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Effects of ethanolic and aqueous leaf extracts of *Bryophyllum pinnatum* on haematological parameters of normal and streptozotocin–induced diabetic rats

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ABSTRACT: The effect of the oral administration of both ethanolic and aqueous leaf extracts from *Bryophyllum pinnatum* (BP) on haematological parameters of normal and streptozotocin - induced (STZ) diabetic rat was investigated. Twenty-five male Wister rats were used and divided into five groups of five rats each. They were designated as (Normal Control – NC, Diabetic Control – DC, Diabetic Ethanolic Extract – DEE, Diabetic Aqueous Extract – DAE, and Normal Aqueous Extract – NAE). Groups NC and DC served as 'control' animals receiving food and water only. Groups DC, DEE and DAE were injected intraperitoneally with 65mg/kg body weight streptozotocin. Induction of diabetes mellitus was confirmed after 48 hours using glucose test strips. The test rats were all treated with 100mg/kgbwt ethanolic and aqueous leaf extracts of Bryophyllum pinnatum for 28days. At the end of the 28days, the rats were sacrificed and whole blood collected for Haematological assay. Results obtained showed a significant difference($P<0.05$) in White Blood Cell (WBC), Red Blood Cell (RBC), Platelet (PLT), Haemoglobin (HBG), Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC) and Lymphocytes (LYM) in treated rats with BP leaf extracts when compared to the diabetic and normal control groups.

Key words: Bryophyllum pinnatum; Haematological parameters; Streptozotocin, Diabetes

Introduction

Human depended on certain plants for healthcare since time immemorial. Centuries of experimentation on the use of plants or products derived from them has led to the development of indigenous systems of medicine that are still respected and used in many societies. Plants have been a source of medicines for humans and livestock and pesticides to protect crops from certain pests and diseases. Despite the immense technological advancement in modern medicine, many people in Africa still rely on traditional healing

practices and medicinal plants for their daily healthcare needs (Worobetz *et al*., 2005). The vegetation and flora biodiversity of Africa provide African traditional healers with an impressive pool of 'natural pharmacy' from which plants are selected as remedies, or as ingredients to prepare herbal medicines (phytomedicines) for a plethora of human and veterinary disorders (Oyewole, 2005). Most of the medicinal plants have traditionally been collected from forest and natural habitats. Indiscriminate deforestation over the years not only reduces their supplies but also endangered some of these valuable species (Principe, 1991; Rao, 1999).

The pharmaceutical industry has come to consider traditional medicine as a source for identification of bioactive agents that can be used in preparation of synthetic medicine (Wink, 1999; Prescott *et al*., 2002; Lang *et al*., 2008). *Bryophyllum pinnatum* is a rapid growing juicy herb with basal rosette. The leaves are thick, fleshy and simple or compound in pairs on reddish stem. Plantlets grow along the notches of the leaf margins which can develop while still attached to the plant or when detached. The plant is classified as a weed, is notorious for its growth potential. Shortly after a leaf falls to the ground, a whole garland of new little plants develops from the notches along the leaf margin. Flowers occur in corymbs / raceme and are about 5cm long, nodding bell-shaped, greenish or yellowish, reddish by the stem. Stem has green flecks, slightly woody, grows about 1m tall.

In view of its importance in the indigenous system of medicine, various groups of workers have investigated the constituent of the leaves of *Bryophyllum pinnatum* and reported the occurrence of organic acids (Oyewole, 2005), hydrocarbons (Erni, 2006), phenolic compounds, flavonoids and sterols (Rajagopal and Sasikala, 2008). Bruneton (1995) examined the crude extract of the leaves and found them to have two or more of the common phytoconstituents like alkaloids, tannins, phenols, glycosides and flavonoids. These major phytocompounds are known to have antimicrobial activity. Oyewole (2005), from the results of his experimental study suggest that the different flavonoids, polyphenols, triterponoids and other chemical constituents of the herb account for the observed antinociceptive, anti-inflammatory, and anti-diabetic properties of the plant. The present investigation is fashioned out to examine the homeostatic changes brought about by *Bryophyllum pinnatum* using ethanolic and aqueous extracts in normal and diabetic rats as a preliminary approach in evaluating its use in clinical practice.

