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Anti-diabetic effect of aqueous fruit extract of *Borassus aethiopum* (Mart.) in alloxan-induced diabetic rats

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Abstract

Introduction: Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia 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.

Objective: The current study was aimed at assessing the phytochemical constituents as well as the antidiabetic and hypolipidaemic effect of fruit extract of *B. aethiopum* in alloxan-induced diabetic rats.

Materials and Methods: The fruits extract was prepared (FEB) and phytochemical constituents evaluated using standard methods. The antidiabetic and hypolipidaemic properties in alloxanized rats was assessed for 7 and 28 days. Normoglycaemic and alloxan-induced diabetic rats were treated with FEB at doses of 100 mg, 250 mg and 500 mg/kg body weight. Observations on body weight, relative organ weight, haematological and biochemical parameters were measured in both acute and sub-chronic circumstances.

Results: The presence of tannins, saponins, glycosides, triterpenoids and alkaloids were detected. 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 mg and 500 mg/kg body weight in sub-chronic studies. WBC and PLT levels were significantly increased after treatment with 500 mg/kg body weight. Urea and ALT levels also reduced significantly in both acute and sub-chronic studies.

Conclusion: The aqueous fruit extract of *B. aethiopum* is antidiabetic. It was also found to be nephron- and hepato-protective as well as boosting the immunity of the animals.

Keywords : *Borassus aethiopum*, Alloxan, Diabetes, hepatoprotection

Introduction

Diabetes Mellitus (DM) is a group of metabolic disorders that results from inadequate insulin action leading to a prolonged increase in plasma glucose levels [1]. 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% [2]. Diabetes is among the first ten causes of mortality in the world accounting 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 [2].

The high prevalence rate of DM is a worry because DM is the main cause of kidney failure, poor eye sight and blindness [3]. 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 [4]. People suffering from DM may require double, or even thrice the health care resources required for other patients. It is also said that an estimated 15% of a nation's health care budget may be allocated to the management of DM [3].

Diabetes management involve three main parts; dietary regulation together with physical activity, oral hypoglycaemic agents and insulin injection [5]. The orthodox medical way of managing DM

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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 [6]. About 80% of the population in Ghana depend on herbal products for their primary health care. 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 [7]. *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 [8]. 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 [9]. 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 [10]. However, no scientific study has been carried out on the antidiabetic activity of the fruit of *B. aethiopum*. This study was designed to principally investigate the antidiabetic activity of the aqueous fruit extract of *B. aethiopum* in experimental rats.

Materials and Methods

Plant preparation and extraction

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 and voucher specimen (KNUST/M2/2016/R003) deposited at the department's herbarium. The extract was prepared by methods previously describe by Amoateng et al [11] and Koffi et al [12] with slight modification. Briefly, the fruits were washed with tap water, and 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 and the resulting powder referred to as an aqueous ripe fruit extract of *B. aethiopum* (FEB).

Phytochemical screening of FEB

Qualitative phytochemical analysis was performed on FEB to ascertain the presence of phytochemicals using standard methods [13-15]. The various phytochemicals tested for, included saponins, tannins, flavonoids, sterols, terpenoids, general glycosides, alkaloids, and anthracenes.

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Preparation of test materials

Preparation of Alloxan solution

Alloxanmonohydrate (CDH, India) was dissolved in normal saline to form an aqueous solution. This was administered at a dose of 150 mg/kg body weight (bwt) intraperitonially to induce diabetes [16].

Preparation of standard drug

Glibenclamide (Diabenol, Thailand) tablets, an oral hypoglycaemic drug [17] were used. This was dissolved in freshly distilled water to form a suspension and was administered at a dose of 10 mg/kg bwt orally.

Experimental Animals

Healthy female albino rats weighing 115-150 g obtained from the Centre for Scientific Research into Plant Medicine, Mampong-Akuapem were 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 hrs. light/dark cycle). Animal were provided freshly prepared distilled water and standard laboratory food (Mash, AgriCare, Kumasi, Ghana) during the study period.

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 [18]. 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.

Experimental Design

Following induction of diabetes, the animals (both diabetic and normal) were divided randomly into nine (9) groups of four (4) body weight matched rats each as follows and treated for 7 days for acute study and 28 days for sub-chronic study.

