KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

ANTI-DIABETIC EFFECT OF AQUEOUS RIPE FRUIT EXTRACT OF *BORASSUS AETHIOPUM* MART. (FAMILY: ARECAEAE) IN ALLOXAN-INDUCED DIABETIC RATS

BY

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MARCH, 2016

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A THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY & BIOTECHNOLOGY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A DEGREE OF MASTER OF PHILOSOPHY IN HUMAN NUTRITION AND DIETETICS

JEREMIAH ISSAKA (BSc)

MARCH, 2016

DECLARATION

I, Jeremiah Issaka, hereby declare that the experimental work described in this thesis is my own work towards the award of an MPhil degree, and that, to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any other degree of this university or elsewhere, except where due acknowledgements have been made in the text.

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ABSTRACT

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels and is among the top ten causes of death in the world. *Borassus aethiopum* Mart. (family Arecaceae) is a plant species of Borassus palm found widely across Africa. It serves an important source of food, providing edible fruits, and nuts, and also has a number of pharmacological uses that have been reported in some parts of the world. This study explored the phytochemical constituents and antidiabetic properties of ripe fruit extract of *B. aethiopum* in alloxanized experimental rats for 7 and 28 days. Normoglycaemic and alloxan-induced diabetic rats were treated with fruit extract of *borassus* (FEB) at doses of 100, 250 and 500 mg/kg body weight. Body weight, relative organ weight, haematological and biochemical parameters were measured in both acute and sub chronic studies. The preliminary phytochemical screening showed the presence of tannins, saponins, glycosides, triterpenoids and alkaloids. Fasting blood glucose was reduced significantly ($p<0.05$) in diabetic rats in acute study at a dose of 500 mg/kg body weight and at 250 and 500 mg/kg body weight in sub chronic studies. White blood cell count (WBC) and platelets (PLT) levels were significantly increased after treatment with 500 mg/kg body weight. Urea and Alanine Transaminase (ALT) levels also reduced significantly in both acute and sub chronic studies. The experiment supports the traditional use of *B. athiopum* as a medicinal plant in the treatment of diabetes mellitus.

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LIST OF ABREVIATIONS

2hrG: 2-Hour Glucose

ADA: American Diabetes Association

ALT: Alanine Aminotransferase

AOW: Absolute Organ Weight

CDH: Central Drug House

CR: Coronary Risk

D: Day

DCCT: Diabetes Control and Complications Trial

DM: Diabetes Mellitus

DPP: Dipeptidyl Peptidase

EDTA: Ethylenediaminetetraacetic acid

FBG: Fasting Blood Glucose

FEB: Fruit Extract of Borassus

GAD: Glutamic Acid Decarboxylase

GDM: Gestational Diabetes

Glib: Glibenclamide

Hb1Ac: Glycosylated Haemoglobin

HCT: Haematocrit

HDL: High Density Lipoprotein

HGB: Haemoglobin

IDF: International Diabetes Federation

LDL: Low Density Lipoprotein

LYM: Lymphocytes

NDIC: National Diabetes Clearinghouse

PLT: Platelets

RBC: Red Blood Cells

ROW: Relative Organ Weight

SEM: Standard Error of Mean

SSTs: Serum Separator Tubes

TC: Total Cholesterol

TG: Triglycerides

VLDL: Very Low Density Lipoprotein

WBC: White Blood Cells

WHO: World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Diabetes Mellitus (DM) is a group of metabolic disorders that results from inadequate insulin action leading to a prolonged increase in plasma glucose levels (Kaku, 2010). It includes several conditions that show symptoms of high plasma glucose levels and presents patients with the likelihood of developing cardiovascular diseases, kidney problems, weakness and numbness among others (Thévenod, 2008).

The World Health Organization (WHO) defines DM based on blood glucose levels as a level of more than 140 mg/dl (7.8 mmol/l) under fasting conditions, or a level of more than 200 mg/dl (11.1 mmol/l) two hours after a person has eaten a carbohydrate food or an equivalent of 75 g glucose (WHO, 1994)

Based upon the aetiology, DM can be grouped into two major types: type 1 DM and type 2 DM. Type 1 DM usually develops during infancy and is primarily caused by a destruction of the insulin secreting β-cells of the pancreas. This destruction is autoimmune mediated and result in a complete lack of insulin in the body. People with type 1 therefore rely on insulin injection to control their blood glucose as well as prevent the development of ketone bodies. Type 2 DM usually develops in adults and is mainly caused by the body's resistance to insulin or the lack of proper secretion of insulin (Thévenod, 2008). People with DM may experience signs such as polyuria, polydipsia, polyphagia, poor eye sight, poor wound healing and recurrent infections (International Diabetes Federation, 2014).

According to the WHO, diabetes management involve three main parts; dietary regulation together with physical activity, oral hypoglycaemic agents and insulin injection **(**WHO, 1994). The inadequacies in the recent modes of diabetes management coupled with the high cost of drugs and the loss of confidence in the orthodox medical management of diabetes have given rise to a fast growing regard for food supplements and plants. The use of plants by people have increased more than thrice in the past decade (Eisenberg *et al*., 1998) but there has not been enough proof to back the several plants used to manage diabetes (Lo *et al*., 2004). It is reported that ancient generations of Africans, Americans, Asians and Europeans practice herbal medicine and that, the medicinal benefit derived from herbal products have been enjoyed by almost all cultures on earth (Wargovich *et al.,* 2001). Medicinal plants are abundant in Ghana and in most instances it serves as the only option available for treatment for some conditions. Approximately one thousand (1,000) medicinal plants exist in Ghana and eighty percent have been documented by base line surveys (Cultural News, 2007). About 80% of the population in Ghana depend on herbal products for their primary health care (WHO, 2008). It is estimated that more than 60% of people in Ghana patronise alternative and complementary medicine, either because it is less expensive, more suitable for use or trusted to be more effective (Voice of America News Bulletin, 2006).

Borassus aethiopum is a plant species of Borassus palm found in Africa. It is often called the African palmyra palm, the African fan palm, among others. *B. aethiopum* is an important food source providing edible fruits, and nuts. The sap obtained from the inflorescence is drunk raw or processed into wine, alcohol or vinegar and also dried into sugar cakes (Hayne and McLaughlin, 2000)

In Ghana and other West African countries such as Ivory Coast, the ripe mature fruits are either boiled or used raw, the mesocarp is mashed, and the thick liquid obtained eaten with or without boiled maize as food **(**Agbo and Simard, 1992**)**. The flowers of *B. aethiopum* are also used to treat conditions such as impetigo and the roots for asthma. Other non-medical uses of the plant have been reported where the leaves are used in the mat and basketry industry, and the trunk used for building and construction of bridges because of its tough and termite –resistant nature (Mshana, *et al*., 2000)

A systematic review of literature showed a knowledge gap in terms of the therapeutic use of *B. aethiopum* especially its hypoglycaemic effects. Amoateng *et al.* (2010) reported that the ripe fruit of *B. aethiopum* has antioxidant and free radical scavenging properties and thus its consumption could therefore be beneficial in preventing diseases that are caused by oxidative stress. An analysis of the chemical constituents of the seed of *B. aethiopum* showed that it contained saponins, flavonoids, reducing compounds, terpenoids, alkaloids and free anthraquinones. Phlobotannin, carotenoids, anthracene and glycosides were however absent (Aguzue *et al.*, 2012). A phytochemical analysis of an ethanolic extract of the fruit of *B. aethiopum* showed that saponins, tannins, alkaloids, triterpenoids and sterols were present. It was also found to possess antioxidant, anti-microbial and antiinflammatory properties (Sarkodie *et al*., 2015). However no research has been done so far on the efficacy of *B. aethiopum* and its effect on blood glucose either in animals or in human subjects. There was therefore the need for a *pre-clinical* trial to test the efficacy of *B. aethiopum* as an anti-diabetic substance. The aim of this research, therefore, was to investigate the anti-diabetic effect of *B. aethiopum* in experimentally induced diabetic rats.

1.2 PROBLEM STATEMENT

According to the International Diabetes Federation (2014), the global diabetes prevalence is 387 million people, representing 8.3%. The prevalence rate for Africa stands at 4.3% (15 million) with Africa recording the highest mortality rate due to diabetes. The prevalence rate for Ghana stands at 3.3% (IDF, 2014). Diabetes is among the top ten causes of mortality in the world; it accounts for about 4.9 million deaths per year in the world, with about 50% of the deaths occurring in persons under 60 years of age. About 612 billion dollars is spent on diabetes worldwide representing 11% of the health expenditure worldwide (IDF, 2014).

The high prevalence rate of DM is a worry because DM is the main cause of kidney failure, poor eye sight and blindness. People suffering from DM may require double, or even thrice the health care resources of those without DM. It is also said that an estimated 15% of a nation's health care budget may be allocated to the management of DM (WHO, 2011).

The orthodox medical way of managing DM with oral hypoglycaemic agents and insulin injection lacks adequacy and compliance, and is also costly. This exposes the patient to a high risk of long-term complications. Some medicinal plants have been proven to be very effective and safe in the management of diabetes (Okeke, 1998). Though some animal studies have proven the anti-diabetic effects of other plants such as *Borassus flabellifer* (Pradeep *et al*., 2014) which is of the same family (Arecaceae) with *B. aethiopum,* there is no study done that really shows the efficacy of *B. aethiopum* as an anti-diabetic plant. Therefore, there was the need for more extensive, appropriate and well-designed *pre-clinical* trial to investigate the effect that *B. aethiopum* has on blood glucose in experimentally induced diabetic rats.

1.3 AIM OF THE STUDY

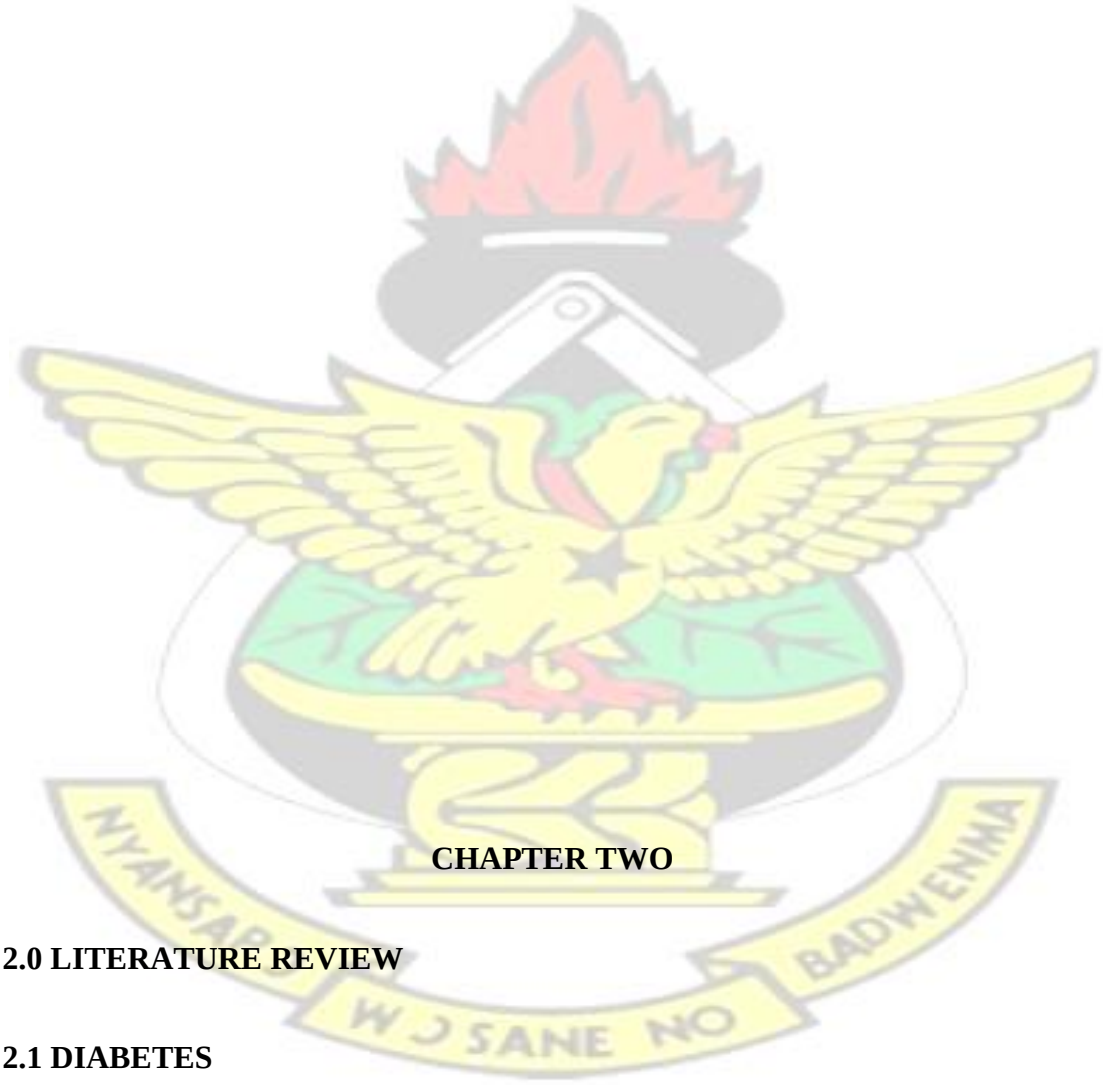
To investigate the anti-diabetic effect of aqueous ripe fruit extract of *B. aethiopum* in experimentally-induced diabetic rats.

1.3.1 Specific Objectives

a. To carry out a preliminary phytochemical analysis of the aqueous ripe fruit extract of *B. aethiopum*.
b. To determine the effects of aqueous ripe fruit extract of *B. aethiopum* on fasting blood glucose in experimentally-induced diabetic and non-diabetic rats.
c. To determine the effects of aqueous ripe fruit extract of *B. aethiopum* on the lipid profile, liver and renal functions of experimentally-induced diabetic and
d. To determine the effects aqueous ripe fruit extract of *B. aethiopum* on the full blood count of experimentally-induced diabetic and non-diabetic rats.

