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ORIGINAL ARTICLE | DIABETES

Biochemical Effects of *Piper Guineense* (*African Black Pepper*) in Female Diabetics: Opportunities for Diabetes Treatment

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ABSTRACT

Objectives: To investigate the biochemical effects of oral doses of *Piper guineense* (*P. guineense*) leaf extract on female diabetics using experimental animals.

Methods: The animals, albino Wistar rats, were divided into six groups (n=7). Animals in group 1 received water and feed only. Animals in groups 2 to 6 were induced with diabetes using alloxan. Methanolic leave extracts of *P. guineense* were administered to groups 2 to 4 in 40 mg/kg, 80 mg/kg and 100 mg/kg body weights representing low, medium and high doses respectively. Group 5 animals were treated with 10 mg/kg body weight of Glibenclamide (Antidiabetic drug) and group 6 animals were left untreated. All treatments were carried out orally and lasted for a period of 14 days. At the end of the 14 days, the animals were humanely sacrificed through cardiac puncture and the blood samples collected for the analyses of some liver and kidney function parameters using assay kits.

Results: The results showed that the oral doses of methanolic leave extract of *P. guineense* had no negative alterations on the biochemical parameters analyzed namely, 1) Lipid profile (Triglyceride, Low Density Lipoprotein, Total Cholesterol and High Density Lipoprotein levels), 2) electrolytes profile (Sodium, Potassium, Chloride, Bicarbonate) 3) Urea, and 4) Creatinine levels. Furthermore, there was a significant reduction in the urea levels of treated animals and marked but insignificant reduction in the total cholesterol level and increase in High Density Lipoprotein at $p<0.05$.

Conclusion and Implications for Translation: The reported antidiabetic *P. guineense* leaf extract caused no adverse biochemical changes in female diabetic rats. This implied that the extract may not distort the lipid and electrolyte profiles of female diabetics and could be pharmacologically safe in the management of female diabetics. It further implied that the *Piper guineense*, or Uziza, commonly taken after childbirth by nursing mothers in some tropical countries may maintain the lipid and electrolyte balance and consequently, prevent hypercholesterolemia and hypertension.

Keywords: Diabetes • *Piper guineense* • Lipids • Electrolytes • Methanolic Extracts • Black Pepper

1. Introduction

1.1. Background of the study

The use of plants as medicine predates written human history. Many of the *herbs* and *spices* used by humans to season food also yield useful medicinal compounds.[1] In recent times, a large number of plants have been credited with medicinal potentials and have been used in many parts of Africa and the rest of the world.[2] The inherent medicinal potentials of these plants lie in their bioactive constituents which includes nutrients such as minerals, vitamins, and non-nutrients such as phytochemicals.[3,4,5,6] One such plant with medicinal potential is *Piper guineense*.

Piper guineense, popularly known as African black pepper, hot leaf, or Uziza (in South East Nigeria), is widely consumed, on account of its nutritional and medicinal properties, in some parts of West Africa, especially in Nigeria and Ghana.[7] Studies have shown that apart from the use of these plants as spices and condiments, they have several other wide applications in the local treatment and management of many diseases.[1,8,9,10,11,12] Medicines developed from plants are comparably safer than their synthetic counterparts thus rendering enormous therapeutic benefits at an economical treatment rate.[13] *P. guineense* is one of such beneficial plants.

The phytochemical analysis of *P. guineense* showed the presence of alkaloids, flavonoids, saponins, tannins, resins and essential oils which have numerous pharmacological properties in diseases such as diabetes mellitus.[14] Diabetes mellitus is one of the most common metabolic disorders resulting from abnormal high blood sugar level. Furthermore, hyperlipidemia and hypercholesterolemia are some of the criteria indicating metabolic syndrome and these conditions are closely associated with insulin resistance.[15] Lipids and lipoproteins abnormalities are well known risk factors of heart diseases.[16]

