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Serum lipid levels of experimental diabetic rats treated with leaf extracts of *Bryophyllum pinnatum*

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ABSTRACT: Serum lipid in the blood levels of rats experimentally induced with diabetes mellitus were estimated after 28 days of treatment with ethanolic and aqueous extracts of *Bryophyllum pinnatum* (BP) leaf. The male Wistar rats were divided into five groups 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 rats served as 'control' animals receiving food and water only. Groups DC, DEE, and DAE were injected intraperitoneally with 65mg/kgbwt streptozotocin (STZ). 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 blood collected for serum lipid profile assay. Induction of diabetes mellitus in groups DC, DEE, and DAE with STZ resulted in hyperglycaemia, hypoinsulinaemia, and increased triacylglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and significantly decreased ($P<0.05$) high density lipoprotein (HDL). BP treated groups DEE, DAE and NAE showed significant decrease ($P<0.05$) in elevated blood glucose, TC, TG, LDL, VLDL as compared to the control groups DC and NC. Furthermore, BP treatment significantly increased ($P<0.05$) serum HDL in the test groups as compared with the control groups. These results established that the inducement produced adverse serum lipid changes in the rats that validate diabetes mellitus as a devastating disease that has claimed many lives if not properly understood and managed.

Keywords: Serum lipid levels, Diabetes mellitus, Streptozotocin.

Introduction

Diabetes is characterized by elevated blood glucose or sugar level. When a person eats, his blood sugar (glucose) rises and the pancreas senses that the blood glucose level has risen and produces a hormone called insulin which is released into the blood to regulate this sugar level. There is a mechanism that does this. In people with diabetes, the mechanism works abnormally. Although diabetes has been recognized since antiquity, the treatments of various efficacy have been known in various regions since the Middle Ages,

and in legend for much longer, pathogenesis of diabetes has only been understood experimentally since about 1900 (Patlak, 2002). *Bryophyllum pinnatum* is suspected to have hypoglycaemic and anti-diabetic properties (Siddhartha and Nag Chandhuri, 1991; Ojewole, 2005). Despite the availability of treatment, diabetes has remained a major cause of long-term complications and death (Trivedi *et al*., 2004). There is still no known cure for diabetes. The patients are only managed until their death. The findings from this research work would complement other plants used as anti-diabetic therapy.

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 40.56grams of crude extract was obtained while 20.85grams 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 (28 + 2^{0}C), 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 – 206grams. 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 for serum lipid profile assay. Sera were obtained from the blood by centrifugation using a bench top centrifuge (MSE) at 3000g for 10 minutes.

Estimation of Serum Lipid Profile

An automated serum lipid profile analyzer, 2016 model, number (SYSMEC XP 300) was used for the analysis of the serum lipid profile.

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

Figure 1 shows the effect of *Bryophyllum pinnatum* leaf extract on serum lipids of normal and STZ-induced diabetic rats. Values are Mean±S.E.M; $n = 5$; ($P \leq 0.05$). The results on the serum lipid analysis is on Figure 1. The figure compares the effect of *Bryophyllum pinnatum* (BP) leaf extract on serum lipids in normal and STZ-induced diabetic rats. It is observed that triacylglycerides in diabetic rats (DEE) decreased when treated with ethanolic extract of BP and increased when treated with aqueous extract (DAE) when compared with diabetic control (DC) group. Triacylglycerides reduced in normal rats (NAE) treated with aqueous extract of BP when compared with the normal control (NC) group.

Total Cholesterol decreased in the diabetic ethanolic extract (DEE) group and decreased in the diabetic aqueous extract (DAE) group respectively as compared with the diabetic control (DC) group. This shows that the aqueous extract has more effect in cholesterol reduction than the ethanolic extract of BP leaf. Total cholesterol in the normal rats (NAE) treated with the aqueous extract of BP increased when compared with the normal control (NC) group.