1.4 JUSTIFICATION

This research will provide sound scientific evidence, based on *pre-clinical* trial, needed to close the knowledge gap in the therapeutic use of *B. aethiopum*. The research will add to literature and will contribute to the knowledge base of the use of *B. aethiopum* as an anti-diabetic plant. It will also provide an alternative to the conventional medical approach of using insulin and oral hypoglycaemic agents in the management of diabetes.



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DIABETES

Diabetes mellitus (DM) is a group of metabolic disorders caused by insufficient secretion of insulin, insufficient action of insulin or both with a main feature of sustained high plasma glucose. The prolonged high blood glucose experienced in DM

is linked with long-term damages to vital organs such as the kidney, the eye, heart, and blood vessels (American Diabetes Association, 2004**).** Symptoms of DM include polyuria, polydipsia, and polyphagia (WHO, 2013). Short-term complications of DM include diabetic ketoacidosis and non-ketotic hyperosmolar coma (Kitabchi *et al.,* 2009). Foot ulcers, chronic renal failure, destruction to the eye, stroke and cardiovascular diseases are some of the long-term complications of DM (WHO, 2013

Diabetes is caused by the lack of adequate production of insulin by the pancreas or the resistance by body cells to the insulin (Shoback and Gardner, 2011**)**. There are many pathogenic processes that lead to one developing DM. Some are caused by deficiency in insulin resulting from the destruction of pancreatic β–cells by autoimmune processes. Others are due to insulin resistance which can lead to the abnormal metabolism of carbohydrate, protein and fat. The insufficient action of insulin experienced in DM is as a result of an insufficiency in the secretion of insulin or the lack of adequate response to insulin by tissues, or both (ADA, 2004)

2.2 CLASSIFICATION AND AETIOLOGY OF DM

DM is grouped into four main classes: type 1, type 2, gestational, and other specific types. The other specific types are a group of a few individual causes (Shoback and Gardner, 2011).

2.2.1 TYPE 1 DM

The main feature of type 1 DM is the loss of the insulin secreting pancreatic β- cells resulting in the deficiency of insulin. Type 1 DM comprises of two main types; immune mediated type 1 DM and idiopathic. Immune mediated type 1 DM, which

makes up majority of the cases occurs when an autoimmune attack that cause the loss of the β- cells of the pancreas is mediated by a T- cell. This results in the lack of insulin production (Rother, 2007). The presence of autoantibodies of the islet cells, autoantibodies to insulin, glutamic acid decarboxylase (GAD), and that of the tyrosine phosphatases IA-2 and IA-2β are key indicators of immune destruction of β- cells. These autoantibodies are usually found in about 85 – 90% of people who present with high fasting blood glucose (ADA, 2004). In type 1 DM, several genetic as well as poorly defined environmental factors contribute to the autoimmune destruction of the beta cells. Obesity is not common among type 1 DM patients even though type 1 DM can occur in obese individuals. People with type 1 DM are more likely to suffer other autoimmune disorders including Addison's disease, hepatitis, vitiligo, celiac sprue among others (ADA, 2004).

Another form of type 1 DM is often described as idiopathic and usually has no recognised aetiology. Patients, who suffer from this type show no proof of autoimmunity, experience a chronic lack of insulin and are likely to develop ketone bodies (ADA, 2004).

2.2.2 Type 2 DM

Type 2 DM usually develops in adults and is mainly caused by the body's resistance to insulin or the lack of proper secretion of insulin (Thévenod, 2008). It accounts for about 90 – 95% of patients with DM, and patients do not usually require insulin injection for survival (ADA, 2004). Type 2 DM often starts with insulin resistance, a state where the insulin present in the body fails to exert enough action on blood glucose primarily due to overweight and lack of exercise (WHO, 2013).

Lifestyle and genetic factors account primarily for type 2 DM (Risérus *et al.*, 2009). Some factors such as obesity (BMI > 30), physical inactivity, poor diet, stress and urbanization are central to the development of type 2 DM (Shlomo *et al.,* 2011). In Japan and China, about 30% of type 2 DM cases are linked to excess fat in the body. In Africa and Europe, 60 – 65% type 2 DM cases are associated with excess body fat. In situations where patients are not obese, a high waist to hip ratio is observed (Shoback and Gardner, 2011).

According to Malik *et al.* (2010), diet is central to the development of type 2 DM, and that excessive intake of drinks sweetened with sugar increases the risk of developing the condition. Fat has also been implicated in the development of type 2 DM with mono and polyunsaturated fats reducing the risk while trans and saturated fats increases the risk (Risérus *et al*., 2009). Excessive consumption of white rice also increases the risk of type 2 DM (Hu *et al.*, 2012**).** Low levels of physical activity or a lack of it is believed to contribute to about 7% of type 2 DM cases (Lee *et al*., 2012).

In type 2 DM, ketoacidosis is rarely experienced by patients. The condition mostly go undetected for a long time because the development of the hyperglycaemia is often slow and does not show symptoms of DM. Patients however, are at a risk of developing both macro and microvascular complications (ADA, 2004).

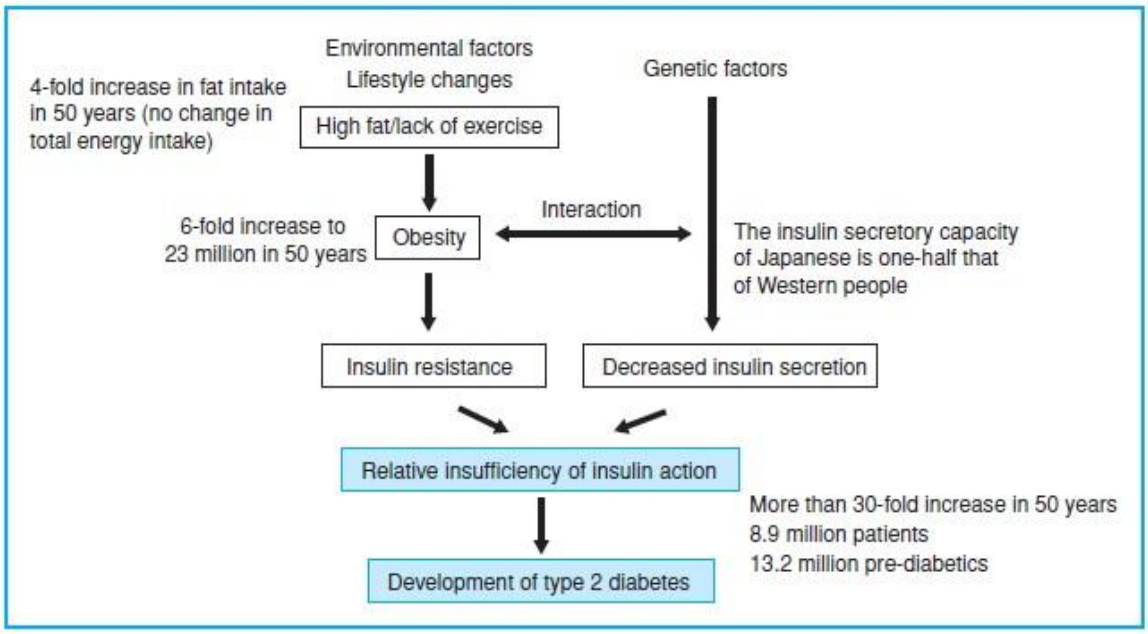


Figure 2.1: Aetiology and pathophysiology of type 2 diabetes (Kaku, 2010).

2.2.3 Gestational diabetes mellitus (GDM)

GDM is described as any level of intolerance to blood glucose experienced during pregnancy and may persist after the pregnancy. Dietary modification and sometimes insulin is used in the management of this condition defined as any degree of glucose intolerance with onset or first recognition during pregnancy. The definition applies regardless of whether insulin or only diet (ADA, 2004). GDM and type 2 diabetes resemble in many ways, with both characterised by inadequate secretion of insulin and responsiveness to it. GDM is said to affect approximately 2–10% of all pregnancies and the condition may get better or even disappear after delivery (National Diabetes Clearinghouse, NDIC, 2011). It is estimated that, 5–10% of women with GDM continue to live with diabetes, usually type 2 after the pregnancy (NDIC, 2011). GDM can be completely treated and patients need cautious supervision by medical staff throughout the pregnancy. Women who fail to manage their GDM stand a greater risk

of developing type 2 DM after pregnancy, and also stand a greater chance of developing pre-eclampsia and a Caesarean section (Yogev *et al*., 2004)**.**

Some other specific types of DM are as a result of the following; genetic defects of β cell function, genetic defects in insulin action, diseases of the pancreas, endocrinopathies, drug- or chemical-induced and infections (ADA, 2004)

2.3 Diagnostic Criteria for Diabetes Mellitus

DM has chronic high plasma glucose as its major symptom. Patients are diagnosed based on the level of their blood glucose in the fasting state, two hour post prandial and also using the glycated haemoglobin (Hb1Ac) levels (WHO, 1999). Table 2.1 summarises the diagnostic criteria for DM.

Table 2.1: Diagnostic criteria for DM based on laboratory indicators (WHO, 2006)

Condition	Fasting Glucose mmol/l	2 hr. glucose mmol/l	HbA1c mmol/mol	DCCT%
Normal	< 6.1	<7.8	<42	<6.0

Impaired fasting glucose	≥6.1 and <7.0	<7.8	42-46	6.0–6.4
Impaired glucose tolerance	<7.0	≥7.8	42-46	6.0–6.4
Diabetes mellitus	≥7.0	≥11.1	≥48	≥6.5

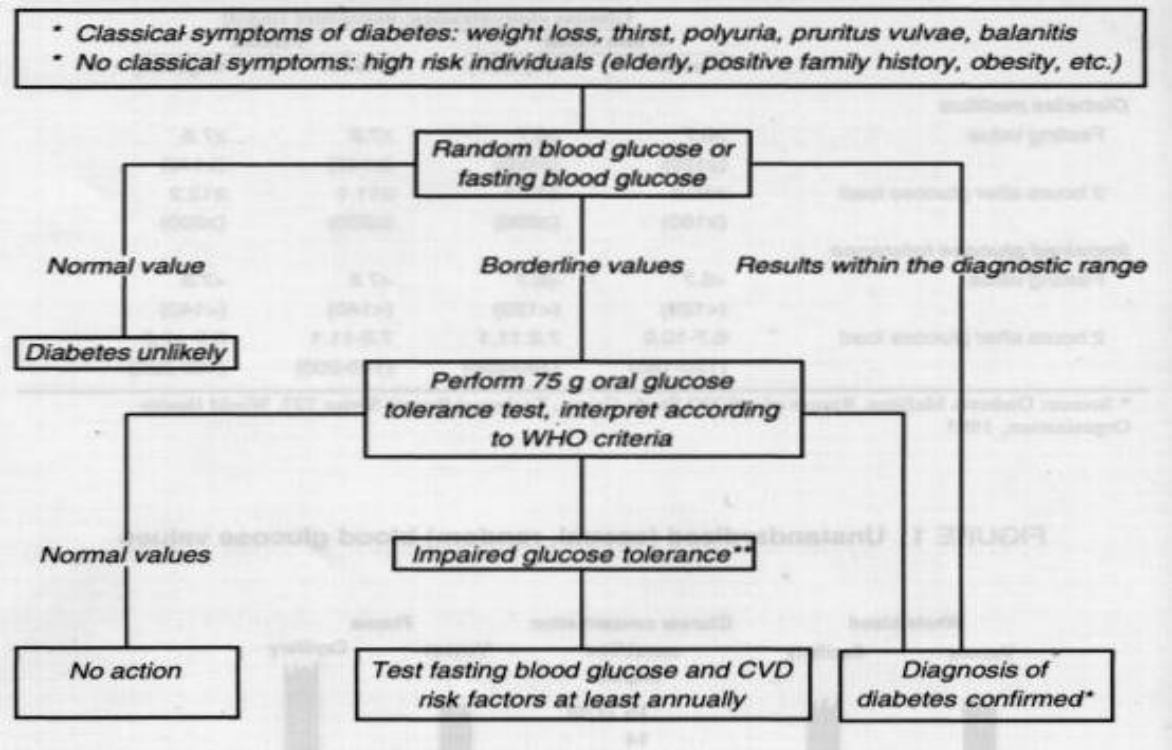


Figure 2.2: Simplified scheme for the diagnosis of DM (WHO, 1994)

2.4 Management of DM

According to the WHO, diabetes management involve three main parts; dietary regulation together with physical activity, oral hypoglycaemic agents and insulin injection. Also, providing diabetes patients with the requisite knowledge about the condition forms an essential part of its management **(**WHO, 1994). Acquisition of knowledge about diabetes and active participation in its management is essential in

preventing complication, since they are less severe in patients with well-controlled blood glucose levels (Nathan *et al*., 2005) (figure 2.3).

2.4.1 Treatment with synthetic drugs

Several drugs have been employed in the treatment of DM. According to Bennett *et al*. (2011a, b) the six classes of drugs used in treating DM function differently but they all ultimately reduce blood glucose levels and help to reduce the symptoms of hyperglycaemia. The six groups of diabetes drugs are; the sulfonylureas (glyburide, glimepiride, glipizide), biguanides (metformin), the alpha-glucosidase inhibitors (acarbose, miglitol), the thiazolidinediones (actos, avandia), the meglitinides (nateglinide, repaglinide) and the DPP-inhibitors (januvia, onglyza) (Bennett *et al*., 2011a, b)

These oral diabetes drugs come with some disadvantages to patients who use them, as reported by Bennett *et al*. (2011a, b). The sulfonylureas (glyburide, glimepiride, glipizide) cause weight gain and an increased risk of hypoglycaemia. The Biguanides (metformin) has a higher risk of gastrointestinal side effects (nausea and diarrhoea), has less convenient dosing and is not suitable for diabetic patients with renal diseases or heart failure due to the risk of lactic acid build-up. The alpha-glucosidase inhibitors (acarbose, miglitol) are expensive, have inconvenient dosing, less effective and have higher risk of gastrointestinal side effects. The thiazolidinediones (Pioglitazone, Rosiglitazone) causes weight gain, have a higher risk of heart failure and increases LDL cholesterol and triglycerides. It is also linked to a higher risk of anaemia and oedema as well as upper and lower limb fractures. It also has a slow onset of action and increases the risk of bladder cancer.

