International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:8, No:1, 2014

Anti-Diabetic Effect of *Bryophyllum pinnatum* Leaves

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***Abstract*—**Diabetes is a chronic metabolic disorder that affects the quality of life in terms of physical health, social and psychological well-being. In spite of the enormous progress in the treatment of diabetes using existing commercial drugs, such as, insulin and oral hypoglycemic agents, the quest and search for new drugs is imperative due to several limitations of the commercial drugs. In addition, the existing diabetic drugs are expensive and unaffordable by the rural populace in the developing countries. The present study demonstrates the anti-diabetic property of aqueous extract of *Bryophyllum pinnatum (BP)* leaves using diabetic rats (albino rats) as models. At the same time, the anti-diabetic effect of the aqueous extract was compared to that of a sample containing a mixture of the extract and a commercial diabetic medicine, glibenclamide. A specified dosage of aqueous extract of *Bryophyllum pinnatum (BP)* leaves was administered on the experimental diabetic rats, and their BGL was measured and recorded. The results showed a significant drop in the BGL of the diabetic rats to a value close to normal blood glucose level within 120 minutes when only aqueous extract from *BP* leaves was used. When a sample containing a mixture of the aqueous extract and glibenclamide was administered, a further drop in BGL was observed. Therefore, the results reveal that aqueous extract of *Bryophyllum pinnatum* leaves have significant anti-diabetic properties, and that the performance of the existing drugs (glibenclamide) could be enhanced with the use of the aqueous extract.

***Keywords*—**Anti-diabetics, *Bryophyllum pinnatum*, Blood glucose level, albino rats.

I. Introduction

THE American Diabetic Association (ADA) defines diabetes (especially diabetes mellitus) as a group of metabolic disorders/diseases typified by hyperglycemia resulting from defects in insulin secretion, insulin action or both [1], [2]. Diabetes mellitus (DM) is one of the major degenerative diseases in the world today. Many deaths of diabetic patients have been attributed to its vascular diseases. In particular, hyperglycemia, which is the primary clinical manifestation of diabetes, is thought to contribute to diabetic complications by altering vascular cellular metabolism, vascular matrix molecules and circulating lipoproteins.

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Diabetes has been classified into four different categories, namely: Type 1, Type 2, MODY and Gestational diabetes [3]. Out of these types of DM, type 2 diabetes (T2DM) is the most common form of diabetes [3]. Recent studies predict the epidemics of DM to increase by 42% in the developed countries by 2025, while a 170% increase was predicted for the developing countries [3]. In developing countries, people living with DM are in the age range of 45-64 years, while the age range for people with DM in the developed countries is ≥65 years [4].

In spite of the immense progress in the treatment of diabetes using existing commercial drugs, such as, insulin and oral hypoglycemic agents, the quest and search for new drugs has intensified tremendously due to several limitations, such as high costs and affordability associated with the existing synthetic drugs [5]. Therefore, the limitations associated with the orthodox diabetic drugs have stimulated the search and research for alternatives like natural resources that display anti-diabetic properties and are readily available and affordable. Studies have shown that phyto-constituents of natural resources, such as medicinal plants, have anti-diabetic effects [6], [7]. The demonstrated results from these studies have culminated into concerted research efforts on the investigation of anti-diabetic properties and activities of some traditional plants and/or anti-diabetic performance of the combination of the traditional plant and existing orthodox anti-diabetic drugs [7]. One of such traditional medicinal plants is the *Bryophyllum pinnatum* leaves from *Bryophyllum pinnatum* plant.

Bryophyllum pinnatum (*BP*) (synonym: *Kalanchoe pinnata*, Lam.) is a perennial herb growing widely and used in folkloric medicine in tropical Africa, India, China, Australia and tropical America [8], [9]. In a developing country like Nigeria, *BP* is classified as a weed that flourishes throughout the Southern part of the country [15]. These plants are usually found in gardens rich in organic manure and sufficient moisture.

