academicJournals

Vol. 11(41), pp. 526-533, 8 November, 2017 DOI: 10.5897/AJPP2017.4835 Article Number: 389C1FE66758 ISSN 1996-0816 Copyright © 2017 Author(s) retain the copyright of this article http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Antidiabetic potential of ethanol leaf extract of *Bryophyllum pinnatum* on alloxan-induced diabetic rats and their haematological profiles

Casmir U. Ezeagu¹, Joshua Parker Elijah¹, Ukegbu Chimere Y¹*, Eze Chuma S.² and Nwodo O. F. C.¹

¹Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nigeria. ²Department of Applied Biology and Biotechnology Enugu State University of Science and Technology, Nigeria.

Received 17 August, 2017; Accepted 12 October, 2017

Bryophyllum pinnatum is a known herb found in the tropical region of Africa and other parts of the world. In this study, the ethanol extract of the plant was investigated for possible anti-diabetic effect on alloxan induced hyperglycaemic wistar albino rats. Phytochemical studies revealed that Flavonoids and alkaloids were highly abundant compared to steroids and terpenoids which were slightly present while glycosides and reducing sugar were moderately present. Diabetes was induced via intraperitoneal injection using alloxan monohydrate. Groups 2 to 5 were induced while Groups 1, 6 and 7 were not. Group 1 was negative control group; Group 2 was positive control group, while Group 3 was the standard control. Groups 4 to 7 were the treatment groups. The results of anti-hyperglycaemic effect of the extract showed a significant (p<0.05) reduction in the glucose level of alloxan-induced diabetic rats in Groups 4 and 5, respectively treated with 200 and 400 mg/kg body weight of the extract when compared with the positive control. However, there was a significant increase (p<0.05) in some haematological parameters such as red blood cell count, haemoglobin count, packed cell volume of groups 4, 5, 6, and 7 when compared with the positive control. There was a significant decrease (p<0.05) in the malondialdehyde, low density lipoprotein, triacylglycerol and total cholesterol concentrations of groups 4, 5, 6, and 7 when compared with the positive control. The result of the study showed that the extract of B. pinnatum exhibited anti-hyperglycaemic activities in alloxan-induced diabetic wistar albino rats.

Key words: Brohyllum pinnatum, phytochemistry, Diabetes, haematological parameters.

INTRODUCTION

Tropical forests are biologically lavished with diverse ecosystem of plants whose potential value as natural pharmacy is yet to be discovered (Cohen-Kohler, 2007). The native people have used plants as medicine for

*Corresponding author. E-mail: ukegbuchimere@gmail.com. Tel: +2348067299158.

Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> centuries. Folk medicine comprises the use of herbs, minerals, animal parts, etc., in prevention and treatment of diseases. However, herbal medicine is the most widely used of the three. Right from the beginning, all or parts of most plant species have been used as herb. There has been an improved acceptance of traditional medicine in recent times. The major reason for this acceptance is the high cost of contemporary medicines which makes it difficult for beneficiaries especially those from poor countries. As regards to folk medicine, there has been a mistaken impression that herbal medicine has fewer side effects (Chan, 2009); however, this is only true for some natural product while some are toxic. In spite of the fact that most of the claims ascribed to herbal medicine are yet to be proven scientifically, some medicinal plants have however been extensively studied, thus warranting their use as alternative to or complements of conventional medicines. One crucial denunciation related with the usage of herbal medicine is the lack of scientific assessment of their safeness, having known that some of these herbs have shown several toxic effect (Ernst, 2005; Yeung et al., 2008). Approximately, 80% of the entire world population rely wholly or partially on drugs from plant origin (WHO, 1996). Bryophyllum pinnatum is an incessant medicinal plant that grows in the wild and used as a traditional medicinal as well as ornamental plant in tropical America and Africa, India, Australia, and China. The genus belongs to the family of Crassulaceae and is cultivated as ornamental house plant on rocks or in the garden (Kulka, 2006). The present herb under study is routinely called an air plant, Canterbury bell, cathedral bell, life plant and resurrection plant. It has a sweet in post digestive effect, is astringent, it has a hot potency and sour in taste. It is also known for its haemostatic and wound healing properties (Khan et al., 2004). The leaves are simple, or compound in pairs, thick and fleshy, on cerise stems. B. pinnatum can propagate out of fortuitous shoots from immature leaf bases. The Creoles in America use the slightly roasted leaves in the treatment of inflammations, cancer, and a leaf infusion for fever. The Palikur in India mix the leaf juice with coconut oil and then rub it on the forehead for migraines and headache. In Mexico and Nicaragua, it is used to promote menstruation and assist in child birth (Gwehenberger et al., 2004). In Nigeria and other West African countries, disorders such as bruises, boils, wounds, insect bites, rheumatism, headaches and body pains, arthritis, etc., are cured using the fleshy leaf (Khan et al., 2004). It is employed in the treatment of gastric ulcers, edema of the legs, kidney stones, skin disorders and burns. The pulp of the leaves or the juice is used externally on traumatic injuries to stop the bleeding as it contracts the minute arterioles and boost the healing without leaving a scar. The herb is great in bleeding disorder such as menorrhagia and piles. However, the present study is geared towards investigating the antidiabetic activity of the plant under review.

