



Anti-diabetic Efficacy of Dietary Vegetable Mixtures in Alloxan Induced Diabetic Guinea Pigs.

Nwafor Arthur¹, Akunneh-Wariso Chris C*¹, Ojeka Sunday Ogbu¹, Agbai Emmanuel Onuka²

1.Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, PMB 5323, Choba, Port Harcourt;

2.Department of Physiology, Faculty of Basic Medical Sciences, Madonna University, PMB 407 Ihiala, Elele Campus, Rivers State.

ABSTRACT

The effect of Nigerian dietary vegetable mixtures composed of *Vernonia amygdalina*, *Ocimum gratissimum*, *Gongronema latifolium*, *Solanum melongena*, *Phyllanthus amarus*, and *Piper nigrum* acclaimed as potent anti-hyperglycaemic agents were evaluated in accordance with local finished medicine products and practices in alloxan-induced diabetic guinea pigs. Blood glucose level analysis was used as diabetic indices. Taken together, the results of the present study, suggests that the strong cooperative additive/synergistic effects of the dietary vegetable mixtures (50/50/50) significantly ($p < 0.05$) with reference to the base value ameliorated alloxan-induced elevated blood glucose level by 24% to 26% after 14 days when compared with that of the positive control (glibenclamide) of 20% or negative control (normal saline) of 41.6%. The demonstration in the present study of the anti-hyperglycaemic activities of the dietary vegetable mixtures is in resonance with its local application and may have important clinical implications in reducing health risks, specifically, type 2 diabetes mellitus and its complications.

Keywords: Diabetes, dietary vegetables, phytochemical/antioxidants, micronutrients, blood glucose

*Corresponding Author Email: arthur.nwafor@uniport.edu.ng

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INTRODUCTION

Although diabetic mellitus (DM) is a chronic disorder of carbohydrate metabolism characterized by increased fasting blood glucose level, the pathophysiological basis is poorly understood. In their estimate the prevalence of diabetes in 2010 among adults (aged 20-79 years) is about 6.4%, affecting 285 million, and will increase to 7.7% and 439 million adults by 2030. Even between 2010 and 2030, there will be a 69% increase in the number of adults with diabetes in sub-Saharan African countries and a 20% increase in developed countries¹ Of interest, the prevalence of diabetes in sub-Saharan African countries, particularly the populations from the Niger Delta region of Nigeria has been suggested as high as 7 to 9% ² Aside, the high prevalence of diabetes in Nigeria has been ascribed to Westernized lifestyle, aging and/or urbanization²; and on the other hand, to environmental degradation and pollution-associated exposure to prolonged and chronic low-level of oil and gas flared contaminants which is capable of giving raised pathogenesis of several diseases including diabetes mellitus ^{3,4} Accordingly, the environmental associated contaminants possibly modulate significantly oxidative changes and lipid metabolism with subsequent induction of hyper-glycaemic and its complications such hypertension and atherosclerosis⁵ which can lead to end-organ damage. In recent times, apart from the conventional diabetes therapy, there is growing interest in medicinal plant-based preparations used in traditional medicine acclaimed by traditional physicians to having beneficial effects in diabetic patients. This partly may be due either that none of the antidiabetic drugs could give a long term glycaemic control without causing any adverse side effects or to low cost than the synthetic anti-diabetic drugs and/or partly to ensure the use of safe, effective and quality products and practices, based on available evidence⁶, taking cognizance of the claims of natural medicine benefits and accessibility, is strongly encouraging research for the need of a better anti-hyperglycaemic drugs for primary health care. Hitherto, it has been suggested that oxygen free-radical generations, non-enzymatic protein glycosylation and alteration in antioxidant enzymes participate in endothelial dysfunction and various pathological conditions such as diabetes and its complications. And, importantly, antioxidants produced by secondary metabolites in medicinal plants – flavonoids, alkaloids and tannins are capable of neutralizing free radicals, consequently reduces severity of diabetic and various complications ^{4,7,8,9,10}

