

THE EFFECT OF *PHYLLANTHUS AMARUS* AQUEOUS EXTRACT ON BLOOD GLUCOSE IN NON-INSULIN DEPENDENT DIABETIC PATIENTS

*Mainen J. Moshi¹, Janet J.K. Lutale², Gerald H. Rimoy³, Zulfikar G. Abbas², Robert M. Josiah² and Andrew B.M. Swai².

¹ Institute of Traditional Medicine, MUCHS, Box 65001, Dar es Salaam, Tanzania.

² Department of Internal Medicine, MUCHS, Box 65001, Dar es Salaam, Tanzania.

³ Department of Clinical Pharmacology, MUCHS, Box 65001, Dar es Salaam, Tanzania.

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*Corresponding author

ABSTRACT

The glycaemic response to 124.5 ± 9.3 (mean \pm SD) g of pancakes was monitored in 21 non-insulin dependent diabetic (NIDDM) patients while on oral hypoglycaemics, after a one week washout period and after a one week twice daily treatment with 100 ml of an aqueous extract from 12.5 g of powdered aerial parts of *Phyllanthus amarus*. After the one week washout period, fasting blood glucose (FBG) and postprandial blood glucose increased significantly compared to when on oral hypoglycaemics ($P \leq 0.05$). After one week herbal treatment no hypoglycaemic activity was observed. Both FBG and postprandial blood glucose remained very similar to that recorded after the washout period ($P > 0.05$). Both liver and renal functions based on alanine transaminase (ALAT) and serum creatinine, respectively, were not significantly affected by the use of the extract. Although lymphocyte and monocyte levels were significantly decreased ($P \leq 0.05$) and granulocyte level was significantly increased after treatment ($P \leq 0.05$) overall total white blood cell (WBC) count and haemoglobin (Hb) were not significantly affected by the one week herbal treatment. We conclude that one week treatment with the aqueous extract of *Phyllanthus amarus* was incapable of lowering both FBG and postprandial blood glucose in untreated NIDDM patients.

Key words: *Phyllanthus amarus*, Euphorbiaceae, aqueous extract, NIDDM, treatment.

INTRODUCTION

Phyllanthus amarus (Schum) Thonn (Euphorbiaceae) is a herb which is widely spread in the tropics (Chhabra and Mahunnah, 1994). It is known among the Wazaramo in Tanzania by the name "mzalia nyuma". An aqueous extract of the aerial parts is used by some traditional healers in Tanzania, in the management of diabetes mellitus; while in other areas the same extract is drunk and the leaves are chewed against persistent coughs and stomachaches (Chhabra and Mahunnah, 1994). The entire plant is used in India to treat hepatitis, dysentery, irritating sores (Reddy *et al.*, 1993) and jaundice (Raja Reddy, 1988). It is also used in the West Indies to prevent intestinal worms in children (Morton, 1977) and in Rarotonga (Cook Islands) to treat earache (Holdsworth, 1991), while in Papua New Guinea the fresh entire plant decoction is drunk to treat migraine (Holdsworth and Balun, 1992). A water extract of the dried entire plant is used in Nigeria to treat diarrhoea (Obasi *et al.*, 1993). Extracts of the plant have shown positive antibacterial activity (Verpootre and Dihal, 1987; Macrae *et al.*, 1988; and Blumberg *et al.*, 1990), antifungal, antitumour, and antiviral activity against Cytomegalovirus and Sindbis virus (Macrae *et al.*, 1988), Woodchuck virus (Unander *et al.*, 1993), hepatitis B virus (Brook, 1988; Mehrotra *et al.*, 1991; Munshi *et al.*, 1993), Rous-sarcoma virus (Yeh *et al.*, 1993), human immunodeficiency virus (HIV) and the Maloney-leukaemia virus (Venkateswaran *et al.*, 1990). Extracts of the plant have also been shown to be antihepatotoxic (Thyagarajan *et al.*, 1990), antimutagenic (Gowrishanker and Vivekanandan, 1994), antipyretic and diuretic (Teothong *et al.*, 1951). They also inhibited viral DNA polymerase (Blumberg *et al.*, 1990; Unander and Blumberg, 1991) and reverse transcriptase (Shead *et al.*, 1992).

An aqueous extract of *P. amarus* at a dose of 0.1 and 1.0 g/kg body weight, enhanced the clearance of an oral glucose load from the blood of normal white albino rabbits but did not significantly lower FBG (Moshi *et al.*, 1997). Since there are no reports in literature of the

clinical use of extracts of this plant in diabetes, this work was done as an attempt to substantiate its traditional uses using NIDDM patients.

