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Interactive Effect of Combined Aqueous Leaf Extracts of *Ocimum gratissimum* and *Vernonia amygdalina* on Fasting Blood Glucose in Rabbits

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ABSTRACT

The leaves of *Ocimum gratissimum* (OG) and *Vernonia amygdalina* (VA) had been of medicinal interest mainly due to their respective antioxidant and antihyperglycaemic properties. A daily dose of 0.5gKg⁻¹ body weight aqueous leaf extracts of OG, VA and 50:50 (OG+VA) was orally administered to three different rabbit groups for 28 days, fed *ad libitum*. The control group was given only 2mlKg⁻¹ of normal saline daily. Fasting blood glucose concentration was monitored with a digital glucometer. From the SPSS (version 15) multivariate analysis (p=.05), results showed that the mean fasting blood glucose concentrations of normal rabbits used was 5.98±0.5 mMolL⁻¹. Only VA lowered fasting blood glucose within 24 hours and maintained the hypoglycaemia for 72 hours; VA potentiated OG after 72 hours and later synergistically lowered fasting blood glucose concentration. This study suggested that VA and (OG+VA) were time-dependent hypoglycaemic: while consumption of VA had acute effect, (OG+VA) could preferably be used for longer prophylaxis of diabetes. Therefore, further researches are recommended on the efficacy of this herbal combination therapy.

Keywords: *Ocimum gratissimum*, *Vernonia amygdalina*, Interactive effects, blood glucose, Rabbits

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INTRODUCTION

Diabetes mellitus, a metabolic disorder found in all nations of the world, is one of the most prevalent epidemics of the 21st century.¹ Recent global estimates indicated that the total number of patients affected by diabetes in 2004 was close to 190 million, a figure likely to have reached 325 million by 2005, that is, an increase of more than 70%.² World Health Organization (WHO) and African regional data estimates of 2007 showed that about 7 million people had diabetes as at the year 2000 and estimated that 18 million are expected to come down with the disease in 2030. This rapidly increasing prevalence is a significant cause for concern.³

Some wild herbs and spices have been shown to be most effective, relatively non-toxic and have substantial scientific documentation to attest to their efficacy in diabetes management.⁴ Considerable evidences had been accumulated to implicate cellular damage arising from reactive oxygen species (ROS), at least in part, in the aetiology and pathophysiology of human diseases such as diabetes.^{5,6} Current therapies seem to be insufficient to prevent diabetic complications in type 2 diabetes, with a two- to four-fold likelihood for developing cardiovascular complications.⁷ The value of current therapies is unequivocal, yet inadequate.⁸ Little scientific evidence exists to support the numerous herbs used to improve diabetes-related metabolic disorders.⁹

Ocimum gratissimum (OG) is commonly called *African basil* or 'scent leaf' in English-speaking countries, *basilic* in French-speaking countries, *ncho-anwu* or *ahuji* in Igbo (South Eastern Nigeria) *Efinrin ajase* by Yoruba tribe of South-Western Nigeria and *Aai doya ta gida* or *Daidoya* in Hausa (Northern Nigeria). *Vernonia amygdalina* (VA), identified as 'bitter leaf', is called *onugbo* in Igbo; *Chusar doki* or *fatefate/ mayemaye* in Hausa and *ewuro* in Yoruba¹⁰

Therapeutic effects of OG have been attributed to the peptides, alkaloids, essential oils, phenols and flavonols which are major components in this plant.^{11, 12, 13} Flavonoids are known to exhibit antioxidant activity and are effective scavengers of super oxide and anions.^{14, 15} It is believed that these secondary metabolites or phytochemicals may be effective in combating or preventing disease due to their antioxidant effect.^{16, 17}

The leaves of VA had been used to treat diabetes locally in Nigerian folk medicine for their antihyperglycaemic effect.^{18, 19} VA exerts antibiotic action against drug resistant micro organisms and can prevent or delay the onset of breast cancer and diabetes.²⁰ Major constituents of the extract from the leaves of VA include: sesquiterpene lactones and steroid glucosides.²¹ It has been reported that a sesquiterpene lactone isolated from the extract of *Ambrosia maritima* is an effective hypoglycaemic agent. It has also been suggested that the hypoglycaemic effect of

aloes and its bitter principle may be mediated through stimulating synthesis and/or release of insulin from the beta-cells of Langerhans.²²

Consumption of the plant extracts is also beneficial to primary and secondary DM such as pregnancy-induced (gestational) DM since dietary factors have been implicated for the rising incidence of diabetes, and most non-insulin dependent DM (NIDDM) occur commonly in obesity and insulin disorder.³⁵ Apart from weight-reduction and weight maintenance diets used in diabetic therapy, leaves of VA, OG and (OG+VA) in foods could possibly be useful. Diabetes is predicted to threaten the health of over 10 million 'normal' Africans within 2 decades³; and therefore necessitates for urgent interventional researches.