The meglitinides (nateglinide, repaglinide) are said to have a high cost and an inconvenient dosing. They are less effective on HbA1c and pose a risk of hypoglycaemia and weight gain. The DPP-inhibitors (Sitagliptin, Saxagliptin) also have a high cost, are less effective on HbA1c and are linked to the inflammation of the pancreas (Bennett *et al*., 2011a, b)

2.4.2 Treatment with natural products

Several plant species have been used traditionally to treat DM in Africa but only a few of such plants have been scientifically proven and documented to posess such therapeutic effects. Discussed below are some of the plants and plant extracts that have been proven and documented to have anti-diabetic effects in experimentallyinduced diabetic rat models.

When chloroform extract of *Acacia Arabica* bark was administered at doses of 250 and 500 mg/kg body weight for two weeks in alloxanized diabetic rats, serum glucose levels were reduced significantly. Parameters of lipid profile (total cholesterol [TC], triglycerides [TG], high density lipoprotein [HDL] and low density lipoprotein [LDL]) levels were also restored (Patil *et al*., 2011)**.** According to Geetha *et al*. (2011**)** plasma glucose was significantly lowered in alloxanized diabetic rats after treatment with extracts *of Achyranthes rubrofusca* (Amaranthaceae) leaves. Also, treatment of diabetic rats with 100 and 200 mg/kg body weight for 30 days with ethanol extract of *Andrographis paniculata* (Acanthaceae) significantly lowered blood glucose levels (Ravikumar *et al.*, 2010).

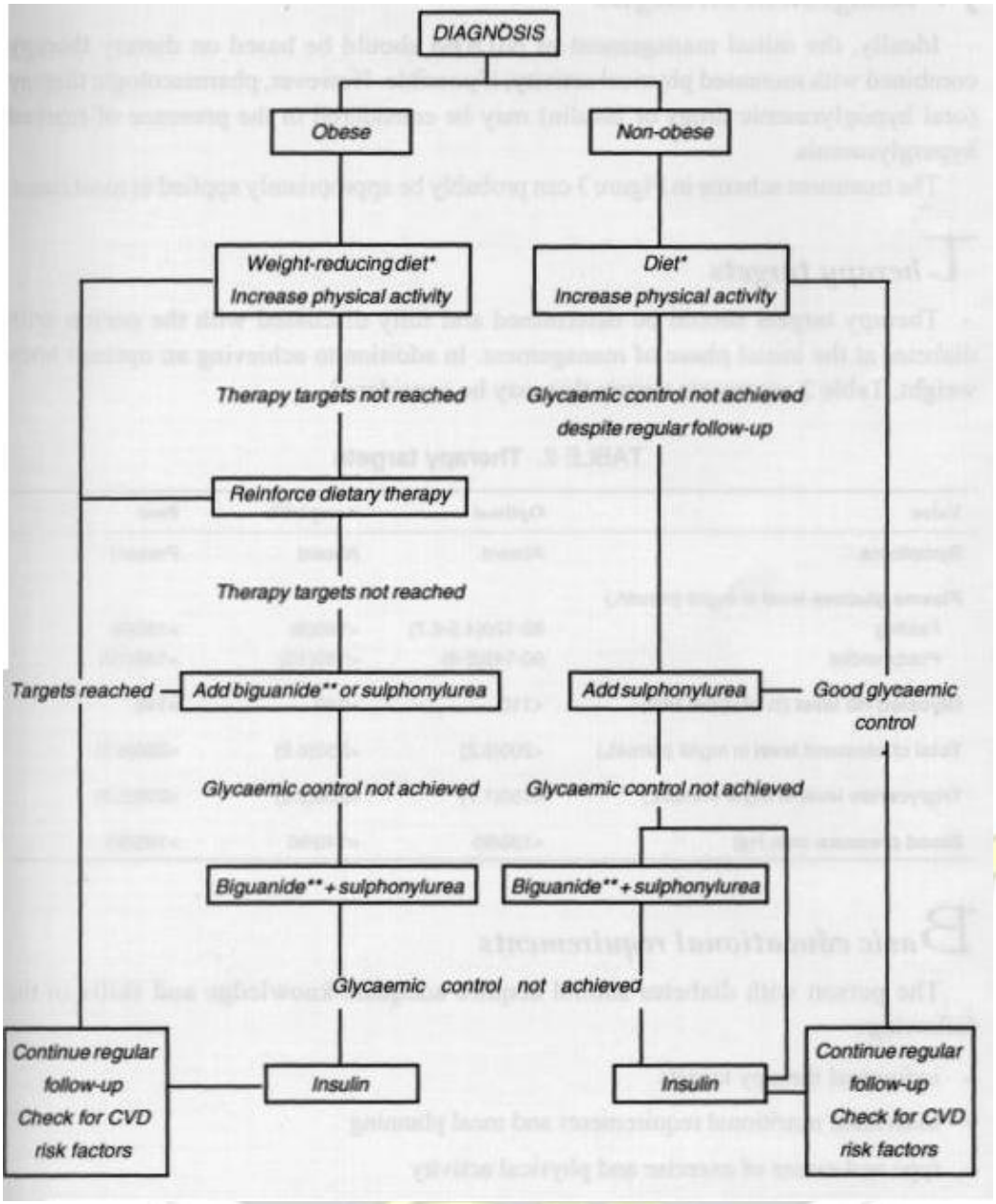


Figure 2.3: Simplified scheme for the treatment of type 2 DM (WHO, 1994)

It has also been reported that, root and leaf extract of *Barleria prionitis* significantly reduced plasma glucose and glycosylated haemoglobin levels after diabetic rats were treated with 200 mg/kg body weight for two weeks. It further increased serum insulin and liver glycogen levels significantly (Dheer and Bhatnagar, 2010)**.** Another study by Nabeel *et al.* (2010) investigated the antihyperglycaemic effect of leaf extract of *Ceriops decandra*. The extract was given at doses of 30, 60, 120 mg/kg body weight

for a period of 30 days. The dosage of 120 mg/kg was very significant in reducing blood glucose levels in the diabetic rats.

Also, when the anti-diabetic potentials of *Ficus bengalensis* was investigated in type 1 and type 2 diabetic rats the blood glucose and lipid levels were decreased significantly following treatment with 1.25 g/kg daily for a period of four weeks showing the anti-diabetic potential of *F. bengalensis* (Chaturvedi and Sharma, 2010).

2.5 *Borassus aethiopum* Mart.

2.5.1 Description

Borassus aethiopum is a plant species of Borassus palm found in Africa. It is often called the African palmyra palm, the African fan palm, among others. *B. aethiopum* grows straight reaching 20 metres in height and has a characteristic crown of up to 8 metres wide. The young *B. aethiopum* are usually covered with stalks of leaves while the mature palms have their trunks swollen at about 12 – 15 metres from the ground. The older palms have a smooth pale-grey bark and leaves that are usually broad, fan – shaped and blue-green in colour. The fruit which has a diameter of approximately

15 centimetres is ovoidally shaped and orange in colour when ripe. The pulp has three hard kennels and also has albumen which hardens up when it is ripe (Orwa *et al.*, 2009).

Figure 2.4: B. aethiopum (A) Whole plant (B) Fruit (C) Nut (Source: www.en.wikipedia.org)

2.5.2 Habitat and Distribution

B. aethiopum is found in many places across Africa. Some of the countries that inhabit the plant are: Cameroon, Burkina Faso, Benin, the Central African Republic and the Comoros. Others include Ghana, Zaire, Senegal and the Northern Provinces (Bayton, 2007; Orwa *et al.*, 2009).

2.5.3 Uses of Borassus aethiopum

B. aethiopum has many uses. It serves an important source of food, providing edible fruits, and nuts. The sap that is obtained from the inflorescence is usually drunk in its raw state or processed into wine, alcohol or vinegar. It is sometimes also dried into sugar cakes (Haynes and McLaughlin, 2000). In Ghana and other West African

countries like Ivory Coast, the ripe mature fruits of *B. aethiopum* are usually eaten raw or boiled. The fleshy mesocarp is often hand mashed to obtain a thick liquid. The liquid obtained is usually taken alone or with boiled maize as food (Agbo and Simard, 1992)

According to Mshana *et al.* (2000), several other uses of *B. aethiopum* have been reported where some mat and basketry industries make use of the leaves of the plant. The trunk is also used for building and construction of bridges due to its tough and termite resistant nature. A number of pharmacological uses of the *B. aethiopum* plant have been reported in some parts of the world. In the northern region of Ghana, the leaves and roots of *B. aethiopum* are used for the treatment of headache (Ziblim *et al*., 2013)

The roots of *B. aethiopum* are reportedly used as treatment for epilepsy by some people in Togo. The roots are macerated and used to bath by people suffering from epilepsy (Tchacondo *et al*., 2012). In Ghana, the seeds of *B. aethiopum* are used for scarification by the Hausa people living in the country (van Andel *et al*., 2012)

2.5.4 Physico-chemical properties of Borassus aethiopum fruit

Fresh mature *B. aethiopum* fruits are usually orange in colour (Koffi *et al.*, 2010) According to Ali *et al.* (2010), a mature fruit of *B. aethiopum* weights between 1.2 - 1.4 kg. The pulp, from which the juice is extracted constitute about 37.53 – 39.67% of the entire weight of the fruit (Table 2.2). Koffi *et al*. (2010), reports that *B. aethiopum* juice is rich in sugars such as glucose, sucrose and fructose. The sugar with the highest concentration was glucose (62.4 mg/ml). Sucrose had a concentration of 53.4 mg/ml while fructose had a concentration of 33.6 mg/ml. The juice also contained

nonidentified high molecular weight oligosaccharides. The fresh fruit juice has a pH of about 3.74 and has a sweet bitter taste and a desirable aroma.

The juice also contains ethanol (1.47 g/l).

Table 2.2: Physical and morphological properties of a mature fruit of *B aethiopum* (Ali *et al.*, 2010)

	Sources Parameters	
Zone 1	**Zone 2**	
Mean diameter (md) (cm)	13.85 ± 0.32	13.56 ± 0.49
Thickness of pulp (tp) (cm)	0.82 ± 0.03	0.82 ± 0.05
Weight of whole fruit (wf) (g)	1325 ± 86	1270± 117
Weight of pulp (wp) (g)	512 ± 37	449± 46.91
Weight of kernels (wk) (g)	659± 47.57	660± 71.40
Percentage of pulp (%p)	38.60 ± 1.1	35.22 ± 1.2
Weight of peelings (g)	158 ± 32.74	155± 30.32
Weight of cockle (g)	291± 12.32	270 ± 9.26
Weight of almond (g)	396 ± 20.45	380± 16.12

A physico-chemical analysis of the pulp of the fruits of the Palmyra palm (*B. aethiopum*) presented high water content (79.13 ± 0.64 to 81.38 ± 1.94 g/100 FM, with respect to the origin). Total sugar content of fruits from were between 5.62 ±

1.13 g/100g FM and 4.47 ± 1.07 g/100 g FM depending on the source. The soluble sugars were 81.00 to 84.11% of the total sugars (Ali *et al*., 2010).

Table 2.3: Physico-chemical properties of the pulp of *B. aethiopum* (Ali *et al*., 2010)

	Sources

Parameters	Zone 1	Zone 2
Water content (%)	79.13 ± 0.64a	81.38 ± 1.94a
Total ash (%)	0.73 ± 0.12 a	74 ± 0.01 b
Total sugar (%)	5.62 ± 1.13a	4.47 ± 1.07a
Soluble sugar (%)	4.58 ± 1.12a	3.76 ± 1.03a
Total proteins (%)	0.85 ± 0.13a	0.73 ± 0.10a
Crude fibers (%)	5.72 ± 0.39a	5.18 ± 0.23a
Total lipid (%)	0.16 ± 0.001a	0.15 ± 0.002a
Carotenoïds (mg/100gFM)	27.42 ± 0.90a	26.61 ± 0.83a
Vitamin C (mg/100gFM)	135 ± 3.94a	171 ± 2.62b
Iron (mg/100gFM)	2.05 ± 0.152	15 ± 0.20
Magnesium (mg/100gFM)	20.61 ± 0.25	21.01 ± 0.31
Phosphorous (mg/100gFM)	567± 0.42	567 ± 0.43
Calcium (mg/100gFM)	108 ± 0.20	108± 0.24
Total phenolic compounds (mg/100gFM)	274 ± 0.19	275 ± 0.20

Also, reports indicate that the ripe fruit extract of *B. aethiopum* has antioxidant and free radical scavenging properties thus its consumption could therefore be beneficial in preventing diseases that are caused by oxidative stress (Amoateng *et al*., 2010)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant material

Fresh mature fruits of *B. aethiopum* (orange colour) were harvested from the wild in Kintampo in the Brong-Ahafo Region of Ghana in June 2015. The fruits were authenticated at the herbarium of the Department of Pharmacognosy, KNUST by Mr. Osarfo Asare. The plant sample was given a voucher specimen number KNUST/M2/2016/R003.

3.2 Preparation of aqueous ripe fruit juice extract of *B. aethiopum*

Freshly harvested ripe fruits were washed with tap water and the juice prepared by methods as described by Amoateng *et al*. (2010) and Koffi *et al.* (2010) with slight modification. The pericarps were peeled off with a knife after which the fruit was separated into three with each portion containing a seed. The mesocarp in each portion was mashed in one litre of freshly distilled water to form thick yellowish syrup. This was then strained to separate the pulp from the juice. The liquid extract was heated to 80^0C for 5 minutes and allowed to cool. It was then freeze-dried using the vacuum freeze dryer (YK-118, Taiwan) at the Crop Research Institute, Fumesua, Kumasi and the resulting powder referred to as an aqueous ripe fruit extract of *B. aethiopum* (FEB). Figure 3.1 shows a flow chart of the processing of the fruit extract.