Lipid abnormality also known as dyslipidemia may be primary and can accompany disease conditions such as hypertension, diabetes mellitus and obesity.[17,18,19,20] *Piper guineense* is useful in the reduction of fasting blood glucose level as it has been reported to have anti-diabetic properties in diabetic albino Wistar rats.[8] However, there is need to investigate its corresponding effects on biochemical parameters thus necessitating this study. This is pertinent because diabetes may cause distorted biochemical parameters which are risk factors for hypercholesterolemia and hypertension.[21]

1.2. Objectives of the study

The objectives of this study are:

1. To study the dose-dependent effects of methanolic leaf extract of *P.guineense* on the lipid profiles in female diabetic albino wistar rats; and
2. To study the dose-dependent effects of methanolic leaf extract of *P.guineense* on the electrolyte profiles in diabetic albino Wistar rats.

Specifically, we sought to further investigate the possible biochemical effects of *P.guineense* when used by diabetic females.

2. Methods

2.1. Collection of plant materials

Fresh leaves of *P. guineense,* were harvested from Umumee Market in Umuokanne in Ohaji Local Government Area of Imo State, Nigeria. The botanical identification was carried out by a plant Biotechnologist from the Department of Biotechnology, Federal University of Technology (FUTO) Owerri, Imo State, Nigeria. The fresh leaves collected were washed and air-dried for a period of one week and ground into powder using electric mill machine. The ground powder was stored in air tight container and ready for extraction.

2.2. Plant extraction

The powdered extract was dissolved in methanol for a period of three days and filtered with Whatman No. 1 filter paper.[6] The filtrate was evaporated to dryness using a rotary evaporator at 45°C and the dried powder was stored in universal sample bottles.[6] Methanol was preferred because of its greater potential for extracting medicinal substances from their crude source.[6]

2.3. Animal care and use

Forty two albino Wistar rats were used in this study. The animals were purchased from a farm in

the Department of Pharmacology, University of Port Harcourt, Rivers State, Nigeria, and housed at room temparature, in well-ventilated, clean cages made of plastic frames and metal netting also in the animal house of the Department of Biochemistry, Federal University of Technology (FUTO) Owerri, Imo State, Nigeria. Their beddings were changed every two days and they were fed with rat feed and water *ad libitum* which were changed daily. [22] The rats were allowed to acclimatize to the new environment for seven days after which the methanoic extract of *P. guineense* leaves was administered by forced feeding.[8]

2.4. Experimental design

The animals were grouped into six groups of seven rats each. Group one served as the normal control and was given water and feed only. Groups 2 to 6 were induced with diabetes. Group 2 served as test 1 and was treated with 40 mg/kg of the extract (low dose). Group 3 served as test 2 and was treated with 80 mg/kg of the extract (medium dose). Group 4 served as test 3 and was treated with 100 mg/kg of the extracts (high dose). Group 5 served as positive control and was treated with 10 mg/kg of glibenclamide (an anti-diabetic drug). Finally, Group 6 served as diabetic control and was left untreated.

2.5. Diabetes induction

The animals were fasted for 24 hours and diabetes induced by a single intraperitoneal injection of a freshly prepared solution of alloxan monohydrate (150 mg/kg) in 0.9% saline (NaCl) solution.[23] The animals were given 2mls of 5% dextrose solution using an orogastric tube immediately after induction to overcome the drug-induced hypoglycemia. About 72 hours later, rats with blood glucose level (BGL) above 200 mg/dl were considered diabetic and selected for the experiment.

2.6. Biochemical assay

At the end of 14 days of treatment, the animals were collected from the cages with the help of baskets and made unconscious by placing them in dessicators with 10% chloroform. A surgical blade was used to sacrifice the animals by cardiac puncture and their blood was taken into the lithium heparin bottle and centrifuged for five minutes. The analysis of the lipid profile and electrolytes were carried out using the standard reagents and methods.[24,25,26] Reagents were obtained from Randox Lipid Profile Reagents for triglycerides, total cholesterol and high density lipoprotein and low density lipoprotein as well as electrolytes such as creatinine and urea produced by Randox Laboratories Limited, United Kingdom. The analysis of Na+, K+, Cl and HCO were carried out using standard TECO assay kits following the procedures prescribed by the manufacturers. The screenings were carried out using an Automatic Biochemistry Analyzer CEL TECH CL 3000M Version 4.2.