Materials and Methods

Plant Sample: Collection, Extraction and Fractionation

Fresh leaves of *Bryophyllum pinnatum* were collected from Ikot Ubo, a village located in Nsit Ubium L.G.A. of Akwa Ibom State, Nigeria. The leaves were identified and authenticated by a Botanist at the Botany Department of the University of Uyo, Uyo, Akwa Ibom State, Nigeria with identification number Ekpo, UUH1481 (Nsit Ubium). The leaves were washed and dried in shade at room temperature for seven weeks. The dried leaves were powdered by using a grinder. This was divided into two parts of 220grams each and packed into Soxhlet column and extracted using 50% ethanol and aqueous solvent respectively for 24hours. The excess of solvent was removed using rotatory flash evaporator maintained at 45^{o}C and the concentrate was further dried at the same temperature in an oven to remove all the water. After drying the aqueous leaf extract 40.56g of crude extract was obtained while 20.85g was obtained from the ethanolic leaf extract after drying. These were each sealed in a 250cm^3 beaker and stored in the refrigerator below 10^{0}C until required for use.

Experimental Animals

Forty male albino rats (110-120g), age 3 (three months old) were used throughout the experiments. The animals were procured from the animal house of the Department of Biochemistry, University of Uyo, Akwa Ibom State, Nigeria. Before initiation of experiment, the rats were acclimatized in the Biochemistry departmental animal house of Michael Okpara University of Agriculture, Umudike for a period of 14 days

in clean metallic cages before being used. Standard environmental conditions such as temperature (26 + 2^0C), relative humidity (45-55%) and 12 hrs dark/light cycle were maintained in the quarantine. All the animals were fed with commercial pelleted rats chow (purchased from Umuahia market, Abia State) and water was allowed *ad-libitum* under strict hygienic conditions.

Streptozotocin induction

Diabetes was induced by intraperitoneal (ip) injection of streptozotocin (STZ; 2-Deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose) (65mg/kg body weight) dissolved in 0.1 M cold citrate buffer (pH 4.5) to the overnight fasted rats (Bedoya *et al*., 1996). Streptozotocin is synthesized by *Streptomycetes achromogenes* and is used to induce both insulin-dependent and non-insulin-dependent diabetes mellitus (IDDM and NIDDM, respectively). After 7 days blood was taken from the tails of the rats and the blood sugar levels were monitored using One Touch® Ultra Glucometer (Lifescan Inc., 1995 Milpitas California 95305, USA). This was also repeated after 14 and 21 days. The animals with blood sugar level more than 200 mg/dl were considered diabetic and included in the experiment.

Experimental design

Forty male Wistar albino rats (110-120g), age, 3 months old were taken to the animal house to be quarantined for the experiment. To study the homeostatic changes in experimental diabetic rats using various extracts, grouping and dosing schedule in the rats were followed. Eighteen of these rats were induced with diabetes using streptozotocin (STZ). These animals were monitored for 22 days to ascertain their diabetic levels.

The diabetic animals were randomly distributed into three groups of five animals each and two other non-diabetic groups of five animals each were also added to the experiment. The weights of the animals in each group were taken before the experiment began. They weighed between 160 – 206g. The five groups were labelled as follows:

a) Group NC: Normal (Control)
b) Group DC: Diabetic (Negative) Control
c) Group DEE: Diabetic rats administered *Bryophyllum pinnatum* 100mg/kg b.w ethanolic extract
d) Group DAE: Diabetic rats administered *Bryophyllum pinnatum* 100mg/kg b.w aqueous extract
e) Group NAE: Normal rats administered *Bryophyllum pinnatum* 100mg/kg b.w aqueous extract

Group NC was the normal control group. The rats were not induced with diabetes but were given food and water without the extract for twenty-eight (28) days.

Group DC was the diabetic (negative) control group. The rats were induced using STZ with diabetes, given food and water but without the extract for twenty-eight (28) days.

Group DEE was the diabetic ethanolic extract group. The rats were induced using STZ with diabetes, given food and water plus the ethanolic extract of *Bryophyllum pinnatum* leaves daily for twenty-eight (28) days.