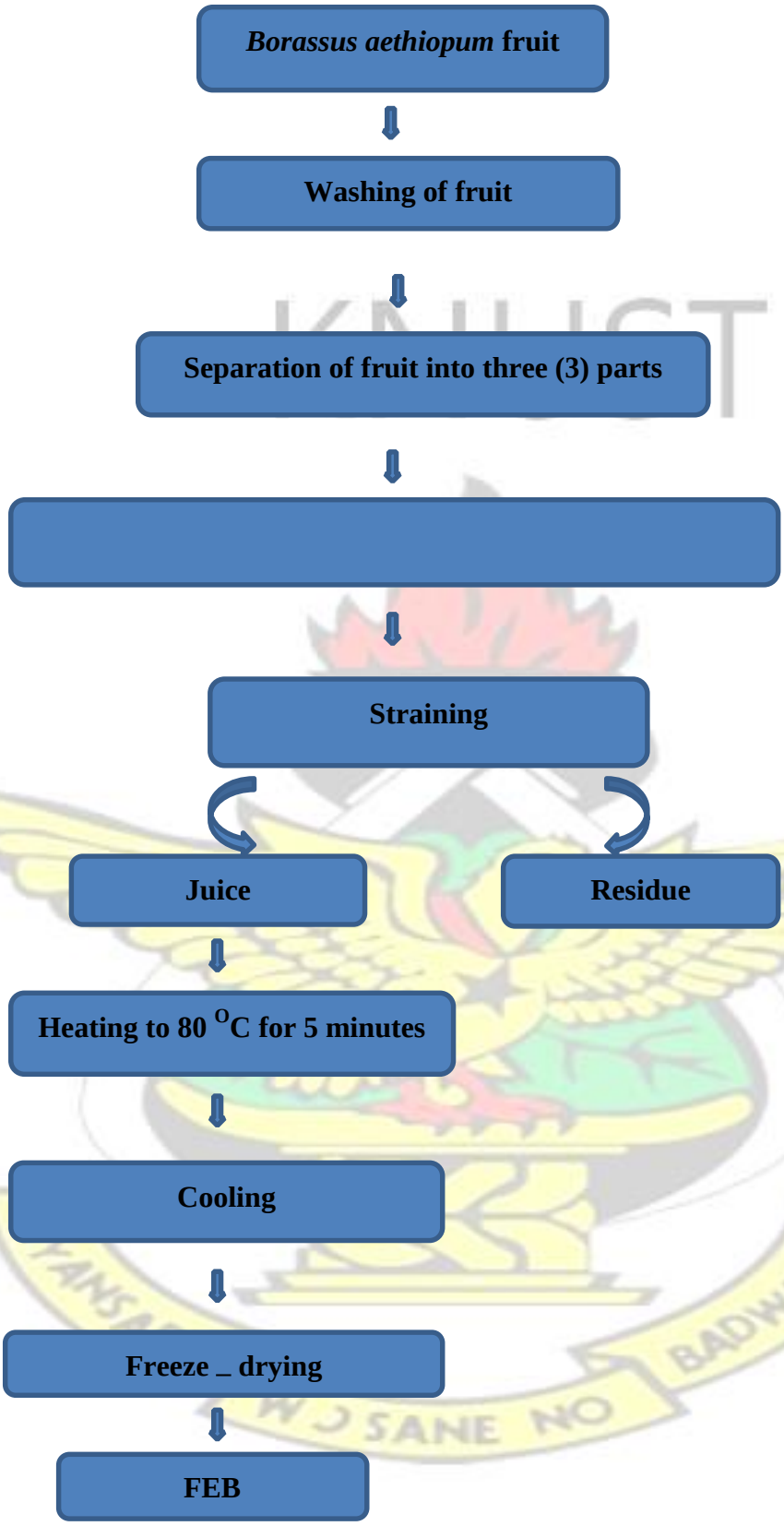

Borassus aethiopum fruit
Washing of fruit
Separation of fruit into three (3) parts
Mashing of mesocarp into water to form thick syrup
Straining
Juice
Residue
Heating to 80 ^{O}C for 5 minutes
Cooling
Freeze _ drying
FEB

Figure 3.1: Processing of ripe fruit extract of *B. aethiopum* (FEB). (Adopted from Koffi *et al.*, 2010 and Amoateng *et al.*, 2010)

3.3 Preliminary Phytochemical screening of FEB

Qualitative phytochemical analysis was performed on the extracts of *B. aethiopum* to ascertain the presence of phytochemicals. Standard procedures as described by Harborne (1998), Trease and Evans (1989), and Sofowora (1993) were employed. The various phytochemicals tested for included saponins, tannins, flavonoids, sterols, terpenoids, general glycosides, alkaloids, and anthracenes.

Briefly, the methods were as follows:

3.3.1 Tannins

A portion of the extract was dissolved in water, after which the solution was clarified by filtration. 10% ferric chloride solution was then added to the resulting filtrate. The appearance of a bluish black colour indicates the presence of tannins.

3.3.2 Saponins

One millilitre of distilled water was added to 1 ml of the extract and shaken vigorously. Astable persistent froth indicated the presence of saponins.

3.3.3 Flavonoids

Two millilitres of dilute sodium hydroxide was added to 2 ml of the extract. The appearance of a yellow colour indicates the presence of flavonoids.

3.3.4 Alkaloids

0.5 g of the extract was stirred in 5 ml of 1% HCl on a steam bath and filtered while hot. Distilled water was added to the residue and 1 ml of the filtrate was treated with a few drops of Wagner's reagent. A reddish brown precipitate indicates the presence of alkaloids.

3.4.5 Triterpenoids

A few mg of the residue was dissolved in chloroform. To this was added few ml of acetic anhydride and two drops of conc. H_2SO_4 from the side of the test tube. The red or violet colour indicated the presence of triterpenoids.

3.4.6 Sterols

The amount of 0.5 g of the extract was dissolved in 10 mL anhydrous chloroform and filtered. Two ml of concentrated sulphuric acid was carefully added to the chloroform solution, so that the sulphuric acid formed a lower layer. A reddish

-brown colour at the interface indicates the presence of a steroidal ring.

3.4.7 Glycosides

Diluted sulphuric acid was added to the extract in a test tube and boiled for 15 min. Then 10% sodium hydroxide and mixed Fehling's solution were added. The formation of brick red precipitate indicates the presence of glycoside.

3.4.8 Anthracenes

2 cm^3 of chloroform was added to the extract in a test tube and allowed to separate. To the chloroform layer was added 10% ammonia solution and vigorously shaken

and kept to separate. The formation of brick-red precipitate indicates the presence of anthracenes.

3.4 Preparation of test materials

3.4.1 Preparation of Alloxan solution

Alloxan hydrate (CDH, India) was dissolved in normal saline to form an aqueous solution. This was given at a dose of 150 mg/kg body weight intraperitoneally to induce diabetes (Joy and Kuttan, 1999).

3.4.2 Preparation of standard drug

Glibenclamide (Diabenol, Thailand) tablets, an oral hypoglycaemic drug (Bennett *et al*., 2011a) was dissolved in distilled water to form a suspension and was administered at a dose of 10 mg/kg body weight orally.

3.5 Experimental Animals

Healthy female albino rats weighing 115-150 g were obtained from the Centre for Scientific Research into Plant Medicine, Mampong-Akuapem and used for the study. They were acclamatized for 14 days in the animal holding facility of the Department of Biochemistry and Biotechnology, KNUST. The animals were housed in aluminium cages under standard husbandary conditions (12 h. light/dark cycle). Animal were provided distilled water and standard laboratory food (Mash, Agricare, Kumasi, Ghana) during the study period.

3.6 Experimental Design

3.6.1 Induction of experimental Diabetes in Rats

Diabetes was induced by a single intraperitonial injection of alloxan hydrate (150 mg/kg b.wt). The rats were given access to 5% glucose solution overnight to withstand the alloxan-induced hypoglycaemia (Pradeep *et al.*, 2014). Diabetes was confirmed 72 hours after induction by measurement of blood glucose levels using OneTouch Select glucometer (USA) and test strips by tail puncture. Animal whose fasting blood glucose was equal to or greater than 11mmol/l were selected as diabetic and subsequently used for the research.

3.6.2 Acute antidiabetic study

After diabetes was fully induced, the animals (both diabetic and normal) were divided randomly into nine (9) groups of four (4) rats each as follows and treated for 7 days.

Group I – Normal rats served as normal control and were given 0.5 ml distilled water per day orally.

Group II – Alloxan-induced diabetic rats served as diabetic control (given 0.5 ml) distilled water per day orally.

Group III - Normal rats given 100 mg/kg body weight of FEB per day orally.

Group IV – Normal rats given 250 mg/kg body weight of FEB per day orally.

Group V - Normal rats given 500 mg/kg body weight of FEB per day orally.

Group VI – Alloxanized diabetic rats given 100 mg/kg body weight of FEB per day orally.

Group VII – Alloxanized diabetic rats given 250 mg/kg body weight of FEB per day orally.

Group VIII- Alloxanized diabetic rats given 500 mg/kg body weight of FEB per day orally.

Group IX – Alloxanized diabetic rats given 10 mg/kg body weight of glibenclamide per day orally.

3.6.3 Sub – chronic antidiabetic study

After diabetes was fully induced, the animals (both diabetic and normal) were divided randomly into nine (9) groups of four (4) rats each as follows and treated for 28 days.

Group I – Normal rats served as normal control and were given 0.5 ml distilled water per day orally.

Group II – Alloxan-induced diabetic rats served as diabetic control (given 0.5 ml) distilled water per day orally.

Group III - Normal rats given 100 mg/kg body weight of FEB per day orally.

Group IV – Normal rats given 250 mg/kg body weight of FEB per day orally.

Group V - Normal rats given 500 mg/kg body weight of FEB per day orally.

Group VI – Alloxanized diabetic rats given 100 mg/kg body weight of FEB per day orally.

Group VII – Alloxanized diabetic rats given 250 mg/kg body weight of FEB per day orally.

Group VIII- Alloxanized diabetic rats given 500 mg/kg body weight of FEB per day orally.

Group IX – Alloxanized diabetic rats given 10 mg/kg body weight of glibenclamide per day orally.

3.6.4 Effect of treatment on Fasting Blood Glucose (FBG)

Anti-diabetic effects were assessed by collecting blood samples from the rats by tail puncture for fasting blood glucose determination. This was done before treatment with extract of FEB and at intervals of 2, 4, 8 hours after administration of extract. This continued daily for 7 days with fasting blood glucose levels determined before administration of extract for acute anti-diabetic study. In the sub-chronic antidiabetic study, fasting blood glucose levels were determined before administration of extract of FEB, and subsequently at intervals of 7, 14, 21 and 28 days. The blood glucose levels were determined using the OneTouch Select Simple Glucose metre and the results reported as mmol/l.

3.6.5 Effect of treatment on body weight

Body weights of animals were determined on day 0, 4 and 7 for acute ant-idiabetic study and on day 0, 4, 8, 12, 16, 20, 24 and 28 for sub-chronic anti-diabetic study.

The percent change in body weight was calculated using the formula:

$$\text{Percentage change in body weight} = \frac{Weight_n - Weight_{initial}}{Weight_{initial}} \times 100\%$$

where Weight $_{initial}$ is the weight measured on the first day (D0) while Weight $_{n}$ is the weight measured at the end of D4 and D7 for acute study, and D4, D8, D12, D16, D20, D24 and D28 for sub-chronic study.

3.6.6 Effect of treatment on Haematological Parameters

At termination of the experiment, animals were fasted overnight after which they were sacrificed by cervical dislocation. Incisions were quickly made in cervical region of the sacrificed animals using of a sterile blade and blood samples collected from the heart into ethylenediaminetetraacetic acid (EDTA) tubes for haematological analysis using the Sysmex XP-300 Automated Haematology Analyser (USA) according to manufacturer's procedure. Determinations included haemoglobin concentration (HGB), red blood cell (RBC) count, platelets (PLT) count, white blood cell (WBC) count, haematocrit (HCT) and lymphocytes (LYM)

3.6.7 Effect of treatment on biochemical parameters

Part of blood samples were put into Serum Separator Tubes (SSTs) and centrifuged at 704 X g (R = 7 cm) for 15 minutes. The sera were separated into eppendorf tubes and stored at 4 ^{0}C prior to analysis. The Flexor Junior Automated Chemistry Analyser (Japan) was used to analyse the samples according to manufacturer's procedure. Total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and the coronary risk (CR) were measured as part of the lipid profile. Serum creatinine and urea levels were determined as markers of kidney function. Alanine transaminase (ALT) levels were also measured as a biomarker for liver function using manufacturers' methods.

3.6.8 Effect of treatment on organ weight

The main organs of sacrificed animals, liver, kidney and pancreas were excised, cleaned with buffered saline and weighed to obtain the absolute organ weight (AOW). Relative organ weights (ROW) were computed using the formula stated below.

$$Relative\ Organ\ Weight = \frac{\text{Absolute Organ Weight}}{\text{Body Weight at Sacrifice}} \times 100\%$$

3.7 Data analysis

Results were expressed as mean ± SEM. The data were statistically analysed with one-way ANOVA followed by Tukey HSD as a *post hoc test* for multiple comparison between groups. The values of $p<0.05$ were considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical content of FEB

The phytochemical screening of the fruit extract of *Borassus aethiopum* (Table 4.1) showed the presence of tannins, saponins, glycosides, triterpenoids and alkaloids. Flavonoids, anthracenes and sterols were absent. **Table**

4.1: Phytochemical content of FEB

Phytochemicals	Presence
Tannins	+
Saponins	+
Flavonoids	
Glycosides	+
Anthracene	
Alkaloids	+
Triterpenoids	+
Sterols	

Where ( ) indicates present and ( ) indicates absent.

