



PHYTOCHEMICAL SCREENING AND HYPOGLYCEMIC EFFECT OF METHANOL LEAF EXTRACT OF *Phyllanthusamarus* IN ALLOXANINDUCED DIABETIC RATS



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Abstract: The aim of this study is to investigate the phytochemical constituents and determine the hypoglycemic effect of methanol leaf extract of *Phyllanthusamarus* in alloxan induced diabetic rats. Diabetes was induced after the rats were fasted for 12 h with a single dose of 120 mg/kg body weight of alloxan. The effects of the extract on body weight and blood glucose level were determined. Alkaloids, saponins, tannins, flavonoids, glycosides, reducing sugar, phlobatanins and phenolics were found to be present in the leaf extract. The fasting blood glucose level of all the treated groups significantly reduced compared to the diabetic not treated group. The body weight for the non-induced treated group increased significantly while that for the induced and treated groups decreased significantly compared to their initial bodyweights. *Phyllanthusamarus* leaf extract possess the potential of reducing blood glucose level and maybe useful as an anti-diabetic agent.

Keywords: Alloxan, diabetes mellitus, hypoglycemic, methanol, *Phyllanthusamarus*, phytochemicals

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterised by glucose intolerance and chronic hyperglycemia. It is one of the fastest growing global diseases which accounts for about 10 % expenditure on health care in many countries (Sharma and Gupta, 2017). In sub-Saharan Africa, it is on a rapid increase with Nigeria having the highest number of these cases (Dahiru *et al.*, 2016). Diabetes mellitus is associated with life threatening complications such as renal failure, heart attack or stroke. Type 1 DM or insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease marked by β -cell destruction. It results from the body's inability to produce enough insulin. Alloxan induced diabetes has a similar characteristic as Type 1 DM (Ighadaro *et al.*, 2017).

Medicinal plants are sources and agents of therapeutic importance useful in management of diseases due to their phytochemical content which are majorly responsible for their actions on the living system. Traditionally, medicinal plants have a long history of usage as medicine and its extracts or active ingredients make up about one quarter of prescribed drugs (Gupta and Vaghela, 2019). Recently there has been a growing interest in natural products possibly because of their little or no side effects (El Omari *et al.*, 2019).

Phyllanthusamarus popularly called 'carry me seed' belong to the family of euphorbiaceae and it has been found to grow in the tropics including Nigeria and Ghana. The species of *Phyllanthus* has been found to be effective and a rich source of biochemical compounds used in food and cosmetics industries. In traditional medicine, *Phyllanthusamarus* has been reported to possess nephroprotective, anti-inflammatory, antiviral, hepatoprotective, antioxidant and anti-hyperglycemic activities (Harikrishnan *et al.*, 2018; George *et al.*, 2019). The purpose of this study is to determine the phytochemical constituents of methanol leaf extract of *Phyllanthusamarus* and its effect on blood glucose level and body weight of alloxan induced diabetic rats.

Materials and Methods

Plant collection

The plant *Phyllanthusamarus* was collected from Bida, Niger state. It was identified and authenticated in the Department of Biological Sciences, Federal University of Technology, Minna, Niger State.

Extraction of plant materials

The powdered leaf (50 g) was extracted in 400 ml of 70% methanol and refluxed for 3 h at 65°C. The extract was

filtered using a muslin cloth. The filtrate was evaporated using a rotary evaporator and concentrated further using water bath (45°C). The extract was collected, weighed and stored in a sterile air tight container and kept in the refrigerator until required for use.

Phytochemical screening of methanol leaf extract of *Phyllanthusamarus*

Phytochemical screening of methanol leaf extract of *Phyllanthusamarus* was carried out by the method described by Sofowora (2006).

Animals

Albino rats weighing between 120 to 150 g were used in the study. The rats were obtained from the animal house of Biochemistry department, Ahmadu Bello University, Zaria. The rats were kept in well ventilated plastic cages and at standard environmental condition. The animals were maintained on commercial pelleted Growers mash (vital feeds) and given water *ad libitum* for two weeks to acclimatize before the commencement of the treatment.

Experimental design

25 rats were randomly grouped into 5 groups with 5 rats in each group and designed as:

Group A: Alloxan-induced diabetic rats treated with 500 mg/kg body weight of methanol extract.

Group B: Alloxan-induced diabetic rats treated with 0.5 mg/kg body weight of metformin.

Group C: Alloxan-induced diabetic rats but untreated.

Group D: Non induced rats but treated with 500 mg/kg body weight of methanol extract.

Group E: Normal rats.

Diabetes induction and treatment

Prior to diabetic induction, the blood glucose level of the animals were taken. The rats were fasted for 12 h and a single dose of 120 mg/kg bodyweight of alloxan was administered. After 6 hours of induction the rats were given 20% of glucose solution for 24 h (Stanley *et al.*, 2001). After 72 h of alloxan-induction, the animals with blood glucose level above 180 mg/dl were selected for the experiments. Treatment with extract followed once daily for a period of five weeks.