Group DAE was the diabetic aqueous extract group. The rats were induced using STZ with diabetes, given food and water plus the aqueous extract of *Bryophyllum pinnatum* leaves daily for twenty-eight (28) days.

Group NAE was the normal aqueous extract group. The rats were not induced but were given food and water plus the aqueous extract of *Bryophyllum pinnatum* leaves daily for twenty-eight (28) days.

The animals were properly fed twice a day except on days they were made to fast over-night for eighteen (18) hours for their blood sugar levels to be taken the following day. Water was ad libitum made available to all the animals in their cages.

Every morning the weights of the rats in each group were taken and the extract (both ethanolic and aqueous leaf extract of *Bryophyllum pinnatum*) was given to the groups as appropriate. The volume of extract given to each rat was calculated in line with the body weight of the rat taken each day.

Administration of the extract

The 'test' compound [i.e., *Bryophyllum pinnatum* leaf ethanolic and aqueous extract (BP, 100 mg kg-1 day-1 p.o.)] was administered orally by intragastric intubation to Groups DEE, DAE and NAE rats. Group DEE rats received the ethanolic leaf extract administration of *Bryophyllum pinnatum* (100 mg kg^{-1}) while Groups DAE and NAE rats received the aqueous leaf extract administration of *Bryophyllum pinnatum* (100 mg kg^{-1}). Groups NC and DC received water only. Commencement of extract administration was from the 22nd day of post STZ injection and continued for the next 28 consecutive days. The reason for the delay in extract administration was because not all the rats induced were diabetic at the same time. Some took a shorter period while others took a longer period. But on the 22nd day all the rats induced were diabetic. The experimental animals were kept under surveillance and observed for physical and morphological changes.

Animal sacrifice and sera preparation

All the experimental animals were sacrificed 24hours after the last administration of the extracts. They were starved for 18hours before the sacrifice.

Procedure

A little knock was given to the rat on the head to daze it and this was placed on the dissecting board with pins fastened to its hands and legs to hold it to the board. Blood samples for sera preparation were collected by cardiac puncture into sterile plain bottles for clinical chemistry analysis and EDTA bottles for haematological analysis. Sera were obtained from the blood by centrifugation using a bench top centrifuge (MSE) at 3000g for 10 minutes.

Haematological Test

Whole blood samples were used in the determination of White Blood Cell (WBC), Red Blood Cell (RBC), Platelet (PLT), Haemoglobin (HBG), Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC) and Lymphocytes (LYM). An automated haematological analyzer, 2016 model, number (SYSMEC XP 300) was used for the analysis of the haematological parameters.

Statistical Analysis

The data obtained were expressed as means (±SEM), and analyzed by using repeated measures of variance. The statistical tool used was SPSS STATISTICS 17.0. The differences between the means of groups were analyzed statistically with one-way analysis of variance (ANOVA); 95% confidence interval). Values at $P<0.05$ were taken to imply statistical significance.

Results and Discussion

The results of the effects of *Bryophyllum pinnatum* (BP) leaf extracts on haematological parameters in normal and STZ-induced diabetic rats are shown in Figure 1. It was observed from the bar chart that all the rats treated with the leaf extracts both ethanolic and aqueous showed an increase in the levels of WBC, PLT, RBC and HGB as compared to the diabetic and normal control (DC and NC) groups. The diabetic rats (DEE and DAE) groups treated with both extracts showed an increase in the PCV levels when compared with the diabetic control (DC) group. The normal rat (NAE) treated with the aqueous extract of BP leaf experienced a decrease in the PCV levels when compared with the normal control (NC) group. The rat groups (DAE and NAE) treated with the aqueous extract of BP leaf experienced a decrease in their MCV, MCH, MCHC and LYM levels when compared with the diabetic and normal control groups while aqueous extract of BP leaf decreased the LYM levels of the normal rats (NAE) when compared with the normal control (NC) group.

