



CODEN (USA): IAJPB

ISSN: 2349-7750

**INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES**<http://doi.org/10.5281/zenodo.290415>Available online at: <http://www.iajps.com>

Research Article

**ANTIOXIDANT AND HYPOLIPIDAEMIC EFFECTS OF
PHYLLANTHUS FRATERNUS METHANOLIC LEAF
EXTRACT ON STREPTOZOTOCIN- INDUCED DIABETIC
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Received: 31 December 2016

Accepted: 31 January 2017

Published: 05 February 2017

Abstract:

Treatments of diabetes with available agents comes with one or more side effects, hence, there is need for continual search of alternative treatment agents from medicinal plants. This study was designed to evaluate the antioxidant property and hypolipidaemic effect of *Phyllanthus fraternus* methanolic leaf extract in diabetic rats. The antioxidant property was evaluated using DPPH free radical scavenging activity and ferric reducing antioxidant power (FRAP) assay. Thirty rats were used out of which twenty four were diabetic. Diabetes was induced by administration of streptozotocin (STZ) intraperitoneally at 60 mg/kg body weight (bw). Five experimental groups (Group A-E) of six rats each were used, the treatment lasted for 28 days; group A was normal control, B was diabetic control while C was standard control treated with metformin at 5mg/kg bw., group D and E received (200 and 300) mg/kg bw of the extract respectively. The IC₅₀ for free radical scavenging activity of *P. fraternus* and ascorbic acid were 43.98 mg/ml and 47.04 mg/ml respectively, while that of ferric reducing antioxidant power (FRAP) were 48.17mg/ml and 51.95mg/ml for *P. fraternus* and ascorbic acid respectively. Treatments with different doses of methanolic leaf extract of *Phyllanthus fraternus* and metformin were shown to significantly ($p < 0.05$) decrease the hyperlipidemia observed in the diabetic control group. Similarly, the level of HDL was observed to be significantly ($p < 0.05$) higher in all the treatment groups when compared to the diabetic untreated group. The reversal of abnormalities observed in lipid profile by methanolic extract of *P. fraternus* and its antioxidant potency offered a potential source of new orally active agent that can halt and reverse most complications due to diabetes.

Key words: Antioxidant, hypolipidaemia, Streptozotocin, *Phyllanthus fraternus*, extract**Corresponding Author:**

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Please cite this article in press as Nadro, M.S. and Elkanah, G, *Antioxidant and Hypolipidaemic Effects of Phyllanthus Fraternus Methanolic Leaf Extract on Streptozotocin- Induced Diabetic Rats*, Indo Am. J. P. Sci, 2017; 4(01).

INTRODUCTION:

Diabetes mellitus is described by world health organization (WHO) as a group of metabolic diseases in which there are high blood sugar levels over a prolonged period (WHO, 2014) [1]. This prolonged high blood sugar levels arises either because insulin production is insufficient, or because the body's cell do not respond properly to insulin, or both. Patients with high blood sugar will typically experience frequent urination (polyuria), increasingly thirsty (polydipsia) and distinctly hungry (polyphagia) [2].

Diabetes can cause many complications if left untreated (WHO, 2013) [3]. Acute complications include diabetic ketoacidosis and non ketotic hyperosmolar coma [4]. Serious long-term complications include cardiovascular disease, stroke, kidney failure, foot ulcers and damage to the eyes (WHO, 2013). Management concentrates on keeping blood sugar levels as close to normal ("euglycemia") as possible, without causing hypoglycemia. This can usually be accomplished with a healthful diet, exercise, and use of appropriate medications (insulin in the case of type 1 diabetes; oral medications, as well as possibly insulin, in type 2 diabetes). In spite of these advances and effort made towards treating, managing, and perhaps preventing the health, economic and social effects of diabetes mellitus, the prevalence of the disease globally is on the increase. As of 2014, an estimated 387 million people have diabetes worldwide as contained in the report of International Diabetes Federation (IDF, 2014), with type 2 diabetes making up about 90% of the cases. This is equal to 8.3% of the adult population, with equal rates in both women and men [5]. In the years 2012 to 2014, diabetes was estimated to have resulted in 1.5 to 4.9 million deaths per year respectively (WHO, 2013, IDF, 2014)[1,3]. The number of people with diabetes is expected to rise to 592 million by 2035 (IDF, 2014) [6]. The global economic cost of diabetes in 2014 was estimated to be \$612 billion USD (IDF, 2013) [7]. Hence, there is an urgent need for new therapeutic drug with high efficacy, low cost, little or no side effects and wider availability if this trend must be reversed. Many plants have been studied in search for antidiabetic activity, some components isolated, but with respect to *Phyllanthus fraternus*, there has been little scientific record to support its anti diabetic activity and to some extent, its active components.