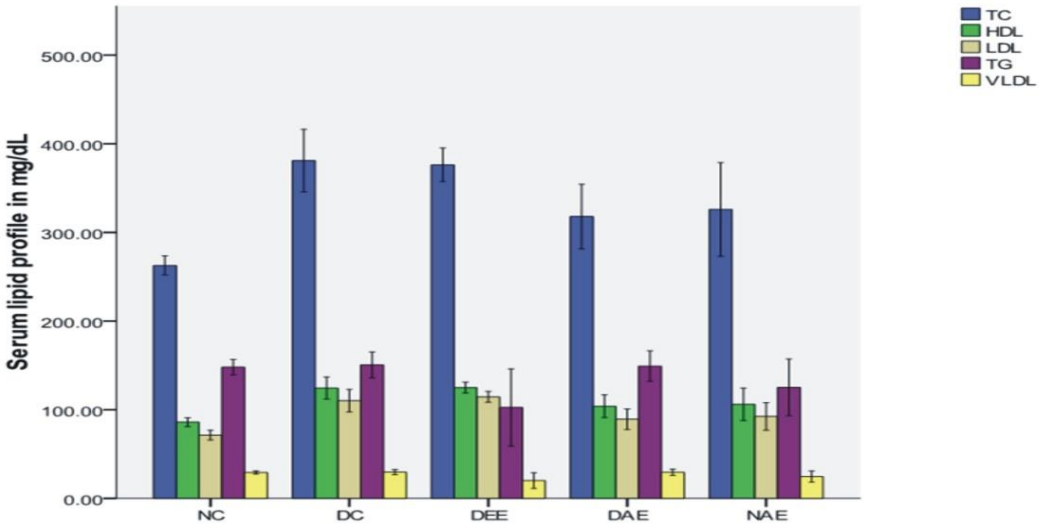

Fig. 1: Effect of *Bryophyllum pinnatum* leaf extract on serum lipids of normal and STZ-induced diabetic rats

High density lipoprotein (HDL) increased in the diabetic rats treated with ethanolic extract (DEE) of BP and decreased in the diabetic rats treated with aqueous extract (DAE) of BP respectively as compared with the diabetic control (DC) group. HDL increased in normal rats (NAE) treated with the aqueous extract of BP when compared to normal control (NC) group.

Low density lipoprotein (LDL) increased in the diabetic rats treated with ethanolic extract (DEE) of BP and decreased in the diabetic rats treated with aqueous extract (DAE) of BP respectively as compared with the diabetic control (DC) group. This implies that aqueous extract of BP leaf is positively predictive of LDL than the ethanolic extract of BP leaf. Also, normal rats (NAE) treated with the aqueous extract of BP experienced an increase in LDL when compared with the normal control (NC) group.

Very low density lipoprotein (VLDL) decreased drastically in the diabetic rats treated with ethanolic extract (DEE) of BP and slightly decreased in the diabetic rats treated with aqueous extract (DAE) of BP as compared with the diabetic control (DC) group. Moreso, normal rats (NAE) treated with aqueous extract had their VLDL increased when compared to the normal control (NC) group without BP leaf extract.

Discussion

Cholesterol is essential for the biosynthesis of several hormones as well as bile acids in animal and human cells. Individuals acquire cholesterol from two major sources, namely that synthesized by the body and dietary intake (Hilsden and Shaffer, 2005). Hypercholesterolemia refers to high level of cholesterol in the blood especially the low density lipoprotein (LDL) which has been implicated as a major risk factor associated with coronary heart disease. The ability to maintain serum cholesterol at a desirable level is one of the major preventive strategies for the disease (Abd El-Gawad *et al*., 2005). The result of this study clearly indicated that the aqueous and ethanolic extracts of *Bryophyllum pinnatum* not only had hypoglycaemic effects but also hyperlipidaemic effects in streptozotocin-induced diabetic rats. A significant reduction ($P \leq 0.05$) in the blood glucose level of all treated groups was observed. This agrees with those of other researchers (Akah *et al*., 2004; Nimenbo-Uadia, 2003; Gyang *et al*., 2004).

The high plasma total cholesterol (TC) concentration observed in the diabetic animals treated with the extract clearly demonstrated the presence of hyperlipidaemic agents in the extract. There was a significant decrease in both triacylglyceride (TG) and LDL-cholesterol levels while significant increase in HDL-

cholesterol levels was observed. It could be interpreted that the extract had some beneficial effects on cardiovascular risk factors. These observations support the local use of *Bryophyllum pinnatum* as hypoglycaemic agent. An increment in HDL cholesterol and a reduction in LDL and total cholesterol could be considered beneficial in the long-term prognosis of diabetic subjects. The treated animals showed significant increase in the levels of HDL-cholesterol compared to the negative control. The observed abnormalities of triglyceride and HDL metabolism are in accordance with reports on early manifestation of insulin resistance, the precursor to diabetes (Otvos *et al*., 2002).

Conclusion

In the present study, it is noticed that inducement of diabetes mellitus in groups DC, DEE, and DAE with STZ resulted in increased TG, TC, LDL and VLDL. STZ significantly decreased ($P<0.05$) HDL. However, in this study it was observed that BP treated groups, DEE, DAE and NAE showed significant decrease ($P<0.05$) in elevated TC, TG, LDL and VLDL. Furthermore, BP treatment significantly increased ($P<0.05$) serum HDL.

Recommendations

The use of *Bryophyllum pinatum* in the treatment of diabetes mellitus should be encouraged since it has the ability to reduce the bad cholesterol but with caution. Also in further study another herbal plant should be combined with *Bryophyllum pinnatum* to prevent the increased levels of LDL and TC in patients when treated with the ethanolic extract of BF.

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