In Nigeria traditional and folkloric medicine, among the promising medicinal plants commonly employed by traditional physicians in the management and treatment of endocrine disease-related diabetes (as well as some specific causes of deaths including cardiovascular disease, cancer, hypertension and even as hepatoprotective agents) are the combinations, in various

ratios, of *Vernonia amygdalina* (bitter leaf), *Ocimum gratissimum* (scent leaf), *Gongronema latifolium* (utazi), *Solanum melongena* (garden egg leaf), *Phyllanthus amarus* (stone breaker, black pepper), and *Piper nigrum* (uziza). Literature is scarce that pushes the impact of hypoglycaemic–antioxidant-higher-fibre quantities of plant foods potencies of Nigerian traditional medicinal plants based on tests done to evaluate the safety, efficacy, and effectiveness of the products and practices. Notwithstanding, the present study was therefore undertaken in an effort to further search for a better curative and safe agents for the treatment and management of diabetes. This was to investigate and compare the therapeutic efficacy and safety in the cooperative additive/synergy of indigenous vegetables used in preparations of many delicacies, acclaimed locally to have reduction in glucose utilization significantly and employed as anti-diabetic agents in traditional system of medicine, in alloxan induced-diabetic guinea pigs. Additionally, the present study was also carried out with the aim of producing an inventory of the plants used locally in the management, treatment or prevention of diabetes, simultaneously included those plants combined which are scientifically justified as anti-diabetic agents. The study also allow isolation and identification of the bio-active constituents from these plants, preparation of standardized dose and dosage regimen that can play a significant role in improving type 2 diabetic patients.

MATERIALS AND METHODS

Preparation and identification of plant materials:

The plant leaves were identified and authenticated at the Department of Crop Science Technology, School of Agriculture and Agriculture Technology, Federal University of Technology, Owerri, Nigeria. A voucher specimen was deposited at the Herbarium for reference purposes. The fresh leaves of *Vernonia amygdalina* (VA), *Ocimum gratissimum* (OG), *Gongronema latifolium* (GL), *Solanum melongena* (SM), *Phyllanthus amarus* (PA), and *Piper nigrum* (PN) were dried for 5 minutes in an oven at temperatures in the order of 60°C or below to stop enzymatic activity¹¹ and air dried to constant weight and milled to fine powder and the subjected aqueous extraction was used for administration. Significant degradation in free radical-scavenging activities in medicinal plants occur in samples maintained at temperatures in excess of 60°C¹²

Drug Administration:

Guinea pigs of both sexes with average weight of 250g were employed in this study. Each animal was made diabetic using a single dose of 200mg/kg body weight of Alloxan

administered intraperitoneally using a 2ml syringe. (Alloxan induced diabetic with either 100mg/kg or 150mg/kg body weight revealed mean fasting blood sugar level less than 1.0mmol/L). While the negative control groups received 2.0ml/kg body weight of normal saline, the positive control groups were administered 0.25mg/day glibenclamide a known oral hypoglycaemic drug and the test groups received 0.5g/kg body weight of the dietary vegetable mixtures in the ratio 50:50:50 orally via cannula.

Determination of fasting blood sugar level:

Using a 2ml syringe, a 12 hour fasting blood samples were collected from the ears of the experimental animals by venepuncture. Pretreatment samples were used to estimate the physiological baseline values of the fasting blood glucose level. Blood samples were spotted on Ultra easy one touch glucometer (Johnson and Johnson Company, UK) strip (Code No.9) and the values were recorded using the glucose monitor.

Statistical Analysis

The results were analyzed using the multivariate analytical statistical package for social sciences (SPSS), version 15. The results were further subjected Z-test and ANOVA analysis. Additionally, the percentage differences in the test groups were also compared with control. $P < 0.05$ was accepted for significant difference.

RESULTS AND DISCUSSION

It is well recognized that diabetic mellitus is one of the commonest and prevalent diseases affecting both men and women worldwide (Patel *et al.*, 2012, Shaw *et al.*, 2010). It has therefore become imperative that the development of alternative methods principally from plants having antidiabetic activity rather than insulin and oral hypoglycemic agents for the treatment of diabetes mellitus, which will minimize the complications associated with diabetes and related disorder is of clinical interest. The present study was thus undertaken to investigate the anti-diabetic effects of the dietary vegetable mixtures, using alloxan induced diabetic rabbit model. Since the quantification of these nutrients in-vivo is costly and time consuming, in the present study, the mean fasting blood glucose level in alloxan induced guinea pig diabetic for two weeks and followed by the administration of the test substances are compared with oral hypoglycaemic drug, glibenclamide.

The anti-diabetic activities of the dietary vegetables are shown in table 1. The percentage reduction of blood glucose level after alloxan induction of DM for the positive control group administered glibenclamide and *phyllanthus amarus* was 20% respectively while that for

vernonia amygdalina and *gongroema latifolium* was 19% and 18 % respectively; and *ocimum gratissimum* and *solanum melongena* 10% respectively and that for *piper nigrum* was 1%. Alloxan induced 41.6% increase in mean fasting blood glucose level in the negative control groups administered normal saline. With the exception of the groups administered *piper nigrum* ($p > 0.05$), ANOVA or Z-test revealed statistically significant differences ($p < 0.05$) in the mean fasting blood glucose level of alloxan induced diabetes and after substance treatments.