MATERIALS AND METHODS

Plant

The fresh leaves of *B. pinnatum* were collected from OPI markret in Nsukka LGA of Enugu State, Nigeria. Identification of the leaves was done by Mr Alfred Ozioko of Bioresources Development and Conservation Programme (BDCP), Nsukka, Nigeria.

Experimental animals

A total of thirty male albino rats with an average weight of 170 ± 16 g were obtained from the Animal House of Department of Zoology, University of Nigeria, Nsukka. Acclimatization of the animals to laboratory conditions lasted for one week under a standard condition with 12 h light and dark cycles and maintained on a regular feed diet and water *ad libitum*. The mice used for LD₅₀ were 8 to 10 weeks (56 to 70 days) old with average weight of 16 ± 1.4 g.

Preparation of extract

The fresh leaves of *B. pinnatum* were washed with distilled water and spread on a mat in a well-ventilated room with regular turning to avoid decay. This process lasted for four weeks after which they were pulverized. 1125 g of dry leaves were coarsely ground. The ground leaves were soaked for 48 h in 95% absolute ethanol with occasional shaking after which they were filtered using muslin sieve. The filtrate was later evaporated to dryness using rotary evaporator (R-300 Rotavapor manual lift) at 60°C.

Determination of LD₅₀ of the extract

The median lethal dose (LD_{50}) of the ethanol leaf extract of the plant was determined by the method of Lorke (1983). Six groups of three adult albino-mice each weighing between 13 and 20 g were used for this experiment.

Experimental design

In this experiment, the rats were divided into seven groups of five rats each. Group 1 was administered 1 ml of 3% tween 80 solution which represented the vehicle. The treatment was carried out (per oral) using gastric gavage.

Group 1: Normal rats were Non diabetic and no treatment was given (negative control);

Group 2: Positive control, Diabetic rats without treatment;

Group 3: Diabetic rats treated with standard drug (2.5 mg/kg body weight of glibenclamide).

Group 4: Diabetic rats treated with 200 mg/kg body weight of the extract

Group 5: Diabetic rats treated with 400 mg/kg body weight of the extract.

Group 6: Non diabetic rats treated with 200 mg/kg body weight of the extract.

Group 7: Non diabetic rats treated with 400 mg/kg body weight of the extract.

Diabetes mellitus induction

On the seventh day of acclimatization, the rats were starved overnight prior to induction of diabetes mellitus with a view to

Weight of dried sample (g)	Percentage yield of extract (%)	Weight of extract (g)
500.00	4.00	20.00

enabling the alloxan to penetrate the vital organs of the rats. Diabetes was induced by intraperitoneal injection of a single dose of 150 mg/kg body weight of 1% alloxan monohydrate dissolved in freshly prepared normal saline. After 24 h, all the rats that have blood glucose level of 200 mg/dl and above were considered diabetic and were selected for the experiment.

Phytochemical analysis of the fresh and dry leaf extact of *B. pinnatum*

The preliminary analysis of the phytochemical composition of the ethanol extract was carried out according to the method of Harborne (1989) which involved testing for the presence of the following plant constituents and their relative abundance. They include the following: alkaloids, flavonoids, carbohydrates, glycosides, proteins, saponins, steroidal aglycone, tannins, oil, acids, resins, and terpenoids.

