extract on body weight of normal and diabetic rats shows (Figure 4.3) no significant difference in body weight between the diabetic control and the diabetic group treated with aqueous and boiled extract. The same is applied to the normal control group compared to the normal control treated with extract. The increase in body weight in all the study groups is found to be negligible and statistically non-significant (p -value >0.05).



Figure 4.3 Effect of *M. oleifera* leaf extracts on body weight (g) levels in normal and diabetic rats.

4.3.4 Effect of *Solanum melongena* fruit extract on body weight

The effect of *S. melongena* fruit extracts on body weight of control and experimental groups of rats are shown in Figure 4.4. Body weight varied from 153g to 194g. The experimental groups of rats show no significant difference in body weight when compared to their respective control group of rats (p -value >0.05).

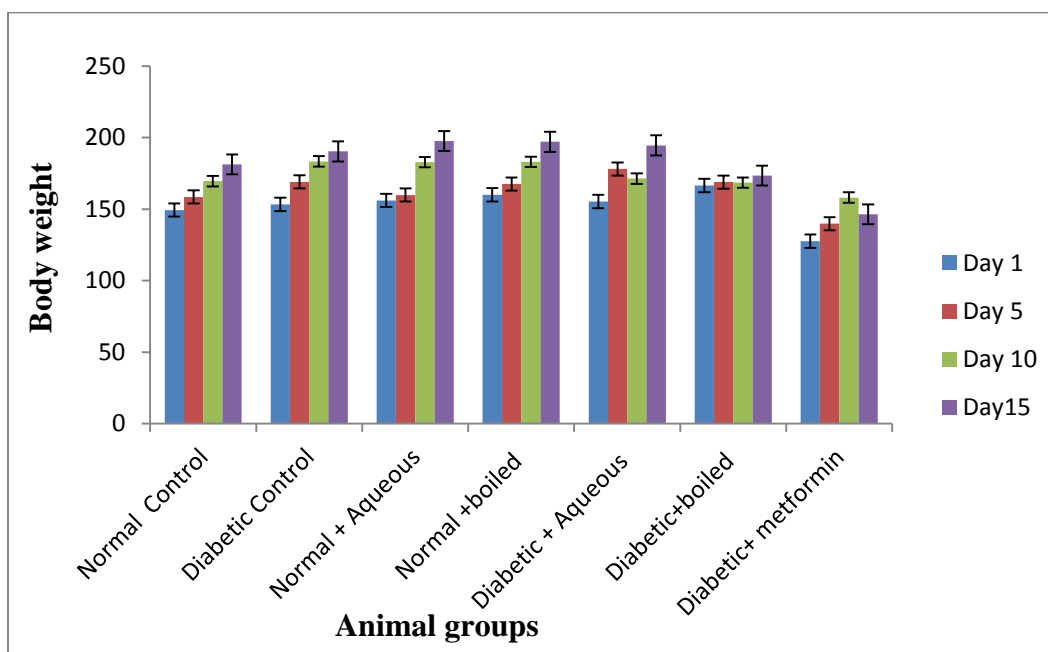


Figure 4.4 Effect of *S. melongena* fruit extracts on body weight (g) levels in normal and diabetic rats.

4.3.5 Effect of all 4 plant extracts on body weight

The results on effects of all the 4 plant extracts together in 1:1 ratio on body weight level in normal and diabetic rats is given in the figure 4.5. The increase in body weight is found to be significantly low in diabetic rats treated with boiled extract compared to the diabetic control group (p - value <0.05). There is no statistical significant difference in body weight among the remaining study groups compared to their control.

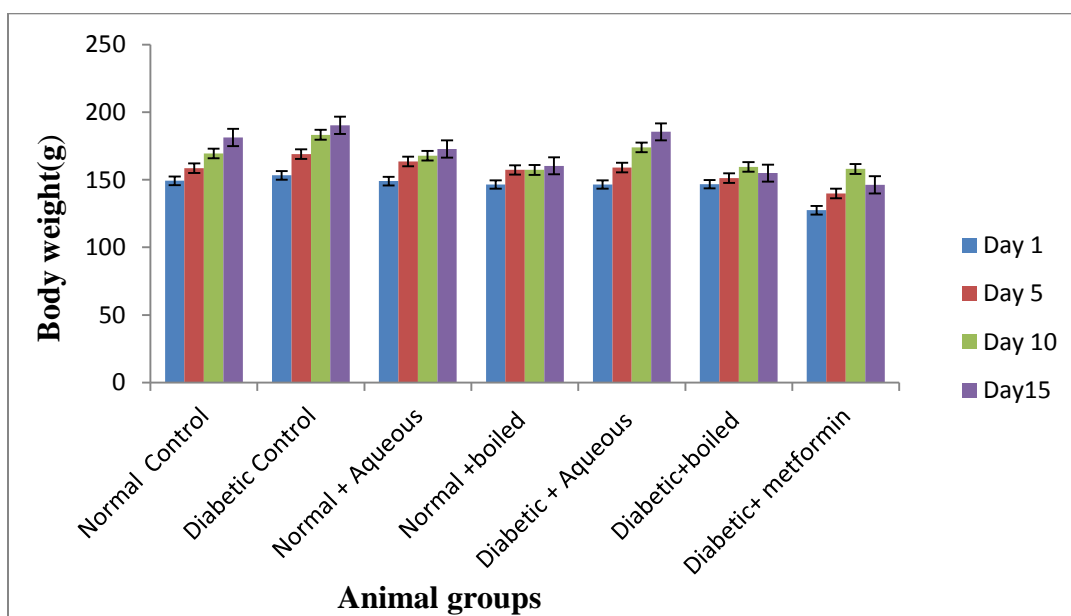


Figure 4.5 Effect of all 4 plant extracts together (1:1 ratio) on body weight (g) levels in normal and diabetic rats.

4.4 Hypoglycemic effects of *Momordica charantia* fruit extract on normal & alloxan induced diabetic rats

4.4.1 Effect of *M.charantia* extracts on blood sugar:

The figure 4.6 shows the effect of *M.charantia* extracts on blood glucose levels in normal and diabetic rats. The diabetic groups show a significant reduction in the blood glucose level at the end of the 15th day of treatment with maximum reduction being achieved in those receiving the aqueous extract ($p\text{-value} < 0.05$). Boiled extracts proved to be less effective than the overnight aqueous extract and metformin in lowering blood glucose. The glucose level in the diabetic control group is higher than their respective normal control groups with high statistical significance ($p\text{-value} < 0.001$). The raised blood glucose levels of the diabetic rats declined sharply after oral feeding of aqueous extract of *M.charantia* than Metformin treated groups.

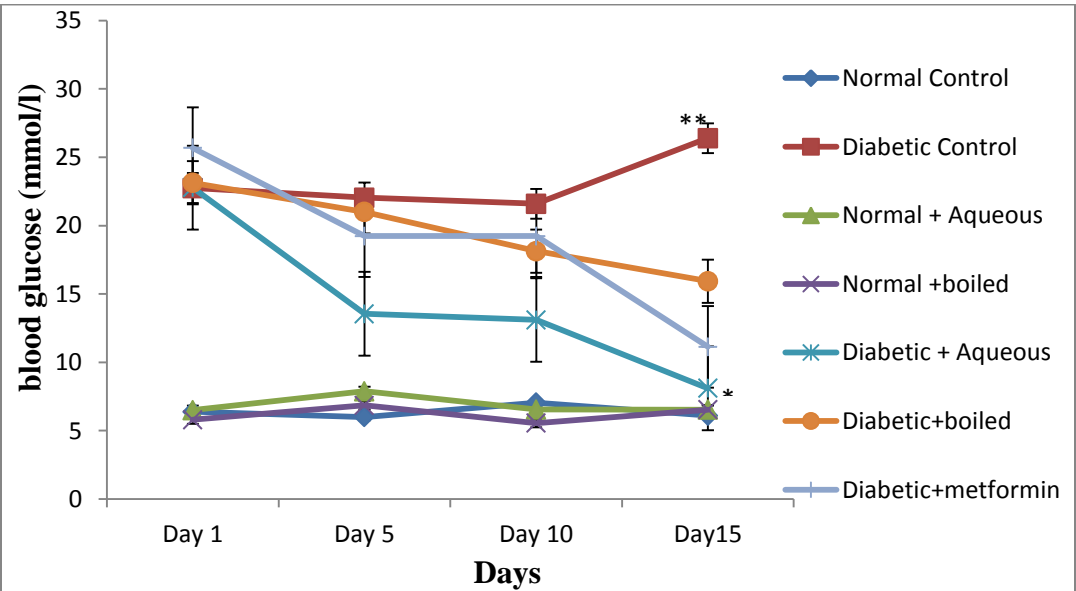


Figure 4.6 Effect of *M.charantia* extracts on blood glucose (mmol/L) levels in normal and diabetic rats.

4.4.2 Effect of *M.charantia* extracts on triglyceride

The figure 4.7 shows the effect of *M.charantia* extracts on triglyceride levels in normal and diabetic rats. The triglyceride levels of the diabetic treated groups were significantly lower than the diabetic control group by the 15th day. There is no significant difference in the triglyceride levels on the 15th day between the normal control and normal experimental groups.

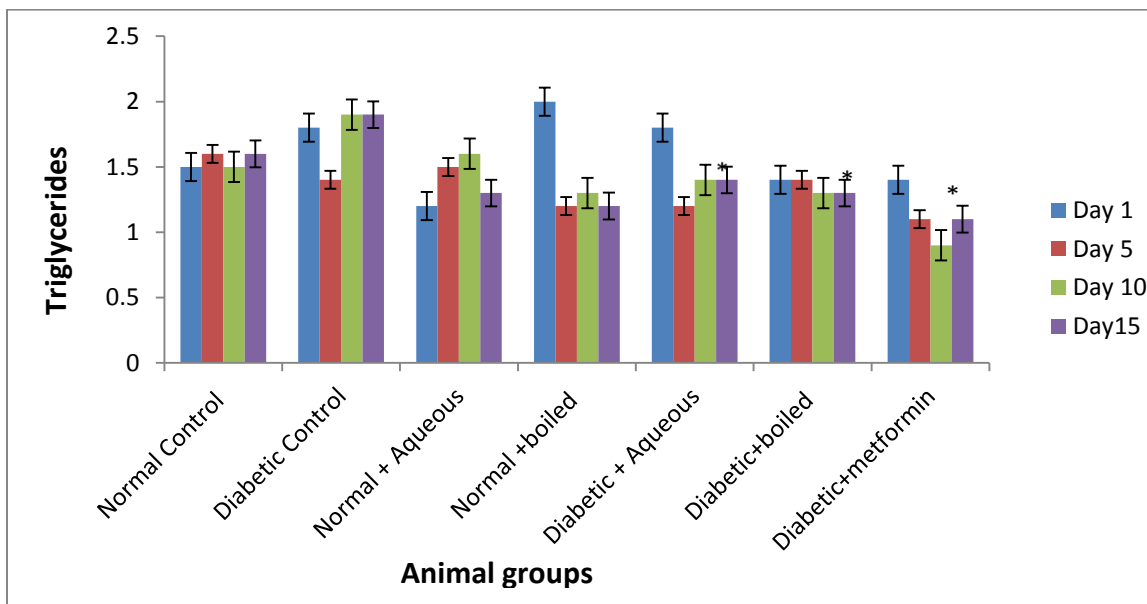


Figure 4.7 Effect of *M.charantia* extracts on triglyceride (mmol/L) levels in normal and diabetic rats.

4.4.3 Effect of *M.charantia* extracts on cholesterol

The figure 4.8 shows the effect of *M.charantia* extracts on cholesterol levels in normal and diabetic rats. Cholesterol levels of the study groups range from 3.7 to 4.2 mmol/L respectively. There is no significant difference in cholesterol levels among the study groups (p -value >0.05). The *M.charantia* extracts has no effects on cholesterol level of the study groups.

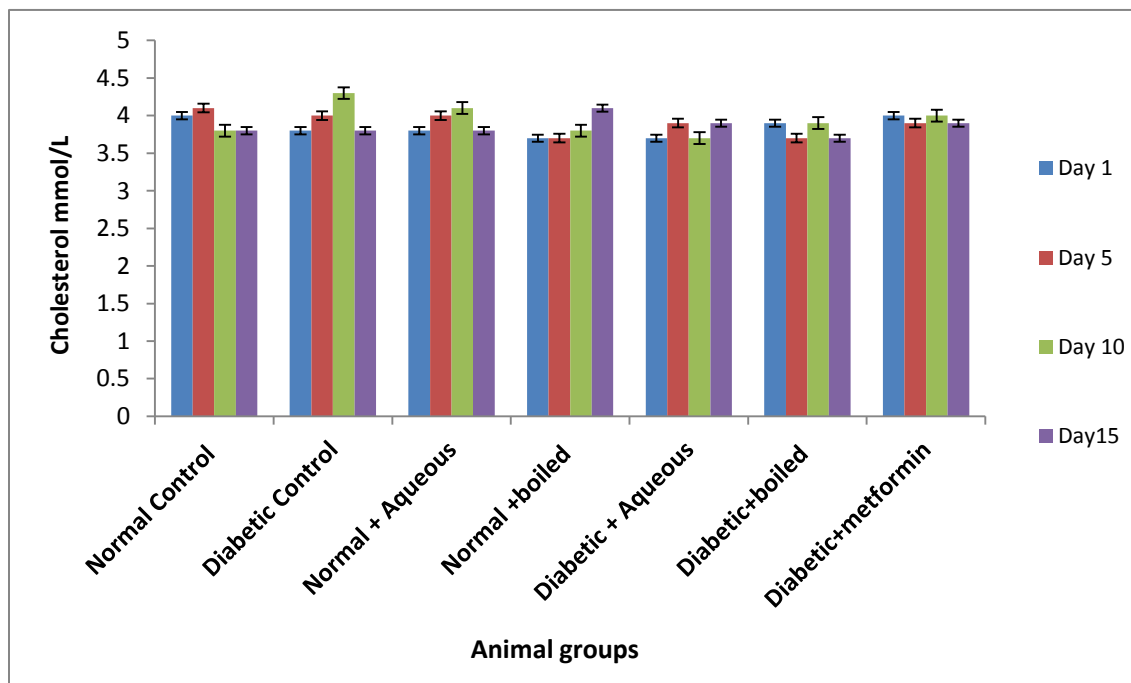


Figure 4.8: Effect of *M.charantia* extracts on cholesterol (mmol/L) levels in normal and diabetic rats.

4.5 Anti-diabetic effects of freeze-dried, overnight aqueous and boiled extracts of *Abelmoschus esculentus* (okra) fruit on normal and diabetic rats.

4.5.1 Anti diabetic effects of *A.esculentus* fruit extracts on blood glucose levels.

Figure 4.9 shows the anti-diabetic effects of *A.esculentus* fruit extracts on blood glucose level of diabetic group treated with aqueous extract and it shows a significant reduction in the blood glucose level by the end of the 2nd week of treatment (**p-value* <0.05). The sugar level of the aqueous extract treated group reduces from 22.2mmol/L to 13.3 mmol/L. The boiled extract is not effective in lowering the blood glucose level. The blood glucose level of the diabetic group treated with boiled extract increases from 18 mmol/L to 29.5 on day 5 and decreases to 23.6 mmol/L after 15 days of treatment. In aqueous and boiled extract treated groups of normal rats, no significant difference is shown in blood glucose levels when compared to their respective normal control. There is a significant high blood glucose level in the diabetic control group compared to the normal control rats.

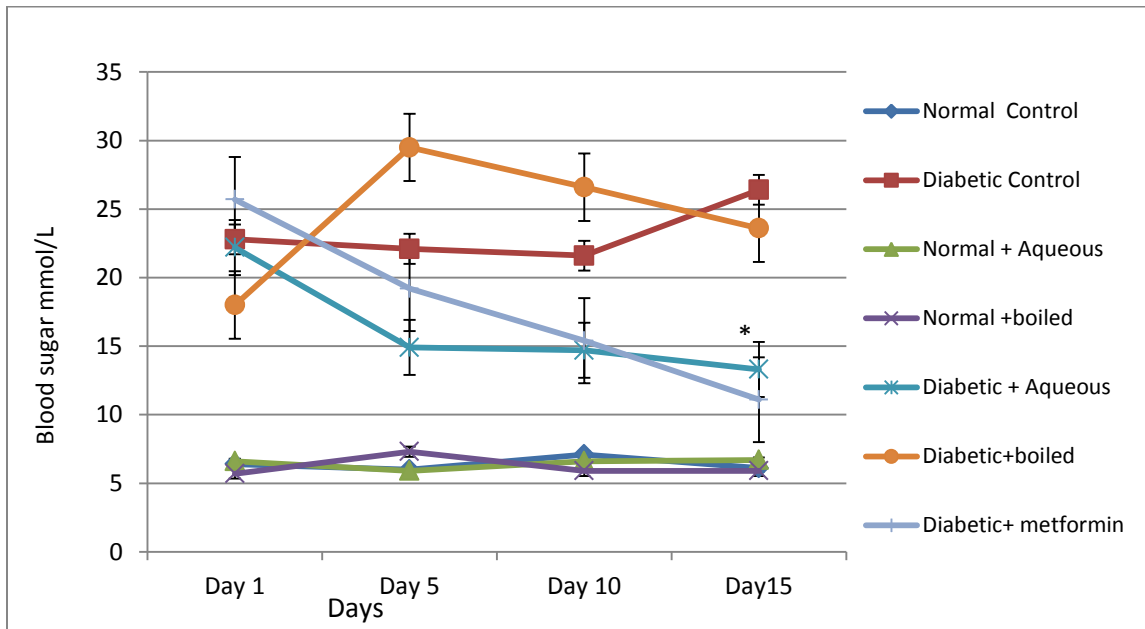


Figure 4.9 Effect of *A. esculentus* fruit extracts on blood glucose (mmol/L) levels in normal and diabetic rats. .

