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INVESTIGATION OF ANTI-DIABETIC PROPERTIES OF *BORASSUS FLABELLIFER* L. (ROOTS) ON TYPE-2 DIABETIC RATS

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Abstract

Treatment of diabetes using plants based medicines is an adjunct therapy for controlling glycemic status with lesser side effects. The present study investigated the antidiabetic potential of *Borassus flabellifer* (BF) root extract in alloxan induced type-2diabetic rats. Sprague Dawley rats of either sex were used. Type-2 diabetes was induced by a single IP injection of alloxan monohydrate (120mg/kg) in rats and was divided into 5 groups of 6 animals each. Ethanolic extract of roots from *Borassus flabellifer* (BF 250 and 500mg/kg) and glibenclamide (10mg/kg) were orally administered once daily for 21 days in the treatment and standard group respectively. At the end of the treatment, rats were sacrificed and blood samples were collected for the estimation of total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), high density lipoproteins (HDL), S. creatinine level. After the study period of 21 days, BF extract improved the oral glucose tolerance ($p<0.001$) and fasting blood glucose level very highly significantly ($p<0.001$) at 500mg/kg b.w. as compared to the diabetic control group. The BF extract significantly lowered TC ($p<0.001$) and TG ($p<0.001$) and serum creatinine ($p<0.001$) level when compared to the diabetic control group. Our investigations suggested that ethanolic extract of *Borassus flabellifer* roots has antidiabetic properties.

Keywords: *Borassus flabellifer,* antidiabetic effect, hypolipidemic effect, alloxan induced diabetes, type-2 diabetes mellitus.

Introduction

Diabetes Mellitus (DM) is a metabolic disorder characterized by hyperglycemia with deficiency of secretion or action of endogenous insulin and no definite cause [1-3]. It is a multifactorial illness with lipoprotein abnormalities, high basal metabolic rate, and high oxidative stress induced damage [4-6]. Chronic hyperglycemia may lead to complications of diabetes like changes in metabolism, nerve, kidney, foot ulceration, and vascular tissue. Protein glycation, the most important source of free radicals, contributes to the progression of these complications in both types 1 and 2 diabetes and mediates the pathogenic effects [7,8].

Many therapeutic approaches have been utilized for treatment of diabetes including insulin and oral hypoglycemic agents. Most of the drugs in current use have been reported with serious side effects and cost of treatment is high [9]. Therefore, to face these challenges, plants can be used as the major source of drugs for the treatment of diabetes mellitus (DM) which have been used in Indian medicine and other ancient systems in the world for a long time [10]. World ethnobotanical information about medicinal plants reports that almost 800 plants could be used to control DM [10,11]. The World Health Organization (WHO) estimates that 80% of the worlds' populations use traditional medicine.The continued use of traditional medicines is linked to their low cost and a general belief that they have minimal side effects [12]. The biodiversity of flora of Bangladesh is very broad and several native Bangladeshi medicinal plant species have a long tradition of use with great phytotherapeutic potential [13]. So, research in medicinal plants is a vital sector for the discovery of promising drugs in Bangladesh [14].

Borassus flabellifer L. (Family: Arecaceae, Local name: **Tal** in Bengali) is a tall palm reaching 12-33 m, a black stem and crown of leaves at the top. It is widely distributed and cultivated in tropical Asian countries such as Thailand, India, Myanmar, Sri Lanka, Malaysia, Bangladesh *etc*. [15]. The plant has been used traditionally as a stimulant, anti-laprotic, diuretic, antiphlogistic. The fruits are useful in hyperdipsia, dyspepsia, flatulence, skin diseases, heamorrhages, fever and general debility. The roots and juice of the plant are useful in inflammatory reactions. It is useful in heart burn, spleenomegaly and in bilious fever [16-18].

The aim of the present study was to evaluate the antidiabetic effect of *Borassus flabellifer* roots extract against alloxan induced type-2 diabetic rats.

Methods

Plant collection and Extraction: The roots of *B. flabellifer* were collected, taxonomically identified and authenticated by the Bangladesh National Herbarium, Mirpur, Dhaka. The collected materials were shed dried at 35° – 40° C for a week and crushed into moderately coarse powder. This powder was extracted using ethanol, dried under reduced pressure and finally extract was obtained.

Experimental Animals: The study was conducted with adult Sprague Dawley (SD) rats (weighing 150-200g) of either sex. They were bred at the Jahangirnagar University animal house maintained at a constant room temperature of 22±5°C, 40-70% humidity conditions and the natural day-night cycle with an *ad libitum* access to food except the day of experimental procedure when animals were used after 12 hrs fasting. The rats had no access to food during the whole period of blood sampling. All protocols for animal experiment were approved by the institutional animal ethical committee.