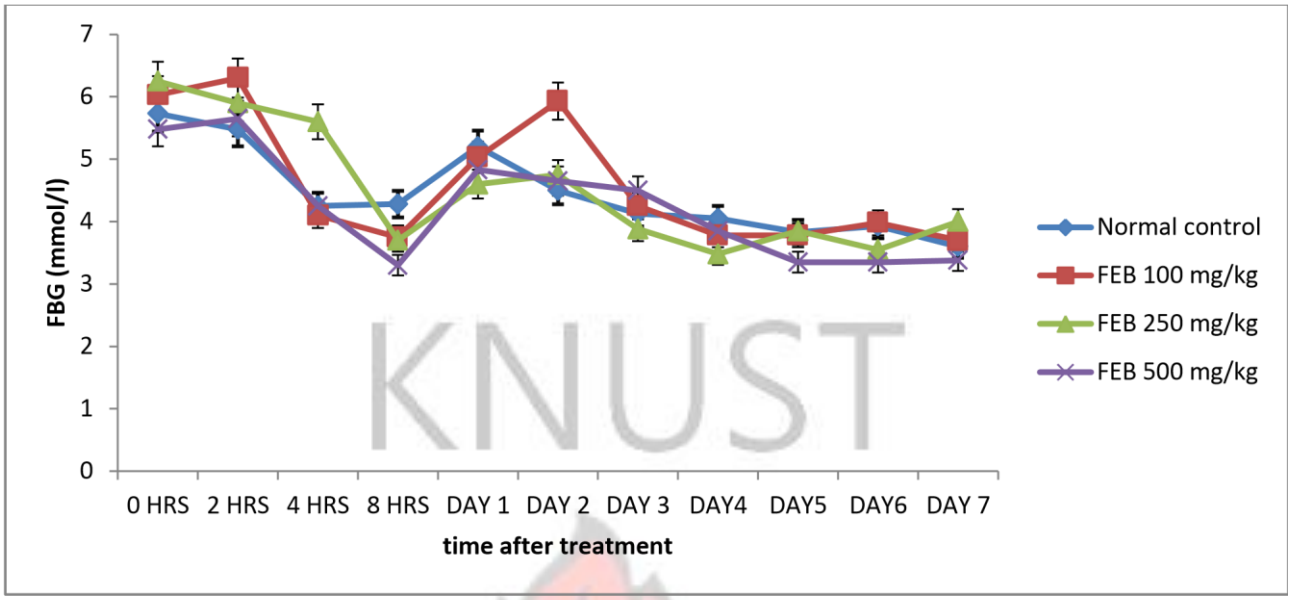
4.2 Acute Antidiabetic Study

The acute antidiabetic study was carried out in normal and alloxan-induced diabetic rats for 7 days.

4.2.1 Effect of treatment on FBG levels of normal and alloxan-induced diabetic rats

FEB (100, 250 and 500 mg/kg body weight) did not reduce fasting blood glucose (FBG) levels significantly in normal rats after 7 days of treatment (Figure 4.1). FBG levels were slightly reduced in all groups after 8 hours of treatment. FEB caused a slight reduction in FBG levels compared to the normal group. After 7 days of treatment, the levels of reduction in FBG were almost the same in all the groups. The mean FBG in the normal rats reduced from 5.73±0.61 mmol/l (D0) to 3.6±0.11 mmol/l (D7), while FEB 100 mg/kg b.wt caused a reduction in fasting blood sugar level from 6.03±0.56 mmol/l (D0) to 3.70±0.33 mmol/l (D7). FEB (250 mg and 500 mg/kg b.wt) caused FBG levels to fall from 6.25±0.54 mmol/l (D0) to 4.00±0.23 mmol/l (D7) and 5.48±0.81 mmol/l (D0) to 3.38±0.17 mmol/l (D7) respectively.

FEB (100 and 250 mg/kg), and glibenclamide 10 mg/kg did not reduce FBG



significantly in diabetic rats after 8 hours and 7 days of treatment (Figure 4.2). The FBG levels of diabetic rats were reduced significantly in rats treated with FEB at 500 mg/kg b.wt. after 2, 4 and 8 hours of treatment. FEB at 500 mg /kg b.wt also reduced FBG levels significantly (P= 0.013) after 7 days of treatment from 25.8±1.33 to 5.23±0.24 mmol/l, as shown in Figure 4.2 below.

Figure 4.1: Effect of treatment on FBG levels (mmol/l) of normal rats. Each point represent a mean of 4 rats

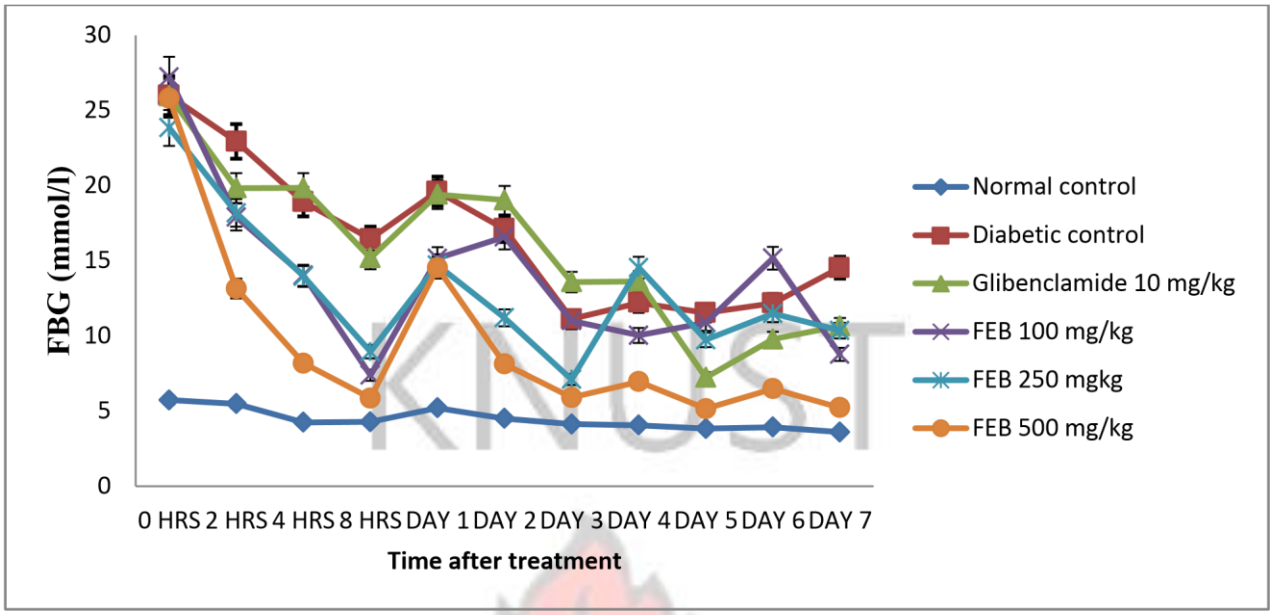


Figure 4.2: Effect of treatment on FBG levels of alloxan-induced diabetic rats.

Each point represent a mean of 4 rats

4.2.2 Effect of treatment on body weight of normal and alloxan-induced diabetic rats

From Figure 4.3 below, there was a reduction in the body weight in normal control rats. All other groups experienced a slight increase in body weight after D0. After D7, the percent change in body weight in all FEB treated rats were almost the same with significant difference ($P<0.05$) observed between those values and that of the normal control group.

A decline in body weight was observed in all the groups after D4 in diabetic rats (Figure 4.4). There were significant differences ($P<0.05$) between the body weights of FEB treated groups at 250 and 500 mg/kg b.wt and that of the diabetic control group. There was however no significant differences in the reduction in body weight in glibenclamide and FEB 100 mg treated rats compared with the diabetic control group

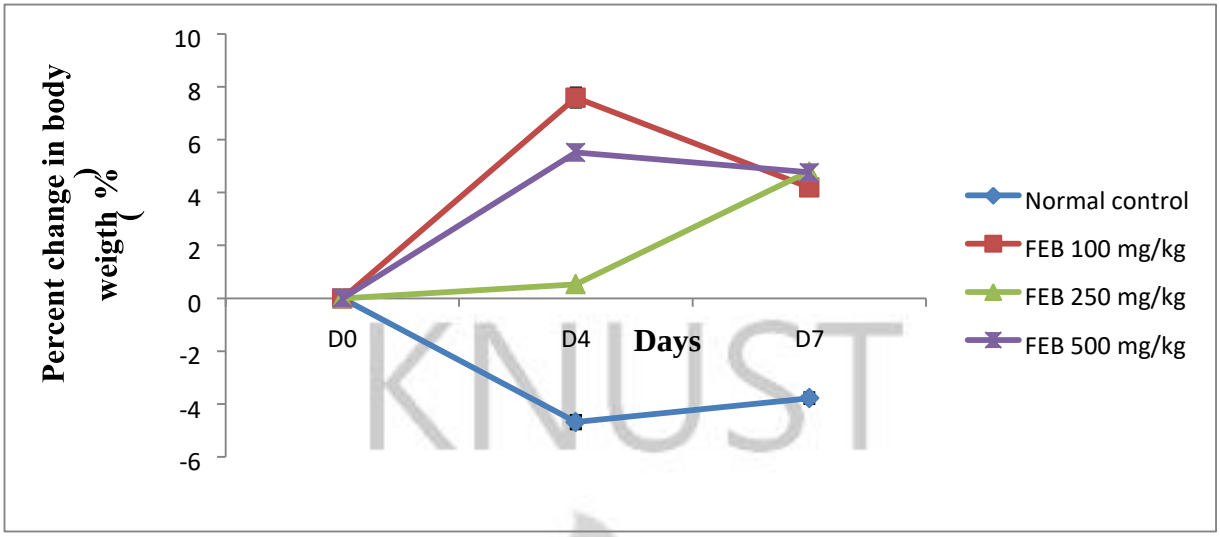


Figure 4.3: Effect of treatment on body weight of normal rats. Each point represent a mean of 4 rats

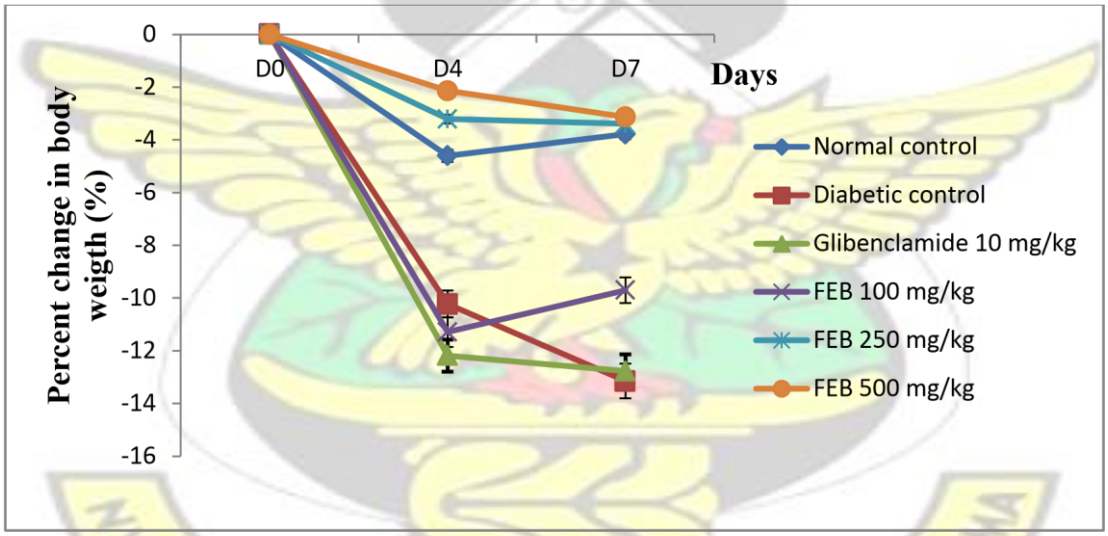


Figure 4.4: Effect of treatment on body weight (g) of alloxan-induced diabetic rats. Each point represent a mean of 4 rats

4.2.3 Effects of treatment on Haematological parameters of normal and alloxaninduced diabetic rats

From Table 4.2 below, the levels of RBC, HGB, HCT and LYM measured in FEB treated normal rats were not statistically different from the normal control group. The values of WBC were significantly increased in FEB 100 mg/kg treated rats while PLT levels were also increased significantly ($P<0.05$) in all FEB-treated groups compared to normal control group.

Among the diabetic rats, the levels of WBC, RBC and HGB did not change significantly in FEB and glibenclamide-treated rats. HCT and LYM levels of treated groups were not statistically different from the diabetic control group. Glibenclamide at 10 mg/kg b.wt and FEB at 500 mg/kg b.wt caused a significant increase ($p<0.05$) in the levels of PLT compared with diabetic control group as shown in Table 4.3 **Table 4.2: Effects of treatment on Haematological parameters of normal rats**

	Treatments					
Parameters	Normal control	Diabetic control	Glib. 10 mg/kg	FEB100 mg/kg	FEB 250 mg/kg	FEB 500 mg/kg

Parameters	Normal control	FEB 100 mg/kg	FEB 250 mg/kg	FEB 500 mg/kg
WBC x 10^3 /µL	3.05±0.61	8.93±2.43*	5.75±0.64	4.65±0.74
RBC x 10^6/µL	7.90±0.38	8.09±0.27	7.78±0.17	7.34±0.35
HGB (g/dL)	13.55±1.05	14.45±0.27	13.70±0.38	13.08±0.88
HCT (%)	42.03±2.38	45.03±1.12	41.85±1.17	39.65±2.05
PLT x 10^3 /µL	527±125.82	664±189.66	629.25±67.57	807.25±121.39*
LYM (%)	63.18±1.17	69.33±1.43	66.14±2.13	63.10±3.90

Mean ± SEM, n = 4, *****$p<0.05$ indicates statistically significant difference versus normal control using one-way ANOVA followed by *post hoc* Tukey HSD analysis.

Table 4.3: Effects of treatment on Haematological parameters of alloxaninduced diabetic rats

WBC x 10^3 /µL	3.05±0.61	4.47±.73	6.35±1.85	6.13±2.23	4.23±1.16	5.25±.83
RBC x 10^6/µL	7.90±0.38	7.99±0.35	7.35±0.39	8.69±0.25	7.20±1.33	7.67±0.23
HGB (g/dL)	13.55±1.05	14.40±0.70	13.00±0.90	15.40±0.17	13.65±2.12	12.78±1.09
HCT (%)	42.03±2.38	43.80±2.33	40.03±1.71	46.30±0.61	40.73±6.49	42.05±1.14
PLT x 10^3 /µL	527.00±125.82	269.00±22.10	852.67±180.11*	349.67±121.46	412.00±147.45	718.75±170.03*
LYM (%)	63.18±1.17	56.80±0.55	46.40±5.20	50.97±15.45	45.70±2.23	68.00±0.10

Mean ± SEM, n = 4, *****p<0.05 indicates statistically significant difference versus Diabetic control using one-way ANOVA followed by *post hoc* Tukey HSD analysis.