Free radical-scavenging ability of antioxidants may reduce the oxidative stress and thus may protect against oxidative damage [8]. Based on observational studies among healthy individuals, antioxidant concentrations were found to be inversely correlated with several biomarkers of

insulin resistance or glucose intolerance[9,10]. Antioxidants may induce beneficial effects on diabetic complications by reducing blood pressure, attenuating oxidative stress and inflammatory biomarkers, improving lipid metabolism and insulin-mediated glucose disposal, as well as by enhancing endothelial function. [10,11, 12].

Phyllanthus fraternus belongs to the family *Euphorbiaceae*, it is commonly called; gulf leaf flower, Chanca piedra, quebra pedra, stone braker, arranca-pedras, carry-me-seed, hurricane weed, paraparai mi, quinine weed (Matur *et al.*, 2009). The plant has been employed for numerous uses such as treatment of blennorrhagia, colic, diabetes, dysentery, fever, flu, tumors, jaundice, vaginitis and dyspepsia. It is also used as a laxative, stomachic, tonic and vermifuge [13]. It has been used for as folk medicine in the treatment of liver, kidney and bladder problem and intestinal parasites [14]. Particularly *Phyllanthus fraternus* herb is bitter in taste and reported to possess diuretic, hypotensive, hypoglycemic effect, antihyperlipemic, antihepatotoxic and anti oxidant activity [15]. An aqueous extract of the leaves lowers blood sugar level in normal and alloxan diabetic rabbits[16]. Different fractions of alcoholic extracts of aerial parts and root of *Phyllanthus fraternus* were screened for antihepatotoxic activity on carbon tetrachloride (CCL4) induced liver damage [17].

The aim of this study is to evaluate the In vitro antioxidant property and hypolipidaemic effect of *Phyllanthus fraternus* methanolic leaf extract on streptozotocin induced diabetic rats.

MATERIALS AND METHOD:**Plant material**

The plant material of *Phyllanthus fraternus* Webster (Leaves) was collected in the month of May, around 6am at Hayin gada, in Girei local Government area of Adamawa State which lies on geographical location 9° 21'53.19''North and 12° 33'28.33''East Google earth (2014). It was authenticated by a botanist in the Department of Biological Sciences, Modibbo Adama University of Technology, Yola, Adamawa State.

Experimental animals

Male albino rats (5 - 6 weeks) weighing 100 – 130 g numbering 30 were obtained from Veterinary Research Institute VOM, Jos, Plateau State and kept in plastic cages with 12 h dark/light cycle, fed with pelletized grower diet (Vital Feeds, UACN) and given water *ad libitum*.

Equipments

The following equipments were used; Electronic balance (Golden mettle-2G2-USA), ACCU-CHEK Glucometer (GC-Roche Diagnostic-

Germany) , ACCU-CHEK Test Strips (Roche Diagnostic-Germany), Spectrophotometer (Vis spectrophotometer 721- PEC Medical USA), Centrifuge (Techmel, USA), Water bath (HH-2 B-Scientific).

Chemicals

Methanol (Sigma-Aldrich Chemie GmbH, Germany), Streptozotocin (Tocris Bioscience London), Metformin, Chloroform, Total Cholesterol (TC), High Density Lipoprotein (HDL) and Triglycerides (TG) (AGAPPE Diagnostic kits, Switzieland). All other chemicals used were of analytical grade.