A number of active compounds, including flavonoids, glycosides, steroids, bufadienolides and organic acids, have been identified in *Bryophyllum pinnatum* [10]-[12]. Therefore, *BP* is used for medicine in ethno practice treatment of wounds of the navel of newly born babies, ear-ache, cough, diarrhea, and dysentery. Generally, the leaves are passed over flames to become soft and thereafter the juice is extracted and administered. In traditional medicine, *BP* leaves have been reported to possess antimicrobial [13], [14], antifungal [15], anti-neoplastic [16], anti-inflammatory and analgesic [17] and antihypertensive [18] activities.

In spite of the enormous medicinal activities of *BP* leaves, little is reported on the anti-diabetic activity of this natural resource. Therefore, the present study investigates and demonstrates the anti-diabetic activity of aqueous extract of *Bryophyllum pinnatum* leaves. The anti-diabetic effect of the aqueous extract was demonstrated using diabetic rats (albino rats) as animal models, because of their similar blood components and physiology when compared to human beings. At the same time, the anti-diabetic effect of the aqueous extract was compared to that of a sample containing a mixture of the extract and a commercial diabetic medicine, glibenclamide (GLI).

II. Experimental

A. Material

Fresh *BP* leaves were collected from a garden situated at Obafemi Awolowo University, Ile-Ife, Nigeria. Other chemicals used in this study were ethanol (80wt %) and glucose-D. Ethanol was used as an extracting agent to obtain the aqueous extract from the *BP* leaves, and Glucose-D was injected into the rats to increase their glucose level. The albino rats used as animal models were purchased from Animal Husbandry farm of Obafemi Awolowo University. Other materials included strips for collecting blood sample for glucometer test, measuring cylinder, weighing balance, syringes, test tubes, rotary evaporator, freeze-dryer and glucometer.

B. Preparation of Aqueous Extract

Fresh *BP* leaves were dried in an oven at 45°C for five days, and the dried leaves grounded to powders. An extraction process with 80% ethanol obtained aqueous extract from the powder (100g) in two stages using 500mL of ethanol in each stage. This extraction was carried out by soaking the powder in the solvent (ethanol) for 3 days with intermittent shaking. The resulting mixture was decanted, and filtered into a sterilized conical flask using filter papers. The filtrate was evaporated using a rotary evaporator, and followed by freeze-drying with a freeze dryer in order to obtain the cake form of the extract with which various concentrations of the aqueous solution used in this study were prepared.

C. Experimental Design and Procedure

Twenty-five (25) albino rats (100-190g) were selected for this study, and they were protected and cared for according to the principle of laboratory animal care [19]. The rats were divided into five (5) groups (I to V) of five (5) rats in each (see Table I). The rats fasted for half-a-day (12h) before the commencement of the experiment. The animals were made diabetic by injecting them with Glucose D at a dose of 3g/kg body weight (see Table I for details). After 20 minutes of injection, the blood glucose level of the diabetic rats was measured at time intervals of 0min, 30min, 60min, and 120 min with the use of a glucometer. Measurement was done by chopping off the tip of tails of the rats, and the collected blood was sensed with the glucometer. For control experiment, the experimental diabetic Albino rats were not injected with the aqueous extract of *BP*. Also for comparison, a mixture of the aqueous extract from the *BP* leaves and glibenclamide (an existing diabetic drug was also administered).

TABLE I
GROUPS OF ANIMALS MODELS EMPLOYED IN THIS STUDY

Groups	Weight (g)	Volume of glucose 3 g/kg body weight (ml)	Vol. of aqueous extract
Group I			
Control	150	4.50	-
	150	4.50	-
	150	4.50	-
	140	4.20	-
	120	3.60	-
Group II			
Aqueous Extract	100	3.00	0.40
(200mg/kg)	130	3.90	0.52
	180	5.40	0.72
	190	5.70	0.76
	140	4.20	0.56
Group III			
Aqueous Extract	120	3.60	0.48
(400mg/kg)	170	5.10	0.68
	160	4.80	0.64
	170	5.10	0.68
	150	4.50	0.60
Group IV			
Aqueous Extract	120	3.60	0.96
(800mg/kg)	170	5.10	1.36
	150	4.50	1.20
	150	4.50	1.20
	150	4.50	1.20
Group V			
GLI (2mg/kg) + 800 mg/kg extract	180	5.40	0.36
	150	4.50	0.30
	140	4.20	0.28
	180	5.40	0.36
	120	3.60	0.24