Anti-diabetic evaluation

Blood glucose concentration was measured on weekly interval to ascertain the hypoglycaemic effect of the extract on the rats being treated. This was done by slightly cutting the tail vein with a sharp scissors. Collection of blood samples was done by nipping and smearing the tail on the indicated portion of glucometer strip until it was fully soaked by blood after it was inserted into the Accu-check glucometer and blood glucose concentration was read within few seconds.

Analysis of biochemical parameters

Packed cell volume (PCV), haemoglobin concentration, red blood cell (RBC) count and total white blood cell (WBC) count were estimated using the standard haematological method as reported by Ochei and Kolhakar (2005). Malondialdehyde (MDA), a product of lipid peroxidation was determined spectrophotometrically using the method of Wallin et al. (1993), while catalase activity was assayed by the method described by Abei (1983). High density lipoprotein (HDL) concentration was assayed for using the method described in Albers et al. (1978), however, low density lipoprotein (LDL) concentration was ascertained according to the method described by Assman et al. (1984). Total cholesterol concentration was determined according to the method described by Allain et al. (1974), while triacylglycerol concentration was determined by the method described by Bucalo and David (1973).

Statistical analysis

Statistical analysis was determined using one way analysis of variance (ANOVA) using Statistical Products and Service Solutions (SPSS). Data obtained from the test groups (mean of triple determination \pm standard deviation [SD]) were considered statistically significant at p<0.05 when compared with their respective controls and the differences.

RESULTS AND DISCUSSION

Percentage yield of ethanol extract of *B. pinnatum* leaves

500g of dried sample of *B. pinnatum* yielded 20 g weight of the extract. Thus, percentage yield is 4%.

This study is a report of the effects of ethanol extract of B. pinnatum on alloxan-induced diabetic rats. Diabetes mellitus is a persistent ailment caused by complete or partial deficiency in insulin levels, which leads to insufficient glucose absorption thus producing a critical and chronic complication. In recent times, the management of diabetes primarily involves lowering of blood glucose level by the use of biguanides, sulphonylureas, diphenyalamine derivatives, thiazolidinediones and glucosidase inhibitors in addition to insulin. Nevertheless, due to some side effects, the efficacies of these compounds are arguable. Therefore, there is a high demand for new compounds with high efficacies and little or no side effects for the management 2003). (Thirunavukkarasu of diabetes et al., Consequently, plants have been suggested to be a source of potentially useful antidiabetic active ingredients due to the high content of potent organic compounds (phytochemicals) that are yet to be investigated scientifically (Saxena and Vikram, 2004).

The percentage yield of the extract is 4% as shown in Table 1. This implies that the plant has a low vield. Table 2 shows the phytochemical composition of the ethanol extracts of the leaves of *B. pinnatum* indicating the abundance of many secondary metabolites including flavonoids, alkaloids, tannins, saponins, glycosides, etc. From the results shown in Table 2, flavonoids, alkaloids, and tannins were found to be highly present in both dry and fresh samples while steroids, hydrogen cyanides and carbohydrates were found in very low concentration in both samples. Also, reducing sugar, saponins, terpenoids and glycosides were moderately present in both samples. Table 3 displays that there was no statistically significant at p>0.05 between difference the quantitative phytochemical constituents of the air dried and fresh samples which shows that no nutrient or phytoconstituent was lost during the process. This also proves that air drying which is the drying technique used in this study is one of the best methods of drying plant materials. The flavonoids, saponins and alkaloids are said to have medicinal properties in animal (Livingston et al., 1997). However, in high concentrations, these secondary metabolites could show some levels of toxicity

Phytochemical	Fresh sample	Dry sample
Alkaloids	++	+++
Flavonoids	+++	+++
Steroids	+	+
Glycosides	++	++
Terpenoids	+	+
Carbohydrates	+	+
Tannins	++	++
Saponin	++	++
Hydrogen cynide	+	+
Reducing sugar	++	++

Table 2. Qualitative phytochemical constituents of the fresh and dry samples of *B. pinnatum*.

+ = slightly present; ++ = moderately present; +++ = highly present.

Table 3. Quantitative phytochemical constituent of fresh and dry samples.