4.2.4 Effect of treatment on various Biochemical parameters of normal and alloxan-induced diabetic rats

In the normal groups total cholesterol (TC) levels of rats treated with FEB (100, 250 and 500 mg/kg) were not different from that of normal control group (Table 4.4).

Triglyceride (TG) levels in FEB-treated rats were not significantlydifferent from normal control group. FEB (100 and 250 mg/kg) slightly increased the levels of HDL while FEB (250 and 500 mg/kg) caused a slight reduction in the levels of LDL. VLDL levels were also slightly increased in FEB treated rats. The mean coronary risk (CR) (3.43, 3.18 and 3.25) in FEB (100, 250 and 500 mg/kg) treated rats were statistically the same as it is in the normal control group (3.15). Creatinine levels were lower in FEB treated rats compared to the normal control group, but this was not significant (Table 4.4). Urea levels in FEB treated groups were not statistical different from the normal control groups. ALT levels were slightly elevated in groups treated with FEB at 100 and 250 mg/kg treated groups but was significantly reduced (P<0.05) in rats treated with FEB at 500 mg/kg.

In the diabetic groups, parameters of lipid profile measured were reduced slightly in diabetic control group compared to normal control group (Table 4.5). Glibenclamide (10 mg/kg) caused an insignificant reduction in all parameters that were measured and FEB (250 and 500 mg/kg) caused an insignificant elevation in diabetic rats and FEB 100 mg/kg caused a slight reduction in TC levels. TG levels in treated groups were not significantly different from the diabetic control group, likewise HDL and LDL levels. The CR was statistically the same for all groups. Creatinine levels were slightly elevated but not to significant levels in glibenclamide and FEB treated rats compared to diabetic control group after 7 days of treatment. The level of urea was significantly reduced in FEB 250 and 500 mg/kg treated rats compared to diabetic control group. ALT levels in treated groups were not significantly different from the diabetic control group

Table 4.4: Effect of treatment on various Biochemical parameters of normal rats

	Treatments			
parameters	Normal control	FEB 100 mg/kg	FEB 250 mg/kg	FEB 500 mg/kg
TC(mmol/l)	2.18±0.11	2.47±0.37	2.25±0.08	2.13±0.10
TG(mmol/l)	0.65±0.07	0.93±0.11	0.90±0.08	0.82±0.13
HDL(mmol/l)	0.96±0.03	1.01±0.13	0.98±0.04	0.90±0.02
LDL(mmol/l)	0.92±0.08	0.94±0.22	0.86±0.04	0.86±0.14
VLDL(mmol/l)	0.30±0.03	0.43±0.05	0.41±0.04	0.37±0.06
CR(ratio)	3.15±0.13	3.43±0.19	3.17±0.05	3.25±0.15
Creatinine(µmol/l)	57.00±3.05	50.72±4.46	48.38±1.07	51.75±3.09
Urea(mmol/l)	8.60±0.50	8.78±0.54	10.18±0.53	8.46±0.39
ALT(U/l)	140.57±18.92	153.70±19.16	150.18±20.78	126.50±13.32*

Mean ± SEM, n = 4, *****p<0.05 indicates statistically significant difference versus normal control using one-way ANOVA followed by *post hoc* Tukey HSD analysis.

	Treatments					
Parameters	Normal control	Diabetic control	Glib 10 mg/kg	FEB 100 mg/kg	FEB 250 mg/kg	FEB 500 mg/kg
TC(mmol/l)	2.18±0.11	1.69±0.11	1.43±0.26	1.21±0.41	1.98±0.25	1.89±0.33
TG(mmol/l)	0.65±0.07	0.39±0.05	0.38±0.15	0.61±0.09	0.61±0.13	0.66±0.09
HDL(mmol/l)	0.96±0.03	0.76±0.05	0.64±0.06	0.78±0.18	0.91±0.10	0.82±0.13
LDL(mmol/l)	0.92±0.08	0.75±0.09	0.63±0.14	0.57±0.09	0.79±0.13	0.78±0.16
VLDL(mmol/l)	0.30±0.03	0.18±0.02	0.17±0.07	0.28±0.04	0.28±0.06	0.29±0.04
CR(ratio)	3.15±0.13	3.08±0.18	3.03±0.35	3.17±0.58	3.00±0.15	3.18±0.06
Creatinine(µmol/l)	57.00±6.10	39.83±8.24	53.33±11.80	47.55±9.15	47.43±6.28	50.83±9.25
Urea(mmol/l)	8.60±0.99	28.71±10.57	19.31±5.15	17.81±3.99	15.24±8.67*	12.58±2.78*

ALT(U/l)	140.58±18.92	115.43±12.63	110.38±9.79	120.13±27.32	142.70±17.39	130.20±15.29

Table 4.5: Effect of treatment on various Biochemical parameters of alloxaninduced diabetic rats

Mean ± SEM, n = 4, *****p<0.05 indicates statistically significant difference versus Diabetic control using one-way ANOVA followed by *post hoc* Tukey HSD analysis.

4.2.5 Effect of treatment on relative organ weight of normal and alloxan-induced diabetic rats

From Figure 4.5 below, there was no significant difference in the relative weights of the kidney and pancreas of treated groups compared with that of the normal control group. The relative weights of the liver in FEB-treated groups were slightly higher, compared with the normal control group. This was however not significant.

In the diabetic groups, the relative weight of the pancreas in diabetic control group was slightly lower than the other groups. This was not significant. The values of the relative weights of the liver and kidney for the treated groups were statistically not different from the control groups (Figure 4.6).

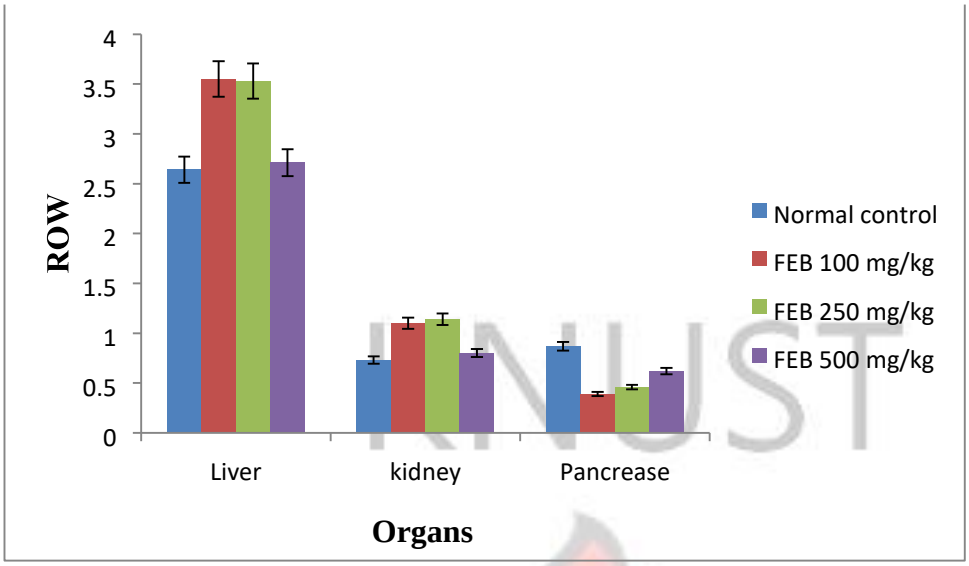


Figure 4.5: Effect of treatment on relative organ weight of normal rats. Each point represents a mean of 4 rats

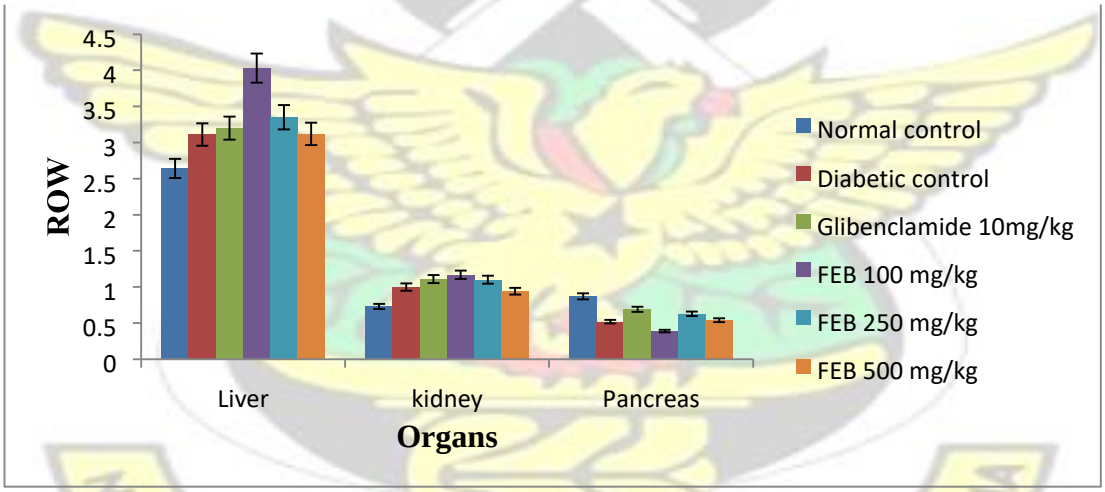


Figure 4.6: Effect of treatment on relative organ weight of alloxan-induced diabetic rats. Each point represents a mean of 4 rats

4.3 Sub chronic antidiabetic study

The sub chronic antidiabetic study was conducted in normal and alloxan-induced diabetic rats for 28 days.

4.3.1 Effect of treatment on FBG levels of normal and alloxan-induced diabetic rats

The FBG of both normal control and FEB treated rats after 28 days were not statistically different. There was very slight reduction in FBG in all the groups after day 28. Figure 4.7 shows the reduction from D0 to D28.

The FBG levels in diabetic groups were significantly high compared to the normal control group following induction of diabetes. FBG levels in diabetic control increased (from 16.83±1.93 to 20.17±0.58 mmol/l) after 28 days. Glibenclamide (10 mg/kg b.wt) reduced FBG levels significantly ($p<0.05$) in diabetic rats after 14 and 21 days. FEB 250 mg/kg b.wt reduced FBG levels significantly ($p<0.05$) in diabetic rats after 21 and 28 days, while FEB 500 mg/kg b.wt caused a significant reduction ($p<0.01$) in FBG after 14, 21 and 28 days Figure 4.8).

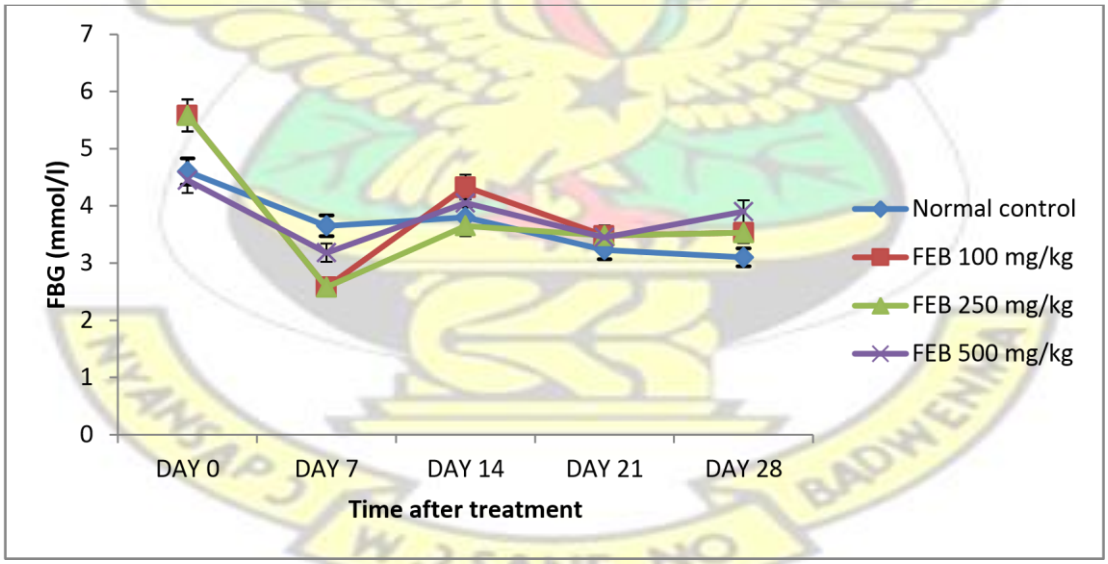


Figure 4.7: Effect of treatment on FBG levels (mmol/l) of normal rats. Each point represents a mean of 4 rats

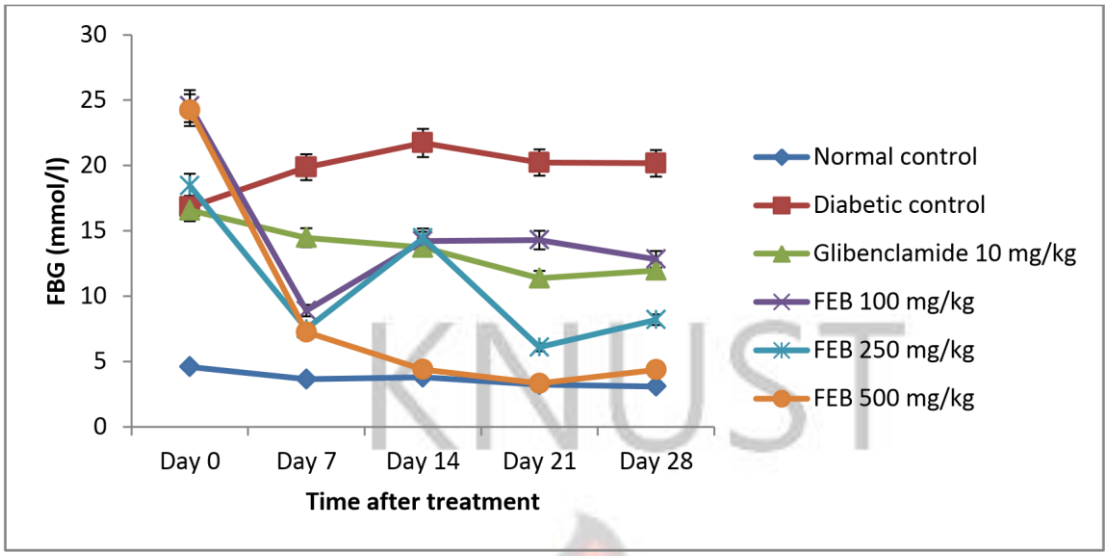


Figure 4.8: Effect of treatment on FBG levels (mmol/l) of alloxan-induced diabetic rats. Each point represents a mean of 4 rats

4.3.2 Effect of treatment on body weight of normal and alloxan-induced diabetic rats

There were weight gain after D0 in both normal control and FEB treated groups. The percent weight gain was significant in FEB treated rats compared to the normal control group ($p<0.05$) (Figure 4.9)

In the diabetic groups, there was weight reduction in diabetic rats following induction, while the normal control rats appreciated in weight. The percent increase in weight in FEB 500 mg/kg treated rats was significant ($p<0.05$) after D16, D20, D24 and D28 when compared with the diabetic control group (Figure 4.10).

