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Evaluation of Hypoglycemic Efficacy of Methanolic Extracts of *Moringa Oleifera* and *Phyllanthus amarus* in Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author IOO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AOT and ONQ managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was undertaken to determine the hypoglycemic efficacy of methanolic extracts of *Moringa oleifera* and *Phyllanthus amarus* in alloxan-induced diabetic rats.
Study Design: Experimental Animal Study.
Place and Duration of Study: Department of Biochemistry, Osun State University, Osogbo Nigeria between September and March, 2013.
Methodology: Twenty four rats sorted into 4 groups were used for the study. Rats in control group (group 1) received distilled water while diabetes was induced in groups 2-4 rats by intraperitoneal administration of alloxan. Animals in groups 3 and 4 were treated with 500 mg/kg bw of methanolic

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leaf extract of *Moringa oleifera* and whole plant extract of *Phyllanthus amarus* respectively for 14 days while group 2 rats were left untreated. Serum glucose and total protein concentrations were measured in the rats after treatment.

Results: The two extracts reversed the alloxan-induced hyperglycaemic condition in rats as there was a significant reduction in blood glucose levels with *Moringa oleifera* having a more pronounced effect. Level of serum total protein was also significantly reduced in rats treated with the two extracts.

Conclusion: This study is a further scientific validation of the widely claimed use of *Moringa oleifera* and *Phyllanthus amarus* as useful ethnomedical treatment for *diabetes mellitus*.

Keywords: Diabetes; Moringa oleifera; Phyllanthus amarus; fasting blood sugar; total protein.

1. INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from insufficient insulin secretion, defects in insulin action or both [1,2]. Presently, there are over 150 millions diabetics worldwide and this is likely to increase to 300 million or more by the year 2025 [3,4]. The underlying goal of all diabetes treatment and management is to maintain an adequate blood glucose concentration. Progress in understanding the metabolic staging of diabetes over the past few years has led to significant advances in regimen for treatment of this devastating disease [5,6]. For centuries, people in many countries have used medicinal herbs as treatment for *diabetes mellitus* but most of the plants prescribed for this ailment are not edible [7-9]. In the light of the above, extensive studies of edible plants which have hypoglycemic effect would be of great value in the dietary management of the disease.

Moringa oleifera is an exceptionally nutritious vegetable tree native to India but now distributed worldwide [10]. It is considered one of the world's most useful trees, as almost every part of the tree can be used for food or has some other beneficial properties [11]. It is traditionally used for the treatment of a number of ailments including diarrhea, paralysis, epilepsy and hysteria [12-14].

Phyllanthus amarus is a small, erect, annual herb having a large number of phytochemicals that are attributed to its leaves, stem and roots [15]. A wide array of studies find *Phyllanthus amarus* plant to posses anti-inflammatory, antimicrobial, antihyperlipidemic, antioxidant, anticancer, hepatoprotective, antidiarrhoeal, chemoprotective, antispasmodic and diuretic properties [16]. The study intend to compare the hypoglycemic efficacy of *Moringa oleifera* leaf and whole plant of *Phyllanthus amarus* in alloxan induced diabetic rats as well as their effects on protein concentration in rats serum.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extracts

Fresh *Moringa oleifera* leaves were obtained from National Centre for Genetic Resources and Biotechnology (NACGRAB) Moor Plantation, Ibadan, Nigeria. Whole plants of *Phyllanthus amarus* were collected in Osogbo, Nigeria. The plants were air dried at room temperature for 3 weeks and grinded into fine powder with kitchen blender. 500 g of the powdered samples were soaked in 2.5 L of 80% methanol for 7 days (to ensure adequate extraction of the phytochemicals) and then filtered using a Whatman filter paper (125 mm). The filtrate was evaporated to dryness in a water bath at 50ºC and kept in air tight container in a refrigerator until used.

2.2 Experimental Design

A total of twenty four (24) albino rats (*Rattus novergicus*) with average weight 135 g were used for the study. They were obtained and raised at the Central Animal House, Osun State University, Osogbo. The rats were housed in plastic cages under standard laboratory conditions in conformity with international guidelines and ethics relating to animal care. They were exposed to 12hours light/dark and fed with rat pellet and clean water *ad libitum*.

The animals were sorted into 4 groups of six rats each as follows; Group 1: Control animals administered distilled water. Group 2: Diabetic rats left untreated. Group 3: Diabetic rats treated with 500 mg/kg bw *Moringa oleifera*. Group 4: Diabetic rats treated with 500 mg/kg bw *Phyllanthus amarus.* The diluent used in

preparing the extract dose is distilled water. Treatment lasted for 14 days. Diabetes was induced by intraperitoneal injection of 150 mg/kg bw of alloxan solution and diabetes was confirmed 72 hours after alloxan injection.

2.3 Collection of Blood Samples and Biochemical Analysis

Rats were sacrificed by decapitation in the 15th day of treatment and their blood collected into clean plain test tubes. The blood was centrifuged at 3000 rpm for 10 min after which the supernatant (serum) was collected and frozen. Serum glucose concentration was estimated by glucose oxidase method [17] while total protein concentration was measured using the Biuret method [18]. Standard kits obtained from Randox Chemicals Limited were used for the analysis. Absorbance measurements were done by the use of Spectrolab UV spectrophotometer (UK).

2.4 Statistical Analysis

Results were presented as Mean ± SD and analyzed using SPSS version 20. Comparism were made between the experimental groups using Duncan's multiple range test and values were considered to be significantly different at $p<0.05$.