Phytochemical constituents <i>B. pinnatum</i> (mg/100g)	Fresh sample (mean of 3 determinations ±SD)	Dry sample (mean of 3 determinations ±SD)
Flavonoids	3.25±0.03	3.26±0.02
Glycosides	3.40±0.25	3.5±0.20
Carbohydrates	0.79±0.01	0.79±0.00
Hydrogen cyanide	0.10±0.00	0.09±0.00
Reducing sugar	500±25	350±25
Saponins	1.7±0.50	1.1±0.15
Steroids	0.27±0.01	0.27±0.00
Tannins	2.25±0.02	2.26±0.02
Terpenoids	0.51±0.01	0.51±0.00
Alkaloids	2.90±0.10	3.25±0.10

Values are expressed as Mean \pm SD. The values shows that both dry and fresh samples of the leaves contain alkaloids, flavonoids, steroids, glycosides, terpenoids, carbohydrates, tannins. There is no (p> 0.05) significant increment in the phytochemical composition of the fresh samples in comparison with the air dried samples of the plant.

and may impair body metabolism (Wieslaw et al., 1999).

The acute toxicity (LD_{50}) test of ethanol leaf extract as shown in Table 4 indicated that at a dose as high as 5000 mg/kg body weight, there was no mortality recorded in the animals and neither did they show any sign of toxicity. This is evidence that the leaves are relatively secure for human and animal consumptions.

Table 5 shows the antidiabetic effect of *B. pinnatum* leaf extract on alloxan-induced diabetic rats. The results displayed that there was a statistically significant (p<0. 05) reduction in the glucose level of the rats in the test group in a dose-dependent manner in comparison with the positive control group. This reduction could be an outcome of the biochemical interactions of the phytochemical constituents of the plant, thereby, enhancing the regeneration of beta cells of the pancrease, thus, stimulating insulin secretion and causing a reduction in the blood glucose level. However, this result is consistent with the report of Ukegbu et al. (2016) which

showed that graded doses of the extract were responsible for lowering of the blood glucose level in diabetic subjects and this reduction could be as a result of the phytochemicals inherent in the plants.

PCV test is a hematological test used to quantify the amount of cells in the blood. The quantity of cells in the blood is stated as a percentage of the total volume of blood; for example, a PCV measurement of 65% signifies that there are 65 ml of cells per 100 ml of blood. The test can be used as a screening tool for anaemia and can also indicate the degree of fluid loss during dehydration. Haemoglobin is the pigment part of the erythrocyte and the oxygen carrying pigment in the red cells. As shown in Table 6, the treatment of the hyperglycaemic rats with the leaf extract produced a significant (p<0.05) increase in the PCV levels and haemoglobin concentration of the treated rats when compared with the positive control. This reduction is probably due to hemolysis and biochemical reactions of the free radicals and this

Phase	Dosage mg/kg body weight	Seed mortality
Phase I		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase II		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

Table 4. Phases I and II of the acute toxicity (LD₅₀) test of *B. pinnatum* leaf extract.

The acute toxicity test (LD₅₀) of the plant extract showed no lethality.

Table 5. The biochemical outcome of ethanol extract of *B. pinnatum* on glucose concentration of alloxan-induced diabetic Wistar albino rats.

Blood glucose level (mg/dl)	Baseline	Day 3	Day 9	Day 15	Day 22
Group 1 (Normal Control)	108.0±5	104.4±3*	102.3±6*	102.3±4*	108.7±3*
Group 2 (Positive Control)	100.4±6	394.7±5	404.0±4	422.3±8	423.9±9
Group 3 (Standard Control)	105.1±3	270.3±7*	237.7±5*	194.3±7*	134.3±5*
Group 4 (Diabetic+200 mg)	85.5±4	357.2±7*	321.1±8*	261.6±5*	174.4±6*
Group 5 (Diabetic+400 mg)	89.7±3	330.7±2*	287.7±6*	258.2±3*	169.5±8*
Group 6 (200 mg ext only)	90.5±5	107.7±4*	104.3±4*	106.7±5*	94.7±5*
Group 7 (400 mg ext only)	95.4±5	113.7±4*	113.2±7*	120.0±6*	93.3±4*

Values are expressed as mean ± standard error of mean; n = 3 animals per group; where P<0.05 is considered significant (*) in comparison with positive control group using ANOVA.

Table 6. The biochemical outcome of ethanol extract of *B. pinnatum* on packed cell volume and Haemoglobin concentration of alloxan-induced diabetic rats.