Methods

Preparation of plant material

The fresh plant material (leaf) was washed with tap water and shade dried for seven days. It was made into powder using mortar and pestle. The powdered plant material was used for the preparation of methanolic extract.

Preparation of methanolic extract

Methanolic extract was prepared by suspending 200 g of the powdered sample in 2 L of methanol for 24 hours with vigorous shaken intermittently, after which it was filtered and then concentrated at 55 °C using a water bath [18].

Determination of in vitro antioxidant properties DPPH (2, 2-diphenyl-1-picryl hydrazyl) radical scavenging assay

The antioxidant activity of the plant extracts was estimated using the DPPH radical scavenging protocol. DPPH solution (0.004% w/v) was prepared in 95% ethanol. A stock solution of methanolic leaf extract of *Phyllanthus fraternus* (MLEP) and standard ascorbic acid were prepared in the concentration of 1 g/100 ml (10 mg/ml). From stock solution 2 ml, 4 ml, 6 ml, 8 ml & 10 ml of this solution were taken in five test tubes respectively. The final volume of each test tube was made up to 10 ml with distill water, given a concentration of 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg /ml & 100 mg/ml respectively. 2 ml of freshly prepared DPPH solution (0.004% w/v) was added in each of the tubes. The reaction mixture was incubated in the dark for 15 min and thereafter the optical density was recorded at 523 nm against the blank. For the control, 2 ml of DPPH solution in ethanol was mixed with 10 ml of ethanol and the optical density of the solution was recorded after 30 min. The test was carried out in triplicates. The

decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%IP) of DPPH radical. The capability of scavenging DPPH radical was calculated using the following equation[19,20,21].

$$\text{DPPH Scavenged (\%)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Where “A control” is the absorbance of the control reaction and “A test” is the absorbance of the sample of the extracts. IC 50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Ferric reducing antioxidant power (FRAP) assay

In ferric reducing antioxidant power assay, 1 ml of test sample of methanolic leaf extract of *Phyllanthus fraternus* (MLEP) in different concentration were mixed with 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide in separate test tubes. The reaction mixtures were incubated in a temperature-controlled water bath at 50 °C for 20 min, followed by addition of 1 ml of 10% trichloroacetic acid. The mixtures were then centrifuged for 10 min at room temperature. The supernatant obtained (1 ml) was added with 1 ml of distilled water and 0.20 ml of 0.1% FeCl₃. The blank was prepared in the same manner as the sample except that 1% potassium ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. The reducing power was expressed as an increase in A700 after blank subtraction[22].

Induction of diabetes mellitus in rats

All the rats were fasted overnight before the administration of Streptozotocin. Diabetes was induced in rats by intra-peritoneal injection of streptozotocin dissolved in distilled water at a dose of 60mg/kg body weight [23]. After the injection, the rats were allowed free access to food and water. To prevent fatal hypoglycaemia due to massive pancreatic insulin release, rats were given 5% glucose solution water in their cages for next 24 h [24].

The animals were tested after 72 hours of streptozotocin administration. The animals with fasting blood glucose more than 300mg/dl were considered diabetic and were used for the experiment [25,26].