3. RESULTS AND DISCUSSION

Fig. 1 show the result of glucose concentration in the serum of control and diabetic animals groups. The result shows that *Moringa oleifera and Phyllanthus amarus* extracts caused a significant decrease ($P<0.05$) in serum glucose concentration in diabetic rats with *Moringa oleifera* having the highest efficacy. Results in Fig. 2 also indicate that the two extracts significantly normalized the elevated serum protein concentration observed in diabetic rats.

A single dose of alloxan injected to rats caused a reproducible elevated serum glucose concentration in the diabetic group within 72 hours. The increase in glucose concentration in the diabetic rats may be due to toxicity of alloxan on the proximal convoluted renal tubules and its ability to destroy the pancreatic islets cells [19]. Treatment of diabetic rats with a dose of methanolic extract of *Moringa oleifera* and *Phyllanthus amarus* caused a dose related significant decrease in blood glucose level (groups 3 and 4) compared with the diabetic untreated (group 2). This may be due to regeneration of destroyed beta-islets following treatment with the extracts. It is likely that the plant extracts caused increased tissue utilization of glucose in the animals either by inhibiting hepatic gluconeogenesis or by promoting glucose absorption into the muscles and tissues [20]. Previous phytochemical analysis revealed that the two extracts contain terpenoids, flavonoids and tannins [21]. These phytochemicals help in stimulating ß-cells and subsequent secretion of preformed insulin in normoglycemic rats [22].

The elevated level of serum total proteins in diabetic rats (Group 2) could be attributed to the adverse effect of diabetes on the kidneys. If diabetes is left untreated, it may result in diabetic nephropathy characterized by a decline in glomerular filtration rate and development of lesions in the glomerular capillaries of the kidneys which allows proteins to escape into the blood [23]. Treatment of animals in groups 3 and 4 with the plant extracts reduced the serum total protein concentration close to that obtained in normal control group. The protein lowering ability of these extracts indicated their favourable effects on kidney structure and function and their ability to reduce severity of diabetes. It may also be due to several reasons like; increased rate of gluconeogenesis, decreased amino acids uptake, and increased conversion rate of glucogenic amino acids to CO_2 and H_2O caused by the extracts. Another group of investigators postulated protein decrease to a decrease in the amount and availability of mRNA, a loss of transitional factor and reduction of ribosomal protein synthesis [24]. Therefore, we can propose in this study that the extracts improved glomerular filtration rate in treated diabetic rats by restoring the thickness of the glomerular basement membrane and therefore prevent protein loss.

4. CONCLUSION

This study showed that methanolic leaf extract of *Moringa oleifera* and whole plant extract of *Phyllanthus amarus* have hypoglycemic properties in alloxan diabetic rats. It was also observed that the plants have total protein lowering effects. These results suggest the presence of biologically active components (phytochemicals) in the plants which are beneficial in the treatment of hyperglycemia and proteinuria.

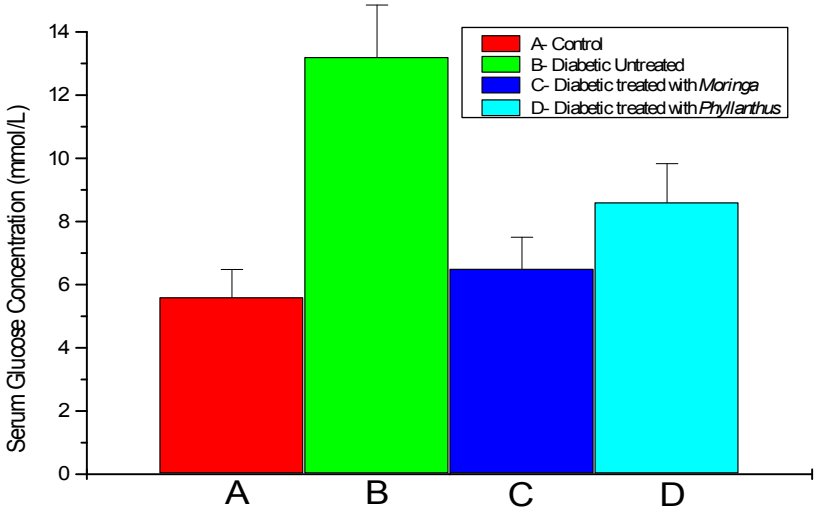

Fig . 1. Serum glucose concentration in diabetic rats treated with *Moringa oleifera* and *Phyllanthus amarus*

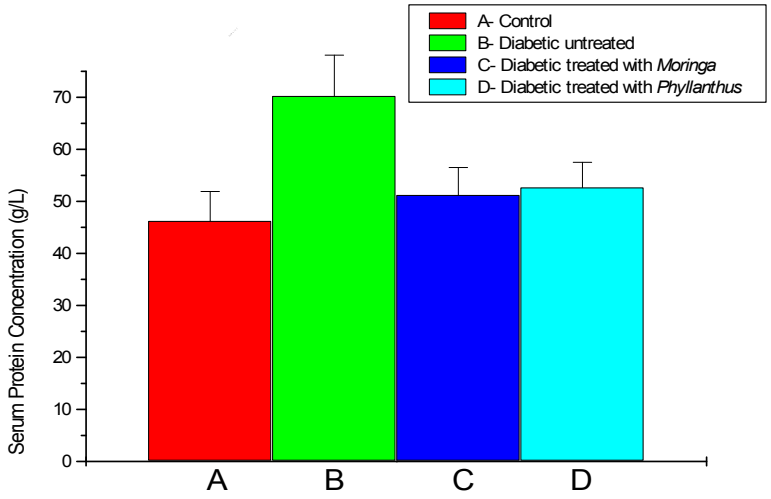

Fig. 2. Serum protein concentration in diabetic rats treated with *Moringa oleifera* and *Phyllanthus amarus*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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