Determination of blood glucose concentration

The blood glucose level was determined using Accu-check Glucometer (Active), after fasting the rats overnight for about 12 h. Blood was taken from a tail vein onto the glucose strip, to obtain blood glucose concentration in mg/dl.

Determination of bodyweight

The rats were weighed using an electric weighing balance (SF-400A) before and after induction and during the period of treatment weekly.

Results and Discussions

Phytochemical screening of methanol leaf extract of *Phyllanthusamarus*

The result of phytochemical screening of methanol extract of *phyllanthusamarus* leaf showed the presence of alkaloids, saponnins, tannins, flavonoids, phenolic compounds, cardiac glycosides, phlobatanins and reducing sugar while steroids and terpenoids were absent (Table 1).

Table 1: Phytochemical screening of methanol leaf extract of *Phyllanthusamarus*

Compound	Methanol extract of <i>Phyllanthusamarus</i> leaf
Alkaloids	+
Saponnins	+
Tannins	+
Flavonoids	+
Glycosides	+
Reducing sugar	+
Phlobatanins	+
Phenolics	+
Steroids	-
terpenoids	-

+ = detected; - = not detected

The presence of these phytochemicals may be responsible for the pharmacological and hypoglycemic properties of the extracts. Flavonoids are one of the most diverse and widespread group of natural compounds that have been shown

to possess a broad spectrum of chemical and biological activities including radical scavenging properties, antiallergic, antiviral, anti-inflammatory, and vasodilating actions. Phenolics are known for their anti hyperglycemic properties (Nkirote *et al.*, 2011) and also possess antioxidant, anti-viral, anti-bacteria and anti-inflammatory effect (Herve *et al.*, 2019). The presence of these phytochemicals in the extract correlates with the work of Shetti *et al.* (2012).

Effect of methanol leaf extract of *Phyllanthusamarus* on blood glucose

The blood glucose level for the diabetic not treated group progressively increased through the 1st to the 5th week compared with the normal control group. The non-induced but treated with extract, induced treated with extract and induced treated with metformin, significantly reduced from week 2 through to week 5 (Fig. 1). The determination of blood glucose concentration showed that there was a reduction in blood glucose concentration in non-induced extract treated rats, induced extracts treated rats and induced metformin treated rats (standard drug). However there was a significant reduction in the blood glucose concentration in induced not treated rats while the normal group maintained a constant variation in blood glucose levels. The significantly reduced blood glucose level demonstrates that the extract contains anti-diabetic activity which may have restored the damaged β -cells of the pancreas and/or stimulated the release of insulin to control the blood glucose level due to the presence of phytochemicals present such as flavonoids and saponins (Gad-Elkareem *et al.*, 2019). Flavonoids have been reported to possess anti-inhibitory activities towards carbohydrate hydrolysing enzymes and also halt pancreatic β -cells damage (El omari *et al.*, 2019).

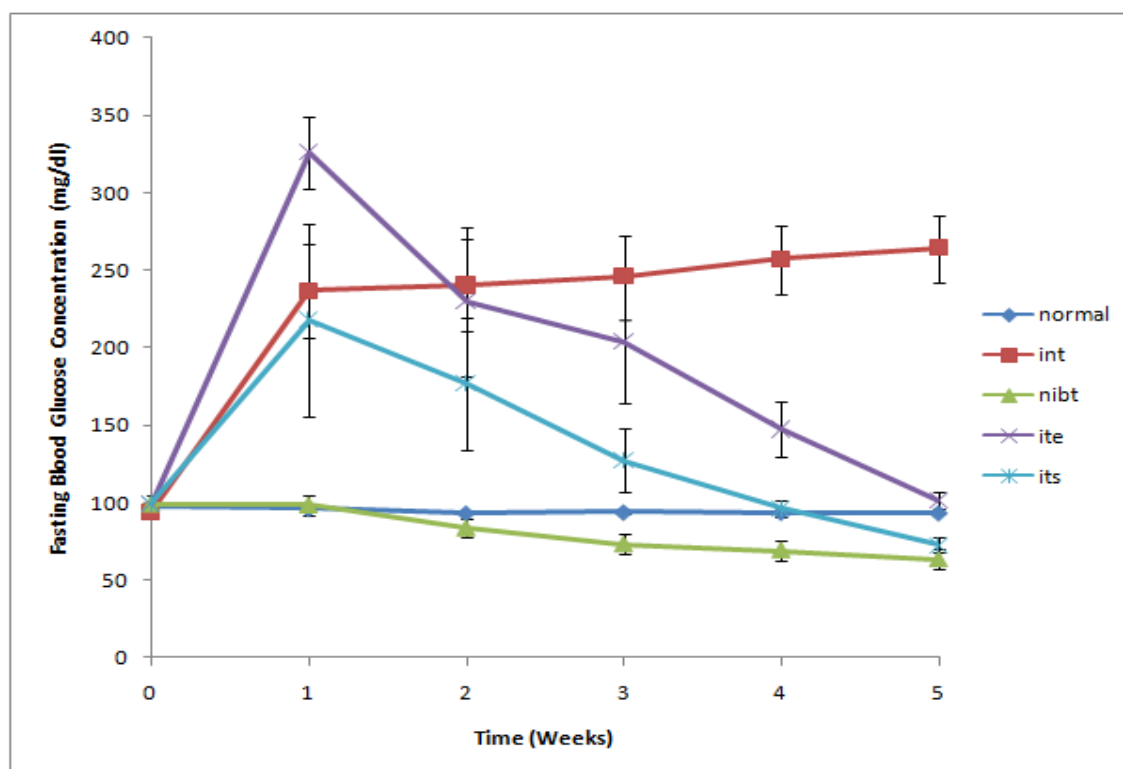


Fig. 1: Effect of methanol leaf extract of *Phyllanthusamarus* on blood glucose of diabetic and non-diabetic rats