Group I – Normal rats control administered 0.5 ml distilled water per day orally.
Group II – Alloxan-induced diabetic rats control administered 0.5 ml distilled water per day orally.
Group III - Normal rats administered 100 mg/kg bwt of FEB per day orally.
Group IV – Normal rats administered 250 mg/kg bwt of FEB per day orally.
Group V - Normal rats administered 500 mg/kg bwt of FEB per day orally.
Group VI – Alloxanized diabetic rats administered 100 mg/kg bwt of FEB per day orally.
Group VII – Alloxaniszed diabetic rats administered 250 mg/kg bwt of FEB per day orally.
Group VIII- Alloxanized diabetic rats administered 500 mg/kg bwt of FEB per day orally.
Group IX – Alloxanized diabetic rats administered 10 mg/kg bwt of glibenclamide per day orally.

Determination of Fasting Blood Glucose (FBG)

Antidiabetic effects were assessed by tail puncture for fasting blood glucose determination at intervals of 0, 2, 4, 8 hours and daily for 7 days for acute antidiabetic study, and at intervals of 0, 7, 14, 21 and 28 days for sub-chronic antidiabetic study. The blood glucose levels were determined using the OneTouch Select Simple Glucose meter and the results reported as mmol/l.

Determination of body weight

Body weights of animals were determined on day 0, 4 and 7 for acute antidiabetic study and on day 0, 4, 8, 12, 16, 20, 24 and 28 for sub-chronic antidiabetic study. The percent change in body weight was calculated using the formula:

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.

Determination of 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 ethylene diamine tetraacetic 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)

Determination of Biochemical parameters

Part of blood samples were put into Serum Separator Tubes (SSTs) and centrifuged at 3000 rpm 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' instructions.

Effect of treatment on organ weight

Organs of experimental 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.

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

Data analysis

Data were expressed as mean ± SEM. The data were statistically analysed using the independent sample t-test with comparisons between experimental groups and control groups. The values of $p<0.05$ were considered significant. Newman-Keuls multiple comparison tests were performed to assess difference between groups.

Results

Phytochemical content of FEB

The phytochemical screening of the fruit extract of *Borassus aethiopum* (Table 1) showed the presence of tannins, saponins, glycosides, triterpenoids and alkaloids. Flavonoids, anthracenes and sterols were absent.

Table 1: Phytochemical content of FEB

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

(+)present;(–) absent

Acute Antidiabetic Study

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

Effect of treatment on Fasting Blood Glucose levels of normal and alloxan-induced diabetic rats

FEB (100 mg, 250 mg and 500 mg/kg body weight) did not reduce fasting blood glucose levels significantly in normal rats after 7 days of treatment (Table 2). The FBG levels of diabetic rats were reduced significantly in rats treated with FEB at 500 mg/kg bwt after 2, 4 and 8 hours of treatment. FEB at 500 mg /kg bwt 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 (Table 3).

Table 2: Effect of treatment on FBG levels (mmol/l) of normal rats

Time after treatment	Normal Control	FEB 100 mg/kg	FEB 250 mg/kg	FEB 500 mg/kg
0 hours	5.73±0.61	6.03±0.56	6.25±0.54	5.48±0.81
2 hours	5.48±0.69	6.30±1.29	5.90±0.83	5.65±0.50
4 hours	4.25±0.16	4.10±0.29	5.60±1.24	4.25±0.15
8 hours	4.28±0.38	3.75±0.18	3.70±0.40	3.30±0.12
1 day	5.20±0.29	5.03±0.26	4.60±0.25	4.83±0.21
2 days	4.50±0.11	5.93±0.60	4.75±0.26	4.65±0.18
3 days	4.13±0.17	4.25±0.39	3.88±0.21	4.50±0.25
4 days	4.05±0.39	3.78±0.34	3.48±0.15	3.85±0.12
5 days	3.83±0.14	3.78±0.15	3.85±0.38	3.35±0.15
6 days	3.93±0.28	3.98±0.09	3.55±0.26	3.35±0.16
7 days	3.60±0.11	3.70±0.33	4.00±0.23	3.38±0.17

Mean ± SEM, n = 4.