Group	PCV (%)	HB (g/dl)
Group 1 (Normal/negative control)	48.67±0.5*	15.07±1.0*
Group 2 (Diabetic not treated - positive control)	32.00±2.5	8.83±0.8
Group 3 (Diabetic + standard drug)	50.33±2.3*	14.0±0.9*
Group 4 (Diabetic + 200 mg/kg body weight of extract)	43.33±2.5*	14.77±0.8*
Group 5 (Diabetic + 400 mg/kg body weight of extract)	44.67±1.1*	15.73±0.4*
Group 6 (Non-Diabetic + 200 mg/kg body weight of extract)	45.00±3.0*	15.63±1.1*
Group 7 (Non-Diabetic + 400 mg/kg body weight of extract)	48.67±1.5*	16.90±0.6*

Values are expressed as Mean ± Standard Error of Mean; n = 3 animals per group; where P<0.05 is considered significant (*) in comparison with positive control group using ANOVA

reduction could be ascribed to low levels of hemoglobin which is a hematological condition that is characterized by low PCV levels. The reduction in haemoglobin level of the hyperglycaemic rats (positive control) might be due to glycosylation of the red blood cells which prevent its frequent interaction with the haem components. This shows that the extract has the ability. The results obtained from this research work is in tandem with the research work done by Oyedemi et al. (2011) where the antidiabetic and haematological effect of aqueous extract of the stem bark of *Afzelia africana* (Smith) on streptozotocin-induced hyperglycaemic Wistar rats was evaluated. The work suggested that plant extracts contains compounds that have the ability to inhibit the

Table 7. The biochemical outcome of ethanol extract of *B. pinnatum* on red blood cell count and total white blood cell count of alloxan-induced diabetic rats.

Group	RBC ×10 ⁹ /L	TWBC (mm ⁻³)
Group 1 (Normal/negative control)	276.67±13*	5200.52±534*
Group 2 (Diabetic not treated - positive control)	150.00±15	1133.33±242
Group 3 (Diabetic + standard drug)	290.00±7*	6066.67±267*
Group 4 (Diabetic + 200 mg/kg body weight of extract)	238.33±11*	5600.00±271*
Group 5 (Diabetic + 400 mg/kg body weight of extract)	241.67±12*	5400.00±487*
Group 6 (Non-Diabetic + 200 mg/kg body weight of extract)	300.00±8*	5066.57±367*
Group 7 (Non-Diabetic + 400 mg/kg body weight of extract)	293.33±17*	5033.67±392*

Values are expressed as Mean \pm Standard Error of Mean; n = 3 animals per group; where P<0.05 is considered significant (*) in comparison with positive control group using ANOVA

Table 8. The biochemical outcome of ethanol extract of *B. pinnatum* on catalase activity and malondialdehyde concentration of alloxan-induced diabetic rats.

Group	CAT (IU/L)	MDA (mg/ml)
Group 1 (Normal/negative control)	5.9±0.08*	5.4±0.20*
Group 2 (Diabetic not treated - positive control)	3.0±0.15	8.4±0.08
Group 3 (Diabetic + standard drug)	6.0±0.09*	6.5±0.12*
Group 4 (Diabetic + 200 mg/kg body weight of extract)	5.9±0.21*	6.6±0.20*
Group 5 (Diabetic + 400 mg/kg body weight of extract)	6.3±0.08*	6.8±0.20*
Group 6 (Non-Diabetic + 200 mg/kg body weight of extract)	4.6±0.16*	5.2±0.30*
Group 7 (Non-Diabetic + 400 mg/kg body weight of extract)	5.2±0.2*	5.4±0.15*

Values are expressed as Mean ± Standard Error of Mean; n = 3 animals per group; where P<0.05 is considered significant (*) in comparison with positive control group using ANOVA.

non-enzymatic glycosylation of RBC membrane proteins which could be through membrane stability activity or through direct inhibition.