Diabetic not treated (int), induced treated with extract (ite), non-induced but treated with extract (nibt), and induced treated with standard, metformin (its).

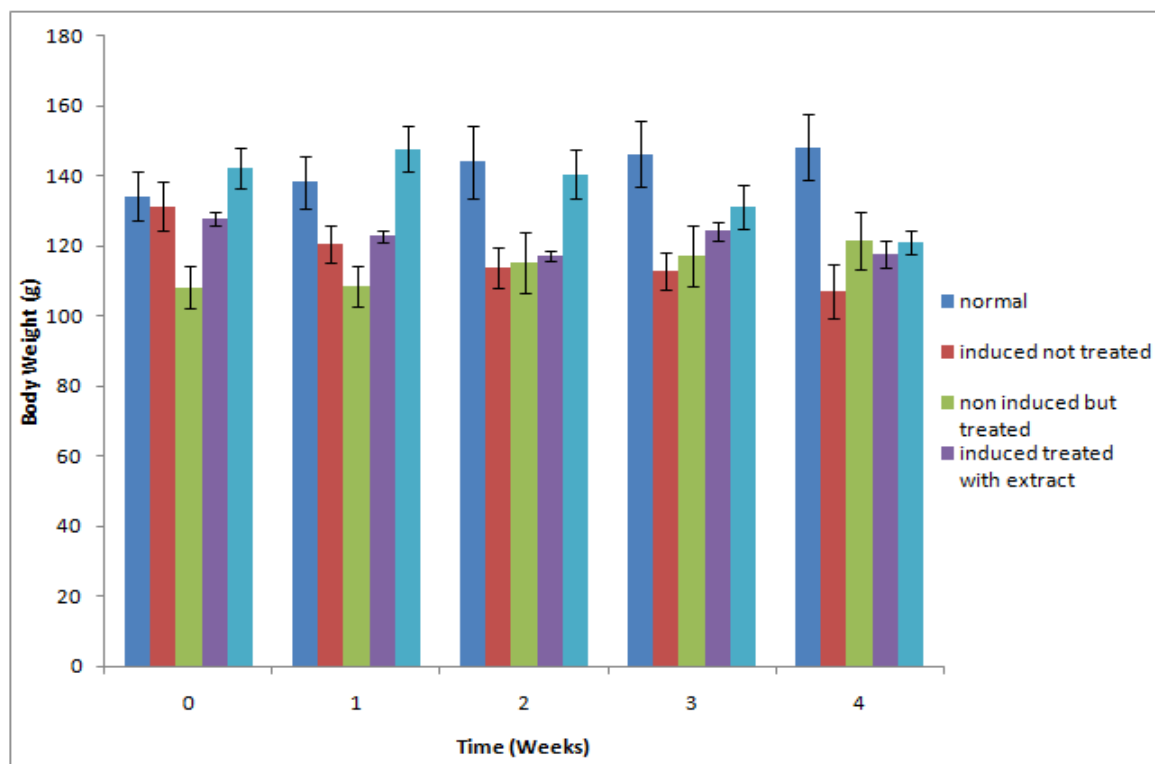


Fig. 2: Effect of methanolic leaf extracts of *Phyllanthusamarus* on bodyweight of diabetic and non-diabetic rats

Diabetic not treated (int), induced treated with extract (ite), non-induced but treated with extract (nibt), and induced treated with standard metformin (its)

Effect of methanol leaf extracts of *Phyllanthusamarus* on bodyweight

The body weight for the induced not treated group reduced gradually compared to the normal control group throughout the 4 weeks while that of the treated groups reduced initially after the administration of alloxan but later gained slight weight (Fig. 2). From the study, the decrease in the bodyweight of the diabetic not treated rats is due to insulin deficiency which prevents the absorption of glucose to cells where they are required and in the process the body alternatively sources for energy from protein and lipid catabolism (Mhya *et al.*, 2019). The rats treated with extracts showed a slight weight gained as that of the standard drug. The extract may have helped to stop or reduce the protein breakdown. This result agreed with the findings of Almalki *et al.* (2019).

Conclusion

The results of this study suggested that *Phyllanthusamarus* possess hypoglycaemic properties which may be due to the phyto-constituents of the plant extract. It may be used in drug development if further investigated.

Conflict of Interest

Authors have declared that there is no conflict of interest in this study.

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