Table 3: Effect of treatment on FBG levels (mmol/l) of diabetic rats

Time after treatment	normal control	Diabetic control	Glib. 10 mg/kg	FEB 100 mg/kg	FEB 250 mg/kg	FEB 500 mg/kg
0 hours	5.73±0.61	25.98±1.82	25.95±1.45	27.20±1.33	23.83±2.48	25.80±1.33
2 hours	5.48±0.69	22.93±2.06	19.83±0.89	17.90±2.74	18.18±2.77	13.15±3.04*
4 hours	4.25±0.16	18.90±3.43	19.83±0.71	14.03±2.65	13.95±3.78	8.18±2.36*
8 hours	4.28±0.38	16.45±5.24	15.18±2.35	7.38±3.85	8.95±3.65	5.85±2.72*
1 day	5.20±0.29	19.60±3.35	19.40±0.68	15.15±4.62	14.70±3.25	14.53±1.63
2 days	4.50±0.11	17.10±3.99	19.03±1.93	16.58±4.93	11.20±2.26	8.13±1.27
3 days	4.13±0.17	11.12±2.93	13.58±3.68	11.00±3.22	7.10±1.69	5.90±0.83
4 days	4.05±0.39	12.20±3.97	12.20±3.97	10.03±3.18	14.53±3.59	6.95±1.04
5 days	3.83±0.14	11.53±2.85	7.23±2.29	10.87±4.17	9.73±3.36	5.15±0.53
6 days	3.923±0.28	12.17±2.55	9.78±3.14	15.17±6.43	11.48±3.14	6.48±1.09
7 days	3.60±0.11	14.53±1.80	10.65±3.26	8.77±4.20	10.35±3.59	5.23±.24*
Mean % reduction after 8 hrs.	25.31	36.67	41.52	72.89	62.43	77.32
Mean % reduction after 7 days.	37.17	44.07	58.96	67.76	56.57	79.73

Mean ± SEM, n = 4, *p<0.05 indicates statistically significant difference versus Diabetic control

Effect of treatment on percent change in body weight of normal and alloxan-induced diabetic rats

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 in the normal rats (Figure 1). 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 in diabetic rats (Figure 2).

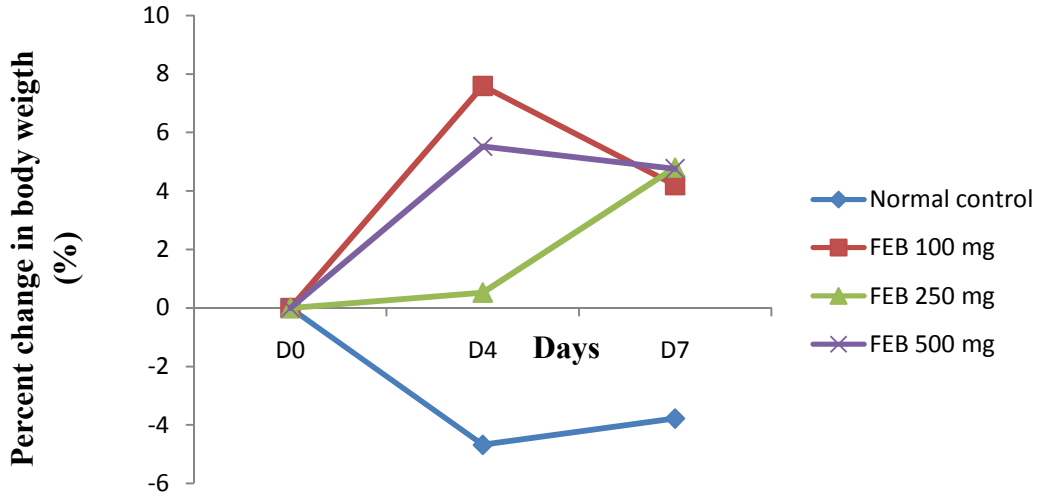

Figure 1: Effect of treatment on percent change in body weight (g %) of normal rats. Each point represents a mean of 4 rats

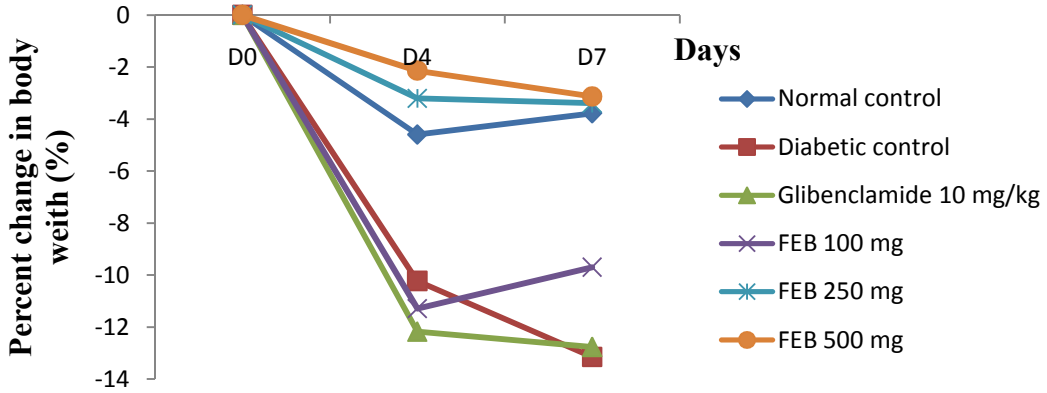

Figure 2: Effect of treatment on percent change in body weight (g %) of alloxan-induced diabetic rats. Each point represents a mean of 4 rats

Effects of treatment on Haematological parameters of normal and alloxan-induced diabetic rats

In the normal rats, 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 FEB 500 mg/kg treated groups compared to normal control group (Table 4).