2.7. Statistical analysis

All data were expressed as Mean ± Standard error of Mean and analyzed using the Analysis of Variance (ANOVA) at $p< 0.05$ levels of significance. The analysis was done with the use of SPSS. Analysis of variance (ANOVA) was used to check if the means of two or more groups are significantly different from each other. ANOVA checks the impact of one or more factors by comparing the means of different samples. We used factorian ANOVA which is an Analysis of Variance test with more than one level of independent variable, or "factor." It can also refer to more than one level of independent variable. For example, an experiment with a treatment group and a control group has one factor (the treatment) but two levels (the treatment and the control).[8]

2.8. Ethical approval

The study followed the principles of laboratory animal care as well as specific national and international laws where applicable.[22] All experiments were reviewed and approved by the School of Health Technology, FUTO ethical committee for the use of laboratory animals.

3. Results

3.1. Dose-dependent effects on lipid profile

The triglyceride concentration showed that there was an increase in the animals treated with 40 mg/kg (14.48 mmol/l), 80 mg/kg reduced significantly (9.91mmol/l), while those treated with the higher dose 100 mg/dl (14.19) and the animals treated with glibenclamide (standard control 14.09)

had a slight increase triglyceride concentration compared with the normal control of 12.9mmol/l as seen in Table 1 below.

It was deduced that the animals treated with the 40 mg/kg of the extract reduced significantly (0.6 mmol/l) those treated with 100 mg/kg and glibenclamide also had a significant decreased of 0.25 mmol/l and 0.17 mmol/l, respectively. However, the animals treated with 80 mg/kg of the extract increased significantly as compare with the control (4.25 mmol/l).

There is a significant increase in HDL concentration in the animals treated with 40 mg/kg and 100 mg/kg of the extract (9.44 mmol/l and 9.1mmol/l). The animals treated with the medium dose of 80 mg/kg had a slight increase (5.32 mmol/l) while the standard control group increased significantly (7.26 mmol/l) as compared with the normal control (4.55 mmol/l).

The result as seen in Table 1 also revealed that there was a significant increase in LDL in the animals treated with 80 mg/kg of the extract (9.04 mmol/l), the animals treated with 40 mg/kg and 100 mg/kg had a significant reduction of (4.44 mmol/l and 4.84 mmol/l), respectively. The standard control group increased significantly (6.83 mmol/l) compared with the control (5.72 mmol/l).

3.2. Dose-dependent effects on electrolyte profile

The result of sodium ion concentration showed significant reduction in the group treated with 80 mg/kg (107.05 mEq/l) whereas the animals treated with 40 mg/kg, 100 mg/kg and glibenclamide had a slight increase in sodium ion concentration (125.22 mEq/l, 124.3 mEq/l, and 130.52 mEq/l) respectively, compared with the normal group (122.74 mEq/l).

There was a significant increase in potassium ion in the animals treated with 100 mg/kg of the extract (5.83 mEq/l), the animals treated with 40 mg/kg and 80mg/kg had a slight increase (4.37 mEq/l and 4.46 mEq/l) respectively, whereas the animals treated with glibenclamide standard control experienced a reduction reduced (3.5 mEq/l) compared with the (3.98 mEq/l).

There was an increase in chloride ion in the animals treated with 80 mg/kg of the extract (124.26 mEq/l). The animals treated with 40 mg/kg and 100 mg/kg had a slight reduction in chloride ion concentration (114.33 and 111.68 mEq/l) respectively, whereas the animals treated with glibenclamide experienced a significant reduction (105.9 mEq/l) compared with the normal control (115.89 mEq/l) as shown in Table 2.The bicarbonate ion as seen in Table 2 showed a significant increase in HCO ion in animals treated with 80 mg/kg. 100 mg/kg and glibenclamide standard control (335.23 umol/l, 399.34 umol/l, and 336.93 umol/l) respectively. Whereas the animals treated with 40 mg/kg of the extract had a slight reduction in HCO ion (255.44 umol/l) compared with the control (272.53 umol/l).