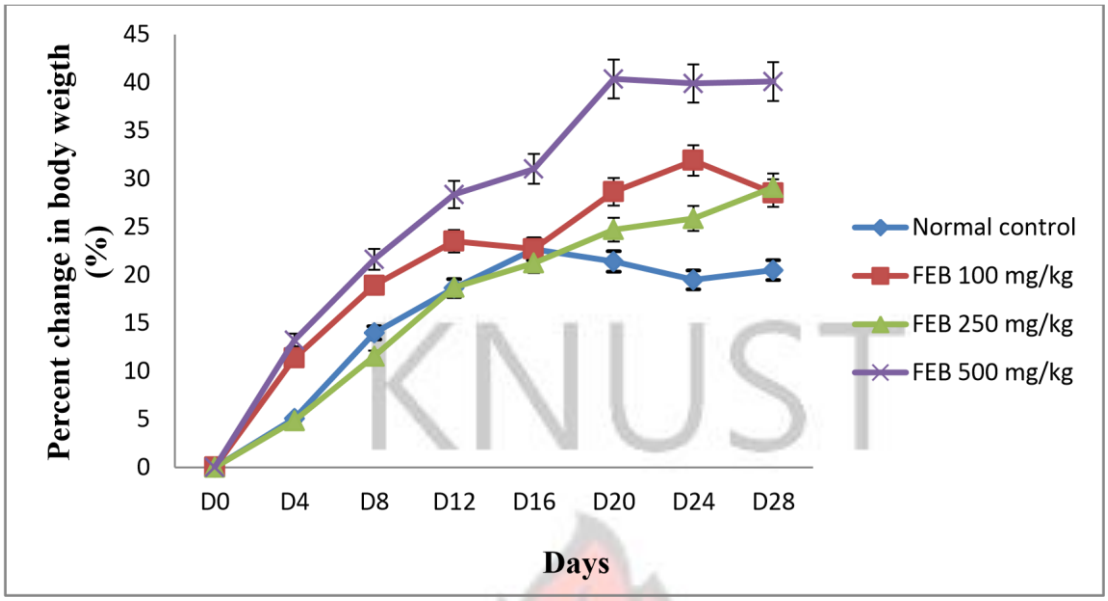


Figure 4.9: Effect of treatment on body weight of normal rats. Each point represents a mean of 4 rats

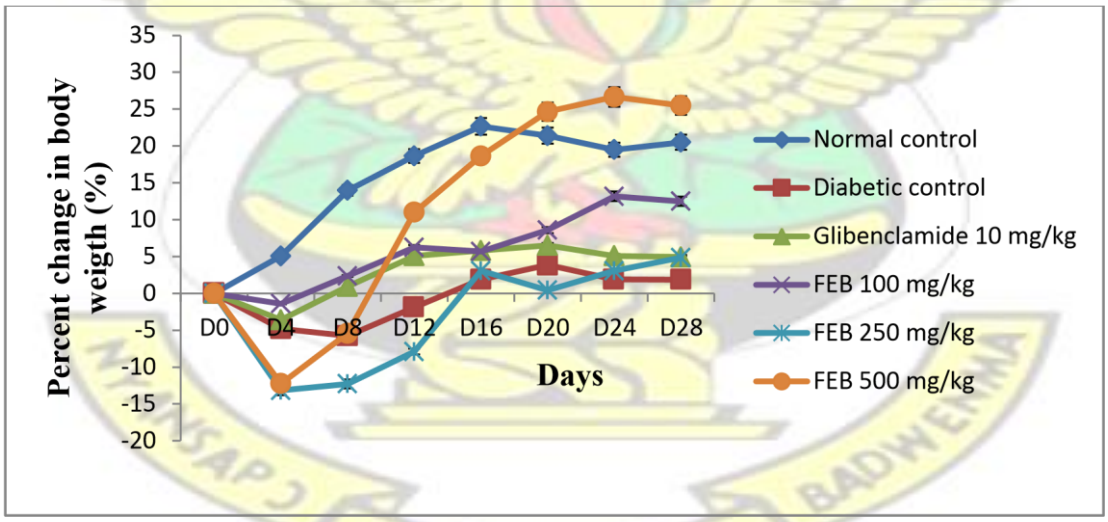


Figure 4.10: Effect of treatment on body weight of alloxan-induced diabetic rats. Each point represents a mean of 4 rats

4.3.3 Effect of treatment on various Biochemical parameters of normal and alloxan-induced diabetic rats

In the normal groups, TC levels were slightly elevated in FEB (100 and 250 mg/kg) treated rats compared to the normal control group while FEB 500 mg/kg reduced it slightly. TG levels in FEB treated groups were not statistically different from the normal control group. HDL and LDL levels were slightly increased in FEB (100 and 250 mg/kg) treated rats. The CR levels were not statistically different (Table 4.6). Creatinine levels were slightly reduced in FEB treated rats. Urea levels in FEB treated rats were also not statistically different from those in the normal control group. The levels of ALT in FEB treated rats were not statistically different from those in the normal control group

In the diabetic groups, glibenclamide (10 mg/kg) increased lipid profile indices insignificantly in diabetic rats. FEB did not cause any significant change in the parameters measured in diabetic rats. The CR for all the groups was statistically the same as shown in Table 4.6. FEB at 500 mg/kg caused a significant reduction ($P<0.05$) in the levels of urea and ALT in diabetic rats compared with diabetic control group. There were no significant difference in the values of creatinine, urea and ALT, in glibenclamide and FEB (100 and 250 mg/kg) treated rats compared with diabetic control group (Table 4.7).

Table 4.6: Effect of treatment on Biochemical parameters of normal rats

Treatments

Parameters	Normal control	FEB 100 mg/kg	FEB 250 mg/kg	FEB 500 mg/kg
TC(mmol/l)	1.94±0.06	2.46±0.29	2.21±0.06	1.82±0.32
TG(mmol/l)	0.87±0.09	1.14±0.18	0.88±0.07	0.92±0.15
HDL(mmol/l)	0.81±0.04	0.94±0.09	0.90±0.057	0.68±0.12
LDL(mmol/l)	0.73±0.05	0.99±0.12	0.91±0.02	0.72±0.18
VLDL(mmol/l)	0.40±0.04	0.52±0.08	0.40±0.03	0.42±0.07
CR(ratio)	3.30±0.09	3.60±0.07	3.40±0.18	3.68±0.03
Creatinine(µmol/l)	56.48±3.27	46.35±1.72	54.78±1.79	43.03±8.01
Urea(mmol/l)	9.17±0.87	11.04±1.71	6.95±0.35	5.39±0.48
ALT(U/l)	130.02±7.23	153.98±30.18	137.48±12.67	127.00±31.71

Mean ± SEM, n = 4,

Table 4.7: Effect of treatment on various Biochemical parameters of alloxaninduced diabetic rat

	Treatments					
parameters	Normal control	Diabetic control	Glib10 mg/kg	FEB 100 mg/kg	FEB 250 mg/kg	FEB 500 mg/kg
TC(mmol/l)	1.94±0.056	1.47±0.14	1.63±0.032	2.26±0.088	1.27±0.06	1.97±0.09
TG(mmol/l)	0.87±0.09	0.42±0.017	0.68±0.01	0.98±0.11	0.29±.02	0.87±0.11
HDL(mmol/l)	0.81±0.04	0.64±0.01	0.73±0.03	0.85±0.09	0.66±.080	0.70±0.02
LDL(mmol/l)	0.73±0.054	0.61±0.14	0.58±.007	0.94±0.08	0.49±0.03	0.92±0.05
VLDL(mmol/l)	0.40±0.04	0.19±0.00	0.30±0.00	0.47±0.05	0.13±0.011	0.39±0.05
CR(ratio)	3.30±0.09	3.03±0.18	3.07±0.18	3.75±0.33	2.80±0.21	3.93±0.26
Creatinine(µmol/l)	56.48±3.27	31.77±1.35	37.43±1.42	50.03±5.25	33.93±1.50	36.43±0.52
Urea(mmol/l)	9.17±0.87	15.41±0.79	10.68±0.37	13.25±2.29	23.69±2.88	4.13±0.13*
ALT(U/l)	130.03±7.24	170.40±1.60	113.47±6.87	144.35±24.18	181.67±3.93	87.03±5.23*

Mean ± SEM, n = 4, *****p<0.05 indicates statistically significant difference versus Diabetic control using one-way ANOVA followed by *post hoc* Tukey HSD analysis.

4.3.4 Effect of treatment on the Relative Organ Weights of normal and alloxaninduced diabetic rats

From Figure 4.11, the values of the relative weights of the organs measured; liver, kidney and pancreas were not statistically different in the normal rats.

In the diabetic groups, there was no significant difference in the values of the ROW between the treated groups and the control groups. The relative weight of the kidney in diabetic control group was slightly higher than the other groups (Fig. 4.12).

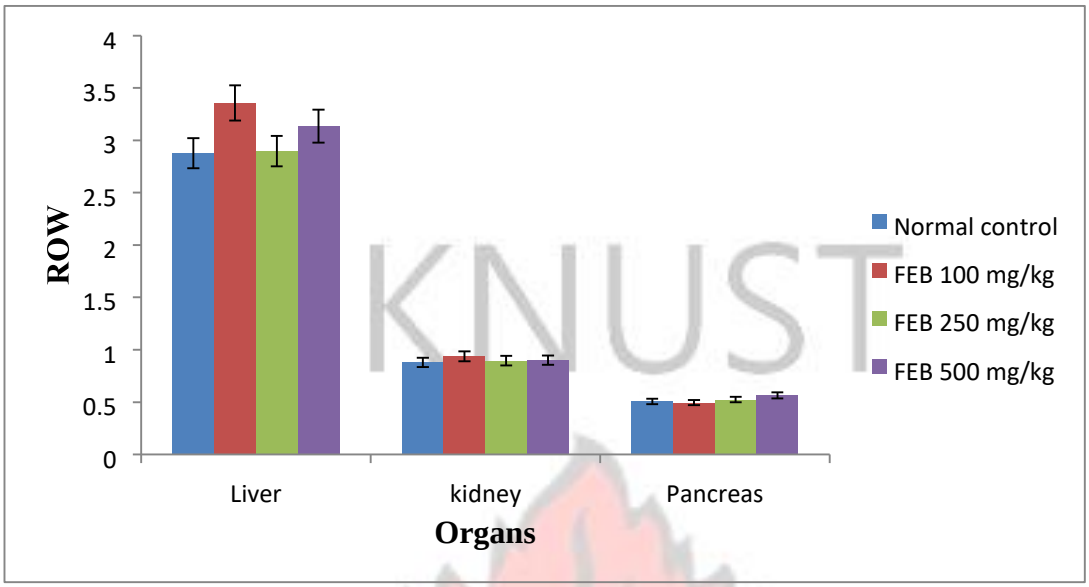


Figure 4.11: Effect of treatment on the Relative Organ Weights of normal rats. Each point represents a mean of 4 rats

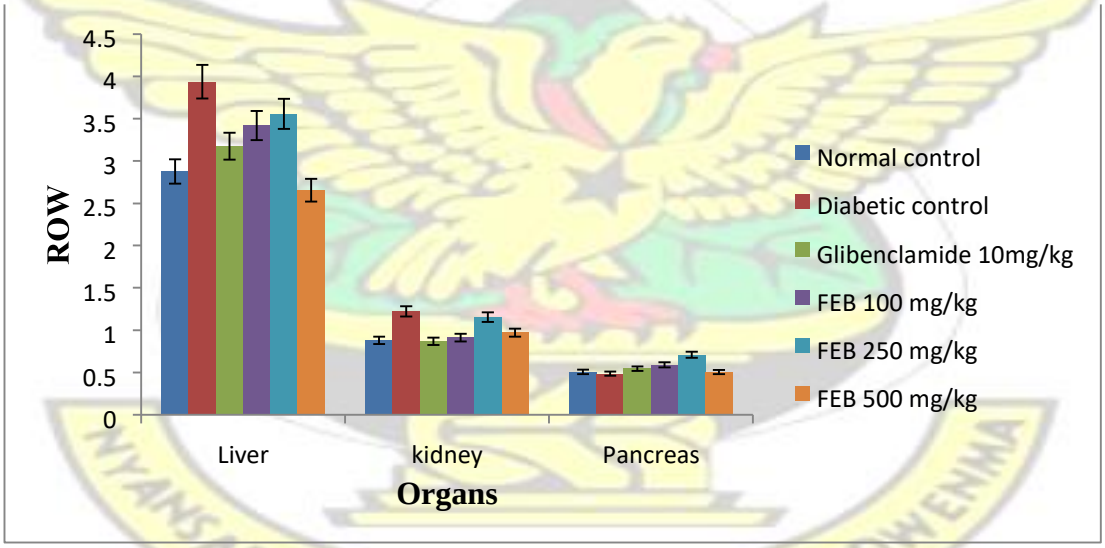


Figure 4.12: Effect of treatment on Relative Organ Weights of alloxan-induced diabetic rats. Each point represents a mean of 4 rats

CHAPTER FIVE

5.0 DISCUSSION

The fruit extract of *B. aethiopum* (FEB) showed the presence of bioactive compounds such as tannins, saponins, alkaloids glycosides and triterpenoids. Flavonoids, anthracenes and sterols were however absent. This is in line with the findings of Sarkodie *et al*. (2015) where the fruit extract of Borassus was found to contain saponins, alkaloids, triterpenoids, tannins and sterols. The slight variation in the results, the presence of glycosides and the absence of sterols in this study, could be due to the different methods employed in the extraction of the fruit. Whereas FEB is an aqueous fruit extract, the extract used by Sarkodie *et al*. (2015) was an ethanolic extract of *Borassus aethiopum*. The presence of these compounds is an indication that the FEB possesses medicinal properties because most of these phytochemicals have been implicated to be medicinal in some studies. Extracts of glycosides and alkaloids reportedly reduced high blood glucose in alloxanized diabetic rats (Okonta and Aguwa, 2007).