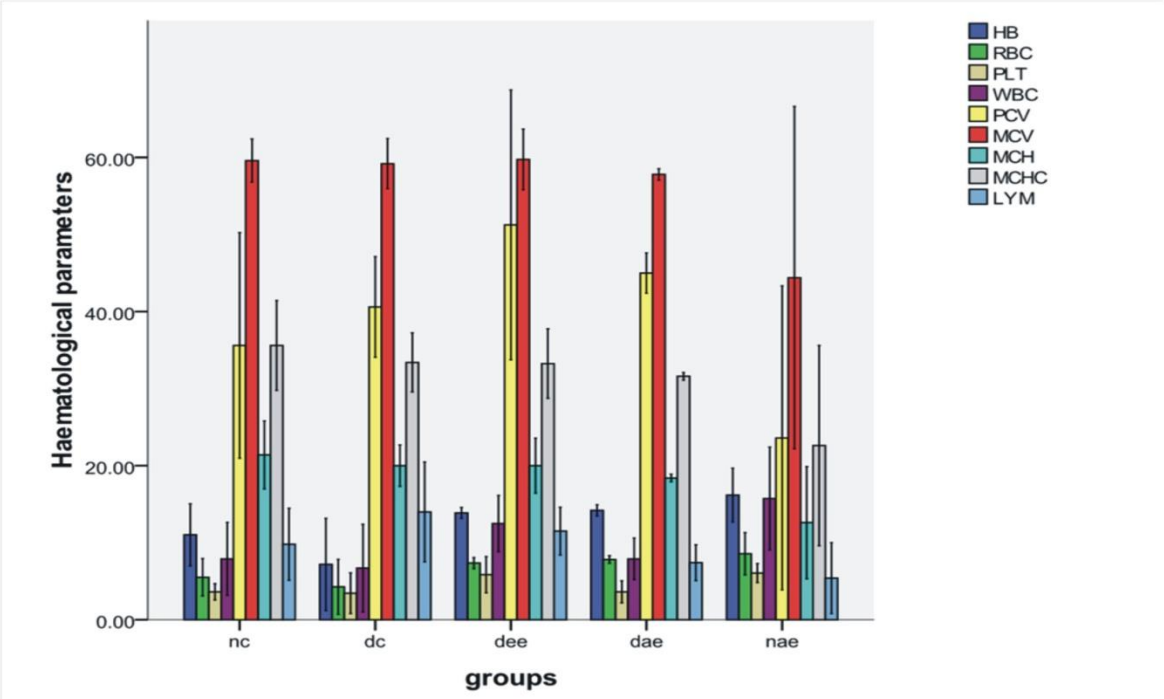

Fig. 1 Effect of *Bryophyllum pinnatum* leaf extract on haematological parameters of normal and STZ-induced diabetic rats

The purpose of determining the haematological indices is to corroborate and correlate the fundermental results obtained in the routine complete blood count (Bry *et al*., 2001). The haematological parameters (WBC, PLT, RBC, HGB, PCV, MCV, MCH, MCHC and LYM) evaluated in this study showed that the levels of WBC, PLT, RBC HBG and PCV treated with both extracts (ethanolic and aqueous) of *Bryophyllum pinnatum* leaf increased appreciably when compared to the diabetic and normal control (DC and NC) groups. There was a negative effect of the BP leaf extracts on PCV, MCH, MCV, MCHC and LYM parameters investigated because there was a decrease in their levels in the rats. Prolong use of the extract may lead to microcytosis and macrocytic anaemia when there is a decrease in MCV and MCHC respectively.

Conclusion

The haematological parameters (WBC, PLT, RBC, HGB, PCV, MCV, MCH, MCHC and LYM) evaluated showed significant difference as compared with the diabetic and normal control groups. The results presented in this investigation showed that the ethanolic and aqueous extracts of *Bryophyllum pinnatum* has an increase in the levels of WBC, PLT, RBC and HBG as seen in the treated wister rats. *Bryophyllum pinnatum* leaf extract has phagocytotic activity by increasing the total white blood count (WBC) which is involved in phagocytosis.

Recommendations

The use of *Bryophyllum pinnatum* in ethnomedicine should be encouraged owing to its immune enhancement potentials but with caution because of likely toxicity. Further research should be carried out to identify the particular phytoreactant that stimulate the immune system in order to eliminate the toxic moiety.

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