The work is to compare the effects of OG, VA and 50:50 OG+VA on the fasting blood glucose concentration in normal rabbits over 28 days. This will suggest the interactive effect of OG and VA when combined in management of diabetes.

MATERIALS AND METHODS

Source and identification of the plant materials

The *Ocimum gratissimum* and *Vernonia amygdalina* were bought from local markets within Owerri capital territory, Imo State Nigeria between the hours of 6-8 am at a prevailing temperature of about 28+ 2°C. All the collections were done in the month of April, 2010. To obtain potent plant extracts, the age of the plant, the extracting solvent, method of extraction and time of harvesting the leaves were considered as reported.^{23, 24} The plant leaves were identified and authenticated at the Department of Crop Science Technology, School of Agriculture and Agricultural Technology in Federal University of Technology Owerri, Nigeria.

Preparation and Storage of Leaf Extracts

Fresh leaves of *OG* and *VA* were thoroughly washed using tap water and rinsed with distilled water. The leaves were dried for 5 minutes in an oven at 60°C to stop enzyme activity (Effraim et al., 2000). They were then air dried to a constant weight, milled with sterile manual grinder (Corona, China) and further milled to a fine powder with the aid of a Binatone blender (Model BLG-401). The powdered material was stocked in a plastic container with water absorbent. The solvent used for the preparation of the extracts was distilled deionised water and the technique was adapted from previous works.^{26, 27}

The aqueous extracts were prepared by weighing out (25 g) of the milled powdered leaves of *OG* and *VA* and added to 250 ml of distilled deionized water respectively in each 500 ml beaker and stirred vigorously with a glass rod. The two mixtures were kept on the laboratory bench for 24

hours before filtering. The extracts were filtered using Whatman no.1 filter paper. Brown- green and dark-green filtrates of OG and VA were obtained, respectively. The filtrates were oven dried at low temperature and the extracts were stored in airtight bottles at 4°C in a refrigerator until ready for use. The stored extracts were allowed to reach room temperature before administration.

Source and Maintenance of Rabbits

The *albino* rabbits of both sexes were bought from the Rabbitry in School of Agriculture and Agricultural Technology, Federal University of Technology Owerri, Nigeria. The 6-8 month old rabbits were acclimatized for two weeks in nearby cages within JONGRES Biomedicals' premises. The rabbits were grouped by weight. Group 1 was untreated control while Groups 2 and 3 were test-groups. The animals were kept under adequate sanitary conditions with natural day-light and dark cycle and free ventilation.

The rabbits were fed on a Nigerian livestock grower mash (feed) containing crude protein, carbohydrate, oil, methionine, fibre, calcium, phosphorus and lysine. The feeding and drinking were carried out *ad libitum*. The rabbitry was cleaned on daily basis. The weights of the animals were taken using weighing machine before and after study.

Administration of the Leaf Extracts

The control Group 1 received orally 2.0 ml/kg body weight of physiological saline daily. A daily dose of 0.50g/kg body weight of each extract (at room temperature of 22 - 26°C) was orally administered as follows: OG, VA and (OG+VA) were given to Groups 2, 3 and 4, respectively. The hypoglycaemic dosage of 0.50g/Kg, oral administration and duration of treatment were chosen based on the earlier reports.^{19, 25, 28, 29, 30}

Monitoring of 12-hour Fasting Blood Glucose

'Ultra easy' one touch meter (Johnson and Johnson Company, UK), a blood glucose monitoring system was used as specified (www.lifeScan.co.uk). The strip code No. was 9. The estimation was made immediately after an ear venepuncture with a sterile needle. Blood glucose level was assayed by allowing a single drop of fresh blood on the strip properly fixed with the glucose monitor. Pretreatment samples were used to estimate the physiologic baseline values of fasting blood glucose. Mean Fasting blood glucose concentrations were monitored on the 1st, 3rd and 28th day of therapy.

RESULTS AND DISCUSSION

Ocimum gratissimum (OG) and *Vernonia amygdalina* (VA) are vegetables used in preparation of many delicacies in Nigeria. Both leaves are locally used in the preparations of assorted sauce or

soup, like yam soup and bitter leaf soup which serve as sauce for the eating of staple foods like yam and *garri* ('*iba*'), respectively. Consequently, aqueous extracts are consumed in foods prepared with these leaves.

OG was reported to have antioxidant effect.^{11, 14, 15} Reports indicated VA as antihyperglycaemic/hypoglycaemic.^{3, 19, 32, 33} Diabetes is a pandemic problem, for instance in UK, diabetes accounts for 30% reduction in life expectancy.³⁴ Evidence-based nutritional approaches to the treatment and prevention of diabetes mellitus (DM) had been considered.¹ Consequently, it became imperative to comparatively evaluate the hypoglycaemic potentials of OG, VA and combined 50:50 (OG+VA). Their hypoglycaemic efficacy could possibly be useful for acute treatment or prophylaxis of diabetes mellitus.