4.5.2 Anti diabetic effects of *A.esculentus* fruit extracts on triglyceride levels

Triglyceride levels of the *A.esculentus* study groups range from 0.9 - 1.9 mmol/L, excluding the diabetic rats given boiled extracts, in which it ranges from 2.5 - 6 mmol/L (Figure 4.10). There is no significant difference in triglyceride levels among the study groups except diabetic rats given boiled extracts.

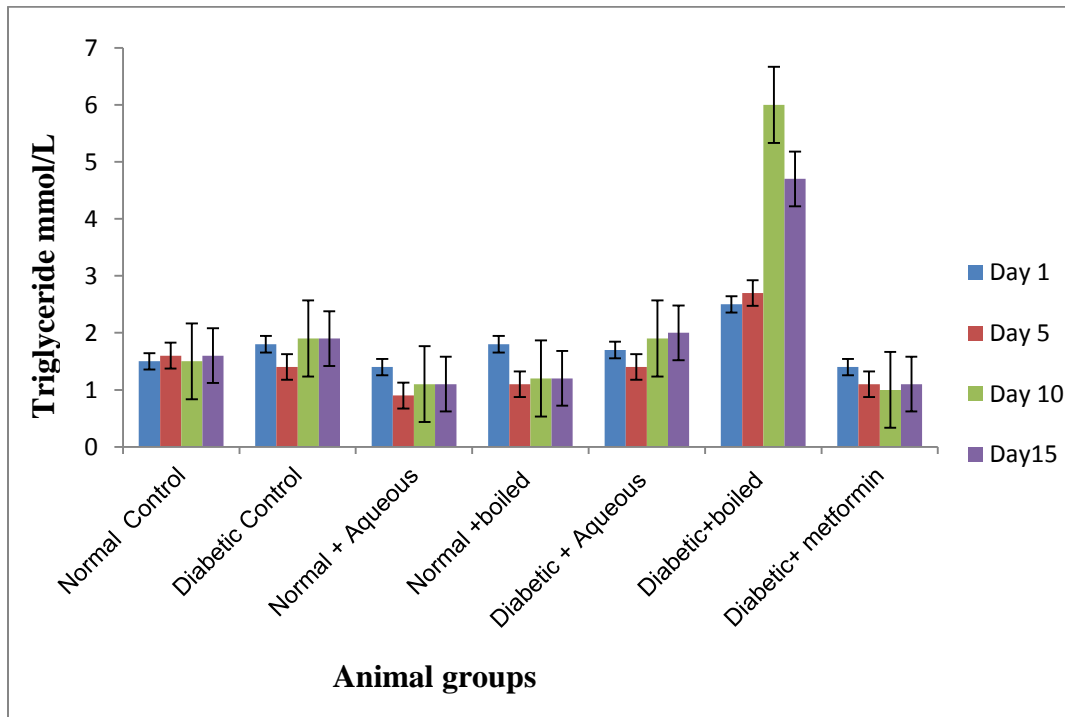


Figure 4.10 Effect of *A. esculentus* fruit extracts triglyceride (mmol/L) levels in normal and diabetic rats.

4.5.3 Anti diabetic effects of *A.esculentus* fruit extracts on cholesterol levels

The cholesterol levels of the study groups range from 3.8 to 4.3 mmol/L (Figure 4.11). There is no significant difference in the cholesterol levels of the different study groups during the course of the experiment. *A.esculentus* maintains the cholesterol levels in the study groups except in the diabetic rats given with boiled extract, which shows maximum cholesterol level of 4.3 mmol/L on 15th day.

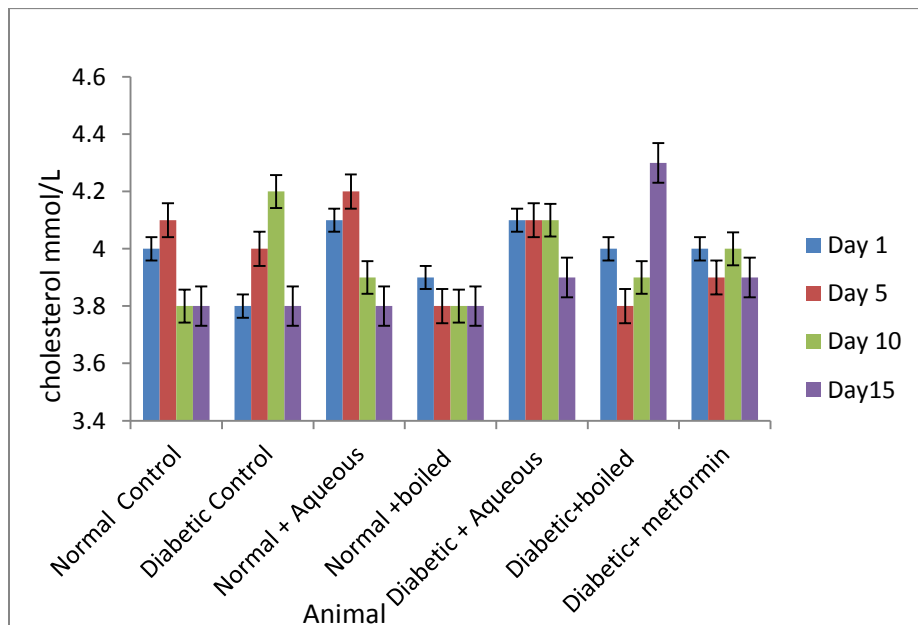


Figure 4.11 Effect of *A. esculentus* fruit extracts on cholesterol (mmol/L) levels in normal and diabetic rats.

4.6 Hypoglycaemic effects of freeze-dried overnight aqueous and boiled extracts of *Moringa oleifera* leaf extracts on normal and diabetic rats.

4.6.1 Hypoglycaemic effects of *Moringa oleifera* leaf on blood sugar

Diabetic treatment groups of *Moringa oleifera* extracts show a reduction in blood glucose levels at the end of 2nd week of treatment (Figure 4.12). Boiled leaf extract proved to be better than aqueous extract in lowering the sugar levels in diabetic rats. There is, however no significant reduction between the diabetic treated groups and diabetic groups. In normal rats treated with aqueous and boiled extracts, no difference was found in blood glucose levels compared to their respective control group. There is a significantly higher blood glucose level in diabetic control groups compared to normal control rats.

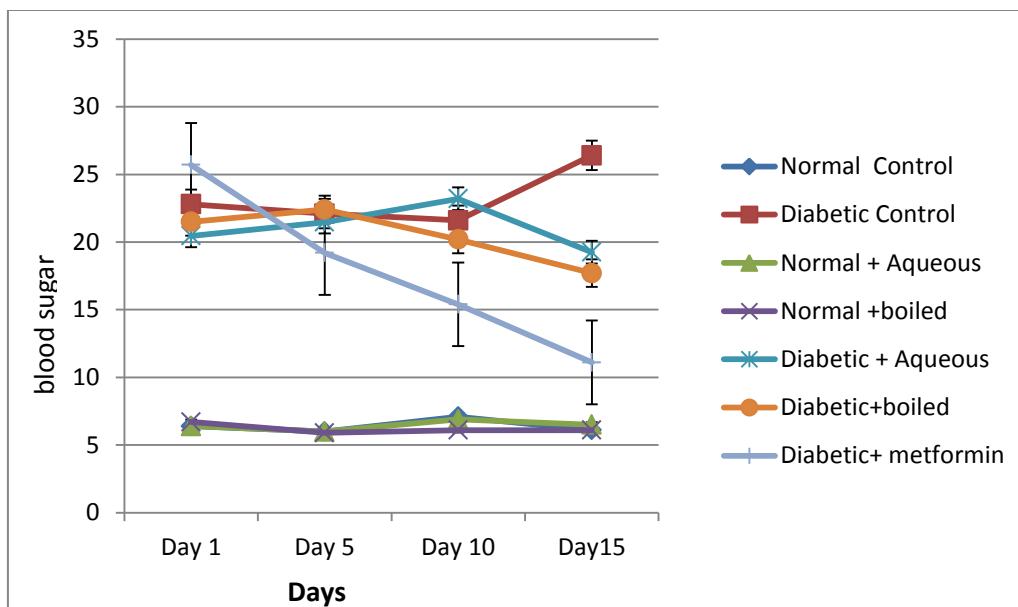


Figure 4.12 Effect of *Moringa oleifera* leaf extracts on blood glucose (mmol/L) levels in normal and diabetic rats.

4.6.2 Hypoglycaemic effects of *Moringa oleifera* leaf on triglyceride

The *Moringa oleifera* boiled extract on diabetic rats shows (Figure 4.13) a reduction of triglyceride from 3.8 to 1.1 mmol/L after the 15th day. Whereas in the normal boiled extract given group there is an increase of triglyceride level from 1.2 to 2.1 mmol/L. There is a significant difference (p -value <0.05) in the lowering of triglyceride level in diabetic rats given aqueous leaf extract compared to the diabetic control group. However, there is no significant difference in triglyceride levels between diabetic rats given boiled leaf extract with those of the diabetic and normal control groups (p -value >0.05).

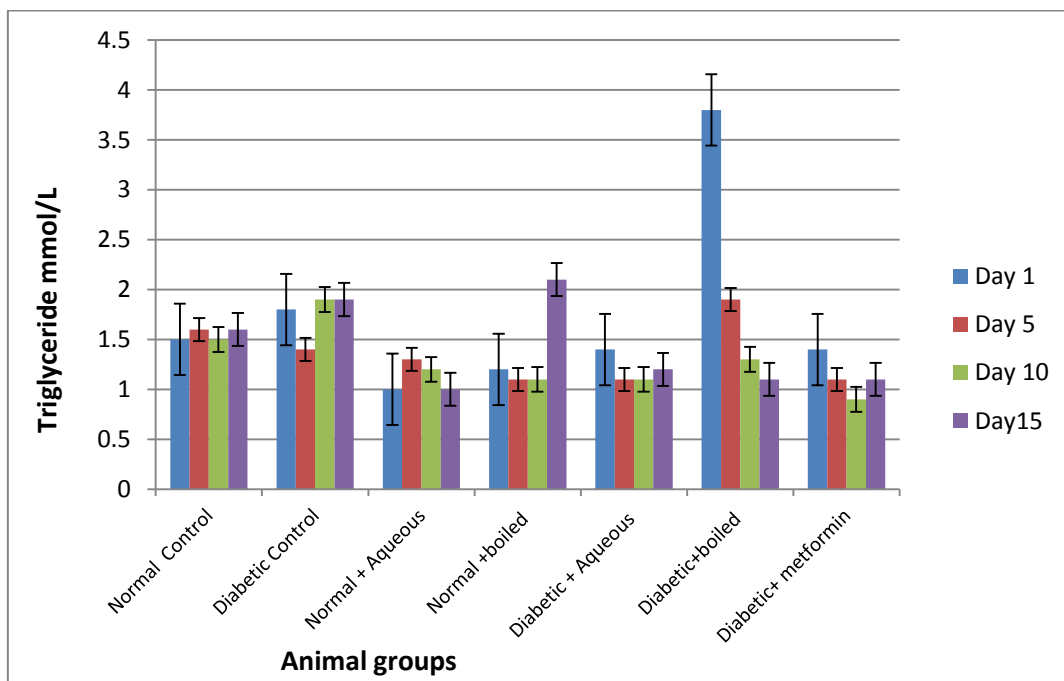


Figure 4.13 Effect of *Moringa oleifera* leaf extracts on triglyceride (mmol/L) levels in normal and diabetic rats.

4.6.3 Hypoglycaemic effects of *Moringa oleifera* leaf on cholesterol

The effects of *Moringa oleifera* leaf extracts on cholesterol levels of the normal and diabetic rats is shown in figure 4.14. The value of cholesterol ranges from 3.7 mmol/L to 4.2mmol/L. There is no significant difference in cholesterol levels between any of the study groups (p -value >0.05).

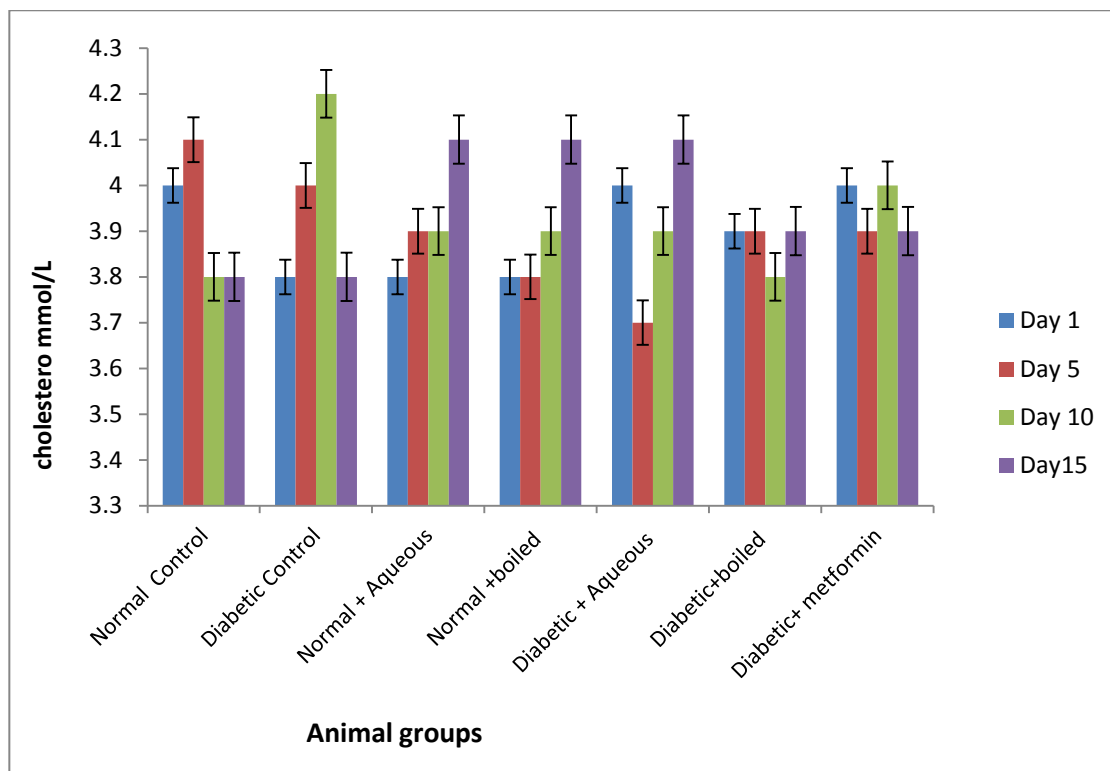


Figure 4.14 Effect of *Moringa oleifera* leaf extracts on cholesterol (mmol/L) levels in normal and diabetic rats.

4.7 Hypoglycaemic effects of freeze-dried overnight aqueous and boiled extract of *Solanum melongena* (brinjal) fruit on normal and diabetic rats.

4.7.1 Hypoglycaemic effects of *S. melongena* on blood glucose level

The figure 4.15 shows that the diabetic rats fed with aqueous fruit extract exhibits statistically significant decrease ($P < 0.05$) in the blood glucose level when compared to the diabetic control group. The decrease in blood sugar level with boiled fruit extract is however, statistically non-significant. Both aqueous and boiled extract did not significantly affect the blood glucose in normal rats.

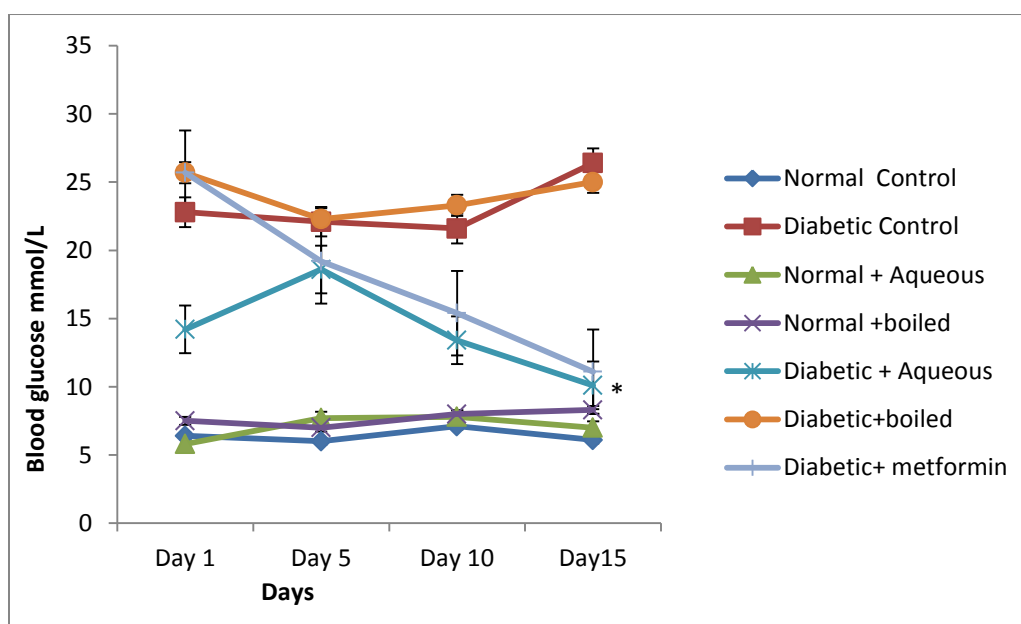


Figure 4.15: Effect of *S. melongena* fruit extracts on Blood glucose (mmol/L) levels in normal and diabetic rats. .