III. Results and Discussion

Table II shows the results of the anti-diabetic activity of the aqueous extract of BP leaves. The blood glucose level (BGL) of the experimental diabetic rats during the experiment was measured at 0 minute, 30 minutes, 60 minutes and 120 minutes after administering the required quantity of the extract to the rats. A decrease in BGL was observed for all the groups during the study. Group I, Group II, Group III, Group IV and Group V show a decrease in BGL from an average value of 10.26 mmol.L^{-1} (at 0 minute) – 4.37 mmol. L^{-1} (at 120 minutes); 20.94 mmol. L^{-1} (at 0 minute) – 5.1 mmol. L^{-1} (at 120 minutes), 11.2 mmol. L^{-1} (at 0 minute) – 4.2 mmol. L^{-1} (at 120 minutes),16.22 mmol. L^{-1} (at 0 minute) – 4.05 mmol. L^{-1} (at 120 minutes), and 21.46 mmol. L^{-1} (at 0 minute) – 4.18 mmol. L^{-1} (at 120 minutes), respectively within a period of 120 minutes (see Table II and Fig. 1 for details). The average percentage reduction in BGL for all the groups of the experimental diabetic Albino rats is depicted in Fig. 2.

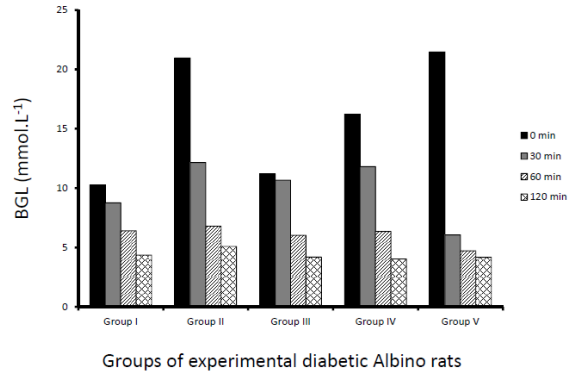

Fig. 1 Anti-diabetic activity of the aqueous extract of *BP* leaves

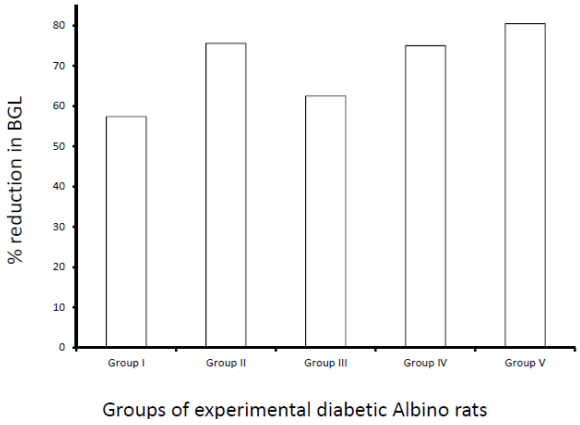

Fig. 2 Percentage reduction in BGL for all groups of experimental diabetic Albino rats during at the end of 120 minutes

For Group I (control experiment), the decrease in BGL occurred naturally without the use of the aqueous extract for all the experimental diabetic Albino rats in this group. The BGL reduced by 57.4% at the end of 120 minutes (see Fig. 2). It is noteworthy to mention that rat 1 , rat 2 and rat 3 have body weight of 150 g each while rat 4 and rat 5 have body weight of 140 g and 120 g, respectively (see Table I). The results show that rat 1 and rat 2 displayed equal amount of BGL at the initial stage (at 0 minute). This observation could be attributed to the equal body weights of the rats. Despite the difference in the body weights of the rats in this group, all of them displayed the same amount of BGL at the end of 120 minutes of treatment, suggesting that reduction in BGL is independent of the body weight but a function of the body metabolism.