In the diabetic rats, 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 (Table 5).

Table 4: Effects of treatment on Haematological parameters of normal rats

	Treatments			
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	527.00±125.82	664.00±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

Table 5: Effects of treatment on Haematological parameters of alloxan-induced diabetic rats

	Treatments					
Parameters	Normal control	Diabetic control	Glib. 10 mg/kg	FEB 100 mg/kg	FEB 250 mg/kg	FEB 500 mg/kg
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

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

In the normal rats, ALT levels were slightly elevated in groups treated with FEB at 100 and 250 mg/kg but was significantly reduced (P<0.05) in rats treated with FEB at 500 mg/kg. All other parameters measured were not significantly different from the normal control group (Table 6). In the diabetic rats, the level of urea was significantly reduced in FEB 250 and 500 mg/kg treated rats compared to diabetic control group (Table 7)

Table 6: 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	2.18±0.11	2.47±0.37	2.25±0.08	2.13±0.10
TG	0.65±0.07	0.93±0.11	0.90±0.08	0.82±0.13
HDL	0.96±0.03	1.01±0.13	0.98±0.04	0.90±0.02
LDL	0.92±0.08	0.94±0.22	0.86±0.04	0.86±0.14
VLDL	0.30±0.03	0.43±0.05	0.41±0.04	0.37±0.06
CR	3.15±0.13	3.43±0.19	3.17±0.05	3.25±0.15
Creatine	57.00±3.05	50.72±4.46	48.38±1.07	51.75±3.09
Urea	8.60±0.50	8.78±0.54	10.18±0.53	8.46±0.39
ALT	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

Table 7:Effect of treatment on various Biochemical parameters of alloxan-induced diabetic rats

	Treatments					
Parameters	Normal control	Diabetic control	Glib 10 mg/kg	FEB 100 mg/kg	FEB 250 mg/kg	FEB 500 mg/kg
TC	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	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	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	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	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	3.15±0.13	3.08±0.18	3.03±0.35	3.17±0.58	3.00±0.15	3.18±0.06
Creatine	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	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	140.58±18.92	115.43±12.63	110.38±9.79	120.13±27.32	142.70±17.39	130.20±15.29

Mean ± SEM, n = 4, *p<0.05 indicates statistically significant difference versus Diabetic control

Effect of treatment on the Relative Organ Weight (ROW) of normal and alloxan induced diabetic rats

In the normal rats, there was no significant difference in the relative weights of the kidney, liver and pancreas of treated groups compared with that of the normal control group (Figure 3). 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).

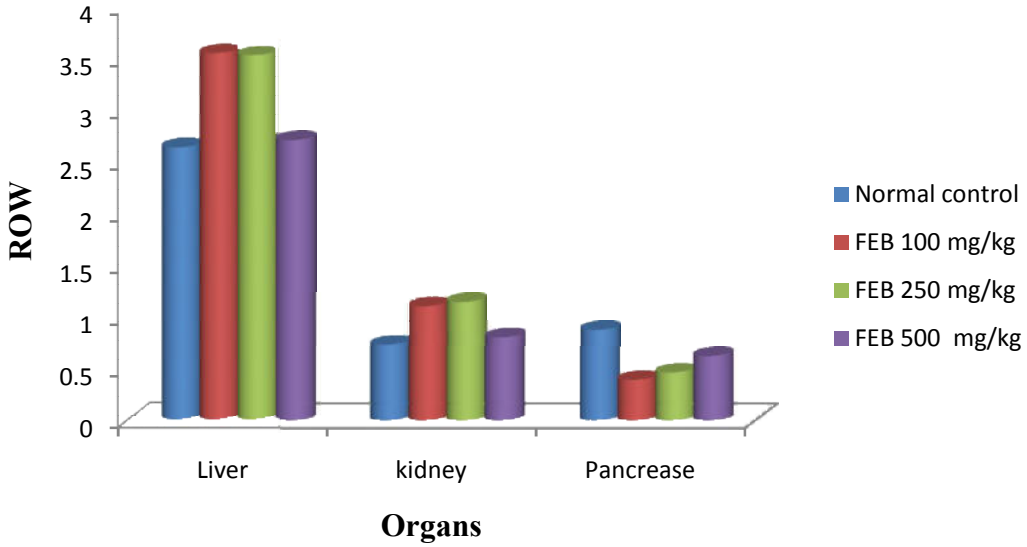

Figure 3: Effect of treatment on ROW of normal rats. Each point represents a mean of 4 rats