4.7.2 Hypoglycaemic effects of *S. melongena* on Triglyceride

The triglyceride levels of diabetic rats given aqueous extract is found to be significantly less compared to the diabetic control rats ($p < 0.05$), as shown in Figure 4.16.

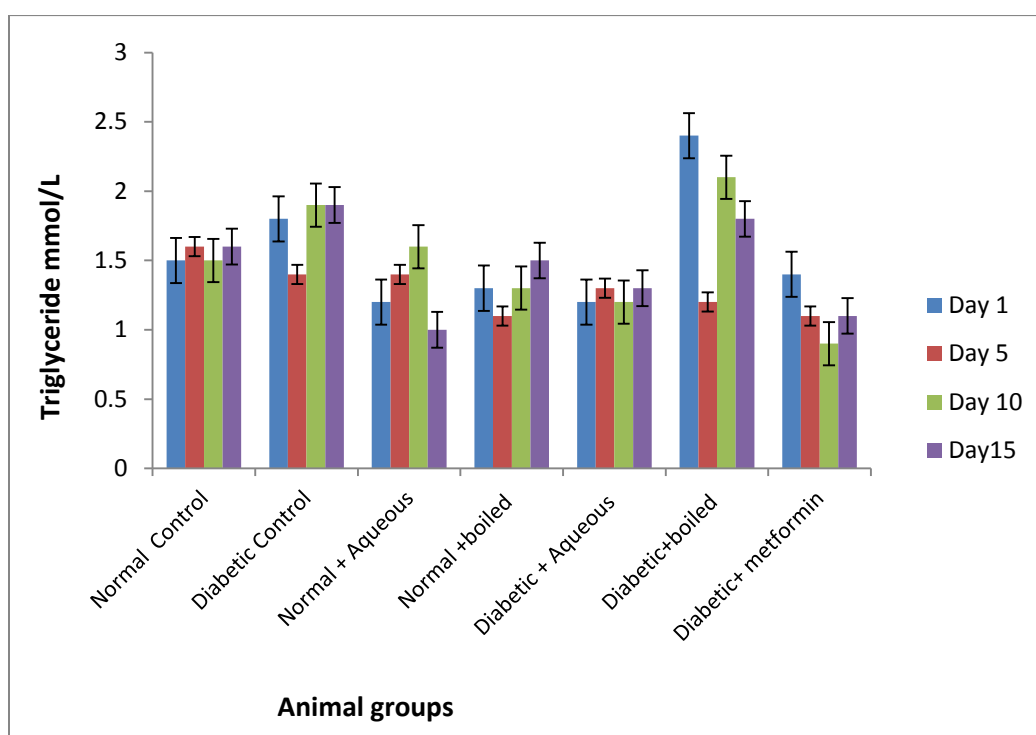


Figure 4.16 Effect of *S. melongena* fruit extracts on triglyceride (mmol/L) levels in normal and diabetic rats.

4.7.3 Hypoglycaemic effects of *S. melongena* on cholesterol

Cholesterol levels vary from 3.6 mmol/L to 4.2mmol/L with no significant difference in cholesterol levels between any of the study groups (p -value >0.05).

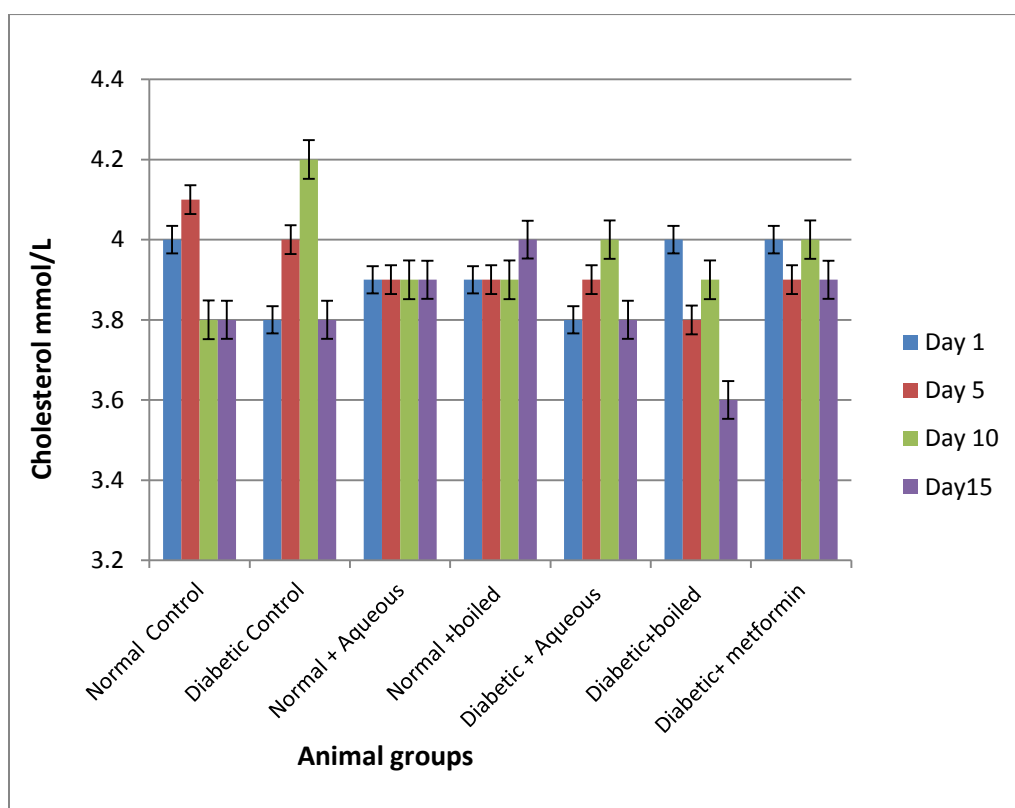


Figure 4.17 Effect of *S. melongena* fruit extracts on cholesterol (mmol/L) levels in normal and diabetic rats.

4.8 Hypoglycaemic effects of freeze-dried overnight aqueous and boiled extracts of all 4 plant extracts together (1:1 ratio) on normal and diabetic rats.

4.8.1 Hypoglycaemic effects of all 4 plant extracts on Blood sugar

The figure 4.18 shows the blood glucose level measured in normal and alloxan-induced diabetic rats. Administration of aqueous extract in diabetic rats significantly decreased blood glucose levels compared to their diabetic control (p -value <0.05). Glucose level is increasing in alloxan induced diabetic rats.

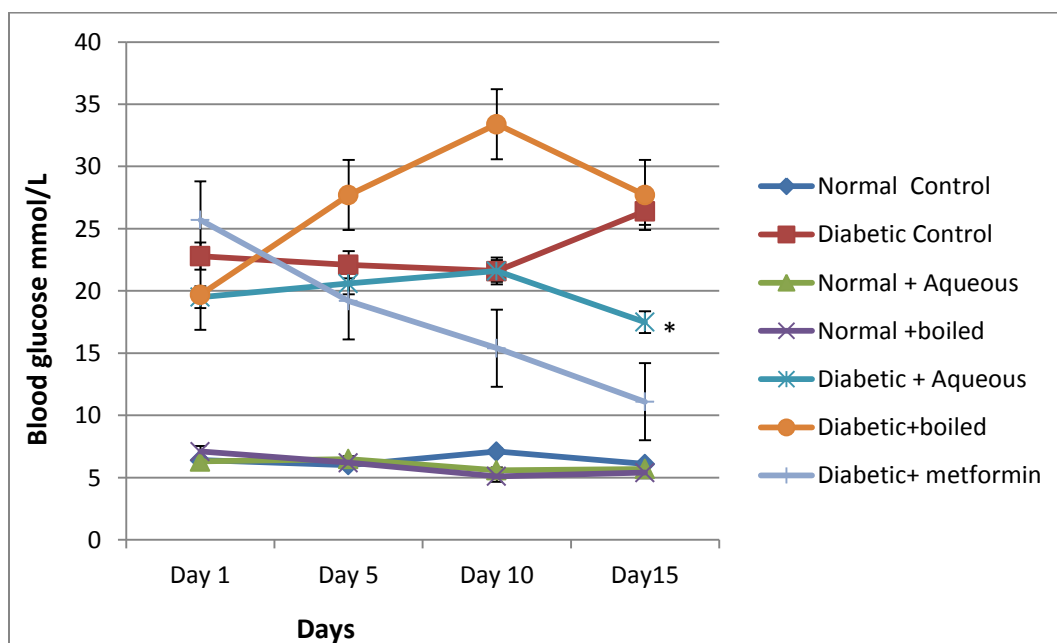


Figure 4.18 Effect of all 4 plant extracts together (1:1 ratio) on Blood glucose (mmol/L) levels in normal and diabetic rats. .

4.8.2 Hypoglycaemic effects of all 4 plant extracts on Triglyceride

Triglyceride levels (Figure 4.19) vary from 0.9 to 1.9 mmol/L. It is noted that the aqueous extract reduces the triglyceride level by 35.8% in diabetic rats when compared to the diabetic control rats (p -value <0.05). No significant difference was shown between the diabetic group given boiled extracts compared to the diabetic control group. The triglyceride level is significantly lower in normal rats fed aqueous extract compared to their respective normal control (p -value <0.001).

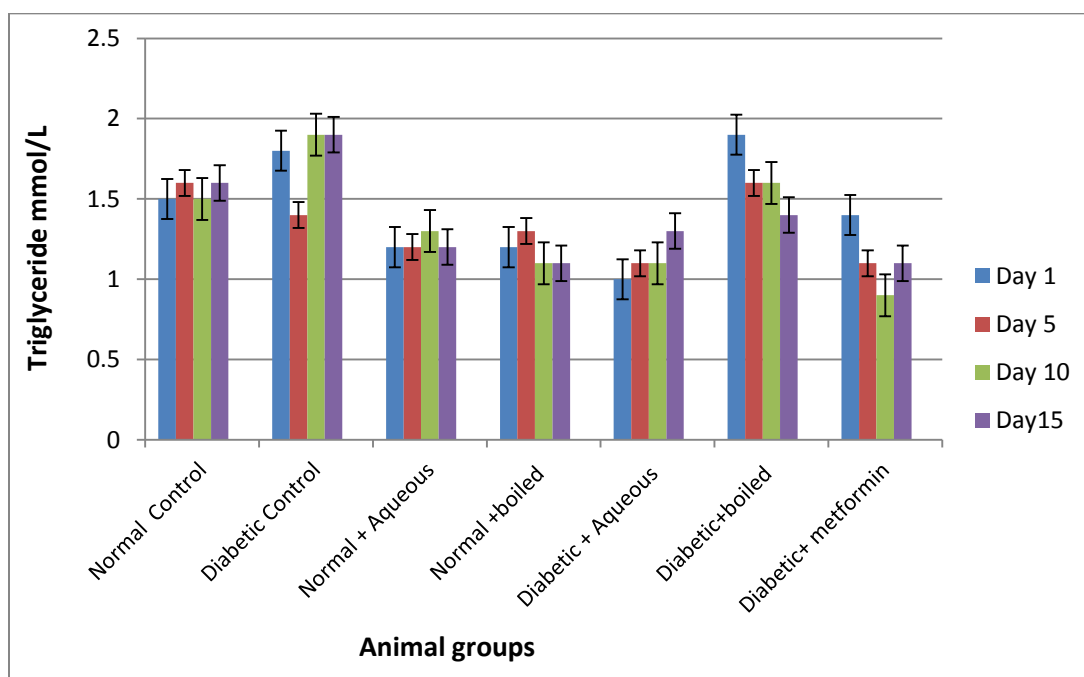


Figure 4.19 Effect of all 4 plant extracts together (1:1 ratio) on triglyceride (mmol/L) levels in normal and diabetic rats.

4.8.3 Hypoglycaemic effects of all 4 plant extracts on cholesterol

Cholesterol level varied from 3 mmol/L to 4.2 mmol/L (Figure 4.20). Compared to their respective control groups, there was no significant difference in cholesterol levels between the diabetic and non-diabetic rats given extracts.

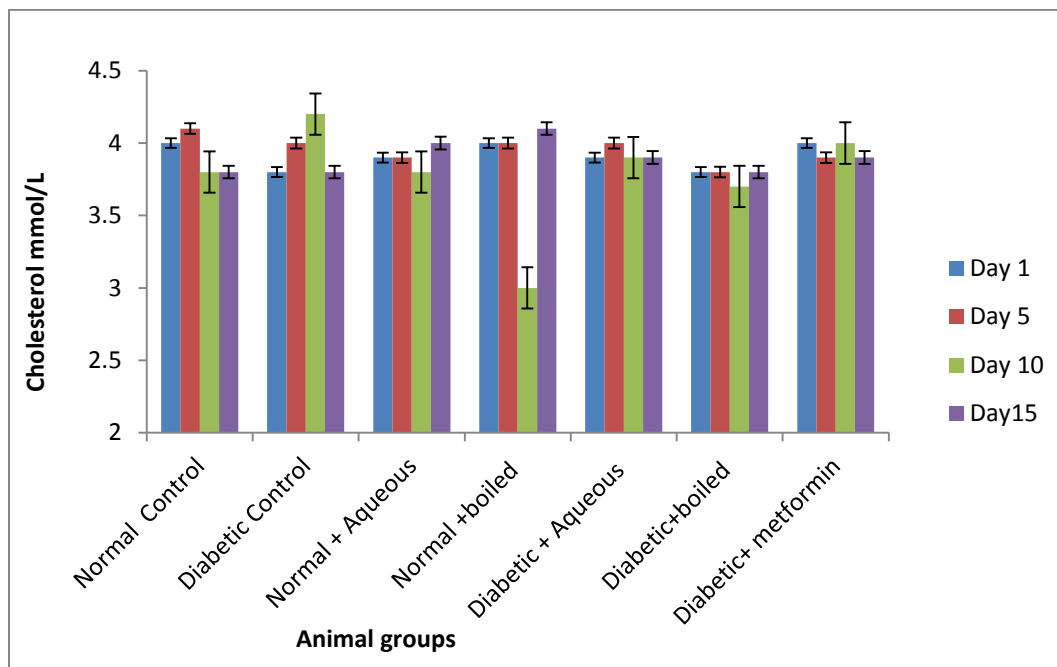


Figure 4.20 Effect of all 4 plant extracts together (1:1 ratio) on cholesterol (mmol/L) levels in normal and diabetic rats.

4.9 Efficacy of all 4 selected plants extracts compared to metformin in blood glucose level of diabetic rats

Table 4.3 Comparison of efficacy of all 4 selected plants extracts with metformin in blood glucose level of diabetic rats

Name of extract	Aqueous/Boiled extract (mmol/L)	Initial (Day1) (mmol/L)	Final (Day 15) (mmol/L)	% reduction	efficacy
<i>M.charantia</i> (Bitter gourd)	Aqueous extract	22.78	8.1	64.4%	√
	boiled extract	23.1	15.9	31.2%	√
Metformin	Metformin	25.68	11.13	56.7%	√
<i>A.esculentus</i> (okra)	Aqueous extract	22.2	13.3	40.1%	√
	Boiled extract	18	23.6	-31.1%	X
<i>M.oleifera</i> (Muringa)	Aqueous extract	20.5	19.3	5.9%	√
	Boiled extract	21.5	17.7	17.7%	√
<i>S.melongena</i> (Brinjal)	Aqueous extract	14.2	10.1	28.9%	√
	Boiled extract	25.7	25	2.7%	√
All 4 (Mc,Ae, Mo, Sm)	Aqueous extract	19.5	17.5	10.3%	√
	Boiled extract	25.7	25	2.7%	√

M.charantia(Mc), *A.esculentus* (Ae), *M. oleifera* (Mo) and *S. melongena*(Sm)

The comparison of efficacy of 4 selected plants with metformin in blood glucose level of diabetic rats is given in table 4.3. It is observed that all the plant extracts used exhibit some hypoglycemic effect except the boiled *A.esculentus* extract. The aqueous extracts of *Momordica charantia* (bitter gourd) and *A.esculentus* (okra), show the greatest antidiabetic potential compared to the other plant extracts. The aqueous extract of *Momordica charantia* (bitter gourd) shows low glucose level in diabetic rats from 22.78mmol/L to 8.1mmol/L on 15th day of treatment whereas, the aqueous extract of *A.esculentus* (Okra) shows reduced blood sugar level in diabetic rats from 22.2 mmol/L to 13.3 mmol/L on the 15th day. Based on the above findings, it was further decided to analyze the apparent mechanism of the hypoglycaemic effects of *Momordica charantia* and *A.esculentus*. Results of the further analysis are given below.

4.10 Determination of the mechanism for the apparent hypoglycaemic effect of the plant extracts (*Momordica charantia* and *Abelmoschus esculentus*) in diabetic rats

4.10.1 *In vitro* transport of glucose across rat's intestine

The inverted intestinal sac method was used to determine the effect of *Momordica charantia* (bitter gourd) and *Abelmoschus esculentus* (okra) on intestinal glucose absorption. Figure 4.21 shows that both *M.charantia* and *A. esculentus* extracts lower the entry of glucose into the intestine. *A.esculentus* was however, more effective in inhibiting the entry of glucose into the intestine than *M.charantia*.