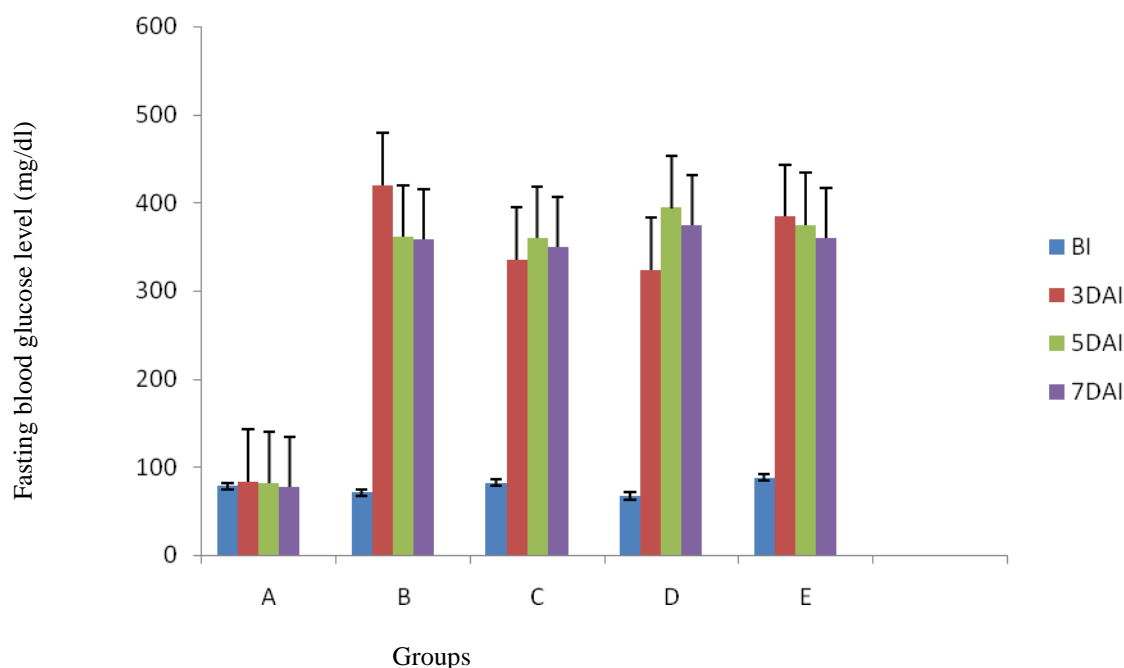


Figure 1: Induction of Diabetes by Intraperitoneal Injection of Streptozotocin (60mg/kgbw) in Albino Rats

Keys
 BI= before induction
 DAI=Days after induction

Experimental Design

In this experiment, a total of 30 rats were used (24 diabetic and, 6 normal). The rats were divided into five groups of six rats each; the grouping/ treatment given are shown below;

Group	Treatment
Group -A (Normal Control)	Normal + no treatment
Group- B (Negative control)	Diabetic + no treatment
Group-C (Positive control)	Metformin 5 mg/kg ⁻¹
Group-D	Extract 200 mg/kg b.w
Group-E	Extract 300 mg/kg b.w

All the animals were allowed free access to food and water *ad libitum*. The treatment lasted for 28days. At the end of the treatment, rats were anaesthetized by placing them in a sealed jar with cotton wool soaked in chloroform. Blood was collected via cardiac puncture from each animal in a plane bottle. The bloods collected were labelled accordingly and were centrifuged at 3000 revolution per minute (rpm) for five minutes to separate the serum from plasma. The serum were carefully collected and transferred to new plane bottles which were subsequently used for biochemical analysis.

Determination of cholesterol

Total Cholesterol and High Density Lipoprotein (HDL) was determined by methods as described by Allain *et al.*, (1974) [27].

Determination of triacylglycerides

The triglycerides were determined as described by Jacobs and VanDemark, (1960) [28].

Statistical Analysis:

Values obtained were expressed as mean ± SEM and data were analysed using analysis of variance (ANOVA) with Bonferroni Post hoc test multiple comparison versus control groups with help of Statistical Package for the Social Sciences (SPSS) software version 21. The values p< 0.05 were considered significant[29].

RESULTS:**Table 1; DPPH radical scavenging activity of *Phyllanthus fraternus* and Ascorbic acid**

Conc (mg/ml)	<i>Phyllanthus fraternus</i>		L. Ascorbic acid	
	% Inhibition	IC ₅₀	% Inhibition	IC ₅₀
20	53.10 ± 0.01		50.04 ± 0.22	
40	72.32 ± 0.40		69.23 ± 0.05	
60	78.34 ± 0.32	43.98 mg/ml	79.49 ± 0.22	47.04 mg/ml
80	88.80 ± 0.04*		81.40 ± 0.02	
100	92.53 ± 0.26*		83.30 ± 0.44	

Values are mean ± SEM (n=3), *significantly higher than L. Ascorbic acid at p<0.05. The IC₅₀ was obtained by linear regression equation.