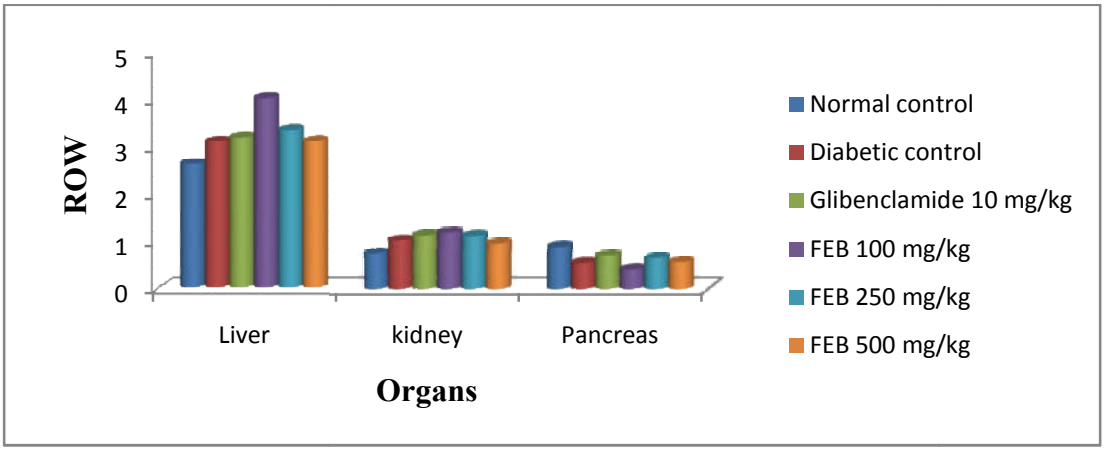

Figure 4: Effect of treatment on ROW of alloxan-induced diabetic rats. Each point represents a mean of 4 rats

Sub chronic antidiabetic study

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

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

In the normal rats, the FBG of both normal control and FEB treated rats after 28 days were not statistically different (Table 8). 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 bwt) reduced FBG levels significantly ($p<0.05$) in diabetic rats after 14 and 21 days. FEB 250 mg/kg bwt reduced FBG levels significantly ($p<0.05$) in diabetic rats after 21 and 28 days, while FEB 500 mg/kg bwt caused a significant reduction ($p<0.01$) in FBG after 14, 21 and 28 days (Table 9).

Table 8: Effect of treatment on FBG levels (mmol/l) of normal rats

Time after treatment	Normal control	FEB 100 mg/kg	FEB 250 mg/kg	FEB 500 mg/kg
Day 0	4.60±0.25	5.58±0.59	5.58±0.59	4.45±0.28
Day 7	3.65±0.33	2.58±0.22	2.58±0.23	3.18±0.09
Day 14	3.80±0.54	4.33±0.34	3.65±0.12	4.05±0.26
Day 21	3.23±0.42	3.48±0.09	3.48±0.09	3.45±0.18
Day 28	3.10±0.29	3.53±0.19	3.53±0.19	3.90±0.25

Mean ± SEM, n = 4.

Table 9: Effect of treatment on FBG levels (mmol/l) of alloxan-induced diabetic rats

Time after treatment	Normal control	Diabetic Control	Glib. 10 mg/kg	FEB 100 mg/kg	FEB 250 mg/kg	FEB 500 mg/kg
Day 0	4.60±0.25	16.83±1.93	16.57±2.61	24.55±1.91	18.45±4.40	24.25±0.54
Day 7	3.65±0.33	19.87±0.50	14.47±2.03	8.90±2.36	6.23±0.39*	7.25±4.10*
Day 14	3.80±0.54	21.73±1.17	13.73±1.30*	14.23±3.20	14.45±1.60	4.40±0.10**
Day 21	3.23±0.42	20.23±1.13	11.37±3.07*	14.30±3.86	6.10±1.14*	3.33±0.22**
Day 28	3.10±0.28	20.17±0.58	11.97±3.10	12.83±3.40	8.20±1.75*	4.37±0.28**
% Mean Reduction	32.61	-19.85	27.76	47.73	55.55	81.97

Mean ± SEM, n = 4, *$p<0.05$, **$p<0.01$ indicates statistically significant difference versus Diabetic control.

Effect of treatment on percent change in body weight of normal and alloxan-induced diabetic rats

There was weight gain after D0 in both normal control and FEB treated groups in the normal rats. The percent weight gain was significant in FEB treated rats compared to the normal control group ($p<0.05$) (Figure 5).