At the end of 120 minutes of treatment with 200 $mg.kg^{-1}$ of the aqueous extract of *BP*, the BGL of Group II decreased by 75.6% (average) (see Fig. 2), and an increase of about 19% over that of Group I. This implies that the use of the aqueous extract has enhanced the reduction of BGL in the experimental diabetic rats. Furthermore, the variation in BGL for members of this group is depicted in Fig. 3. From Fig. 3, the 190g rat has the highest glucose level at the initial stage of the experiment (at 0 minute). The high glucose level could be attributed to the fact that the rat received the highest dosage of glucose due to its body weight. However, the glucose level of all rats of the group decreased with time after administering the aqueous extract of *BP*, but the degree of reduction of BGL does not correspond to the body weights of the rats (see Fig. 3) This observation corroborates the fact that the anti-diabetic activity of the aqueous extract is independent of the body weight.

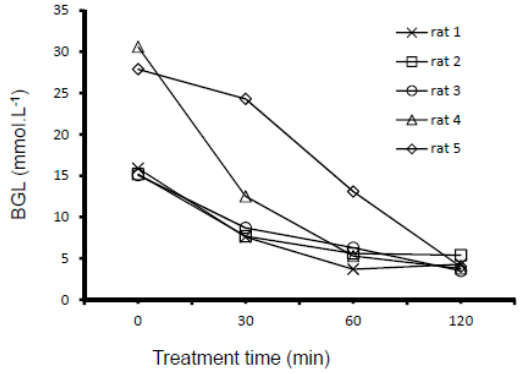

Fig. 3 Reduction in BGL in Group II experimental diabetic Albino rats

Similarly, a decrease of 62.5% in BGL was obtained for experimental diabetic Albino rats of Group III after 120 minutes of treatment with 400 $mg.kg^{-1}$ of the aqueous extract of *BP* leaves. As observed in Table I, rat 2 and rat 4 in this group have the highest body weight of 170g each, thus receiving the same volume of glucose during the period the rats were made diabetic. Despite the similarity in the body weight of rat 2 and rat 4, their BGL differs significantly at 0 minutes (see Fig. 4). This observation is also suggesting that the increase in glucose level could be a function of the body system and not of the body weight. However, after 120 minutes of treatment, all the rats displayed the same BGL of 4.0 $mmol.L^{-1}$. In addition, the reduction in BGL for Group III is about 13% less than that of Group II.

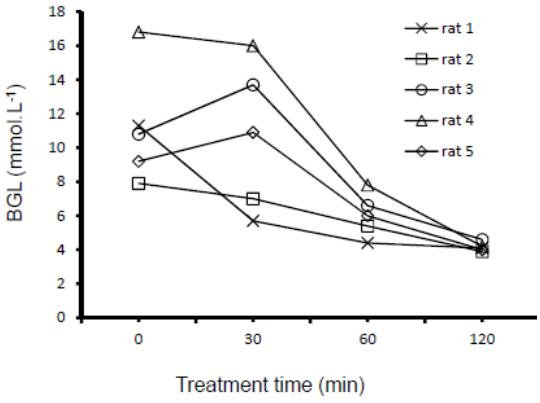

Fig. 4 Reduction in BGL in Group III experimental diabetic Albino rats

Group IV experimental diabetic Albino rats displayed a reduction of 75% in BGL after treatment with aqueous extract