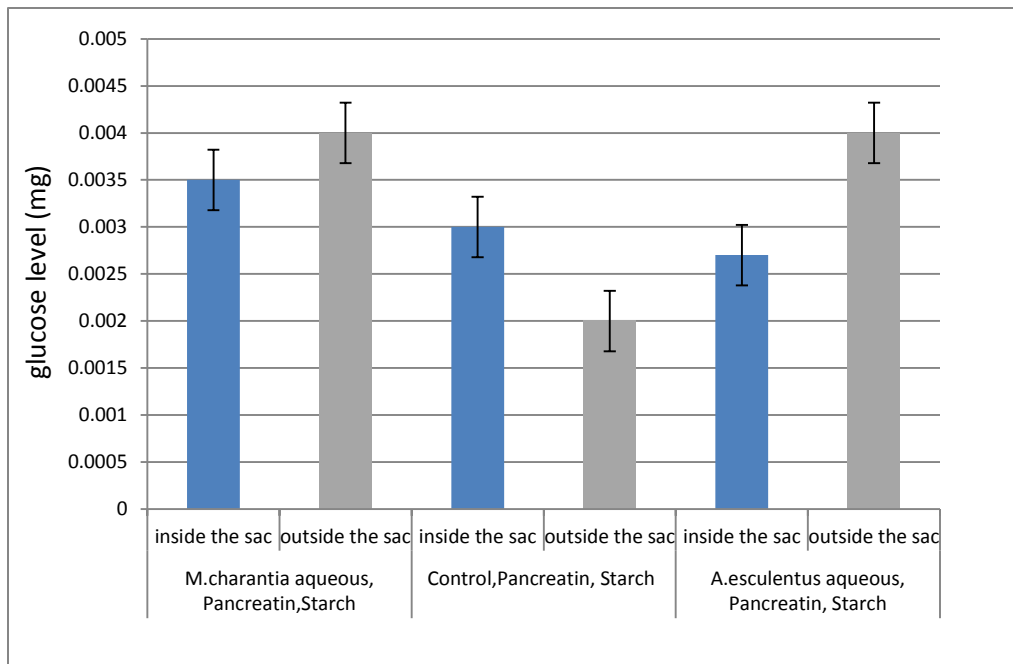


Figure 4. 21. Effect of Extract on glucose uptake in rat's intestine. An inverted segment of the rat's intestinal sac was incubated 37°C for 3 hours in a solution containing 7ml of 1% starch, 1ml of 1% pancreatin and extract.

4.10.2 The effect of extract on Na/K ATPase

The effect of the aqueous extracts of *Momordica charantia* (bitter gourd) and *Abelmoschus esculentus* (okra) on Na⁺/K⁺-ATPase activity is evaluated. The *Abelmoschus esculentus* extract reveals a more statistically significant inhibitory activity (25%) on its functioning compared to *Momordica charantia* (12.5%). *P*-value < 0.05 (Figure 4.22).

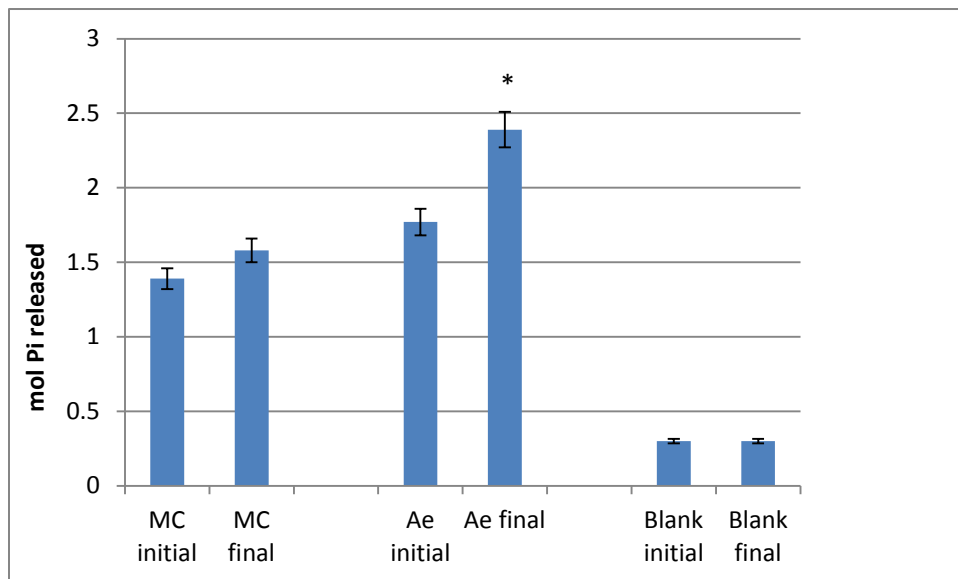


Figure 4.22 Inhibitory activity of *Momordica charantia* and *Abelmoschus esculentus* on Na⁺/K⁺-ATPase (Values are mean ± standard error (n = 3) *Momordica charantia* (Mc) *Abelmoschus esculentus* (Ae))

4.10.3 Assessment of the glycogen content

The glycogen content within the liver was estimated, in non-diabetic control, diabetic control, diabetic *Momordica charantia* aqueous, diabetic *Momordica charantia* boiled, diabetic *Abelmoschus esculentus* aqueous, non-diabetic *Momordica charantia* aqueous, non-diabetic *Momordica charantia* boiled, non-diabetic *Abelmoschus esculentus* aqueous and diabetic metformin as shown in Table: 4.4.

The table shows the changes in hepatic glycogen content in the hepatic tissue of the control and experimental group of rats. In diabetic controls, hepatic glycogen content decreases significantly by 66.2% as compared to non-diabetic controls. Treatment with aqueous *Abelmoschus esculentus* extract leads to 55% increase in hepatic glycogen content in comparison with diabetic controls rats. A significant decrease in liver glycogen content is shown in the diabetic control group of rats which normalized after *Abelmoschus esculentus* aqueous treatment. Treatment with *Momordica charantia* aqueous extract shows 47.3% and *Momordica charantia* boiled extract shows 59% rise in glycogen in comparison with diabetic control. There is no significant difference among the normal treated compared to normal diabetic rats.

Table: 4.4 showing the amount of glycogen present in the liver of different study groups (n=3)

Groups	Glycogen mg/gm
Diabetic Control	9.01 ± 2.25
Diabetic Metformin	23.645 ± 1.13
Diabetic <i>M.charantia</i> aqueous	17.11 ± 0.89
Diabetic <i>M.charantia</i> boiled	22.07 ± 0.45*
Diabetic <i>A.esculentus</i> aqueous	20.04 ± 1.5*
Non-diabetic Control	26.69 ± 1.01*
Non-diabetic <i>M.charantia</i> aqueous	21.395 ± 1.13
Non-diabetic <i>M.charantia</i> boil	18.47 ± 2.48
Non-diabetic <i>A.esculentus</i> aqueous	26.58± 0.45

Values are given as mean ± standard error. Values are statistically significant at * $p < 0.05$. Diabetic control rats were compared with normal control rats. Diabetic Mc aqueous, Diabetic *M.charantia* boiled, Diabetic Okra aqueous, Diabetic Metformin treated rats were compared with diabetic control rats.

4.10.4 Enzyme Glucokinase and Hexokinase

The results of the *in vitro* effect of the vegetables on hexokinase and glucokinase are given in Table 4.5. As compared to non-diabetic control values, the mean levels of enzymes hexokinase and glucokinase values decrease in the diabetic control rats. The percentage of hexokinase and glucokinase values decrease by 66.7 and 83.3 in diabetic control rats respectively. Treatment with *M. charantia* and *A. esculentus* significantly increases these parameters ($P<0.001$) as compared to diabetic controls. On the other hand, non-diabetic treated groups show no effect on glucokinase activity.

Table 4.5 Effect of administration of selected doses of *M.charantia* and *A.esculentus* on enzymes in carbohydrate metabolism in rats.

Group	Hexokinase (units/ml)	Glucokinase (units/ml)
Diabetic control	0.009±0.033	0.191±0.021
Diabetic Mc Aqueous	0.126±0.064**	0.324±0.027 *
Diabetic Mc Boil	0.054±0.089 **	0.446±0.053
Diabetic Ae Aqueous	0.032±0.031 **	0.618±0.029
Diabetic Metformin	0.018±0.079 *	0.135±0.005 **
Non-diabetic control	0.027±0.052 **	1.143±0.779
Non-diabetic Mc Aqueous	0.036±0.049	0.864±0.022
Non-diabetic Mc Boil	0.009±0.058	0.291±0.0075
Non-diabetic Ae Aqueous	0.027±0.004	0.103±0.063

Values are given as mean ± S.E. for groups of four animals each. Diabetic control was compared with the normal. Experimental groups were compared with diabetic control (P-value < 0.01*, P-value < 0.001**).

4.10.5. Histopathological analysis of liver and gastrocnemius muscle tissues of normal and experimental groups of rats.

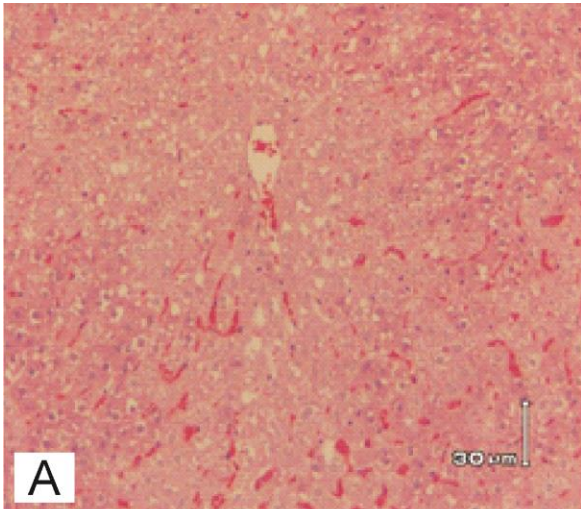
4.10.5.1 Histopathological analysis of liver in normal and experimental groups of rats

The cell structure of the liver and gastrocnemius muscle tissues of normal and experimental *Sprague-Dawley* rats is given in Figures 4.23 A – R.

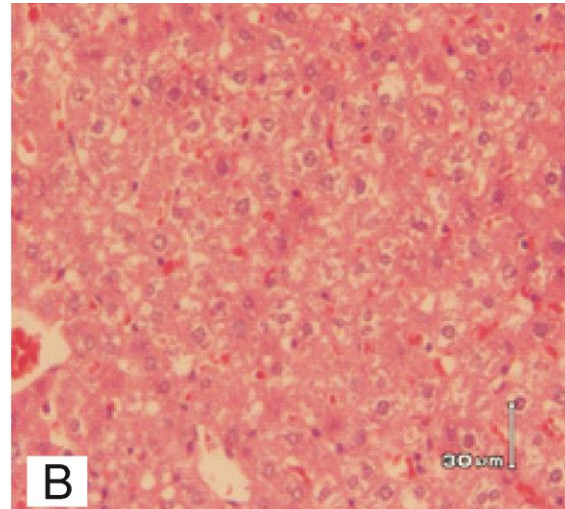
Figure 4.23 A shows red blood cells in sinusoids (congestion), scant hepatocytes showing small suspect lipid vacuoles. Hepatocytes have granular, eosinophilic to fairly vacuolar cytoplasm (possible degenerative changes).

Figure 4. 23 B shows mild diffuse sinusoidal congestion. Hepatocytes show mild hydropic degeneration, which is within normal limits. The central veins appear normal. No architectural disturbances, fibrosis or chronic lesions are seen.

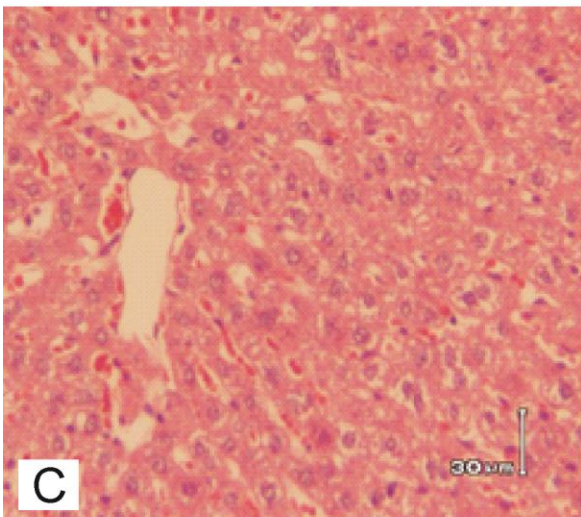
Figures 4.23 C, D, E and F show mild diffuse sinusoidal congestion. Hepatocytes show mild hydropic degeneration, which is within normal limits. No architectural disturbances, fibrosis or chronic lesions are seen.



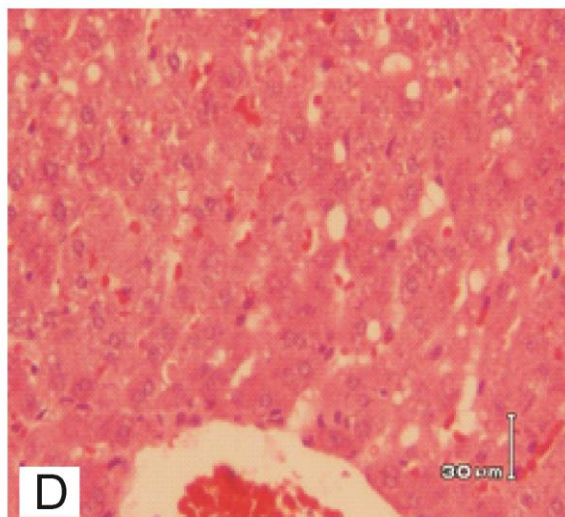
Diabetic control liver



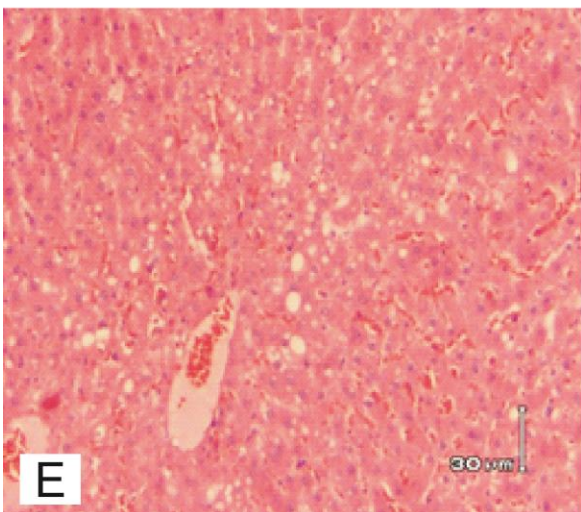
Non-Diabetic control liver



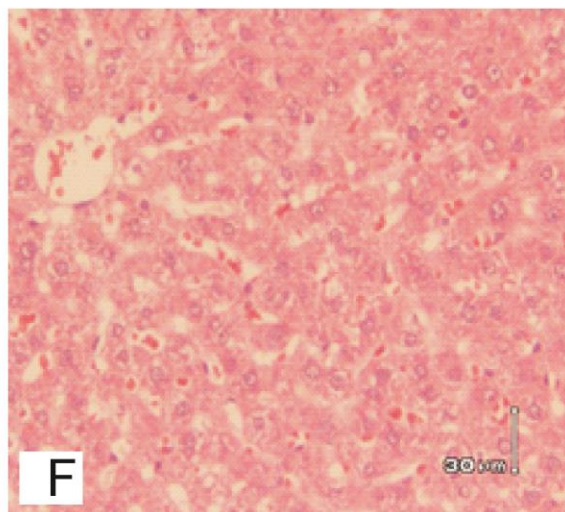
Diabetic *M. charantia* aqueous liver



Diabetic *M. charantia* boiled liver

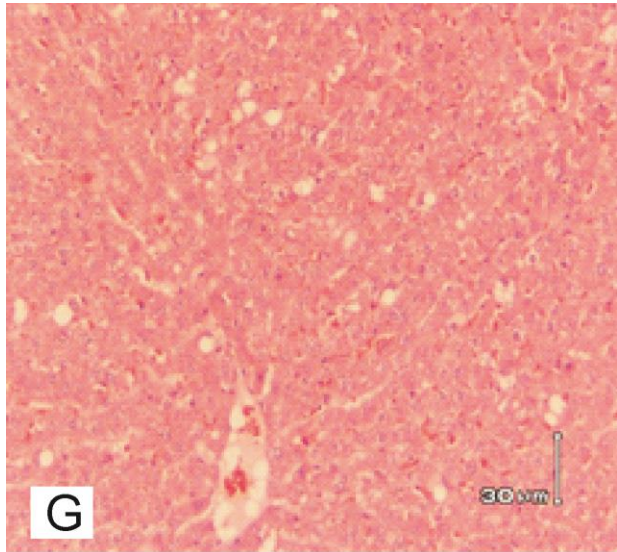


Diabetic *A. esculentus* aqueous liver

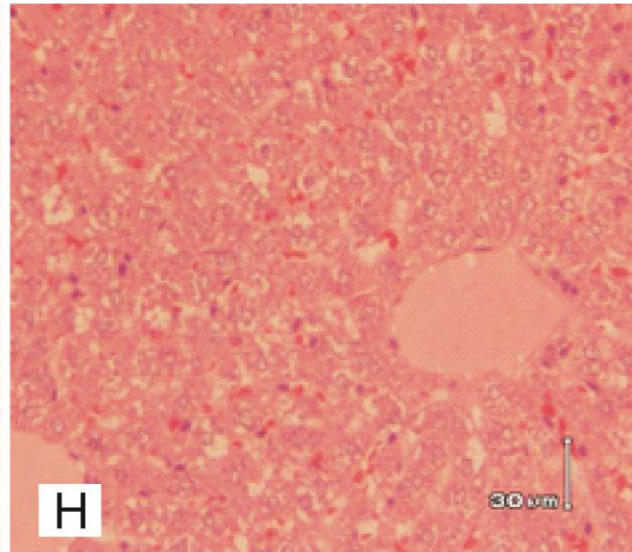


Diabetic Metformin liver

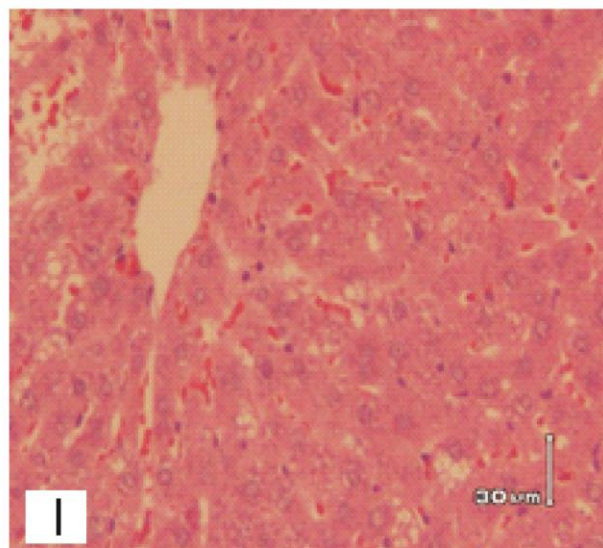
Figures 4.23 G, H and I show mild diffuse sinusoidal congestion. Hepatocytes show mild hydropic degeneration, which is within normal limits. The central veins appear to be normal. No architectural disturbances, fibrosis or chronic lesions are seen.