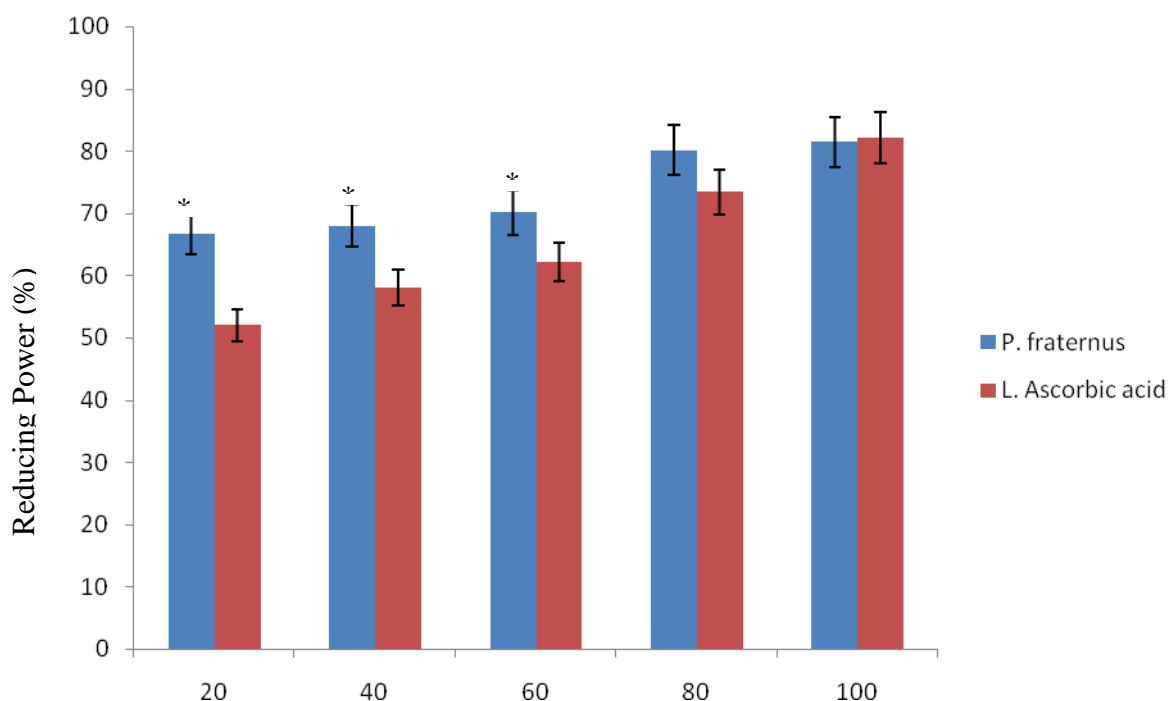


Figure 1: Ferric Reducing Power Concentration (mg/ml) Methanolic Leaf extract of *P. fraternus* and Ascorbic Acid

*Significantly higher compared with L. Ascorbic acid at p<0.05

IC₅₀ for *P. fraternus* = 48.17

IC₅₀ for L. Ascorbic acid = 51.95

Ferric reducing antioxidant power (FRAP) of methanolic leaf extract of *P. fraternus*

The reducing antioxidant power of the methanolic leaf extract of *P. fraternus* (Figure 1) showed a linear increase in the reducing power across a concentration range of 20-100 mg/ml of the extract and ascorbic acid. The reducing power of methanolic leaf extract of *P. fraternus* was significantly (p<0.05) higher than that of ascorbic acid at concentrations of 20, 40 and 60 mg/ml.