Induction of Type 2 Diabetes: Rats were injected with a freshly prepared solution of alloxan monohydrate (i.p.) in saline (300 mM NaCl) at a dose of (120 mg/kg, b.w.). Alloxan injection can provoke fatal hypoglycemia as a result of reactive massive release of pancreatic insulin, so rats were also given orally 5–10 mL of a 20% glucose solution after 6 h. Rats were then kept for the next 24 h on a 5% glucose solution as beverage to prevent too severe hypoglycemia. After 1 week, rats displaying fasting glucose level 8-15 mmol/l were chosen for the experiments [19,20].

Acute study: OGTT was conducted in control and treated groups of rats, 24 h before decapitation of rats. All groups were administrated glucose (3g/kg) by gastric gavages route. Blood glucose levels were determined at 0, 60 and 120 min subsequently to receive glucose and fasting glucose was measured [21].

Chronic study: Hyperglycaemic (Type-2) animals were then divided into five groups of six animals each. Group I and II were treated with saline and

served as normal control and diabetic control. Group III was administered glibenclamide and served as standard. Group IV and V were administered ethanolic extract of *Borassus flabellifer* at 250mg/kg and 500mg/kg body weight by oral route. All doses were continued for 21 days in hyperglyceamic rats. Blood samples were collected from the cut tip of the tail at 0, 7, 14 and 21st day from the respective start of treatments and measured serum glucose. At the end of the experiment rats were sacrificed, blood was collected and serum lipid profile and creatinine, levels were estimated by enzymatic colorimetric method [22].

Statistical analysis: Graphs were prepared by using MS Excel 2007 and data analysis for animal studies were done by SPSS 16.0 for Windows using the One way ANOVA followed by Bonferroni's post hoc test. All the data were presented as Mean±SEM. *(P<0.05), **(p<0.01) and ***(p<0.001) were counted as significant, highly significant and very highly significant respectively as compared to the vehicle treated diabetic control group.

Results and Discussion

Text Diabetes mellitus is a chronic disease which causes millions of deaths worldwide each year as a result of the associated complications [23]. Hyperglycaemia is an independent risk factor in the development of chronic diabetic complications. Therefore the management of type 2 diabetes relies on the maintenance of blood glucose concentration in a normal or near normal level [24]. The present study investigated the effects of a medicinal plant, *Borassus flabellifer* (BF) on body weight, blood glucose, serum lipids and serum creatininein alloxan induced type-2 diabetic model rats. Treatment of diabetic rats with 250mg/kg and 500mg/kg of *Borassus flabellifer* extract in the oral glucose tolerance test (OGTT), improved glucose tolerance at 120 minute which was found to be significant (p<0.001) at 500mg/kg. Glibenclamide (10mg/kg) showed a significant fall in serum glucose level at 120 min (p<0.001).Therefore, the extract of *B. flabellifer* showed significant antihyperglycemic effect at 120 min in fasting rats as well as when fed simultaneously with oral glucose load in type 2 model rats (figure 1). It has been demonstrated that the post-prandial hyperglycemia is an important cardiovascular risk factors in type-2 diabetes [25]. Studies have shown that the post-meal hyperglycemia doubled the risk of heart disease and fatal cardiovascular diseases [26]. In acute test the extract opposed the rise of blood glucose when was fed with simultaneous glucose load.

The effect of ethanolic extract of BF (250mg & 500mg/kg) and standard antidiabetic drug (glibenclamide 10mg/kg) on blood glucose level and other biochemical parameters in alloxan induced type 2 diabetic rats has been depicted after 21 days continuous treatment.

It is known that loss of body weight and decreased growth rate in diabetic rats, is due to increased catabolism of protein [27]. Rats treated with BF extract improved body weight suggesting that the higher dose of BS could be protective against protein degradation by improving glycemic control (figure 2).

At day 0 and day 7 there was no comparable changes in blood glucose level as compared to diabetic control group. At day 14 a significant change was observed by BF extract but highly significant changes by glibenclamide (10mg/kg).

After a 21 days chronic study, the BF extract at both 250mg and 500mg/kg dose on type 2 diabetic rats showed highly significant reduction in serum glucose level (p<0.001) whereas, glibenclamide (10mg/kg) showed very highly significant (p<0.001) reduction of glucose level. Therefore, the extract has comparable antidiabetic activity with glibenclamide (figure 3).