A red blood cell count is a blood test that estimates how many red blood cells (RBCs) in the blood and also detects anemic conditions. Total white blood cell count is a biochemical test used to investigate an infection or inflammatory process and it may also be used to diagnose the presence of other diseases that affect WBCs which includes allergies, leukemia or immune disorders, to name a few. From Table 7, the RBC count showed a statistically significant increase (p < 0.05) in the diabetic rats treated with graded doses of the leaf extract in comparison with the positive control group. However, converselv. the treatment of alloxan-induced hyperglycaemic rats with the ethanol extract of B. pinnatum caused a statistically significant reduction (P< 0.05) in the total white cell count of treated rats when compared with the diabetic untreated rats. This might suggest that the leaf of *B. pinnatum* cannot be used as a blood booster or could contain compounds that inhibit haemolysis through membrane stabilization or through other unknown mechanisms. The elevation of the total white blood cells in the diabetic untreated rats is an indication of inflammation, acute infection or tissue damage. The result of this research work is in agreement with the research work done by Muhammad et al. (2012) where the haematological parameters of alloxan-induced diabetic rats treated with leaf essential oil of *Hoslundia opposita* (Vahl) was evaluated.

Higher levels of malondialdehyde and lower level of catalase activities indicate oxidative stress. The levels of antioxidant defense system are always diminishing in alloxan-induced diabetic rats (Prince and Menon, 2000) which correlate with the results shown in Table 8 where there was a significant reduction (p < 0.05) in the catalase activity of the positive control in comparison with the normal control group and the standard control group. From Table 8, it was observed that the treatment of alloxan-induced diabetic rats with the extract of B. pinnatum leaf extract significantly increased (P< 0.05) the catalase activity of the treated rats when compared with the untreated rats. Calatase is a haemoprotein which catalyses the reduction of hydrogen peroxides and defend the tissues from damage from reactive hydroxyl radicals. A decrease in the catalase activity results in a number of superoxide anion radicals and hydrogen peroxides. The results of this work are in consonance with the works of Mahrukh et al. (2015) where the antidiabetic effect of Sida cordifolia (Aqueous Extract) on

Table 9. The effect of ethanol extract of *B. pinnatum* on low density lipoprotein and high density lipoprotein concentration of alloxan-induced diabetic rats.

Group	LDL (mmol/l)	HDL (mmol/l)
Group 1 (Normal/negative control)	1.5±0.12*	1.3±0.08*
Group 2 (Diabetic not treated - positive control)	2.8±0.09	0.65±0.09
Group 3 (Diabetic + standard drug)	1.4±0.05*	1.4±0.09*
Group 4 (Diabetic + 200 mg/kg body weight of extract)	1.6±0.12*	1.35±0.06*
Group 5 (Diabetic + 400 mg/kg body weight of extract)	1.25±0.11*	1.5±0.08*
Group 6 (Non-Diabetic + 200 mg/kg body weight of extract)	1.7±0.07*	-
Group 7 (Non-Diabetic + 400 mg/kg body weight of extract)	1.8±0.04*	-

Values are expressed as Mean \pm Standard Error of Mean; n = 3 animals per group; where P<0.05 is considered significant (*) in comparison with positive control group using ANOVA.

Table 10. The effect of ethanol extract of *B. pinnatum* on Triacylglycerol and total cholesterol concentration of alloxan-induced diabetic rats.

Group	TAG (mmol/l)	T.CHOL (mmol/l)
Group 1 (Normal/negative control)	1.0±0.06*	3.2±0.13*
Group 2 (Diabetic not treated - positive control)	2.3±0.07	5.1±0.07
Group 3 (Diabetic + standard drug)	1.1±0.08*	3.1±0.06*
Group 4 (Diabetic + 200 mg/kg body weight of extract)	1.2±0.05*	3.5±0.08*
Group 5 (Diabetic + 400 mg/kg body weight of extract)	1.1±0.03*	3.4±0.07*
Group 6 (Non-Diabetic + 200 mg/kg body weight of extract)	1.15±0.07*	3.4±0.09*
Group 7 (Non-Diabetic + 400 mg/kg body weight of extract)	1.1±0.06*	3.4±0.04*

Values are expressed as Mean \pm Standard Error of Mean; n = 3 animals per group; where P<0.05 is considered significant (*) in comparison with positive control group using ANOVA.

diabetes induced in wistar rats using streptozotocin was evaluated.

Lipid peroxidation is one of the major features of chronic diabetes (Satheesh and Pari, 2004) which is characterized with high breakdown of intra-cellular macro molecules. In this study as shown in Table 8, a significant increase (p<0.05) in the concentration of malondiadehyde (MDA), the product of lipid peroxidation, was observed in the diabetic untreated rats (positive control) when compared with the diabetic rats treated with B. pinnatum ethanol leaf extract. The significant (p<0.05) decrease in the level of malondiadehyde seen in the treatment group could be as a result of the actions of the organic compounds (phytochemicals) inherent in the extract in mopping out free radicals thus inhibiting the breakdown of the lipid layers of intracellular micro and macro molecules. Thus, the results of this research work is in tanden with the works of Selvan et al. (2008) where the antidiabetic and antioxidant effect of methanol extract of Artanema sesamoides in streptatozocin-induced diabetic rats was determined.