There was a significant decrease in urea concentration in all the animals treated with 80 mg/kg and glibenclamide (1.12 mmol/l and 1.09mmol/l). The animals treated with 40 mg/kg and 100 mg/kg had a slight reduction in urea (3.28 and 2.55 mmol/l) respectively compared with the control (13.16 mmol/l).

Creatinine concentration reduced significantly in the animals treated with 80mg/kg (150umol/l). There

Table 1: Differences in Lipid Profile Parameters across Experimental Groups

Groups	Triglycerides (Mean/SE)	TC (Mean/SE)	HDL (Mean/SE)	LDL (Mean/SE)
1	12.90±0.87	4.25±3.4	4.55±1.72	5.72±1.11
2	14.48±0.08	0.60±0.17	9.44±0.96	4.44±1.14
3	9.91±4.58	5.07±2.95	5.32±2.35	9.04±2.38
4	14.19±0.09	0.25±0.13	9.10±1.46	4.84±1.53
5	14.04±0.39	0.17±0.13	7.26±6.55	6.83±6.62

TC=Total Cholesterol, HDL=High Density Lipoproteins, LDL=Low Density Lipoproteins Group 1=Normal Control, Group 2=Treated with 40 mg/kg, Group 3=Treated with 80 mg/kg, Group 4=Treated with 100 mg/kg, Group 5=Positive Control

Table 2: Results of the Electrolyte Profile across Experimental Groups

Groups	Na+ (mEq/L)	K+ (mEq/L)	Chloride (mEq/L)	HCO (umol/L)	Urea (mmol/L)	Creatinine (umol/L)
1	122.74±8.42	3.98±0.11	115.89±9.65	272.53±91.04	13.16±6.79	270.00±60.93
2	125.22±7.84	4.37±0.05	114.33±4.35	255.44±120.90	3.28±2.20	202.50±12.99
3	107.05±15.86	4.46±0.28	124.26±7.43	335.23±164.70	1.12±0.48*	150.00±75.00
4	124.20±4.87	5.83±1.64	111.68±8.85	399.34±137.75	2.55±1.77*	373.50±176.68
5	130.52±5.94	3.50±0.48	105.90±2.82	336.93±209.67	1.09±0.17*	202.50±22.50

*Significantly reduced at p<0.05, Group 1=Normal Control, Group 2=Treated with 40 mg/kg, Group 3=Treated with 80 mg/kg, Group 4=reated with 100 mg/kg, Group 5=Positive Control

was a slight reduction in the 40mg/kg and glibenclamide groups (202.5 umol/l), whereas the animals treated with 100 mg/kg (373.5 umol/l) compared with the normal control group (270 umol/l).

3.3. Biochemical effects

All animals in Group 6 (untreated diabetic) rats did not survive after three days, perhaps, due to the high level of blood glucose. Whereas *P. guineense* plant extract restored the high level of blood glucose to its normal level in the treated rats. The results of the lipid profile showed a reduction in total cholesterol and insignificant increase in High Density Lipoprotein. Triglyceride and Low Density Lipoprotein showed no statistical significant difference when compared with the control at p<0.05 after 14 days of treatment, although they are dose-dependent as shown in Table 1 above.

The extract was able to reduce the high electrolyte profile differences in Sodium, Potassium, Chloride, Bicarbonate and Creatinine levels compared to the Control. However there was a significant reduction in the Urea Levels at p<0.05 after 14 days of treatment.