MATERIALS AND METHODS

Collection and preparation of plant material

The aerial parts of *Phyllanthus amarus* were collected from Kibaha, Coast Region. The plant was identified by Mr. E.B. Mhoro, a senior technician at the Institute of Traditional Medicine (Muhimbili University College of Health Sciences). Voucher specimen have been kept in the herbarium of the Institute (Voucher no. TMRU 3156). The plant material was dried in the shade, at room temperature, until completely dry and then ground into a powder. The powder was put into packets each containing 25 g of the plant material and distributed to the study patients. Patients were instructed to soak the 25 g in 200 ml of water overnight, and then boil for 5 minutes. They were further instructed to filter the aqueous extract with a clean piece of cloth and use the extract on the same day. They were instructed to take 100 mls of the extract in the morning and 100 mls in the evening for one week.

Patient selection

The patients used in this study were recruited from the Muhimbili Medical Centre Diabetic Clinic. Several patients attending clinic were interviewed to determine their suitability for inclusion in the study. Those willing to participate were invited to join the study. Patients

confirmed to have NIDDM but having normal renal and liver function, normal blood picture and already stabilized with oral hypoglycaemics were selected for inclusion in the study. A total of 23 patients were chosen, which included 21 males and 2 females of age 47.8 ± 6.3 years (mean \pm SD; range 37 - 59). Their systolic and diastolic blood pressure was 126.2 ± 14.3 and 87.1 ± 9.3 (mean \pm SD) mmHg, respectively; while their BMI was 26.9 ± 2.7 (mean \pm SD) kg m^{-2} .

Two male patients dropped out of the study before starting the herbal medicine. Ethical clearance was obtained from the College Research and Publications Committee (Muhimbili University College of Health Sciences) following which the patients signed an informed consent form before participating in the study. Patients were allowed to stop treatment if for any reason they were dissatisfied with the study.

The protocol and dosing schedule

On each study day the patients came to the diabetic clinic without taking breakfast. Baseline data were collected on the first study day which included FBG, ALAT, creatinine and cholesterol levels. Urine samples to determine the presence of glucose or red blood cells (RBCs) were also collected. All patients found with glucose in urine were excluded; none had RBCs in urine. The patients were then given a standard meal consisting of 2 pancakes with a mean (SD) weight of 124.5 (9.3) g and one mug of white tea without sugar (340 ml). The tea and pancakes were prepared in the same way and by the same person throughout the study. Blood samples were collected from the antecubital vein before the meal (i.e time 0) and at 0.5, 1, 1.5, 2, 3 and 4 h after the meal, for measurement of blood glucose. On the same day the patients were instructed to stop medication to allow a washout period of 7 days.

After the washout period the patients were again given the standard meal following an overnight fast and blood samples collected before the meal and at 0.5, 1, 1.5, 2, 3 and 4 h after the meal for the measurement of blood glucose. The patients were then given packets containing 25 g of the plant material and instructed to take 100 mls of the extract twice daily and report to the clinic after one week. On the day of the visit they reported to the clinic without taking breakfast. Blood samples were collected for measurement of FBG, ALAT, creatinine, cholesterol and for doing complete blood count (CBC). The patients were then given the standard meal and after 0.5, 1, 1.5, 2, 3 and 4 h, blood samples were collected for the measurement of blood glucose.

Measurement of blood glucose

Blood glucose was measured in mmol/l using a Yellow Spring glucose analyser model 23 A (Ohio, USA) which is based on the glucose oxidase method.

Assessment of response

Response to herbal treatment was evaluated based on the ability to lower FBG and the ability to lower blood glucose values over a four hour period following ingestion of the standard meal.

Complete blood count (CBC), ALAT, creatinine and cholesterol levels

Blood samples were collected in ethylene diamine tetraacetic acid (EDTA) vacutainer tubes (Becton Dickinson) between 9.00 and 12.00 h and haematological parameters measured on the whole blood within 6 h of collection on a Microdiff 18 Coulter cell counter (Coulter Corporation, Miami, Florida, USA). ALAT, creatinine and cholesterol levels were measured using the Ames Technicon analyser model no. RA-50 (Ames product code no. 7424, Germany). Urine samples were checked for the presence of proteins and blood using Ames multistix (Bayer, Germany).

Data analysis

The data for blood glucose profiles were analysed by comparing mean blood glucose values on days 0, 7 and 14 using one way analysis of variance (one way ANOVA). The Neuman-Keuls range test was used to determine differences between the mean glucose values. Differences were considered significant when $P \leq 0.05$. FBG, ALAT and creatinine levels were compared using one way ANOVA. Haematological data before and after herbal treatment were compared using the Student's paired t-test. Differences were considered significant at $P \leq 0.05$.