Effects of Methanolic Leaf Extract of *Phyllanthus fraternus* on Lipid Profile in Streptozotocin Induced Diabetic rats

The study revealed (Table 2) an increase in serum total cholesterol (TC), triacylglycerides (TG) and low density lipoprotein (LDL) with decrease in high density lipoprotein (HDL) in the diabetic untreated group when compared with the normal control. Treatments with different doses of methanolic leaf extract of *Phyllanthus fraternus* and metformin were shown to significantly (p<0.05) decrease the hyperlipidemia observed in the diabetic control group. The low level of HDL observed in diabetic control group was shown to significantly (p<0.05) increase in all the treatment groups when compared to the diabetic untreated groups.

Table 2: Effects of Methanolic Leaf Extract of *Phyllanthus Fraternus* on Lipid Profile (mg/dl) in Streptozotocin Induced Diabetic Rats

Treatment	TC	TG	HDL	LDL
Normal Control	160.74 ± 1.83	77.33 ± 1.76	45.00 ± 1.73	100.27 ± 0.89
Diabetic Control	240.33 ± 1.45 ^a	184.00 ± 2.08 ^a	17.66 ± 0.88	185.87 ± 0.92 ^a
Met.5mg/kg-b.w.	188.33 ± 0.88 ^{ab}	88.00 ± 1.15 ^{ab}	36.66 ± 0.88 ^c	133.74 ± 0.61 ^{ab}
ME 200mg/kg b.w.	194.00 ± 2.08 ^{ab}	94.00 ± 1.52 ^{ab}	28.00 ± 0.57 ^c	147.20 ± .18 ^{ab}
ME 300mg/kg b.w.	183.33 ± 1.76 ^{ab}	89.66 ± 0.88 ^{ab}	30.00 ± 1.15 ^{cd}	135.39 ± 0.31 ^{ab}

Values are mean ± SEM; n=5.

Different letter(s) superscript along the same column means there is significant difference at p<0.05;

TC = total cholesterol, TG= triglycerides, HDL= high density lipoprotein, LDL= low density lipoprotein,

ME= methanolic extract

Keys:

a= significantly higher compared to normal control

b= significantly lower compared to diabetic control

c= significantly higher compared to diabetic control

d= significantly lower compared to standard control

DISCUSSION:

Oxidative stress (imbalance between the pro-oxidants and antioxidant defense system of the body as a result of steady state reactive oxygen species (ROS)) has been shown to be responsible for damage to biomolecules [30,31], at least in part for pancreatic β -cell dysfunction caused by glucose toxicity in hyperglycaemia. During these processes, ROS are produced and cause tissue damage [32,33]. Therefore, the radical scavenging activity and reducing power of *Phyllanthus fraternus* observed in this study can help in tackling tissue damage and onward complications associated with diabetes mellitus [8]. This scavenging activity and reducing power is associated with the presence of phenols and flavonoids in the plants which have been previously reported to be responsible for various antioxidant activities [34].

Furthermore, prolonged treatment (28 days) with methanolic extract of *phyllanthus fraternus* (MEP) (200 & 300 mg/kg body weight) and metformin (5mg/kg b.w) showed continual decrease of blood glucose, suggesting long term maintenance of blood glucose level in diabetic rats. Several medicinal plants have been reported to restore activity of key enzymes of glucose and glycogen metabolism which are strongly disturbed in streptozotocin diabetic rats [35,36]. Hypoglycaemic effect of MEP may arise from the inhibition of hepatic glucose production, or insulin signalling [37].

Diabetic untreated rats showed increased plasma lipids which is the major risk factor for different cardiovascular diseases associated with diabetes mellitus [38]. An increased level of serum cholesterol indicates hypercholesterolemia, hyperlipidemia, hypothyroidism, uncontrolled diabetes, and nephritic syndrome and cirrhosis. This high serum lipid is due to increase in the mobilization of free fatty acids from the peripheral fat depots and due to lipolysis caused by lipolytic hormones [39]. The present study showed increase in total cholesterol (TC), triglycerides (TG), and

low density lipoprotein (LDL) in the diabetic untreated group with decreased level of high density lipoprotein HDL. Treatment with MEP showed significant reduction of the hyperlipidaemia observed which may be due to the control of blood glucose level and thereby control of the lipolytic hormones [38].

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