4. Discussion

The high rate of mortality recorded in Group 6 animals could be due to the effects of diabetes on the animals which may have resulted in dyslipidemia involving elevated plasma levels of triglycerides, total LDL-cholesterol, VLDL-cholesterol and low level of HDL-cholesterol[27] that plays an important role in diabetic atherosclerosis.[28] Increased triglyceride level due to insulin deficiency results in hyperglycemia in which fatty acids from adipose tissues are mobilized for energy purpose followed by accumulation of the excess fatty acids in the liver which are converted to triglyceride.[29]

The marked reduction in Total Cholesterol levels of treated animals could be due to the cholesterol lowering ability of *P. guineense* containing alkaloids.[30] Alkaloids are made up of heterocyclic nitrogen that has anti-malarial, antihypertensive, antiarrhythmic and anticancer properties.[31] The cause for a reduction may be due equally to the antioxidant ability of *P.guineense*, which are rich in Vitamin C and E, and with its ability to prevent lipid peroxidation in both plasma and tissues.[32] High cholesterol in blood is associated with an increased risk of various disorders, such as atherosclerosis, stroke, and others cardiovascular diseases.[33] *P. guineense* has been shown to have anti-lipidemic effects.

The increase in the High Density Lipoproteins Cholesterol (HDL-C) may be associated with reduced risk in coronary heart disease. This may be due to the ability of *P. guineense* to enhance reverse cholesterol transport and scavenging excess cholesterol from peripheral tissue followed by esterification through lecithin cholesterol acyltransferase thereby delivering it to the liver and steroidogenic organs for lipoproteins and eventual elimination from the body.[34,35]

P. guineense is potent in increasing the HDL-C levels which could be attributed to the active constituents in the leaf extract, thereby reducing the risk of coronary heart disease caused by diabetes and other metabolic diseases. The higher the HDL-C the better and low levels of HDL-C raise the risk of heart disease.[35]

P. guineense had been reported to be a potent antioxidant which had hepato-protective properties.[9] It has equally been reported to also contain cardiac glycosides in a significant amount which is useful in

the management of diseases associated with the heart.[33]

The blood electrolytes measurements were carried out to evaluate and monitor electrolytes imbalances induced by diabetes. The renal system plays a primary role in the regulation of electrolyte/fluid balance, the pH buffer system and in the elimination of waste products.[26] The *P. guineense* leaf extract significantly reduced the excess serum urea levels at ($p<0.05$) associated with diabetes. The presence of increased blood urea nitrogen (BUN) may be due to pre-renal causes (cardiac de-compensation, water depletion due to decreased intake and excessive loss, increased protein catabolism, and high protein diet), renal causes may be due to (acute glomerulonephritis, chronic nephritis, polycystic kidney disease, nephrosclerosis, and tubular necrosis) and post renal causes may include all types of obstruction of the urinary tract, such as stones, enlarged prostate gland, and tumors. [26] *P.guineense* could be a safer anti-diabetic herb for female diabetic patients and further studies should explore the phytochemical analysis.

5. Conclusion and Implications for Translation

Oral doses of *P.guineense* have been shown not to induce any adverse effects/alterations on the biochemical parameters of female diabetic rats. Thus, its current uses as a flavoring and anti-hyperglycemic agent pose no adverse effects on the biochemical parameters but rather can be useful in reducing the risk of cardiovascular disorders and some other diabetic complications.

Compliance with Ethical Standards

Conflict of Interest: *Authors have declared that they have no conflict of interest.* ***Financial Disclosure:*** *The finances were used according to plan and were disclosed to all the authors.* ***Funding/Support:*** *There was no special funding for the project. The funding was done by the authors.* ***Ethics Approval:*** *All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the laid down ethical standards in School of Health Technology Federal University of Technology, Owerri.* ***Acknowledgments:*** *None.* ***Disclaimer:*** *None.*

Key Messages

- Flavoring and anti-hyperglycemic effects of *P. guineense* do not pose any adverse effects on the biochemical parameters.
- *P. guineense* plant extract restored high level of blood glucose to normal level in treated rats; the lipid profile results demonstrated a reduction in total cholesterol but insignificant increase in High Density Lipoprotein.

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