Table 1 Mean fasting blood glucose level before and after alloxan induction of hyperglycaemia (ranges are parenthesis)

Substance	Fasting blood glucose before alloxan induction of DM (mmol/L)	Fasting blood glucose after alloxan induction of DM (mmol/L)	Fasting blood glucose following substance administration (mmol/L)
Normal saline (0.9%)	4.98±0.08 (4.88-5.10)	6.97±0.04(6.9-7.00)	7.05±0.05(7.0-7.10)
Glibenclamide	5.02±0.12(4.90-5.13)	7.0±0.12(6.8-7.10)	5.02±0.12(4.83-5.10)
<i>Vernonia amygdalina</i> (VA)	5.02±0.03(4.98-5.05)	7.0±0.10(6.6-7.10)	5.07±0.04(5.03-5.10)
<i>Ocimum gratissimum</i> (OG)	5.07±0.48(5.0-5.10)	6.67±0.10(6.5-6.8)	5.42±0.36(5.38-5.53)
<i>Gongronema latifolium</i> (GL)	4.94±0.05(4.88-5.0)	6.95±0.12(6.8-7.1)	5.23±0.07(5.23-5.40)
<i>Solanum melongena</i> (SM)	4.47±0.57(4.88-5.03)	7.0±0.05(7.0-7.1)	6.02±0.25(5.73-6.40)
<i>Phyllanthus amarus</i> (PA)	4.98±0.07(4.9-5.10)	6.95±0.42(6.9-7.0)	5.02±0.02(5.0-5.04)
<i>Piper nigrum</i> (PN)	5.03±0.09(4.90-5.13)	6.96±0.67(6.9-7.0)	6.85±0.05(6.8-6.90)

Table 2 compares the mean fasting blood glucose levels in alloxan induced guinea pig diabetic for two weeks followed by the administration of the dietary vegetable mixtures in the ratio 1:1:1. The dietary vegetable mixtures potentiated 23.9% to 26.1% lowering of blood glucose level after alloxan induction of DM compared to the positive control values of 20%. ANOVA or Z-test showed significant statistically differences ($p < 0.05$) in all the test groups.

Table 2. Blood glucose lowering effects of the combination of the medicinal plants in alloxan diabetic rats (ranges are parenthesis)

Substance	Fasting blood glucose before alloxan induction of DM (mmol/L)	Fasting blood glucose after alloxan induction of DM (mmol/L)	Fasting blood glucose following substance administration (mmol/L)
(VA)+(PA) +(GL)	5.01±0.62(4.93-5.07)	6.96±0.10(6.8-7.10)	4.79±0.06(4.77-4.90)
(OG)+(PA)+(GL)	4.97±0.08(4.87-5.07)	7.02±0.07(6.9-7.10)	4.89±0.03(4.87-4.90)
(VA)+(PA)+(OG)	5.03±0.0.25(5.0-5.07)	6.93±0.05(6.9-7.0)	4.89±0.17(4.83-5.03)
(VA)+(GL)+(OG)	5.11±0.48(4.83-5.97)	6.91±0.08(6.8-7.0)	4.98±0.45(4.93-5.03)

Hitherto, the results of the present study is suggestive plausibly that the higher quantities of the combinative bio-active constituents of the dietary vegetable mixtures significantly lowered alloxan elevated blood glucose levels in guinea pigs in contrast either when employed singularly or when applied in the ratio 50/50 as in the previous studies^{13,14}. Further experiments on the cooperative additive/ synergistic potency are needed to confirm the idea of more central mechanisms of action whether the anti-diabetic effect of the dietary vegetable mixtures plausibly regulated the release of increase of insulin level in the body, increase the body's sensitivity to insulin or decrease glucose absorption in the intestine (and thus increase the body's ability to repair it). It is plausible that the strong cooperative antioxidant/phytochemicals of the dietary vegetable mixtures are capable of neutralizing free radicals, and thus reduce the severity of diabetic complications^{4,7,8,9} together with the higher fibre contents of the plant based diets play the crucial role in the reduction of blood glucose level. Our observations if it should be extrapolated to the human medicine, they are in resonance with its local applications; the increased consumption of the dietary vegetable mixtures as demonstrated, may have important clinical implications by ameliorating oxidative stress and this may consequently avert complications especially in type 2 diabetes mellitus (and obviously may be beneficial to those who are also at much greater risk of developing heart disease). Apparently, traditional physicians plausibly explore the strong cooperative synergistic/additive relationship in the higher antioxidant/ phytomedicine of the dietary vegetable mixtures in the treatment and management of diabetes and its complications.

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