Non-diabetic *M.charantia* aqueous liver



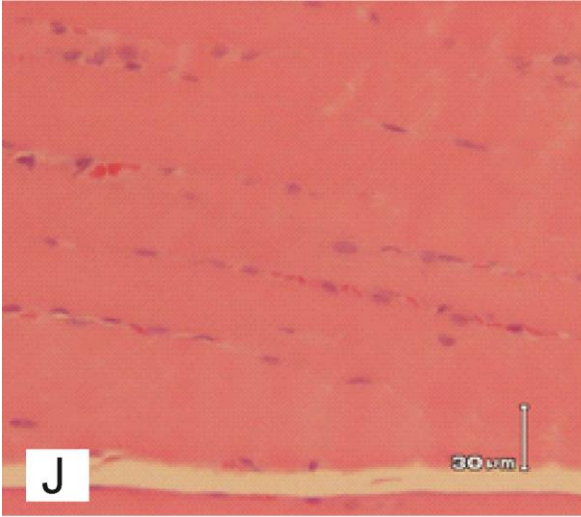
Non-diabetic *M.charantia* boiled liver



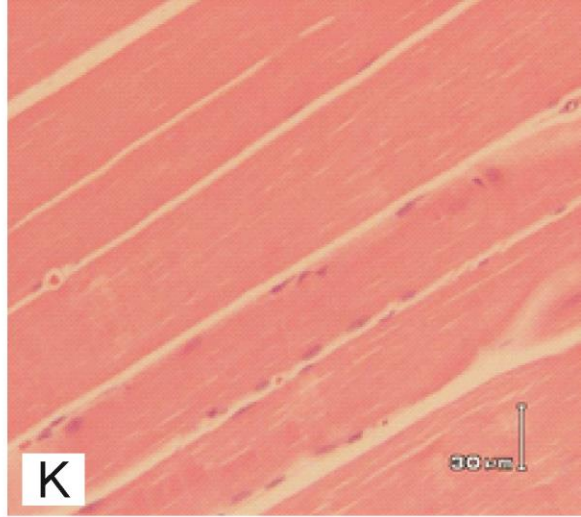
Non- diabetic *A.esculentus* aqueous liver

4.10.5.2 Histopathological analysis of Muscle tissues in normal and experimental group of rats

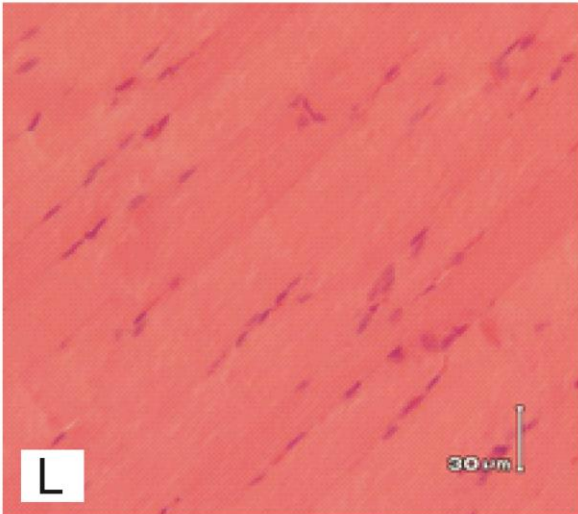
Figure **4.23 J** appears within normal histological limits, muscle fibres with normal eosinophilic staining sarcoplasm and nuclei underneath sarcolemma which are elongated and hyperchromatic. Figures **4.23 L, M, N** and **O** appear within normal histological limits.



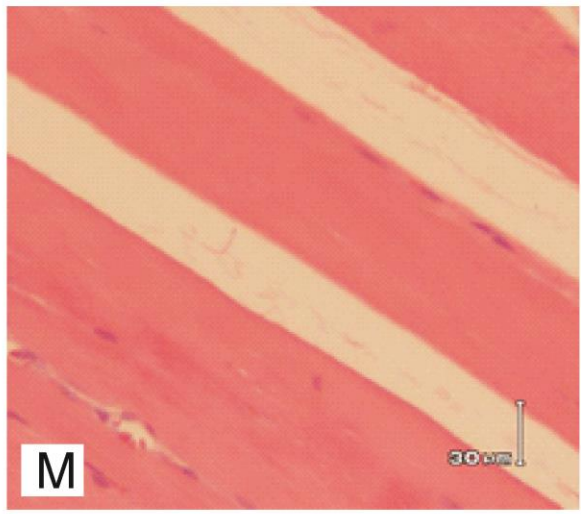
Diabetic control Muscle



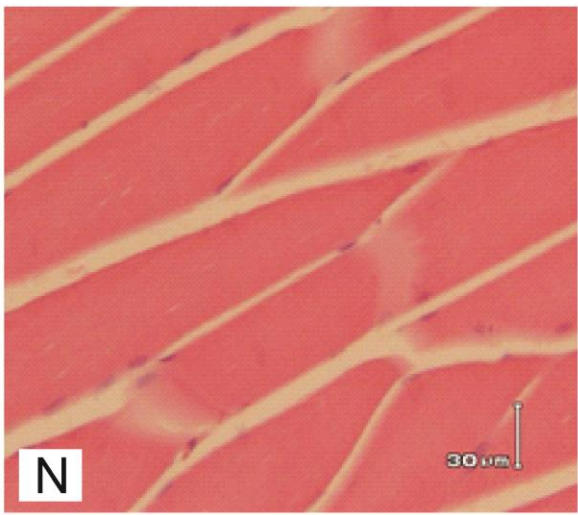
Non-diabetic control Muscle



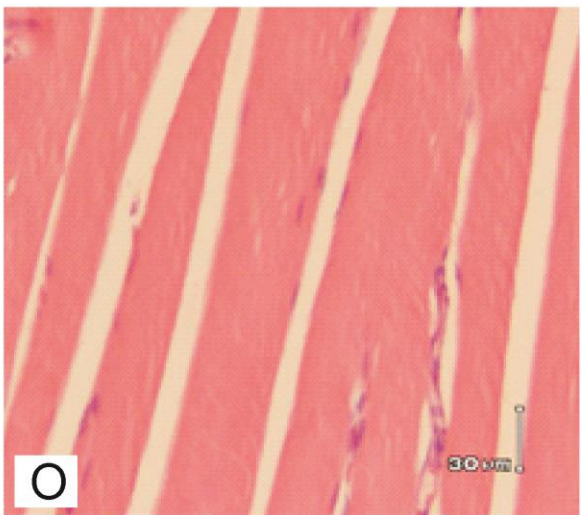
Diabetic *M. charantia* aqueous Muscle



Diabetic *M. charantia* boiled Muscle

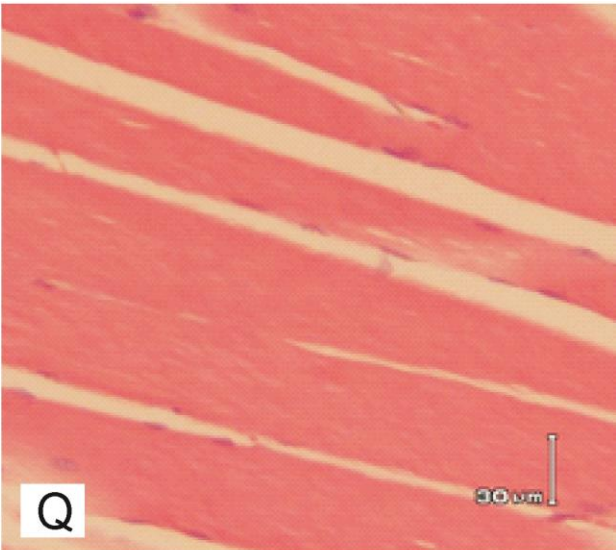
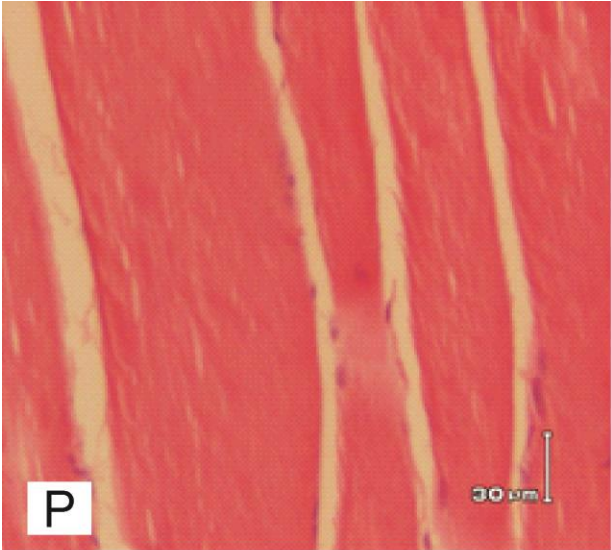


Diabetic *A. esculentus* aqueous Muscle



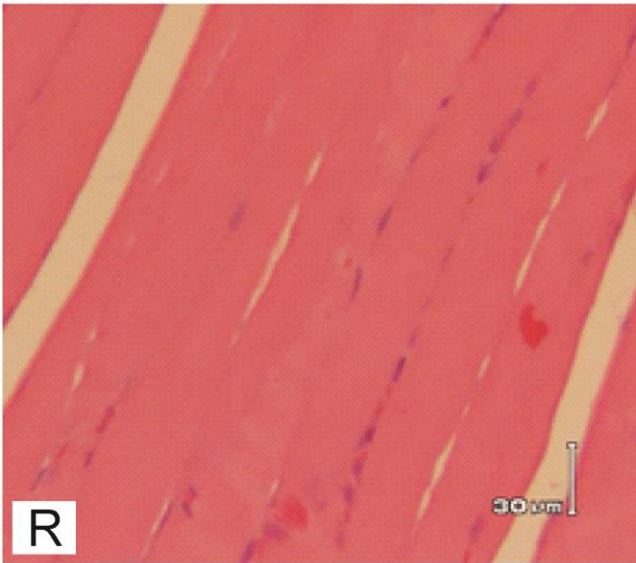
Diabetic Metformin Muscle

Figures 4. 23 K, P, Q, and R appear within normal histological limits. At the end of a 15 day treatment period, sections of liver and gastrocnemius muscle tissues of normal rats showed no changes in the histology of the tissues.



Non-diabetic *M.charantia* aqueous Muscle

Non-diabetic *M.charantia* boiled Muscle



Non-diabetic *A.esculentus* aqueous Muscle

CHAPTER 5

DISCUSSION

Diabetes mellitus (DM) is a metabolic disorder marked by high blood sugar levels as a result of the pancreas not producing enough insulin or ineffective use of the produced insulin. According to WHO (2010) more than 221 million people worldwide were suffering from DM, of which 95% were type 2 diabetics. By 2025, the worldwide prevalence of diabetes is expected to be 300 million (Venkat Narayan *et al.*, 2000; Mckinlay and Marceau, 2000) of which 228 million are estimated to be in developing countries and 72 million in developed countries (Mckinlay and Marceau, 2000).

Medicinal plants play a vital role against various diseases. A number of plants have been used for the treatment of diabetes throughout the world (Rathi *et al.*, 2002; Viridi *et al.*, 2003; Shekelle *et al.*, 2005; Kwon *et al.*, 2007; Erasto *et al.*, 2005; Hussain *et al.*, 2009). This study was carried out to investigate nutritional content and the antidiabetic properties of *Momordica charantia*, *Abelmoschus esculentus*, *Moringa oleifera* and *Solanum melongena* on alloxan induced diabetic rats. The study also aimed to determine the apparent mechanism by which plant extracts lowered the sugar levels in rats.

The inorganic and organic constituents of the hypoglycaemic plants have medicinal properties (Kar *et al.*, 2003). As seen in Table 4.1, all four plants were reported to have protein, fats, glucose and starch. In *S.melongena*, a higher value of protein was reported

by Agoreyo *et al.* (2012). The amount of protein found in *A.esculentus* was in agreement with the values reported by Adenipekun and Oyetunji, (2010). *Moringa* leaves appeared to be a good source of protein. The protein content of these vegetables can help to contribute to daily protein intake requirements. Indian varieties had the least protein content while SA varieties had the highest; this could be due to genetic variation (Aja *et al.*, 2013) and also due to the fact that the Indian seeds were cultivated in a different climatic conditions. The low starch and glucose levels in these vegetables are beneficial to diabetic individuals and individuals who are watching their weight. The low range of fat was reported in *A.esculentus* (Hussain *et al.*, 2010). The low fat content in *A.esculentus* makes it an ideal vegetable for individuals with increased serum lipid levels, high blood pressure, heart diseases and diabetes.

Minerals are important constituents of human diet as they serve as co-factors for many physiological and metabolic processes. Deficiencies of certain micronutrients correlate to diabetic complications (Mooradian and Morley, 1987). As seen in Table 4.2, all the four plants are rich in minerals. The amount of Calcium in *M.oleifera* correlates with the findings of Moyo *et al.*, (2011). This differed from the findings of Sodamade *et al.*, (2013). Calcium plays an important role in the development of Type 2 diabetes mellitus as insulin secretion is calcium dependent; therefore, inadequate calcium intake can have adverse effects on β -cell secretory function (Anastassios *et al.*, 2007). Cytochrome C oxidase, superoxide dismutase, lysyl oxidase and tyrosine oxidase are the major enzymes that require copper for their activity. Deficiency is associated with impaired

glucose tolerance and increased cholesterol levels (Mooradian *et al.*, 1994). The amount of cobalt content in *A. esculentus*, *M. charantia* and *S.melongena* differs from the findings by Hussain *et al.* (2011) and Ismail *et al.* (2011). Adequate dietary intake of iron, zinc and copper is essential to human health. Iron deficiency has no significant effect on glucose homeostasis, but it is associated with increased serum triglyceride levels (Mooradian and Morley, 1987). Potassium is the most abundant intracellular cation in the body and contributes to intracellular osmolality. Potassium is also necessary for insulin secretion. Enzymes involved in glycolysis and oxidative phosphorylation are potassium dependent (Arivalagan *et al.*, 2013). Magnesium is an important co-factor of many regulatory enzymes. Rajendran *et al.* (2007) mentioned that the decreased manganese levels in blood can cause pancreatotomy and diabetes in animals. Manganese acts like insulin and transports glucose into fat tissue. A low sodium diet has been reported to be beneficial in the prevention of high blood pressure (Agoreyo *et al.*, 2012). Zinc is required for protein and carbohydrate metabolism (Arivalagan *et al.*, 2013). Zinc is a major constituent of enzymes in metabolic pathways. Zinc deficiency causes a decrease in insulin secretion; increased tissue resistance to insulin and glucose intolerance (Mooradian *et al.*, 1994). Crystalline insulin contains 0.5% zinc (Mooradian and Morley, 1987, Mooradian *et al.*, 1994).

This study shows that there is a wide variation in the levels of the minerals investigated in the vegetables. The variation in the nutritional values will differ for a wide range of reasons, such as the region in which it is cultivated, growing conditions, the nature

of the soil, seasonal changes, genetically different cultivars, the period of analysis (Jongrungruangchok *et al.*, 2010, Ogbe & John 2011/2012, Morabad *et al.*, 2012), the use of fertilizers and the state of the plants maturity at harvest (Ekholm *et al.*, 2007). If the cultivated soil is deficient in certain trace metals, then the cultivated crop would ultimately be deficient for those metals (Ismail *et al.*, 2011).

All the plant extracts (*M. charantia*, *A. esculentus*, *M. oleifera* and *S. melongena*) except the *A. esculentus* boiled extract showed hypoglycemic effects within the two week study period. Preliminary experiments demonstrated that aqueous extracts of *M. charantia* and *A. esculentus* had maximum antidiabetic potential compared to the other plant extracts (Table 4.3). The higher hypoglycaemic activity of *M. charantia* was supported in a study by Kar *et al.* (2003).