There was no significant change in the FBG levels of normoglycaemic animals in both acute and sub-chronic studies. This showed that the FEB neither caused hyperglycaemia nor hypoglycaemia in normal animals. This is an indication that FEB is safe for consumption by normal animals since it is a good source of antioxidants (Amoateng *et al*., 2010) and phytochemicals (Sarkodie *et al*., 2015). FEB will help keep animals well hydrated since it has high water content (79.13 to 81.38%) (Ali *et al*., 2010). In alloxan-induced diabetic rats, FEB at 100 and 250 mg/kg and glibenclamide 10 mg/kg did not cause a significant reduction in FBG after 7 days of treatment. This could be due to the duration of the study or the doses administered. FEB 500 mg/kg however caused a significant reduction in the FBG of diabetic animals

after 2 h. (P=0.043), 4 h. (P=0.047) and 8 h. (P=0.048) of treatment and after 7 days of treatment (P =0.013).The mean reduction in FBG by FEB 500 mg/kg ($p < 0.05$) was however less than the values recorded by Pradeep *et al*. (2015) ($p < 0.01$) after 6 hours of treatment with 600 mg/kg b.wt with the inflorescence extract of *Borassus flabellifer*, a plant that belongs to the same family as *Borassus aethiopum*. This difference could be due to the fact that these plants are of different species and that FEB is an aqueous fruit extract whereas the study by Pradeep *et al*. (2015) used an ethanolic extract of the inflorescence of *Borassus flabellifer*.

In the sub chronic study, glibenclamide 10 mg/kg b.wt caused significant reduction in FBG levels after 14 (p=0.013) and 21(p=0.043) days (D0 [16.56±2.61], D14 [13.73±1.30] D21 [11.36±3.08]). This could be due to the extended length of treatment that allowed glibenclamide to exert its hypoglycaemic effect. There was a dose-dependent effect of FEB on FBG in diabetic rats. FEB 250 mg/kg reduced FBG significantly D7 (P=0.041), D 21 (P=0.01) and D 28 (P=0.03). This could be due to the duration of treatment since the FEB 250 mg/kg did not reduce FBG levels significantly in the acute study. Also, FEB 500 mg/kg showed anti-diabetic property by significantly reducing FBG levels in diabetic rats throughout the study (after D7 [P=0.015], D14 [P=0.00], D 21[P=0.00] and D28 [P=0.00]). The reduction in FBG by FEB 500 mg was more significant ($p< 0.01$) compared with glibenclamide ($p< 0.05$) after 28 days, but was the same compared with Pradeep *et al*., (2015) ($p< 0.01$), when the antidiabetic property of ethanolic extract of the inflorescence of *Borassus flabellifer* was investigated. This could be due to the fact that both plants belong to the same family (Aracaecea). The antidiabetic property exhibited by FEB could be attributed to the individual or synergistic effects of its phytochemical constituents. These bioactive compounds have been implicated in some animal studies

to possess antidiabetic activities. Extracts of glycosides and alkaloids reportedly reduced hyperglycaemia in alloxanised diabetic rats (Okonta and Aguwa, 2007). The hypoglycaemic activities of saponin extracts were also reported by Yoshikawa *et al*. (1996). No previous study has investigated the anti-diabetic properties of FEB. The significant reduction of the FBG is an indication that FEB is antihyperglycaemic and suitable for the control of high blood glucose especially in type 1 diabetes.

The FEB also had a positive effect on the weight of animals treated. In the acute study, normal animals treated with FEB appreciated in weight while animals in the normal control group lost weight at D4. There was significant difference ($P<0.05$) in the percent body weight in all FEB treated groups compared with the normal control group. In the sub-chronic study, FEB at 500 mg/kg caused a significant increase in percent body weight at D16, D20, D24 and D28.

Weight loss which is seen as a symptom of diabetes (IDF, 2014) was observed among diabetic rats following induction by alloxan. The diabetic groups began to appreciate weight after D4. Glibenclamide 10 mg/kg b.wt did not cause any significant change in the body weights after 28 days of treatment. This is contrary to reports by Bennet *et al*., (2011a) that sulfonylureas cause weight gain. This could be due to the duration of treatment or the dose used in this study. There was weight gain in all FEB treated groups throughout the studies. FEB at 250 and 500 mg/kg caused a significant dose dependent increase in the percent body weight in the acute study while FEB at 500 mg/kg caused a significant increase in percent body weight of diabetic animals in the subchronic study ($P<0.05$). There was no significant change in the percent body weight when animals were treated with FEB 100 mg/kg. This is an indication that normal metabolism of the diabetic animals was not interfered with at this dose. The appetite

levels were not altered and thus feed intake was equally not interfered with. This is an indication that FEB does not induce weight loss. With its anti-diabetic activity established in this study, it could also serve as a good source of nourishment especially for type 1 diabetic subjects who usually suffer weight loss (IDF, 2014). The significant increase in the percent body weight of animals could be attributable to the presence of sugars in the FEB (Koffi *et al.*, 2010; Ali *et al*., 2010). The FEB could also increase feed intake of animals, by elevating their appetite.

The effect of FEB on some haematological parameters of normoglycaemic and diabetic animals was assessed after 7 days of treatment. There was a general lack of significant changes in the values of RBC, HGB, HCT and LYM in both normal and diabetic rats. This is an indication of safety of FEB. In the normal rats, WBC were increased significantly in FEB 100 mg/kg treated rats while PLT levels were also increased in FEB 500 mg/kg treated rats. FEB at 500 mg/kg and glibenclamide 10 mg/kg caused a significant increase in PLT levels in diabetic rats after 7 days of treatment. According to Soetan *et al*. (2013), the main task of the WBC and its differentials are to defend the body against disease invasions by fighting infections and distributing antibodies for the purpose of immune response. Animals with high levels of WBC are able to resist diseases by generating antibodies for immunity.

Those with significantly low counts are exposed and stand the risk of infection (Soetan *et al*., 2013). The observed significant increase in WBC level highlights the beneficial effect of FEB in improving the immunity and general health of the animals.

Aster (2004) reports that, the mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration shows the level of blood in the body. A significantly reduced level indicates the presence of anaemia. The non-significant difference in haemoglobin concentration recorded in this study could imply that FEB at all doses

does not induce anaemia, thereby making it safe. Also, the insignificant changes in the level of the RBC is an indication that the supply of oxygen and carbon dioxide to tissues and lungs respectively in the animals was not interfered with as suggested by Isaac *et al.* (2013); RBCs are implicated in oxygen and carbon dioxide transport in the body. A reduction in the level of RBC therefore means that low levels of oxygen will be carried to tissues and limited carbon dioxide will return from the lungs. According to Purves *et al*. (2003), blood platelets are involved in the clotting of blood. This implies that a low level of platelets will result in prolonged clot formation which will lead to excessive haemorrhage during injuries. There was a significant increase in the level of platelets in FEB at 500 mg/kg b.wt (P=0.039) and glibenclamide 10 mg/kg (P=0.02) treated animals. This is an indication that FEB at a dose of 500 mg/kg b.wt will reduce haemorrhage. This may be due to the presence of antioxidants and phytochemicals in FEB. Also, the insignificant change in the level of the haematocrit is an indication of safety of FEB since it will not interfere with the transport of absorbed nutrients in animals. This is supported by Isaac *et al.* (2013), who reports that the haematocrit (HCT) which is also known as the Packed Cell Volume is involved in the transport of oxygen and absorbed nutrients. FEB therefore, shows a high level of safety and keeps the haematological parameters in safe ranges.

Also, the effect of FEB on various biochemical parameters were measured and there was no significant change in the levels of parameters of lipid profile (TC, TG, HDL, LDL and VLDL) in both acute and sub-chronic studies for normal and diabetic animals. There were no significant changes in the coronary risk of FEB-treated animals. This is an indication that FEB does not alter the lipid profiles of the animals.

This makes it a safe for the animals. The safety of FEB could be due to the presence of phytochemicals such as saponins, tannins, alkaloids and glycosides. The fruit is also reported to be low in total lipids (0.16g/100g) (Ali *et al*., 2010).

There was no significant change in the level of creatinine among normal and diabetic animals in both acute and sub-chronic studies. This is an indication that FEB is safe and does not cause any damage to the kidney since creatinine levels are often seen as a measure of renal function. Urea, which is also used as a measure of renal function, was significantly reduced in diabetic animals in both studies. FEB at 250 (P=0.035) and 500 mg/kg (P=0.026) b.wt caused a significant reduction in urea levels in diabetic rats in acute studies, while FEB at 500 mg/kg b.wt also reduced it significantly (P=0.04) in sub-chronic studies. This is an indication that FEB offers protection for the kidney.

Alanine Aminotransferase (ALT) is usually an indicator of liver function. The liver releases ALT and an elevation of this enzyme in plasma is an indicator of liver damage (Crook, 2006). ALT level was significantly reduced by FEB 500 mg/kg b.wt. in normal animals in acute study (P=0.049) and in diabetic animals in sub chronic study (P=0.04). This is an indication that FEB is hepato-protective. The nephron- and hepato-protective effect of FEB is attributable to the fact that it is rich in antioxidants and exhibits free radical scavenging properties (Amoateng *et al*., 2010). This could also be due to the fact that FEB was not only found to contain antioxidants, but also possesses anti-microbial and anti-inflammatory agents (Sarkodie *et al*., 2015). The presence of bioactive compounds such as tannins, saponins, alkaloids and glycosides could also offer protection for these vital organs.

The relative organ weights of FEB-treated animals were not significantly different from normal control animals and diabetic control animals for both acute and subchronic studies. There was no significant change in the relative weights of the liver, kidney and pancreas. This is an indication that the treatment had no deleterious effect on the organs and for that matter is very safe for the animals. This was evident in one part of this study where FEB was not only found to be safe for the liver and kidney, but also offered protection for these organs by reducing the levels of ALT and urea significantly in alloxanized diabetic rats after 7 days and 28 days of treatment. The likely factors for the safety of FEB to the organs of animals are the presence of antioxidants in the extract. It is also said to possess free radical scavenging properties that can prevent damage to organs due to oxidative stress (Amoateng *et al*., 2010). The anti-microbial and anti-inflammatory properties of FEB reported by Sarkodie *et al*., (2015) could also offer this level of protection for the organs. The presence of the phytochemicals in FEB could also offer some level of protection for the organs of the animals.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The study investigated the anti-diabetic and hypolipidaemic effect of the aqueous ripe fruit extract of *Borassus aethiopum* in (FEB) in alloxan-induced diabetic rats. It sought to assess the phytochemical content of the fruit and to determine its efficacy as an anti-diabetic agent. The hypothesis that the FEB could reduce fasting blood glucose in diabetic rats has been proven. The study has shown that FEB at 500 mg/kg b.wt can reduce fasting reduced FBG in diabetic rats in acute studies. In sub-chronic studies, FEB at 250 and 500 mg/kg b.wt reduced FBG significantly in diabetic rats indicating that it is anti-diabetic. The FEB however, did not reduce FBG in normal rats in both acute and sub-chronic studies. The study has also shown an overall safety of FEB as a fruit for consumption.

It was found that FEB was nephro and hepato-protective in diabetic rats. Urea levels were reduced significantly in acute study while both urea and ALT levels were reduced in sub-chronic study. Parameters of lipid profile measured (TC, TG, HDL, LDL, and VLDL) as well as the coronary risk was not altered in both normal and diabetic rats in both acute and sub-chronic studies.

Administration of FEB also showed a favourable effect on various haematological parameters measured, increasing significantly the levels of platelets (PLT) in both normal and diabetic rats in the acute study. It also increased significantly the levels of PLT and WBC in normal rats in acute studies. It was equally found to be safe for body organs (liver, kidney and pancreas) since it had no deleterious effects on them.

This was evident in the lack of changes in the relative organ weights measured in both acute and sub chronic studies. The body weights of diabetic rats were increased significantly by FEB 250 and 500 mg/kg b.wt in acute study. The same was observed in sub-chronic studies by FEB 500 mg/kg b.wt.

The study therefore shows that FEB is anti-diabetic, nephron and hepato-protective. It is generally safe for consumption and has no deleterious effects on the organs of the experimental rats.

6.2 RECOMMENDATIONS

Following the findings from this study, the following suggestions are made:

1. The hypolipidaemic effect of FEB should be investigated by inducing dyslipidaemia in experimental animals to effectively assess its effects on the parameters of lipid profile.
2. Further studies should be conducted on the antidiabetic properties of FEB by conducting histological studies on the pancreas to determine the level of pancreatic beta cell regeneration after the treatment with FEB.
3. *Borassus aethiopum* fruit should be processed by food processing companies into a fruit juice for consumption by the general public.
4. People living in areas where *Borassus aethiopum* is abundant, as well as the general public should be educated on the enormous health benefits of *B. aethiopum*, and its consumption encouraged.

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