RESULTS

The effect of the aqueous extract on blood glucose

The mean (SD) fasting blood glucose was 7.96 (3.4), 10.03 (3.9) and 10.59(4.4) mmol/l before washout, after washout and one week after herbal treatment, respectively. Figure 1 shows the mean blood glucose profiles following a standard meal. Fasting and postprandial blood glucose increased after a one week washout period as compared to before washout ($P \leq 0.05$). After one week of treatment with the herbal extract, both FBG and postprandial blood glucose levels

remained virtually the same as during the washout period ($P > 0.05$).

The effect of herbal treatment on cholesterol, creatinine, ALAT levels and blood count

Table 1 shows the results for the levels of cholesterol, creatinine, ALAT and haematological parameters before and after herbal treatment. The results show that the values of creatinine, ALAT and cholesterol measured before and after herbal treatment were all approximately within the normal ranges which are 10 - 40 U/l, 53 - 124 $\mu\text{mol/l}$ and 3.5 - 6.7 mmol/l for ALAT, creatinine and cholesterol, respectively (Prabhu *et al.*, 1992). The percentages of both lymphocytes and monocytes were significantly lower after herbal treatment ($P \leq 0.05$), while that of granulocytes was higher after herbal treatment ($P \leq 0.05$). However, total WBC count and Hb were not significantly affected by the herbal treatment ($P > 0.05$).

DISCUSSION

Traditional healers use the aqueous extract of the aerial parts of *Phyllanthus amarus* as treatment for patients with diabetes mellitus. The results from this study show no evidence of a hypoglycaemic effect on NIDDM patients. In a previous work, on normal fasted albino rabbits (Moshi *et al.*, 1997), it was shown that 0.1 and 1 g/kg body weight of an aqueous extract of the plant caused a dose-dependent increase in the clearance of an administered oral glucose load from the blood. However, the extract did not significantly lower FBG. The results from rabbits seemed to suggest that the extract was capable of preventing the escalation of postprandial blood glucose. Certainly the response by NIDDM patients was different. There was no change in mean postprandial blood glucose levels following one week of treatment with the herbal extract. Similarly, the extract did not lower the mean FBG in the NIDDM patients. There are two possible explanations for the observed differences. In the present study we used NIDDM patients while in the previous study we used normal and not diabetic rabbits. This may be the cause of the observed differences. It will be interesting to see how the blood glucose of an NIDDM rodent species would respond following administration of the plant extract. The greater effect observed in rabbits could also be due to species differences, thus suggesting that the dose used could have been too small to have a significant effect on the diabetic patients. The dose was deliberately chosen to be within the range used in rabbits because it was being administered repeatedly. Based on the results from the previous work on rabbits (Moshi *et al.*, 1997) it is tempting to suggest that the response could have been better if a bigger dose of the extract was used. This could also account for the observed. Therefore, we would like to suggest that a comparison of treated and untreated drug free patients and using a bigger dose of the extract may produce the same effect in NIDDM patients as in the rabbits. However, while increasing

the dose is the suggested next step, the question of toxicity is critical. The levels of ALAT, creatinine and cholesterol were all slightly higher but they were not statistically significantly different from the pre-treatment values. Although in previous studies there were no reports of toxicity (Thyagarajan et al., 1990; Thamlikitkul *et al.*, 1991), it will be wise to exercise caution and monitor these parameters closely in any future work using higher doses.

CONCLUSION

One week treatment with the aqueous extract of *Phyllanthus amarus* was incapable of lowering both FBG and postprandial blood glucose in untreated NIDDM patients.

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Figure 1. The mean blood glucose values during a 4 hour period after administration of a standard meal. Each value represents mean \pm SD (n = 21).

Table 1. The mean haematological profile, cholesterol, creatinine and ALAT levels of the study patients before and after herbal treatment. * Significantly lower/higher ($P \leq 0.05$).

Measurement	Before treatment (mean \pm SD)	After treatment (mean \pm SD)
WBC ($\times 10^3$)cells/ml	5.3 \pm 1.9	4.6 \pm 1.1
Lymphocytes %	42.4 \pm 9.5	34.6 \pm 9.0*
Monocytes %	4.8 \pm 2.1	3.2 \pm 1.7*
Granulocytes %	51.8 \pm 9.9	60.0 \pm 9.4*
Hb (g/dl)	13.4 \pm 2.2	12.8 \pm 1.8
Creatinine (μ mol/l)	89.3 \pm 7.7	95.7 \pm 28.4
ALAT (U/l)	15.7 \pm 7.7	16.3 \pm 6.9
Cholesterol (mmol/l)	4.4 \pm 1.1	5.0 \pm 1.4