From the results presented in Figure 4.6 and Figure 4.9, it is confirmed that the aqueous extract of *M. charantia* and *A. esculentus* fruits at a dose of 300 mg/kg body weight possessed significant antihyperglycemic activity in diabetic rats when compared to the diabetic control group. There was no effect with respect to glycaemia in non-diabetic rats given aqueous extract compared to the normal rats. Jayasooriya *et al.* (2000), Vikrant *et al.* (2001), Toshihiro *et al.* (2001), Kameswararao *et al.* (2003), Garau *et al.* (2003), Teoh *et al.* (2010) and Sabitha *et al.* (2011) supported these findings but a study by Ojewole *et al.* (2006) did not correlate with our finding. Ojewole *et al.* (2006) reported that

M.charantia fruit extract had significant hypoglycemic effects in normal rats as well. Saha *et al.* (2011) and Mohammady *et al.* (2012) reported that *A.esculentus* and *M.charantia* extract 300 mg/kg b.w significantly reduced the blood glucose level in diabetic treated rats compared to the diabetic control group. Khatun *et al.* (2011) supported our findings that consumption of water-soluble dietary fibers like *A.esculentus*, reduced blood glucose by reducing the diffusion of glucose and postponing the digestion and absorption of carbohydrates. Said *et al.* (2007), reported that 'glucoselevel' a combination of four anti-diabetes plants lowered the sugar level of diabetic rats from 400 to 200 mg/dl after two weeks. Parkash *et al.* (2002), had isolated charantin from *M.charantia*, a peptide resembling insulin. According to Jayasooriya *et al.* (2000), the hypoglycemic effect was due to inhibition of glucose absorption, conversion of glucose into hepatic glycogen and the insulinogenic activity of *M.charantia* extract. Garau *et al.* (2003), Kameswararao *et al.* (2003), Sathishsekar and Subramanian (2005), Jelodar *et al.* (2007), Singh and Gupta (2007), Singh *et al.* (2011), Bano *et al.* (2011) and Mohammady *et al.* (2012) support the insulinogenic activity of *M.charantia*. Contrary to this theory, the suppression of the gluconeogenic enzymes such as glucose-6-phosphatase and fructose-biphosphatase may be involved in the hypoglycaemic effects of *M.charantia* (Garau *et al.*, 2003). Alloxan is a diabetogenic agent used to induce Type 2 diabetes in animals via the selective destruction of the β cells (Kumar *et al.*, 2006). Alloxan is capable of inducing both type 1 & 2 diabetes mellitus with correct dosage selection (Etuk 2010), but Rao *et al.* (1999), reported that alloxan causes permanent destruction of β cells, thus

M.charantia produced antidiabetic effects in diabetic rats via other mechanisms and not via the release of insulin from the β cells. Aqueous extract of *M. oleifera*, *S. melongena* and *M.charantia* significantly lower the triglyceride levels of diabetic treated groups whereas there was no significant difference in the triglyceride levels of *A. esculentus* treated groups. Cholesterol levels of the study groups ranged from 3.6 to 4.3 mmol/L. There was no significant difference in cholesterol levels between any of the study groups during the course of the experiment. Similar effects i.e., reduced cholesterol levels, were reported by Kameswararao *et al.* (2003), and Ngoc *et al.* (2008). They further mentioned that as the blood sugar level becomes normal in diabetic rats, triglyceride and cholesterol levels decrease. Mohammady *et al.* (2012), reported that increased level of triglyceride and cholesterol in diabetic control rats may be due to insulin deficiency.

The oral feeding of *M. oleifera* leaf and *S. melongena* fruit extract showed no significant difference in body weight whereas the *M.charantia* and *A. esculentus* extract given groups of rats, showed an increase in body weight compared to their corresponding controls. The present findings disagree with the results of Bano *et al.* (2011) who found that the addition of *M.charantia* aqueous extract significantly lowered the body weight of diabetic treated group. According to Singh *et al.* (1989), Sathishsekar and Subramanian (2005), Tripathi and Chandra, (2010), and Mohammady *et al.* (2012), the oral feeding of *M.charantia* extract resulted in an increase in body weight in diabetic rats and a

reduction in body weight in the diabetic control rats. An increase in body weight in the diabetic treated animals is due to the better insulin secretion (Kameswararao *et al.*, 2003). Continuous excretion of glucose as well as gluconeogenesis can reduce the weight loss in diabetics (Viridi *et al.*, 2003; Dheer and Batnagar, 2010).

Figure 4.21 shows that both *M.charantia* and *A. esculentus* extracts lowered the entry of glucose into the intestine. *A. esculentus* was however more effective in inhibiting the entry of glucose into the intestine than *M.charantia*. This was in agreement with previous studies on 'glucoselevel' a combination of four anti-diabetes plants (Said *et al.*, 2007). Mahomoodally *et al.* (2007); Mohammady *et al.* (2012) and Habicht *et al.* (2014) agreed that *M.charantia* decreased the glucose values inside the inverted intestine due to reduced sucrase activity. Similar results were reported by Garau *et al.* (2003) in glucose loaded rats or diabetic mice which showed suppression in the transfer of glucose from the stomach to the small intestine. The present result is in agreement with the result of Khatun *et al.* (2011). The gelatinous soluble dietary fiber of *A.esculentus* reduced the intestinal absorption of glucose in rats.

In the present study, an attempt was made to explain the effect of these plants on hepatic glycogen levels. In diabetic individuals, the glycogen content of the liver drops as it depends on insulin for the entry of glucose (Rathi *et al.*, 2002). In the present study, the glycogen content in the liver was reduced by 62.2 % in diabetic control rats when compared with the non-diabetic controls. Treatment with *M.charantia* and *A.esculentus*

increased the glycogen content by 47.3% (*M.charantia* aqueous), 59.2% (*M.charantia* boiled), 55% (*A.esculentus* aqueous) compared to the diabetic control group (Table 4.4). Grover *et al.* (2000), Kameswararao *et al.* (2003) and Mohammady *et al.* (2012) agreed with our findings. This may be due to the insulin release from the β cells. According to Fernandes *et al.* (2007), Prabhakar and Doble, (2011) the hepatic glycogen content decreased in diabetic rats after the administration of *M.charantia*. Bhaskar and Kumar, (2012) reported that liver glycogen content was reduced in *Punica granatum* extract given groups compared to the diabetic control. The fruit extract of *Physalis peruviana* Linn and metformin improved the levels of glycogen content in liver and muscle tissues (Sathyadevi *et al.*, 2014). This was in agreement with previous studies on *Annona squamosa* (Shirwaikar *et al.*, 2004) and *Terminalia chebula* (Kumar *et al.*, 2006).

In diabetes, glycogen synthesis is reduced in the liver due to reduced glycogenolysis and insulin levels (Dheer and Bhatnagar 2010, Bhaskar and Kumar 2012). The reduced glycogen content in diabetic rats is due to reduced activity of glycogen synthase and increased activity of glycogen phosphorylase (Kumar *et al.*, 2006). Hexokinase is a cytosolic enzyme that catalyzes the phosphorylation of glucose upon entry into the cell (LaPier and Rodnick, 2001). The activity of hexokinase and glucokinase, the main glycolytic enzymes used in the initial phosphorylating steps of glucose metabolism, are reduced significantly under diabetic conditions which are improved by some hypoglycaemic plants (Syiem *et al.*, 2009). Glucokinase plays an important role in

controlling glucose phosphorylation and metabolism in liver and in the pancreatic islets. A deficiency of glucokinase would disturb glucose homeostasis (Matschinsky, 1990).

In the present study the activity of hexokinase and glucokinase in the muscle and liver was reduced in the diabetic control rats as compared to the non-diabetic rats and the condition was returned with *M.charantia* and *A.esculentus* treatment as compared to normal control (Table 4.5). Habicht *et al.* (2014), Grover and Yadav (2004) and Mohammady *et al.* (2012) agreed with our findings on *M.charantia*. This was in agreement with previous studies on *Eugenia jambolana*. Grover *et al.* (2000), and Jung *et al.* (2004) agreed with the activity of glucokinase in diabetic mice. When the glycogen content increased, glucokinase activity also increased in the diabetic treated group (Prabhakar and Dogle, 2011). The present study had similar results; but Ugochukwu and Babady, (2003), reported a higher glucokinase activity in untreated diabetic rats than in the treated group. A low GK activity is due to gluconeogenesis and glycogenesis favouring a high glucokinase activity. They also emphasized that the decreased activity of GK in diabetic animals was due to malfunctioning insulin release.

The reduced activity of hexokinase in the liver of diabetic controls may be due to the reduced ingestion of glucose in the system and high blood sugar levels (Kumar *et al.*, 2006). Na⁺/K⁺ ATPase or the sodium-potassium pump is a member of the P-type class of ATPases and is a critical enzyme found in the membranes of all animal cells. It has an anti-porter like activity, but is not truly an anti-porter as both sodium and potassium ions are transported actively against their concentration gradient with the energy provided by ATP (Morth *et al.*, 2007). The pump consists of 2 subunits – alpha and

beta, which are expressed in different isoforms (Sweeney and Klip, 1998). The Na⁺/K⁺ ATPase enzyme serves multiple purposes, most importantly generating the resting membrane potential as well as allowing for the secondary active transport of other compounds into the cell by using the Na⁺ gradient created by this pump (Sadava *et al.*, 2008).

As seen in the figure 5.1, binding of sodium to the pump occurs at the cytoplasmic side. ATP then phosphorylates this enzyme and the remaining ADP is released. The phosphorylation causes a conformational change in the Na⁺/K⁺ ATPase pump and the 3 sodium ions are released into the extracellular space. The pump then binds 2 extracellular K⁺ ions causing dephosphorylation of the pump and reverting it to its previous conformational state. The dephosphorylated form of the pump has a higher affinity for Na⁺ ions than K⁺ ions thus releasing the K⁺ ions intracellularly. Na⁺ and ATP can then bind again and the process restarts (Guyton and Hall, 2006).

Both *M.charantia* and *A.esculentus* extracts investigated in the current study demonstrated some Na⁺/K⁺ ATPase inhibitory activity (Figure 4.22). The results obtained for the inhibition of Na⁺/K⁺ ATPase indicated that *M.charantia* and *A.esculentus* aqueous extract are capable of inhibiting the action of this enzyme.

The aqueous extract of *A.esculentus* showed a higher inhibition than the *M.charantia* for the enzyme inhibition. This was in agreement with previous studies on *M.charantia* (Garau *et al.*, 2003).

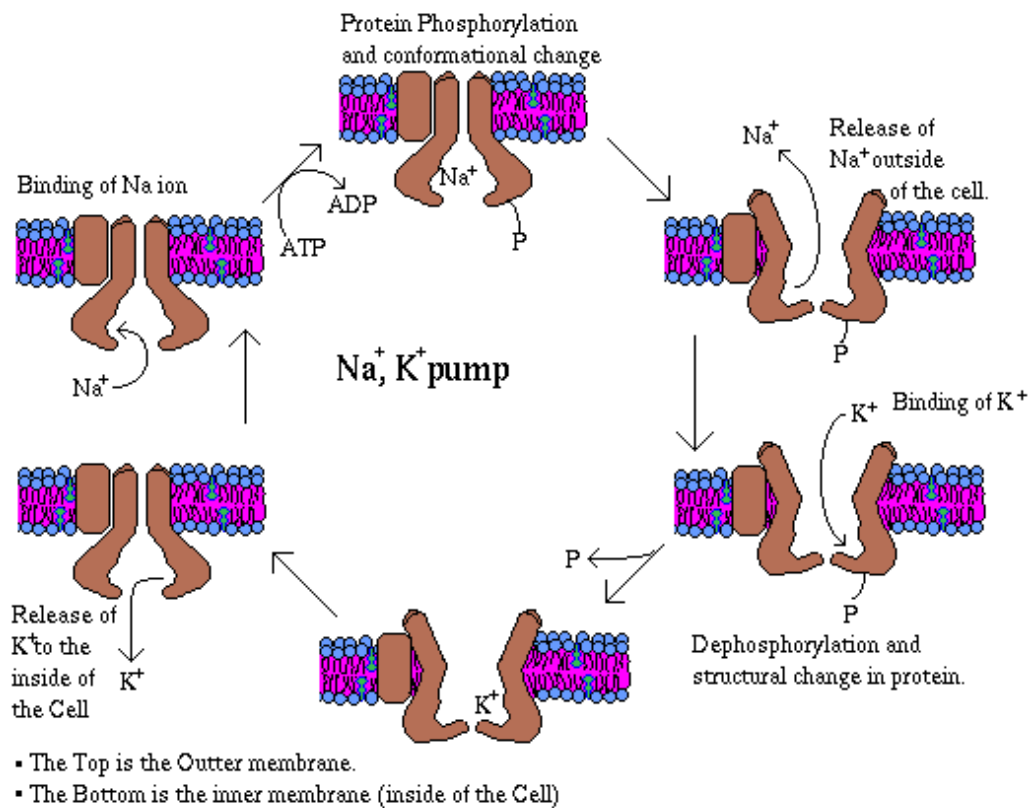


Figure 5.1 Functioning of the Na⁺/K⁺ ATPase pump (Dalton, 2014)

Diabetic control liver (Figure 4.23 A) showed red blood cells in sinusoids (congestion), and scant hepatocytes showing small suspect lipid vacuoles. Hepatocytes have granular, eosinophilic to fairly vacuolar cytoplasm (possible degenerative changes). Diabetic and non-diabetic treated groups (Figure 4.23. B - I) showed mild diffuse sinusoidal congestion. The hepatocytes were within normal limits or showed mild hydropic degeneration. The central veins appeared normal. No architectural disturbances, fibrosis or chronic lesions were seen. Gastrocnemius muscles of diabetic control, diabetic and non-diabetic treated groups, Figure 4. 23 J – R, appeared within normal histological limits. Mohammady *et al.* (2012), reported that the diabetic groups that were given the *M.charantia* extract regained normal appearance of the β cell after 4 weeks of treatment. Viridi *et al.* (2003), reported that oral feeding of *M.charantia* extract for a period of 30 days did not change the histology of the liver and kidney of diabetic and normal rats. Diabetic treated and non-diabetic groups of the liver and muscle of the present study showed similar results.

All the vegetables studied have the potential to provide essential nutrients to human beings. The aqueous extract of *Momordica charantia* (bitter gourd) lowered the glucose level of diabetic rats by 64.4% whereas the aqueous extract of *Abelmoschus esculentus* (okra) reduced the blood glucose of diabetic rats by 40.1% within the 15 day study period.

M.charantia and *A. esculentus* extracts lowered the entry of glucose into the intestine. Treatment with the extracts of *M.charantia* and *A.esculentus* normalized the liver glycogen content with a significant increase in the activity of HK and GK. It also inhibited Na⁺/K⁺ ATPase activity. Histopathological analysis of liver and gastrocnemius muscle tissue of rats given the extracts showed no toxicity; hence, *M.charantia* and *A.esculentus* are advisable for safe consumption, especially in individuals with diabetes.

CHAPTER 6

CONCLUSION

The prevention of diabetes and its potential complications is one of the major challenges of the future. In Ayurvedic medicine, hypoglycaemic plants have been used in natural forms. The inorganic part of a medicinal plant contains various mineral elements including Ca, Zn, K, Mn and Cr which contribute to the medicinal properties of the plant and are associated with the mechanism of insulin release (Kar *et al.*, 2003).

At present, the survey obtained from interviewed local informants, show that Indian mothers used to cook the above mentioned four (4) vegetables regularly. However in recent times, the use of these vegetables has fallen away. The changes in the traditional dietary habits may contribute to the higher frequency of diabetes, type 2, in the population. The vegetables listed in our study can be easily grown in home gardens, dried and frozen, to be made available throughout the year.

A study of two extracts from *Momordica charantia*, *Abelmoschus esculentus*, *Moringa oleifera* and *Solanum melongena* indicate that aqueous extracts of *Momordica charantia* and *Abelmoschus esculentus* at a dose of 300mg/kg body weight can reverse alloxan induced hyperglycemia in rats. Thus, vegetables such as *Momordica charantia* and *Abelmoschus esculentus* can be recommended to diabetic patients, for use on a long term basis, potentially curbing the onset of secondary complications.

The assessment of the antidiabetic and hypoglycaemic properties as well as the mechanism of action of the hypoglycaemic effect of the vegetables used in this study will bring significant evidence and information regarding the use of medicinal plants in the treatment and management of diabetes.

The re-inclusion of these vegetables on a regular basis in the daily diet, together with medication and exercise will improve general health and blood glucose control in diabetic patients. The extracts of the vegetables with proven hypoglycaemic properties will be kept in storage and will be available for future studies aimed at the identification of some active compounds.

Both the boiled and aqueous extract of *M.charantia* showed blood glucose lowering effects, but the aqueous extract showed the maximum efficacy. This study can be considered as the first comparison of the two extracts of *Momordica charantia* from South Africa. This study can be considered as the first composition comparison of *Abelmoschus esculentus* (okra), *Momordica charantia* (bitter gourd), *Moringa oleifera* (drumstick), and *Solanum melongena* (brinjal) of India and South Africa. Our results have indicated that the cultivation area is an important factor influencing a plant's nutrient content. Finally, it was found that the protein content of the South African varieties is significantly higher than those from India. The fruit of *Abelmoschus esculentus* (SA) has a higher Co and Mg content than *A.esculentus* (India). *Momordica charantia* (India) has higher amounts of Ca, Co and Mn than *M.charantia* (SA). *Solanum melongena* (SA) is an important source of Fe and Co compared to its similar counterpart from India. The fat, glucose and potassium content of the Indian variant of

M. oleifera (India), is significantly higher than *M. oleifera* (SA), whereas the protein, starch and Co content are significantly higher in the South African variety. All these vegetables have the potential to provide essential nutrients to human beings. Of note, *M. oleifera* of India and SA, contain superior amounts of starch, glucose, protein, fat and minerals compared to *Abelmoschus esculentus*, *Momordica charantia* and *Solanum melongena* and this may contribute to the nutritional requirements of man when the plant is used for curative purposes in certain diseases.