Thus, the result of chronic antihyperglycemic study on alloxan induced type 2 diabetic rats indicates that, the BF decreases the serum glucose level highly significantly and in a time dependent manner.

Dyslipidemia is one of the complications of hyperglycemia. Untreated diabetic animals showed a significant increase in serum TC, LDL-C and TG concentrations against low levels of HDL-C after alloxan administration [28-30]. The serum lipid profile of rats was evaluated in this study. Treatment of type 2 diabetic rats with ethanolic extract of BF (250 mg & 500mg/kg), improved

dyslipidemia. At day 21, BF 500mg/kg produced significant reduction of total cholesterol ($p<0.001$), triglycerides ($p<0.001$), and LDL-C ($p<0.001$) level whereas HDL-C level was changed insignificantly as compared to the diabetic control group. The effects were comparable to that of the standard drug glibenclamide (figure 4).

These ameliorating effects demonstrated the antihyperlipidemic effect of *B. flabellifer*, and it could also be suggested that this antihyperlipidemic effects of BF pass through a decrease in intestinal cholesterol absorption or a decrease in the biosynthesis of cholesterol specifically by decreasing the activity of HMG-CoA reductase inhibitors [31].

Hyperglycaemia is considered as major risk in the development of diabetic nephropathy. There are different mechanisms by which increased blood glucose level causes nephropathy. It produces the oxidative stress [32]. Serum creatinine level is a biomarker of renal function.

Oral administration of *B. flabellifer* extract reduced the serum creatinine level at both doses but significantly ($p<0.001$) at 500mg/kg dose (figure 5). A decrease in creatinine level by BF extract suggests an improvement in renal function and reinforcement from oxidative stress.

In the present study, the *B. flabellifer* roots were selected for the evaluation of possible antidiabetic activity. The above results suggest that, it has antihyperglycemic activity both at acute and chronic study. The lipid profile was found to be significantly improved. The alcoholic extract also has prominent effect on serum creatinine level.

Conclusion

The overall findings of this study focused that treatment of alloxan-induced diabetic rats, with the extract of *Borassus flabellifer* for 21days, could restore normal bioactivities by shifting lipid and carbohydrate metabolism homeostasis. Furthermore, BF showed significant nephroprotective action. Therefore, it can be concluded that alcoholic extract of this plant can be successfully utilized for the management of diabetes.

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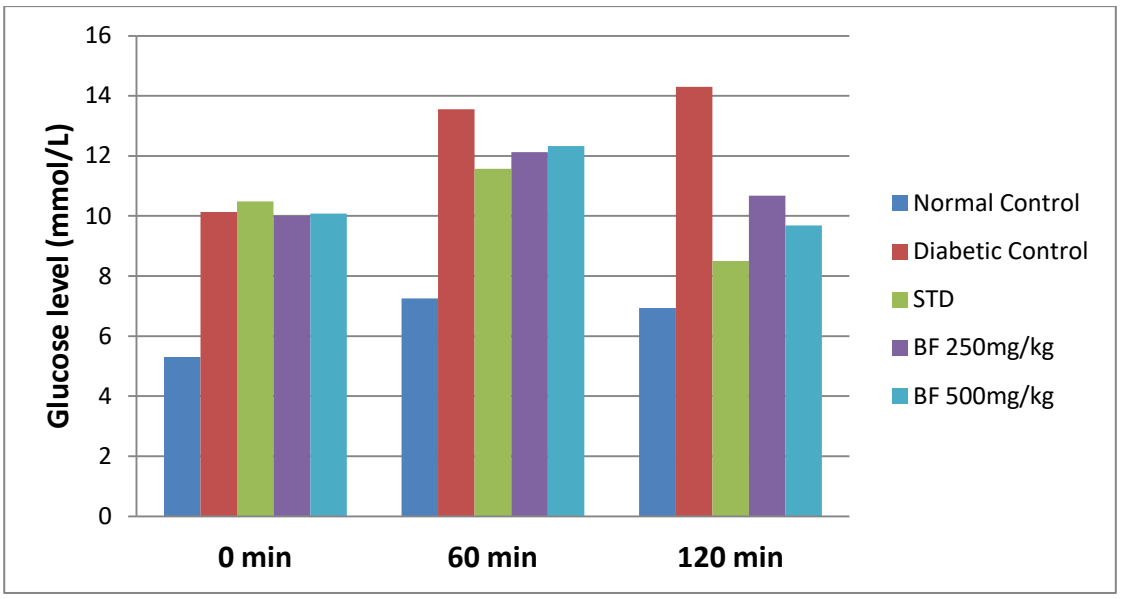

Figure 1: Effect of ethanolic extract of *Borassus flabellifer* on the OGTT in type-2 diabetic rats.