Results from Tables 9 and 10 show that oral administration of ethanol leave extract of *B. pinnatum* for 21 days at various doses showed a statistically significant (p<0.05) reduction in the low density lipoprotein,

triacylglycerol and total cholesterol concentration in diabetic rats. Prolonged diabetes mellitus is a risk factor for coronary heart diseases. The abnormal increased level of serum lipids such as LDL, TAG and total cholesterol shown in Tables 9 and 10 is primarily as a result of the spontaneous actions of lipolytic enzymes on the fat depot mainly due to insufficiency in insulin levels (Ei-soud et al., 2007). At a healthy state, insulin triggers the enzyme lipoprotein lipase, which breakdown triglycerides via hydrolysis while in a diabetic state; lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia. Alteration of serum lipid profile is known to occur in diabetes and this is likely to increase the risk of coronary heart diseases. A significant reduction (p<0.05) in serum lipids, particularly, the LDL. total cholesterol and triacylglycerol concentrations achieved through the administration of the extract was considered as being profitable for the long term prognosis of the disease (Chattopadhay and Bandyopadhay, 2005). The ethanol extract of B. pinnatum leaves produced a significant reduction (p<0.05) in the total cholesterol, low density lipoprotein and triacylglycerol concentration, while conversely, high density lipoprotein levels showed a significantly increase (p<0.05) in the diabetic rats treated with the extract when compared with untreated diabetic

rats (positive control group). The significant (p<0.05) decrease in cholesterol and LDL levels achieved by the administration of *B. pinnatum* leaf extract indicates a possible protection against hypercholesterolemia with its complications. Insulin deficiency may also be responsible for dyslipidaemia because insulin has an inhibitory action on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-COA) reductase, a key rate limiting enzyme responsible for the metabolism of cholesterol rich LDL particles.

Conclusion

The results presented in this study have shown that the ethanol extract of *B. pinnatum* has hypoglycemic effect. The graded doses of the extract equally boosted the hematological parameters of the treatment justifying its use in the treatment and management of diabetes mellitus and its related complications at the traditional level among the people of the South Western and South Eastern Nigeria and many other countries of the world.

REFERENCES

- Abei HE (1983). Catalase. In: Methods of Enzymatic Analysis. 3rd edn. 273-285.
- Albers JJ, Warnick GR, Cheung MC (1978). Dertermination of High Density Lipoprptein. Lipids. 13:926-932.
- Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC (1974). Enzymatic determination of serum cholesterol. Clin. chem. 20(4):470-475.
- Assman G, Jab HU, Hormert U (1984). LDL- Cholesterol determination in blood following precipitation of LDL with polyvinyl sulphate. Clin. Chem. Acta. 140(1):77-84
- Bucalo G, David H (1973). Quantitative determination of serum triacylglycerol by use of enzymes. Clin. chem. 19(5):476-482.
- Chan TYK (2009). Potential risks associated with the use of herbal antiobesity products. Drug Safe. 32(6):453-456.
- Chattopadhay RF, Bandyopadhay M (2005). Effect of *Azadirachta indica* leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats. Afr. J. Biomed. Res. 8(2):101-104.
- Cohen-Kohler JC (2007). The morally uncomfortable global drug gap. Clin. Pharmacol. Therap. 84(5): 610-614.
- Ei-soud NH, Khalil MY, Oraby FS, Farrag AR (2007). Antidiabetic effects of fenugreek alkaloid extract in streptozotocin induced hypoglycaemic rats. J. Appl. Sci. 3(10):1073-1083.
- Ernst E (2005). The efficacy of herbal medicine-an overview. Fund. Clin.Pharmacol.19(4):405-409.
- Gwehenberger B, Rist L, Huch R, Von-Mandach U (2004). Effect of *Bryophyllum pinnatum* versus fenoterol on uterine contractility. Europ. J. Obst. Gynecol. Reprod. Bio. 113(2):164-171.
- Harborne JB (1989). Phytochemical methods. A Guide to Modern Technology of Plant Analysis, 3rd Ed. Chapman and Hall, New York. pp. 88-185.
- Khan M, Patil PA, Shobba JC (2004). Influence of *Bryophyllum pinnatum* leaf extract on wound healing in albino rats. J. Nat. Remedies 4(1):41-46.
- Kulka RG (2006). Cytokinin inhibits epiphyllous plantlet development on leaves of Bryophyllum (Kalanchoë) Marnierianum Exp. Bot. 57(15):4089-4098.
- Livingston AL, Whitehand LC, Kohler GO (1997). Microbiological assay for saponin in alfalfa products. J. Asso. Off. Anal. Chem. 60(4):957-958.