In conclusion, the present study calls attention to the use of *Momordica charantia* and *Abelmoschus esculentus* in diabetes mellitus. The results of the study revealed that *Momordica charantia* and *Abelmoschus esculentus* have numerous anti-diabetic properties such as decreasing serum glucose level, increasing serum insulin level and decreasing intestinal glucose absorption. *Momordica charantia* and *Abelmoschus esculentus* did not cause hypoglycemia when given to normal rats, so it is safe for consumption in non-diabetic individuals for its other potential beneficial effects. As *Abelmoschus esculentus* is an organic product, aqueous *A. esculentus* extract is not harmful to humans. Experiments in rats showed beneficial anti-diabetic activity.

Better glycemic control can prevent and slow down many of the complications associated with diabetes, thus improving the overall quality of a patient's life. Lifestyle modification is the key factor to achieving this control. Exercise, healthy dietary choices and compliance to treatment are the essentials for maintaining good glycemic control.

As part of necessary lifestyle changes, individuals with diabetes should be encouraged to include these medicinal plants in their daily diet in an attempt to obtain improved glycaemic control.

Suggestions for further studies

Further *in vivo* investigations, over an extended study interval, may be carried out to assess the effect of the different extracts of *Momordica charantia* (Bitter Gourd), *Abelmoschus esculentus* (Okra), *Moringa oleifera* (Drumstick), and *Solanum melongena* (Brinjal) on blood sugar, triglyceride and cholesterol levels over a longer term. Such studies may also identify longer term benefits or adverse effects that may have been overlooked in our study.

Since *M.charantia* and *Abelmoschus esculentus* have been shown to have the greatest anti-diabetic properties, a large human-based clinical study may be useful in confirming the efficacy of these plant samples in patients with diabetes.

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Appendix A

Details of preparation of some reagents

1. Tris buffer (pH 7.4) (final concentration per liter: 100mmol of NaCl, 20 mmol of KCl, 2.5mmol of MgCl₂, 0.5 mmol of EGTA, 50mmol of Tris-HCl (pH 7.4), 1 mmol of ATP, 1mmol of phosphoenolpyruvate, 0.16mmol of NADH, 5 kU of pyruvate kinase, 12kU of lactate dehydrogenase).
2. Homogenisation buffer (pH 7.4) for GK (final concentration per liter: 50mM Tris/HCl, 100 mM KCl, 10 mM DTT and 1mM EDTA.
3. Reaction Mixture for GK (final concentration per liter)
100mM Tris-HCl pH 7.4, 7.5 mM MgCl₂, 5 mM ATP, 1 mM NAD, 5.5 units of glucose-6-phosphate dehydrogenase (exogenous enzyme), liver extract 10 μ l, and the desired concentration of glucose.
4. Homogenisation buffer (pH 7.5) for HK (final concentration per liter: 50mM Tris/HCl, 2mM MgCl₂, 100 mM KCl, 30 mM DTT and 1mM EDTA.
5. Reaction Mixture for HK (final concentration per liter)
40mmol HEPES, 10mmol Creatine phosphate (2Na)
0.8 mmol EDTA, 0.9 international units (IU)/ml Creatine phosphokinase
7.5mmol MgCl₂, 0.7 IU/ml glucose 6 phosphate dehydrogenase
1.5 mmol KCl, 0.4 mmol β nicotinamide adenine dinucleotide
2.5mmol ATP(2Na) phosphate (NADP)
6. Potassium phosphate buffer pH 6.9

3.5g dipotassium phosphate (K_2HPO_4) made up to 100ml with distilled water. 2.7g potassium dihydrogen phosphate (KH_2PO_4) to 100ml with distilled water. Mix 55ml K_2HPO_4 and 45ml KH_2PO_4 . This buffer was stored at $4^{\circ}C$ until further use.

4. 30% KOH solution: 100% KOH = 35.29 g KOH/100 ml water 30% KOH = 30% above KOH solution + 70% water.

5. Anthrone solution: Dissolve 1g anthrone in 500ml of 72% H_2SO_4 (72ml conc. H_2SO_4 pour into 28ml water to make 100ml x 5 to make 500ml. So add 360 ml Conc. H_2SO_4 into 140 ml water.

6. Nessler's reagent: This was prepared by dissolving 5.5g of red mercuric iodide (HgI_2) and 4.125g of potassium iodide in 25ml of distilled water. To this mixture was added a cooled solution of 14.50g of sodium hydroxide in 50ml of distilled water. This was made up to 100ml and kept in a dark reagent bottle.

7. 10 % buffered formalin 1.75 g of sodium hydrogen orthophosphate ($Na_2HPO_4 \cdot 2H_2O$) and 3.25 g of di-sodium hydrogen orthophosphate (Na_2HPO_4) was dissolved in 25 ml of boiling water. 50 ml of 40 percent formalin was added and the resulting mixture was made up to 400ml with distilled water.

8. Preparation of Standard solutions of glucose and starch 0.1, 0.2,0.6mg of maize starch was dissolved in 20ml of perchloric acid each and shaken thoroughly after which anthrone reagent was added and used to plot the standard starch curve. 1,2,3 10 mg of glucose was dissolved in 50ml of 80% ethanol each and shaken vigorously. Anthrone was added and used to plot the standard glucose curve.

Appendix B

Details of Methodology

B.1. Proximate Analysis

B.1.1. Determination of Moisture Content

Moisture analysis was carried out using the drying oven method (AOAC, 2003). Two grams of sample was transferred to a previously dried and weighed crucible and placed in the oven thermostatically controlled at 105°C for 24 hours. It was then removed, placed in a dessicator to cool and then weighed. The difference between the final and initial weights was recorded as the moisture content and expressed as a % of the weight of the original sample.

$$\text{Moisture content (\% of dry weight)} = \frac{\text{loss in weight (g)} \times 100}{\text{original sample weight}}$$

B.1.2 Determination of Ash Content

Ash content was analysed as per the method outlined in the AOAC (2003). Two grams of sample was transferred into a previously dried and weighed crucible and ignited for 4 hours in a furnace pre-heated at 600°C. It was subsequently cooled and weighed. The recorded weight gave the ash content of the sample expressed as the percentage of the original weight.

$$\text{Total ash (\% dry weight)} = \frac{\text{weight of ash (g)} \times 100}{\text{original weight of sample}}$$

B.1.3. Determination of Protein Content

Total protein was determined by the Kjeldahl method (AOAC, 2003), involving digestion, distillation and titration. One gram of the sample was digested in a 30 ml Kjeldahl flask with 20 ml of concentrated sulphuric acid and two Kjeldahl tablets. The content was boiled in a Kjeldahl apparatus. The digestion was continued until the dark mixture became very clear and then continued for 30 extra minutes. The flask was allowed to cool and the content transferred into a 200 ml volumetric flask and diluted to the mark with distilled water. One millilitre was pipetted into a 50ml calibrated flask and diluted to 30 ml with distilled water. 4ml of Nessler's reagent was added and the mixture diluted to the mark with distilled water. The absorbance was read at 530 nm (Fig B.1 Standard curve for total protein). Such values were multiplied by 6.25 to obtain the total protein concentration.

The standard curve was prepared by dissolving 4.7162 g of $(\text{NH}_4)_2\text{SO}_4$ in 1 litre of distilled water. Aliquots containing 0.1-0.6 mg of nitrogen was diluted to approximately 30 ml. 4ml of Nessler's reagent was added, allowed to stand for about 20 minutes for colour development. The absorbance was read at 530 nm. A plot of absorbance versus concentration gave the standard nitrogen curve (Table.B.1)

Test tube No.	1	2	3	4	5	6	7
Ammonium sulphate	0.0	0.1	0.2	0.3	0.4	0.5	0.6
Nessler's reagent (ml)	4	4	4	4	4	4	4
Distilled water (ml)	50.0	49.9	49.8	49.7	49.6	49.5	49.4
Total (ml)	54	54	54	54	54	54	54

Table B.1 Protocol for the standard curve for Nitrogen

B.1.4. Determination of fat Content

Fat determination was determined using the soxhlet extraction method (AOAC, 2003). The dried sample for moisture determination (1g) was transferred to a 22 x 88mm paper thimble which was then plugged with cotton wool to avoid sample loss, and placed in the Soxhlet extractor. One hundred and eighty millilitres of n-hexane was poured into a previously dried and weighed round bottom flask, which was then attached to the bottom of the extractor. The heating mantle was switched on till the hexane reached boiling point and the heating was continued for another 16 hours. The flask and its contents were then removed and dried to a constant weight (under vacuum) in a rotary evaporator at 40⁰C. The flask was cooled and weighed. The amount of fat extracted was calculated from the difference between the weight of the flask and contents before and after extraction.

$$\text{Crude fat (\% dry weight)} = \frac{\text{weight of lipid (g)}}{\text{Original sample weight}} \times 100$$

B.1.5 Carbohydrate Analysis

Carbohydrate content was determined using the Hansen and Moller (1975) method. One gram of sample was quantitatively transferred into a burette previously stuffed with glass wool. The sample was then soaked with 2 ml of 80% ethanol and stirred to remove air bubbles in order to avoid channel formation. An air space was created by inserting a stopper of glass wool as shown in Fig 3.1. This was done to prevent carbohydrate diffusion into the solvent. Twenty five millilitres of 80% ethanol was used to percolate the soluble carbohydrates at a percolation rate of approximately 1.5ml/hr. After the ethanol percolation, the residue was thoroughly mixed with 2ml perchloric acid and again percolated with 25 ml of 35% perchloric acid.

From test solutions containing the starch or the glucose 2ml was pipetted into a pyrex glass tube and kept at 0°C. Ten millilitres of anthrone reagent which had been cooled to 0°C was added to the 2ml test solution. The reaction mixture was mixed thoroughly and heated for exactly 10 minutes at 100°C in a water bath. After this treatment, the tube was rapidly cooled to 0°C and the absorbance read at 630nm, against a distilled water blank after an hour. The amount of starch and soluble carbohydrate was then estimated from standard solutions (Appendix A. 8) prepared to give the standard graphs (Table B2 and B3).

Test tube No	1	2	3	4	5	6	7	8	9
Glucose solution (mg/50ml)	0.0	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Anthrone reagent (ml)	10	10	10	10	10	10	10	10	10

Table B.2 Protocol for standard glucose curve

Test tube No	1	2	3	4	5	6	7	8	9
Starch solution (mg/20ml)	0.0	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Anthrone reagent (ml)	10	10	10	10	10	10	10	10	10

Table B.3 Protocol for standard Starch curve

B.2 Mineral analysis:

All metals (Na, K, Fe, Mn, Mg, Cu, Ca, Co, Zn) were analysed by the Varian Inductively Coupled Plasma (ICP) method.

Instructions for sample preparation for Varian ICP- OES analysis.

Sample preparation

A 0.5 g of ashed sample was weighed into a pre-cleaned beaker. Concentrated nitric acid 20 ml was added, the beaker covered with a watch-glass and the sample boiled gently on a hot-plate until digestion was complete. This process took approximately 3 h. The digested sample was then allowed to cool before being transferred quantitatively into a clean 100 ml volumetric flask and made up to the mark with de-ionized water. The

samples were filtered thoroughly and only the clear aqueous solutions were used for mineral analysis. All assays were carried out in triplicate.

Instrument run/Preparation of standards

Table B.4 Preparation of working standard solutions

Standard Concentration, ppm	Final Volume of Standard solution, ml	Volume of 100ppm pipetted, ml
0.50	500	2.5
1.00	500	5
2.00	500	10
5.00	500	25
10.00	500	50
25.00	500	125

A 10% nitric acid blank solution was prepared and used for the analysis.

A certified Merck-ICP Multi Element Standard Solution IV was purchased and used for the quantification of metals in the samples. Fifty millilitres of a certified Merck-ICP Multi Element Standard Solution IV was pipetted out into a 500 ml volumetric flask and made up to the mark.

Table B.5 Parameters

Instrument Make and Model	Varian ICP-OES 735
Power, kW	1.00
Plasma flow, L/min	15.0
Auxiliary gas flow, L/min	1.5
Nebulizer pressure, kPa	200
Replicates & read time (seconds)	3 & 5
Sample Uptake delay, seconds	15
Rinse time, seconds	15
Calibration mode	Quantitative

B.3 To determine the hypoglycaemic mechanism of the plant extracts in diabetic rats

B.3.1 Effect of the extract on intestinal glucose absorption

Effect of the extract on intestinal glucose absorption was determined using the inverted intestinal sac model (Said et al., 2008). A stainless steel rod (350mm, 1.5mm) was used to push into the ileal end of the small intestine until it reached the duodenal end. The inverted intestine was then slipped off the steel rod and placed in saline in a dish. One end of the inverted intestine was tied (Mahomoodally et al., 2007) and 1ml of buffer (Potassium phosphate, pH 6.9) was placed in the inverted intestocrofttinal segment, which was then immersed into a beaker containing 1ml of 1% Pancreatin, 7ml of 1% starch and plant extract (0.075mg/ml). In the control, the extract was replaced with the buffer. The sacs were incubated at 37⁰C for 3 hours. After 3 hours, glucose obtained from both outside and inside the sac was taken to represent starch digestion and glucose absorption (Said et al., 2008). Glucose determination was done using the Glucose (GO) Assay kit.

B.3.2 The effect of extract on Na/K ATPase

The rat's small intestines were homogenised in 0.1 M sodium phosphate buffer pH7.0. The homogenate was centrifuged at 1200 rpm for 15 min. The supernatant was collected and kept at -20°C until use. The reaction mixture containing 20 µl of sample, 20 µl of homogenate was added to 380 µl of reagent 1 [final concentration per litre: 100 mM NaCl, 20 mM KCl, 2.5 mM MgCl₂, 0.5 mM EGTA, 50 mM tris-HCl (pH 7.4), 1 mM ATP, 1 mM phosphoenolpyruvate, 0.16 mM NADH, 5 kU pyruvate kinase and 12 kU of lactate

dehydrogenase; all from Sigma] and mixed well. The mixture was incubated at 37°C for 5 min after which 5 µl of 10 mM ouabain (Reagent 2) was added to inhibit ouabain-sensitive ATPase activity. Absorbance was read at 340 nm, Na⁺/K⁺ ATPase activity was calculated from the difference in absorbance (final – initial) at 340 nm.

Percentage inhibition = $\frac{\text{final} - \text{initial}}{\text{final}} \times 100$

B.3.3 Assessment of the glycogen content

One millilitre of solution was put into a test tube and kept at 0°C. Five millilitres of anthrone reagent was cooled to 0°C and then added to the 1ml solution in the test tube. The reaction mixture was shaken thoroughly and heated for 10 minutes at 100°C in a water bath. The tube was rapidly cooled to 0°C and the absorbance read at 625 nm against a distilled water blank within an hour. Glycogen content was estimated from standard glycogen treated with the anthrone reagent.

Preparation of Standard glucose:

Stock- Dissolve 100 mg glucose in 100ml water.

Working standard: 10ml of stock diluted to 100 ml with distilled water.

Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard.

'0' serves as blank. Make up the volume to 1ml in all the tubes including the sample tubes by adding distilled water. Cool the contents of the tubes on ice before adding ice cold anthrone. Then add 4ml of anthrone reagent. Heat for eight minutes in a boiling water bath. Cool rapidly and read the green to dark green colour at 630 nm.

Draw a standard graph by plotting concentration of the standard on the X- axis versus

absorbance on the Y- axis. From the graph calculate the amount of carbohydrate present in the sample tube.

Calculation

$$\text{Amount of carbohydrate present in 100mg of the sample} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

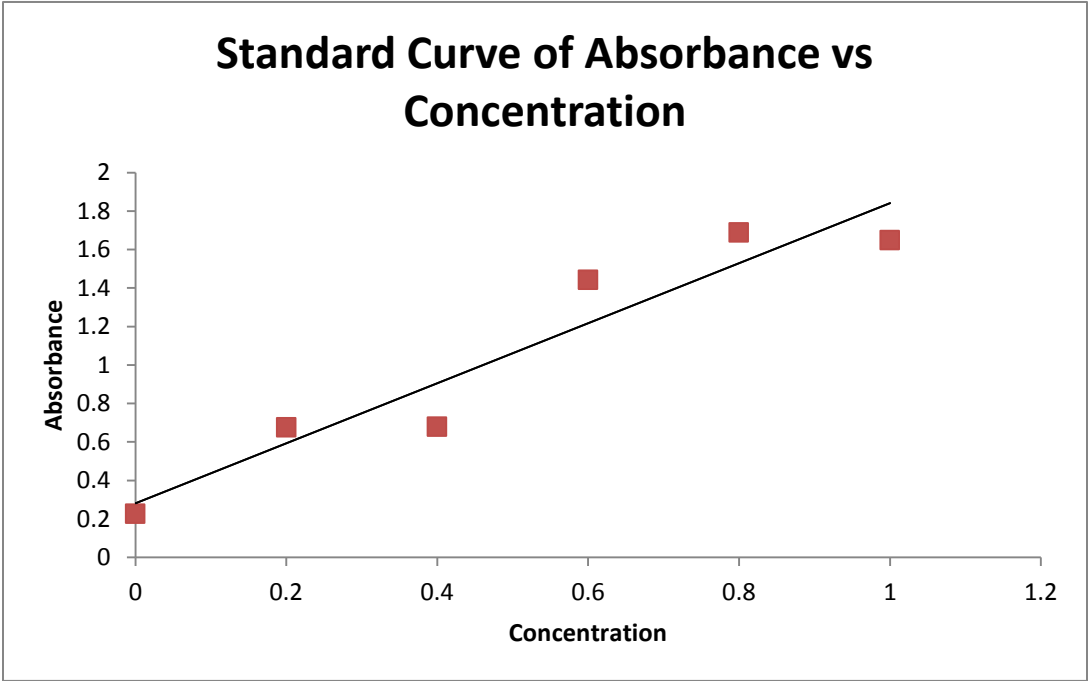


Figure B.1: Standard graph calculated the amount of carbohydrate

B.3.4 Glucokinase Activity in Liver

The reaction mixture consisted of 100mM Tris-HCl pH 7.4, 7.5 mM MgCl₂, 5 mM ATP, 1 mM NAD, 5.5 units of glucose-6-phosphate dehydrogenase (exogenous enzyme) (Newgard et al.,1983).