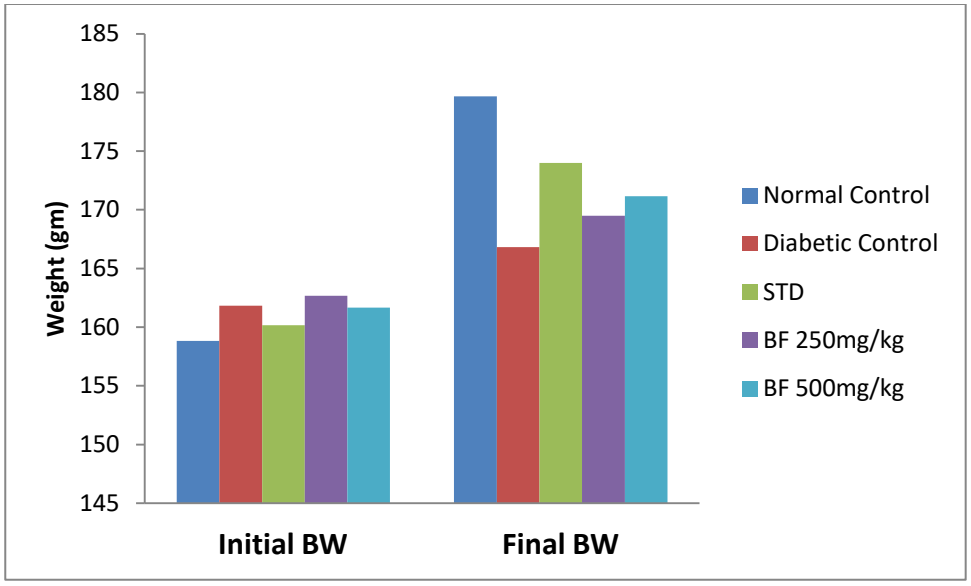

Figure 2: Effect of ethanolic extract of BF on the body weight after 21 days feeding in alloxan induced type-2 diabetic rats.

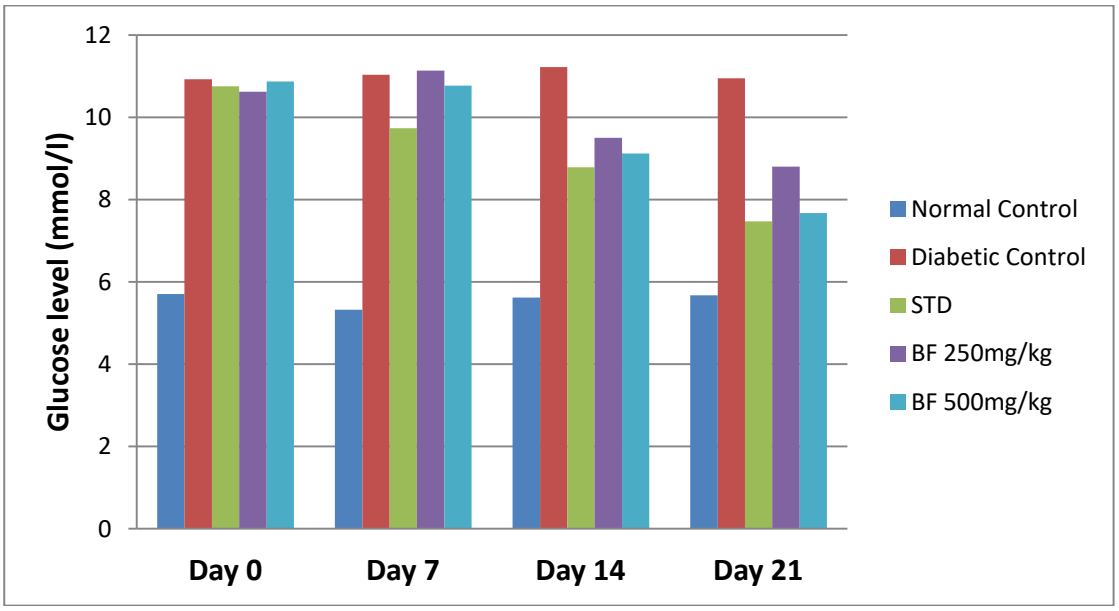

Figure 3: Effect of ethanolic extract of BF on the fasting blood glucose level after 21 days feeding in type-2 diabetic rats.