of 800 mg.kg^{-1} for 120 minutes. In spite of the difference in the weight of the rats of this group, all the rats attained the same BGL at the end of the 120 minutes treatment with the aqueous extract of the BP leaves. The observation is similar to the trends observed for Group II and Group III. In Group V, the BGL of the experimental diabetic rats decreased by 80.5% during the treatment period of 120 minutes using a mixture of 2 mg.kg^{-1} of GLI and 800 mg.kg^{-1} of the aqueous extract of *BP*. The observation suggests that the addition of GLI to the extract promotes further reduction of BGL in the diabetic rats. In addition, BGL of Group V diabetic rats shows a further decrease of about 5% when compared to 75.6% of Group II. Furthermore, the BGL of rat 1, rat 4 and rat 5 at 0 minutes were the same despite the difference in the body weights of these rats (see Table I and Fig. 5). However, all the rats of this group attained similar BGL at the end of 120 minutes treatment period (see Fig. 5). In summary, the anti-diabetic activity of the aqueous extract of BP leaves in all the groups of experimental diabetic Albino rats investigated in this study follows this order:

GROUP V > **GROUP II** > **GROUP IV** > **GROUP III** > **GROUP I**

It is expected that further reduction of BGL will be achieved in all the groups if the treatment is prolonged beyond 120 minutes.

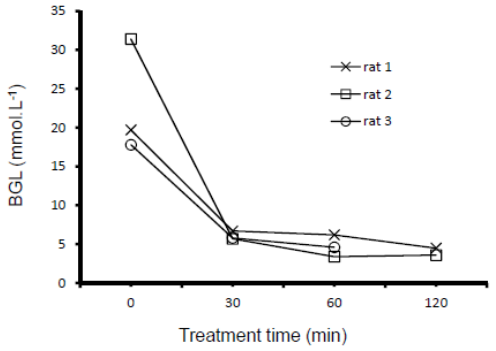

Fig. 5 Reduction in BGL in Group V experimental diabetic Albino rats

IV. Conclusions

The results obtained from the present study have demonstrated the antidiabetic activities of aqeuous extract of *Bryophyllum pinnatum* leaves. For the most of the diabetic animal models treated with the aqeuous extract, the high blood sugar level was reduced to normal after treating for ~120 minutes. Observations at time intervals between 0 minute and 120 minutes in steps of 30 minutes showed that a concentration of 200 mg/kg aqueous extract resulted is a significant drop in blood sugar level for treated samples when compared with the performance of other dosages. In addition, when a mixture of GLI and the aqueous extract was administered on the diabetic rats, higher performance was observed in comparison to a dosage of 200 mg/kg aqueous extract at the same time interval. Also, the use of a mixture of the GLI and the aqueous extract proved more effective and efficient than the use of any single dosage of the aqueous extract; therefore, a mixture of the extract and GLI could be promising for treating diabetes effectively. However, further extensive study, in the area of physiology and pharmaco-kinetic, is essential to establish this natural product as a drug for treating diabetes. Since the cause of DM is genetic-related, in depth understanding of molecular genetics and bioinformatics could pave the way to gaining insights into the biological processes that promote the spread of DM. Additionally, proper understanding of the biological processes could be instrumental to devising tools and therapy required to combat DM clinically [20].

TABLE II
Effect of Aqueous Extract of *Bryophyllum pinnatum* Leaves and Glibenclamide on Different Groups of the Experimental Albino Rats

	BGL (mmol.L^{-1})			
	0 minute	30 minutes	60 minutes	120 minutes
Group I	10.26 ± 0.63	8.76 ± 2.79	6.42 ± 1.48	4.37 ± 0.31
Group II	20.94 ± 3.42	12.16 ± 3.16	6.80 ± 1.63	5.10 ± 0.80
Group III	11.20 ± 1.52	10.66 ± 1.94	6.04 ± 0.57	4.20 ± 0.15
Group IV	16.22 ± 1.60	11.80 ± 1.96	6.36 ± 0.57	4.05 ± 0.25
Group V	21.46 ± 2.50	6.06 ± 0.31	4.73 ± 0.81	4.18 ± 0.26

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