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 treated rats was significant ($p<0.05$) after D16, D20, D24 and D28 when compared with the diabetic control group (Figure 6).

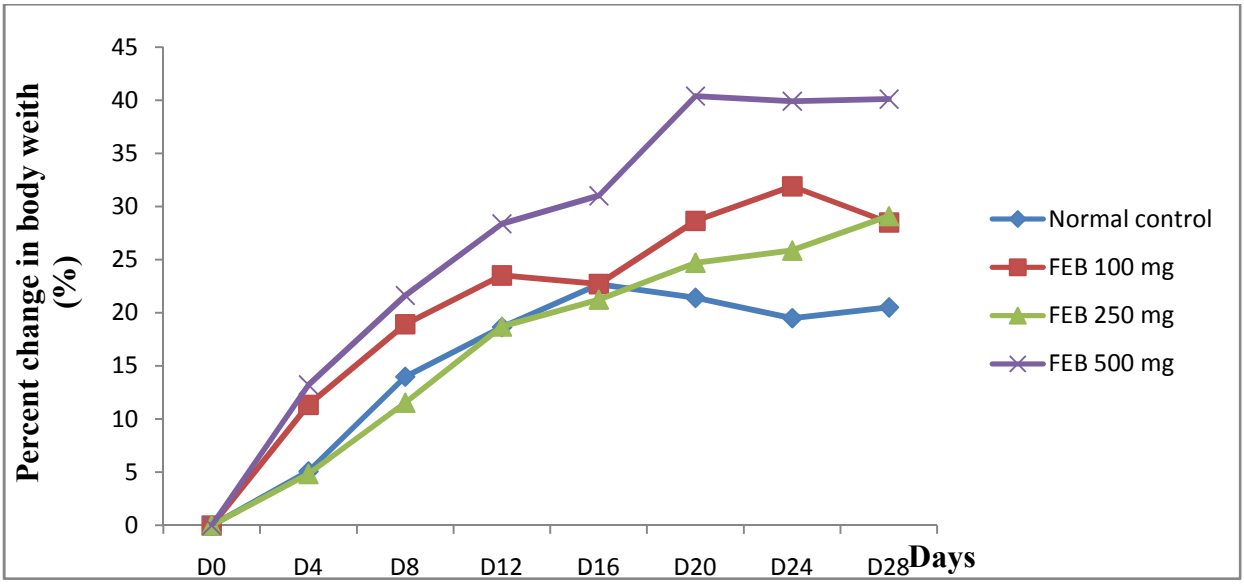

Figure 5: Effect of treatment on percent change in body weight (g %) of normal rats. Each point represents a mean of 4 rats

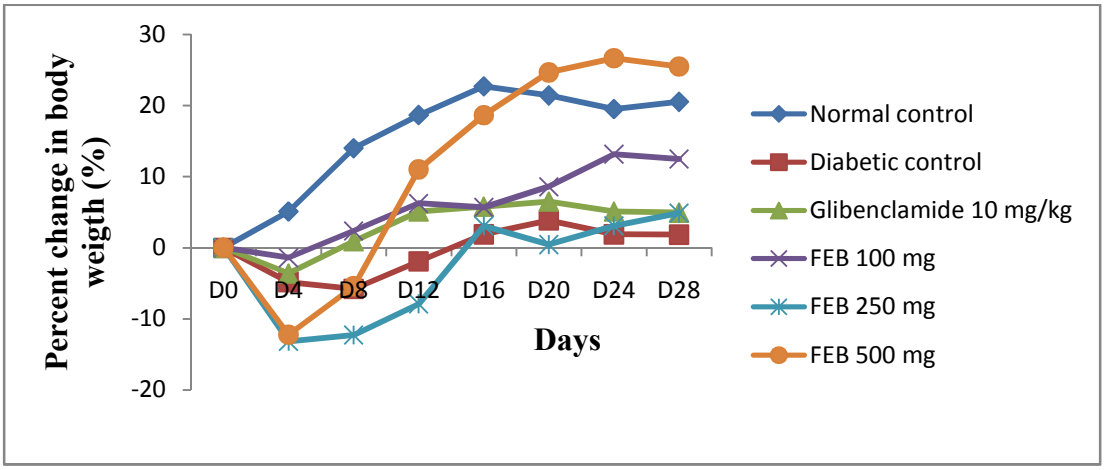

Figure 6: Effect of treatment on percent change in body weight (g %) of alloxan-induced diabetic rats. Each point represents a mean of 4 rats

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

In the normal groups, there were no significant changes in the levels of the biochemical parameters measured (Table 10).