- Lorke D (1983). Determination of acute toxicity. Arch. Toxicol. 53:275.
- Mahrukh A, Shahid P, Mudasir S, Rajinder R, Pawan KV, Azad AA, Nrip KP (2015). Antidiabetic Effect of *Sida Cordifolia* (Aqueous Extract) on Diabetes Induced In Wistar Rats Using Streptozotocin And Its Phytochemistry. Int. J. Pharmaceut. Res. Innov. 8, 11-22.
- Muhammad NO, Akolade JO, Usman LA, Oloyede OB (2012). Haematological Parameters of Alloxan-Induced Diabetic Rats Treated With Leaf Essential Oil of *Hoslundia Opposita* (Vahl). EXCLI J. 11:670-676.
- Ochei J, Kolhatkar A (2005). Medical Laboratory Science: Theory and practice. Tata Mc Graw Hill Publishing Company, New Delhi. pp. 281-283.
- Oyedemi SO, Adewusi EA, Aiyegoro OA, Akinpelu DA (2011). Antidiabetic and haematological effect of aqueous extract of stem bark of *Afzelia africana* (Smith) on streptozotocin-induced diabetic Wistar rats. Asian Pac. J. Trop. Biomed. 1(5):353-358.
- Prince SM Menon VP (2000). Hypoglycaemic and other related actions of *Tinospora* cardifolia roots in alloxan induced diabetic rats. J. Ethnopharmacol. 70(1):9-15.
- Satheesh MA, Pari L (2004). Antioxidant effects of *Boehavia diffusa* in tissues of alloxan-induced diabetic rats. Indian J. Exp. Biol. 42: 669-695.
- Saxena A, Vikram NK (2004). Role of selected Indian plants in the management of type 2 diabetes. J. Alt. Compl. Med. 10(2):369-378.
- Selvan VT, Manikandan L, Senthi GP, Suresh R, Kakoti BB, Gomathi P, Kumar DA, Saha P, Gupta M, Mazumder UK (2008) Antidiabetic and Antioxidant Effect of Methanol Extract of *Artanema sesamoides* in Streptatozocin-Induced Diabetic Rats. Int. J. Appl. Res. Nat. Prod. 1(1):25-33.
- Thirunavukkarasu V, Anuradha CV, Viswanathan P (2003). Protective effect of Fenugreek (*Trigonellafoenum* graecium) seeds in experimental ethanol toxicity. Phytother. Res. 17(7):737-743.
- Ukegbu CY, Odiba A, Edeke A, Anunobi O, Ike C (2016). Anti-diabetic Effect of the Methanolic Leaf Extract of Axonopus compressus (*P. Beauv*) in Alloxan Induced Diabetic Rats. Int. J. Biochem. Res. Rev. 12(1):1-5.
- Wallin B, Rosegren B, Shertzer HG, Caeejo G (1993). Lipoprotein oxidation and measurement of thiobarbituric acid reacting substances, formation in a single microtitre plate: its use for evaluation of antioxidants. Anal. Biochem. 208(1):10-15.
- Wieslaw O, Marta J, Anna S (1999). Determination and toxicity of saponins from *Amaranthus cruentus* seeds. J. Agric. F. Chem. 47: (9) 3685-3687.
- World Health Organisation (1996). WHO guidelines for the assessment of herbal medicines. WHO Expert Committee on Specification for Pharmaceutical Preparation. Technical Report series No 863. Geneva.
- Yeung KS, Gubili J, Cassileth B (2008). Evidence-based botanical research: Applications and challenges. Hematol. Oncol. Clin. North Am., 22(4):661-670.