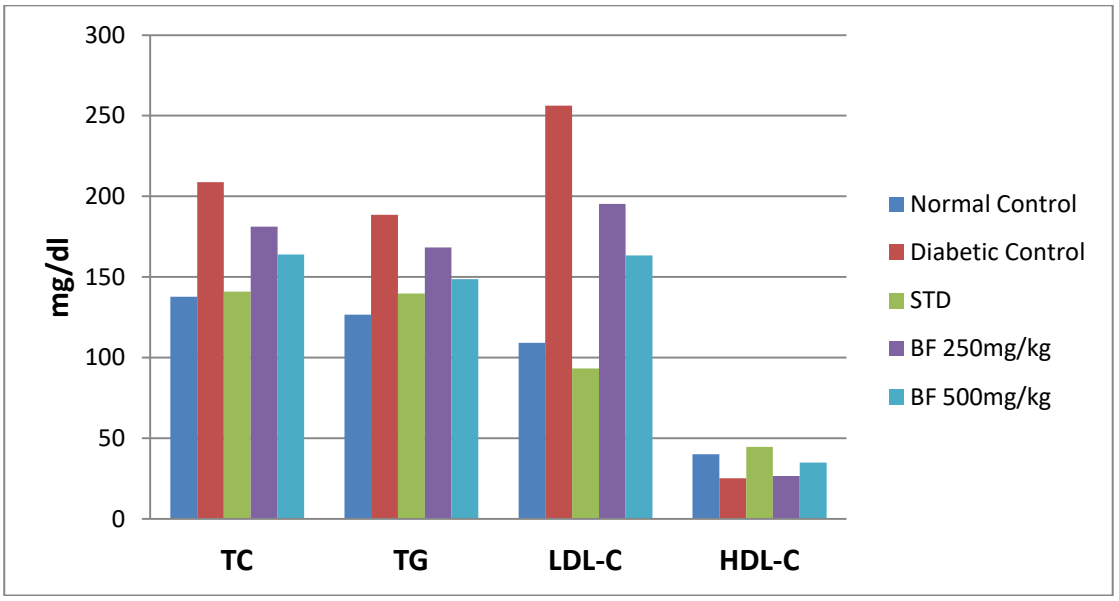

Figure 4: Effect of BF on total cholesterol (TC), triglycerides (TG), LDL-C and HDL-C level after 21 days feeding in type-2 diabetic rats.

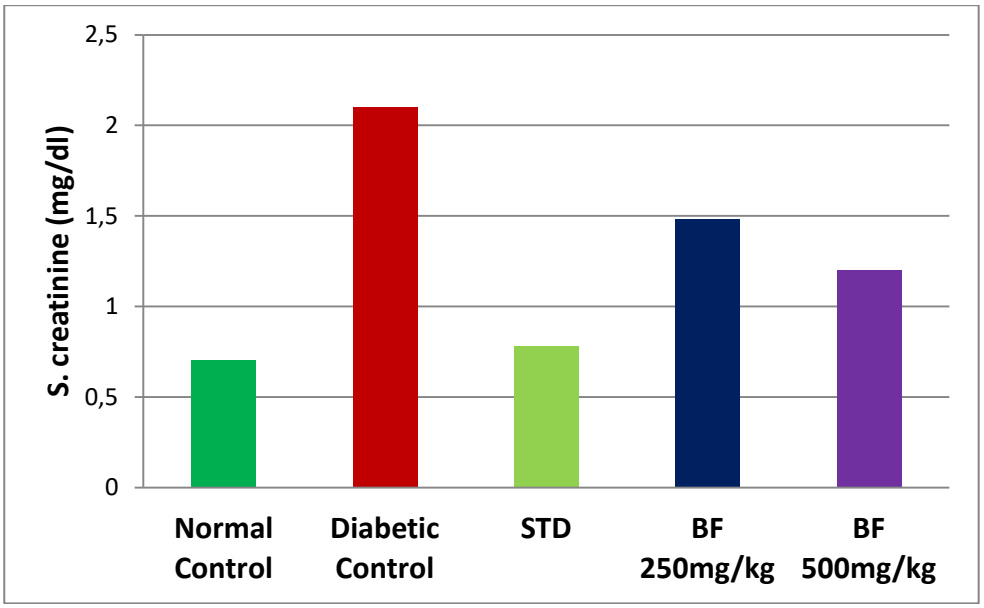

Figure 5: Effect of BS extract on the serum creatinine level after 21 days feeding in type-2 diabetic rats.