In the diabetic groups, FEB at 500 mg/kg caused a significant reduction ($P<0.05$) in the levels of urea and ALT 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 11).

Table 10: Effect of treatment on Biochemical parameters of normal rats

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

Mean ± SEM, n = 4.

Table 11: Effect of treatment on various Biochemical parameters of alloxan-induced diabetic rat

	Treatments					
parameters	Normal control	Diabetic control	Glib 10 mg/kg	FEB 100 mg/kg	FEB 250 mg/kg	FEB 500 mg/kg
TC	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	0.87±0.09	0.42±0.017	0.68±0.01	0.98±0.11	0.29±.02	0.87±0.11
HDL	0.81±0.04	0.64±0.01	0.73±0.03	0.85±0.09	0.66±.080	0.70±0.02
LDL	0.73±0.054	0.61±0.14	0.58±.007	0.94±0.08	0.49±0.03	0.92±0.05
VLDL	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	3.30±0.09	3.03±0.18	3.07±0.18	3.75±0.33	2.80±0.21	3.93±0.26
Creatine	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	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	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

Effect of treatment on the Relative Organ Weights (ROW) of normal and alloxan-induced diabetic rats

From Figure 7, ROW of the organs measured, liver, kidney and pancreas were not statistically different in the normal rats and those treated with FEB only.

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 (Figure 8)

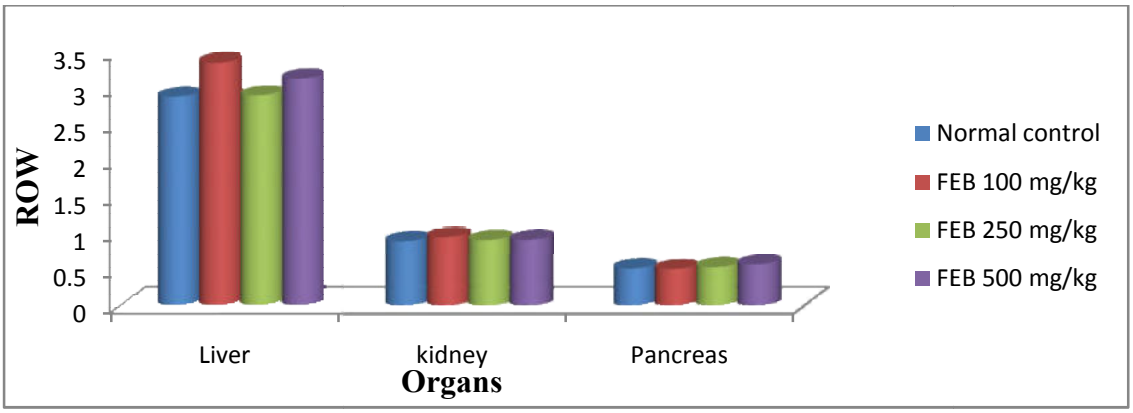

Figure 7: Effect of treatment on the ROW of normal rats. Each point represents a mean of 4 rats

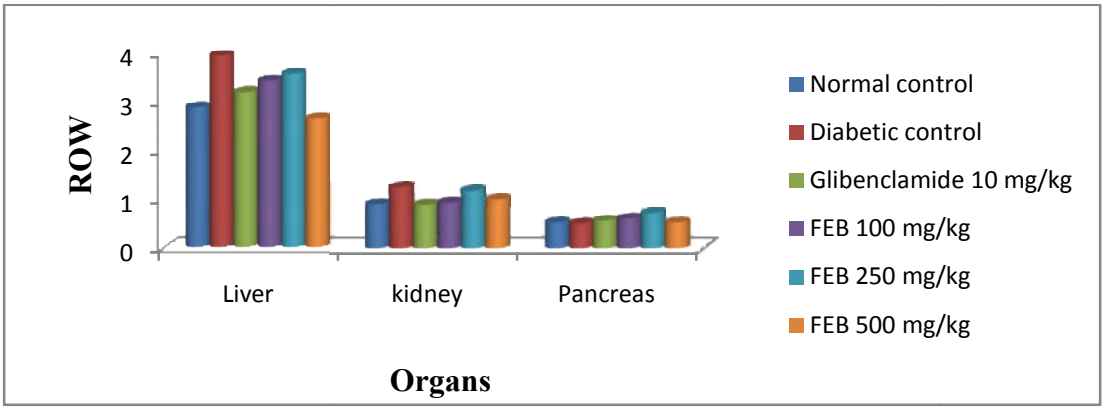

Figure 8: Effect of treatment on ROW of alloxan-induced diabetic rats. Each point represents a mean of 4 rats

Discussions

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 other findings where the fruit extract of Borassus was found to contain saponins, alkaloids, triterpenoids, tannins and sterols [19]. 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 other study used extract used 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 [20]. The hypoglycaemic activities of saponin extracts has also been reported [22].