To 1ml of reaction mixture in a cuvette, 10µl of liver homogenate was added. The reaction was initiated by adding 0.1ml of 10mmol D-glucose (1.0 mmol final concentration), and the change in absorbance was recorded at a wavelength of 340nm for 3 minutes at 30 seconds interval.

The production of NADH was followed at 340 nm. Appropriate controls lacking glucose, glucose-6phosphate dehydrogenase, or both were included for each extract studied (Newgard et al., 1983).

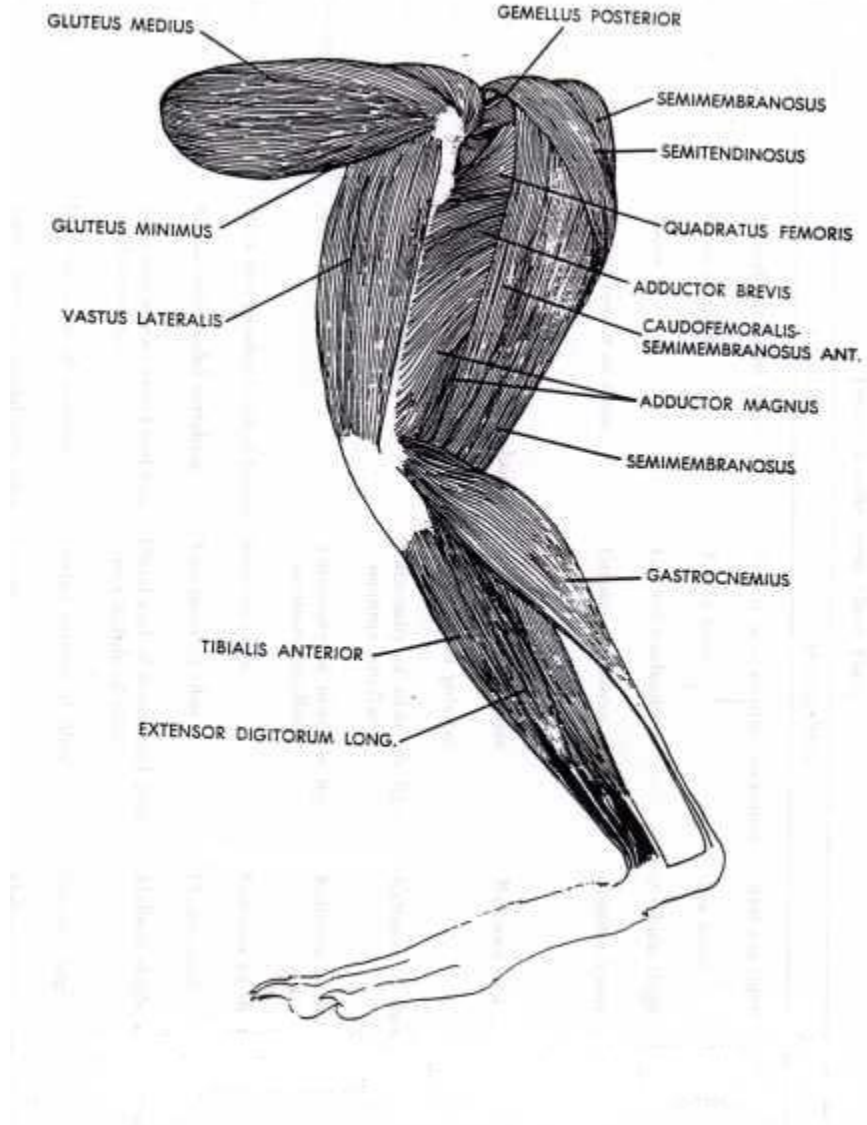
B.3.5 Hexokinase Activity in Muscle

Weighed amounts of muscle were homogenized in 10 volumes of buffer: 50 mM Tris/HCl (pH7.5), 2 mM MgCl₂, 1mM EDTA, 30 mM DTT (Dithiothreitol). This was centrifuged at 20,000g for 15mins at 4⁰C and the supernatants collected in 2 ml micro tubes and stored in at -30⁰C for for the enzyme assay (Lwueke and Nwodo, 2008 and Ugochukwu and Babady, 2003).

The reaction mixture consisted of 40mmol HEPES, 0.8 mmol EDTA, 7.5mmol MgCl₂,1.5 mmol KCl, 2.5mmol ATP (2Na),10mmol Creatine phosphate (2Na), 0.9 international units (IU)/ml Creatine phosphokinase (from rabbit muscle), 0.7 IU/ml glucose 6 phosphate

dehydrogenase and 0.4 mmol β nicotinamide adenine dinucleotide phosphate (NADP) (pH-7.4). Into a cuvette containing 1ml of reaction mixture was added 20 μ l of homogenate. The reaction was initiated by adding 0.1 ml of 10 mmol D-glucose (1.0 mmol final concentration), and the change in absorbance was recorded at a wavelength of 340 nm for 3 minutes at 30 seconds interval.

Figure B.2 Gastrocnemius muscle of rat (google picture, retrieved 20.01.2012)



Appendix C

Ethic Clearance

UNIVERSITY RESEARCH ETHICS COMMITTEE
(Reg No: UZREC 171110-30)



UNIVERSITY OF ZULULAND
Website: <http://www.uzulu.ac.za>

Private Bag X1001
KwaDlangezwa 3886

Tel: 035 902 6645
Fax: 035 902 6222
Email: dviljoen@pan.uzulu.ac.za

ETHICAL CLEARANCE CERTIFICATE

Certificate Number	UZREC 171110-030 PGD 2012/14					
Project Title	The antidiabetic properties of four plants grown in India and KwaZulu-Natal, South Africa suitable for diabetic management.					
Principal Researcher/ Investigator	R Georgekutty					
Supervisor and Co-supervisor	Prof. Opoku					
Department	Biochemistry & Microbiology					
Nature of Project	Honours/4 th Year	Master's	Doctoral	x	Departmental	

The University of Zululand's Research Ethics Committee (UZREC) hereby gives **PROVISIONAL** ethical approval in respect of the undertakings contained in the above-mentioned project proposal and the documents listed on page 2 of this Certificate. Special conditions, if any, are also listed on page 2.

The Researcher may therefore commence with the research as from the date of this Certificate, using the reference number indicated above, but may not conduct any data collection using research instruments that are yet to be approved.

Please note that the UZREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the documents that were presented to the UZREC
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research

The Principal Researcher must report to the UZREC in the prescribe format, where applicable, annually and at the end of the project, in respect of ethical compliance.

The table below indicates which documents the UZREC considered in granting this Certificate and which documents, if any, still require ethical clearance. (Please note that this is not a closed list and should new instruments be developed, these may also require approval.)

Documents	Considered	To be submitted	Not required
Faculty Research Ethics Committee recommendation	X		
Animal Research Ethics Committee recommendation			X
Health Research Ethics Committee recommendation			X
Ethical clearance application form	X		
Project registration proposal	X		
Informed consent from participants	X		
Informed consent from parent/guardian	X		
Permission for access to sites/information/participants	X		
Permission to use documents/copyright clearance			X
Data collection/survey instrument/questionnaire	X		
Data collection instrument in appropriate language		Only if necessary	
Other data collection instruments		Only if used	

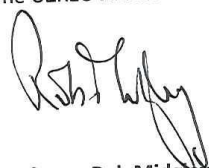
Special conditions: Documents marked "To be submitted" must be presented for ethical clearance before any data collection can commence.

a) An opinion from a veterinarian endorsing the research methodology must be obtained.

The UZREC retains the right to

- Withdraw or amend this Certificate if
 - Any unethical principles or practices are revealed or suspected
 - Relevant information has been withheld or misrepresented
 - Regulatory changes of whatsoever nature so require
 - The conditions contained in this Certificate have not been adhered to
- Request access to any information or data at any time during the course or after completion of the project

The UZREC wishes the researcher well in conducting the research.



Professor Rob Midgley
 Deputy Vice-Chancellor, Research and Innovation
 Chairperson: University Research Ethics Committee
 20 February 2013

Appendix D

Research Output

International Conference

ANTIDIABETIC PROPERTIES OF FREEZE-DRIED OVERNIGHT AQUEOUS & BOILED EXTRACTS OF *Momordica charantia* ON NORMAL & ALLOXAN INDUCED DIABETIC RATS.

Raymol Georgekutty* and Andy Opoku

Department of Biochemistry, University of Zululand, Private Bag x 1001, Kwa-Dlangezwa, 3886, SOUTH AFRICA *Correspondence, email:

GeorgekuttyR@Unizulu.ac.za

INTRODUCTION

Diabetes Mellitus is a metabolic disorder. Pancreas does not produce enough insulin or the insulin produced is ineffective. Insulin is required for the metabolism of carbohydrates, proteins and fats. These compounds are broken down into glucose. Insulin allows glucose entry into cells in order to produce energy.

Type 1 diabetes mellitus (IDDM)/Juvenile onset diabetes. Pancreas: little/no insulin, develops in children, auto immune disease, insulin injections daily. **Type 2 diabetes mellitus** (NIDDM) Adult onset diabetes. Deficiency in insulin / insulin resistance occurs after 45 yrs. (90-95% of diabetes). Medication, diet and exercise. **Gestational diabetes:** glucose intolerance, pregnancy. Resolves after pregnancy, can develop type 2 later.

	<p><i>M.charantia</i></p>	<p>Objective To investigate the hypoglycaemic effect of freeze-dried overnight aqueous extract and boiled extract of (<i>Momordica charantia</i> L) on normal and alloxan- induced diabetic rats. To establish which extract would be the most effective one for the management of diabetes mellitus.</p>	
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Genetic & environmental factors contribute to the onset of the disease.

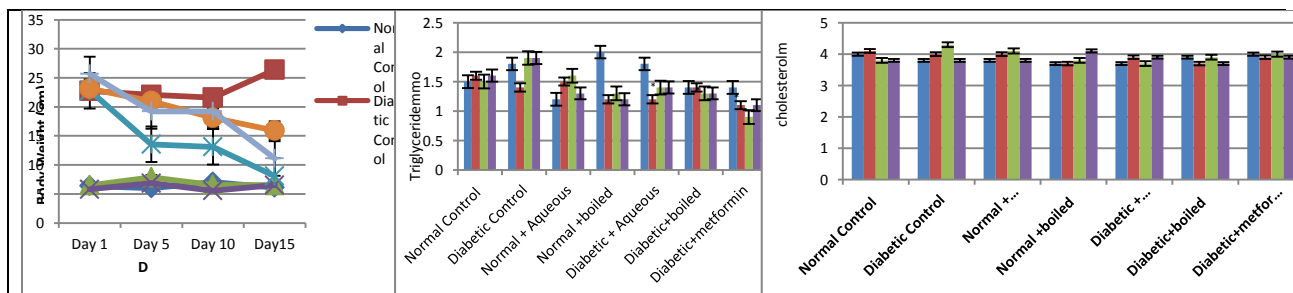
Complications of diabetes Eye disease (retinopathy), Kidney disease (nephropathy), Nervous system damage, Pregnancy complication, Slow healing sores in leg

Materials & Methods

The seeds of *M.charantia* were collected locally and the plants were cultivated.

Preparation of freeze-dried bitter melon juice. Unripe fresh fruit was washed thoroughly with water, cut into small pieces. An electric blender was used to extract the juice from the fruit with water (1:1w/v). The extracted juice was frozen and underwent freeze drying process for 72hrs & the powder was kept in airtight containers at -70°C (Mohammady et al.,2012)

Results Diabetic treatment groups produced significant reduction in the blood glucose level at the end of 2 week of treatment with maximum reduction was achieved in diabetic aqueous extract given groups than the rest. Boiled extract was less effective than overnight aqueous extract and metformin in lowering blood glucose.



Triglyceride level of diabetic treated groups on the 15th day was significantly lower than the diabetic control group of the last day. There is no significant difference in the triglyceride levels on the 15th day of normal control and normal experimental groups. Cholesterol levels of the study groups ranged from 3.7 to 4.3 mmol/L respectively. There is no significant difference in cholesterol levels among the study groups. Body weight The oral feeding of extracts resulted in an increase in body weight. Diabetic animals fed with the aqueous extract showed less weight gain than the corresponding controls. A steady increase in the body weight was observed which was significant in all the groups except in day 5 and day 10 of group 5.

Conclusion Both extract did lower blood glucose but aqueous extract showed the maximum efficacy. This study can be considered as the first comparison of the two extracts of *Momordica charantia* from South Africa. This hypoglycaemic traditional medicinal plant has less side effects, low cost and also play a role in the cure and prevention of diabetes. Individuals with diabetes should be encouraged to include this vegetable in their daily diet to assist with blood sugar control.

Acknowledgement This study was financially assisted by the research committee of the University of Zululand, South Africa

*Fourth Euro-India
International Conference on*
HOLISTIC MEDICINE
ICHM 2014

23 - 26 October 2014

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International and Inter University Centre for Nanoscience and Nanotechnology (IUCNN)

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Dr. A. George

Dr. A. George
Convener

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Arun George

Dr. A. George
Convener





(Venda Conference)

The Proximate Composition of two vegetables from India and South Africa

BY: RAYMOL GEORGE

SUPERVISOR: PROF A. R. OPOKU

Department of Biochemistry, University of Zululand, KwaZulu-Natal, South Africa

Introduction: The aim of this article is to evaluate and compare the nutritional content of <i>Abelmoschus esculentus</i> (L). Moench and <i>Solanum melongena</i> (L) commonly used in India and to compare them with the South African varieties. In SA, these vegetables are not commonly used among the general population. The objective of this study was to compare the South African varieties with the Indian varieties and to analyze the proximate composition, and to determine the variety that had the higher nutritional content. All these plants have beneficial properties with respect to long term control of diabetics and other diseases.			
<i>Abelmoschus esculentus</i> Family: Malvaceae Annual herb 3-7 feet high. The edible tender fruit is a capsule.		<i>Solanum melongena</i> Family: Solanaceae Annual plant. The edible fruit is a fleshy berry, varying in size, shape and colour.	
(India)	(SA)	(India)	(SA)
			

Method: The seeds of *Abelmoschus esculentus* (Okra) and *Solanum melongena* (Brinjal) were collected from India and from Empangeni, KZN, South Africa. The plants were cultivated from the collected seeds in the home garden of the researcher in Empangeni, KwaZulu-Natal.

Proximate Analysis

It was carried out according to the procedure of Association of Official Analytical Chemist (AOAC, 1990). The moisture and ash were determined using weight difference method. Total protein was determined by the Kjeldahl method, involving digestion, distillation and titration. Crude protein was estimated by multiplying the sample percentage nitrogen content by a protein conversion factor 6.25. Crude fat determination involved using the soxhlet extraction method. Carbohydrate content was determined by the Hansen and Moller (1975) method.

Results: The species evaluated in the present study showed variations in the results. *Abelmoschus esculentus* & *Solanum melongena* from South Africa has got a significantly higher fat and protein content than those from India. Glucose content was higher in *Abelmoschus esculentus* of India, whereas in *Solanum melongena* it was higher in the SA variety. The South

African variety of *Solanum melongena* had significantly higher starch content than those from India whereas in *Abelmoschus esculentus* the starch content was higher in the Indian variety.

Table 3: Proximate analysis of *A. esculentus* & *S. melongena* of India and South Africa (n=3)

Plant Species	Country	Moisture%	Ash%	Fat%	Protein %	Glucose %	Starch %
<i>A. esculentus</i>	India	90.02 ± 0.22a	7.20 ± 0.50 a	2.92 ± 0.15 b	1.14 ± 0.09 b	6.80 ± 0.35 a	9.17 ± 0.49 a
	SA	90.20 ± 1.16a	6.47 ± 1.00 a	4.67 ± 0.23 a	1.64 ± 0.14 a	4.20 ± 0.06b	5.62 ± 0.22 b
<i>S. melongena</i>	India	91.80 ± 0.78a	8.47 ± 0.44 a	2.84 ± 0.98 b	1.21 ± 0.05 b	6.00 ± 0.29b	7.60 ± 0.23 b
	SA	92.37 ± 0.44a	6.68 ± 0.53 a	7.87 ± 1.18 a	2.50 ± 0.25 a	6.97 ± 0.15 a	14.40 ± 0.81 a

Values are mean ± standard error. Values with different letters within a column differ significantly (P < 0.05).

Conclusion: This study can be considered as the first composition comparison of *Abelmoschus esculentus* and *Solanum melongena* of India and South Africa. The variation in the nutritional values will differ for a wide range of reasons, such as the region in which it is cultivated, growing conditions, the nature of the soil, seasonal changes, genetically different cultivars & the period of analysis (Jongrungruangchok et al 2010, Ogbé & John 2011/12, Morabad et al 2012). Of note, the South African variety of *Solanum melongena* had significantly higher fat, protein, glucose and starch content than those from India.

Acknowledgement

This study was financially assisted by the research committee of the University of Zululand, South Africa.

Hansen, J; Moller, I (1975) Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. *Analytical Biochemistry*, 68:87-94