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 [11] and phytochemicals [19]. FEB will help keep animals well hydrated since it has high water content (79.13 to 81.38%) [21]. In alloxan induced diabetic rats, FEB at 100 mg, 250 mg and glibenclamide 10 mg did not cause a significant reduction in FBG after 7 days of treatment. This could be due the duration of the study or the doses administered. FEB 500 mg/kg however caused a significant reduction in the FBG of diabetic animals ($P<0.05$), after 2, 4 and 8 hours of treatment and after 7 days of treatment. The mean reduction in FBG by FEB 500 mg ($p < 0.05$) was however less than the values recorded ($p<0.01$) by studies which used the inflorescence extract of *Borassus flabellifer,* a plant that belongs to the same family as *Borassus aethiopum* after 6 hours of treatment with 600 mg/kg b.wt. [18]. In the sub chronic study, glibenclamide at 10 mg/kg caused significant reduction ($P<0.05$) in FBG levels after 14 and 21 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 antidiabetic effect. There was a dose dependent effect of FEB on FBG in diabetic rats. FEB 250 mg/kg reduced FBG significantly ($P<0.05$), after 7, 21 and28 days. This could be due to the duration of treatment since the FEB 250 mg did not reduce FBG levels significantly in the acute study. Also, FEB 500 mg/kg showed antidiabetic property by significantly reducing FBG levels in diabetic rats after 7 days ($p<0.05$) and after 14, 21 and 28 days ($p<0.01$). 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 a study that used the inflorescence extract of *Borassus flabellife* ($p< 0.01$) [18. 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 [20]. The hypoglycaemic activities of saponin extracts were also reported [22]. No previous study has investigated the antidiabetic 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.

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 [2] 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 that sulfonylureas cause weight gain [17]. 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 at this dose. The appetite levels were not altered and thus feed intake was equally not interfered. This is an indication that FEB does not induce weight loss and is safe for diabetics. With its antidiabetic 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 [2]. The significant increase in the percent body weight of animals could be attributable to the presence of sugars in the FEB [12,21]. 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. 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 [23]. The observed significant increase in WBC level highlights the beneficial effect of FEB in improving the immunity and general health of the animals. 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. RBC are implicated in oxygen and carbon dioxide transport in the body [24]. Theinsignificant change 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.

Blood platelets are involved in the clotting of blood [25]. There was a significant increase in the level of platelets in FEB at 500 mg/kg b.wt ($P<0.05$) and glibenclamide 10 mg/kg ($P<0.05$) treated animals. This is an indication that FEB at a dose of 500 mg/kg b.wt will induce blood clotting and thereby reduce haemorrhage.

The haematocrit (HCT) which is also known as the Packed Cell Volume is involved in the transport of oxygen and absorbed nutrients [24]. 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.

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 was no significant changes in the coronary risk of FEB treated animals. This is an indication that FEB does not induce dyslipidaemia neither does it increase the risk of coronary heart diseases. This makes it a safe fruit for consumption. 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) [21].

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 ($P<0.05$) and 500 mg/kg b.wt ($P<0.05$) 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 ($P<0.05$) in subchronic studies. This is an indication that FEB is not only safe for the kidney, but offers protection.

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 [26]. ALT level was significantly reduced by FEB 500 mg/kg b.wt. in normal animals in acute study ($P<0.05$) and in diabetic animals in sub chronic study ($P<0.05$). This is an indication that FEB is hepatoprotective. 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 [11]. 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 [19]. The presence of bioactive compounds such as tannins, saponins alkaloid 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 and diabetic animals respectively for both acute and sub chronic 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. There was no damage to the pancreas since it is more likely to have played a role in the regulation of high blood glucose in the antidiabetic study.

The likely factors for the safety of FEB to the organs of animals are the presence of antioxidants in the extract and its free radical scavenging properties that can prevent damage to organs due to oxidative stress [11]. The anti-microbial and anti-inflammatory properties of FEB [19] could also offer this level of protection for the organs. The presence of the phytochemicals in FEB could also offer some level of safety for the organs of the animals.

Conclusion

Based on the results of the study, 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 antidiabetic. 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 also found that FEB was nephro and hepato-protective in diabetic rats.

Conflict of Interest

The authors declare no conflict of interest.

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