Table 1 below shows the pre- and post treatment values of 12-hour fast blood glucose (FBG) concentrations. The control animals (Group 1) were administered with only normal saline; test animals (Groups 2 and 3) were administered with 0.5g/Kg/day of *Ocimum gratissimum* extract (OG) and *Vernonia amygdalina* extract (VA), respectively; while test animals (Group 4) were given 50:50 OG+VA.

Table 1: Pre- and Post Treatment Mean Concentrations (mMol/L) of 12- Hour Fasting Blood Glucose (FBG).

Drug conc. 0.5g/Kg/day/rabbi Group	Mean FBG ±SD (mMol/L)	Mean FBG ±SD (mMol/L)	PD ₁ %	Mean FBG ±SD (mMol/L)	PD ₃ %	Mean FBG ±SD (mMol/L)	PD ₂₈ (%)
	Pre treatment(DAY 0)	Post treatment DAY 1		DAY 3		DAY 28	
1(control)	6.38± 0.3	6.15±.2	3.61	6.03±.3	5.49	5.48±1.0	14.11
2(OG)	6.08±0.2	5.58±.6	8.22	5.40±.6	11.18	5.18±.5	14.80
3(VA)	5.58±0.6	4.60±.5	17.56	4.03±.3	27.78	4.88±.2	12.54
4(OG+VA)	5.88±0.3	5.60±.6	4.76	4.88±.4	17.00	4.18±1.7	28.91
Total mean	5.98±0.5	5.48±.7	8.36	5.08±.8	15.05	4.93±1.0	17.56
Total mean (2-4 treated)	5.84	5.26		4.77		4.74	

PD₁= Percentage difference between pre treatment and 24 –hour BGC post treatment values;

PD₃= Percentage difference between pre treatment and 3day- BGC post treatment values; PD₂₈=

Percentage difference between pre treatment and 28 –day BGC post treatment values;

SD = Standard deviation

$$PD = \frac{\text{Pre-treatment conc.} - \text{Post-treatment conc.}}{\text{Pre-treatment conc. (mMol/L)}} \times 100\%$$

In table1, the total mean of the fasting blood glucose (FBG) concentration (in mMol/L) decreased with the increasing days of treatment with 0.5g/Kg/day. The Percentage Difference (PD) is greatest in Group 3(VA) on Days 1 to 3, followed by Group 4 (OG+VA) on Day 3. The mean values of FBG increased on the 28th day.

The total mean of the pre-treatment (Day 0) BGC values give the normal (baseline) fasting blood glucose concentration of 5.98 ± 0.5 mMolL⁻¹. This baseline value served as control in determining the hypoglycaemic efficacy of the extracts in Table 2.

In Figures 1 and 2, Group 3 (VA) had the highest hypoglycaemic effect on FBG concentration on day 3; followed by Group 4 (combined extracts (OG+VA)). Group 1 (control) had the lowest effect on FBG compared with all the treated groups.

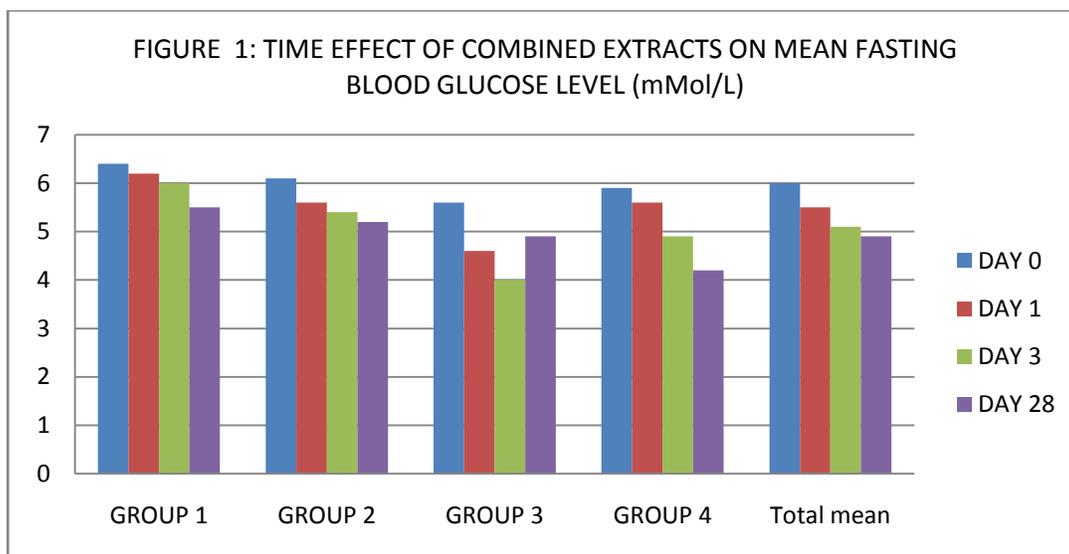


Figure 1 Time effect of combined extracts on mean fasting blood glucose level(mMol/L)

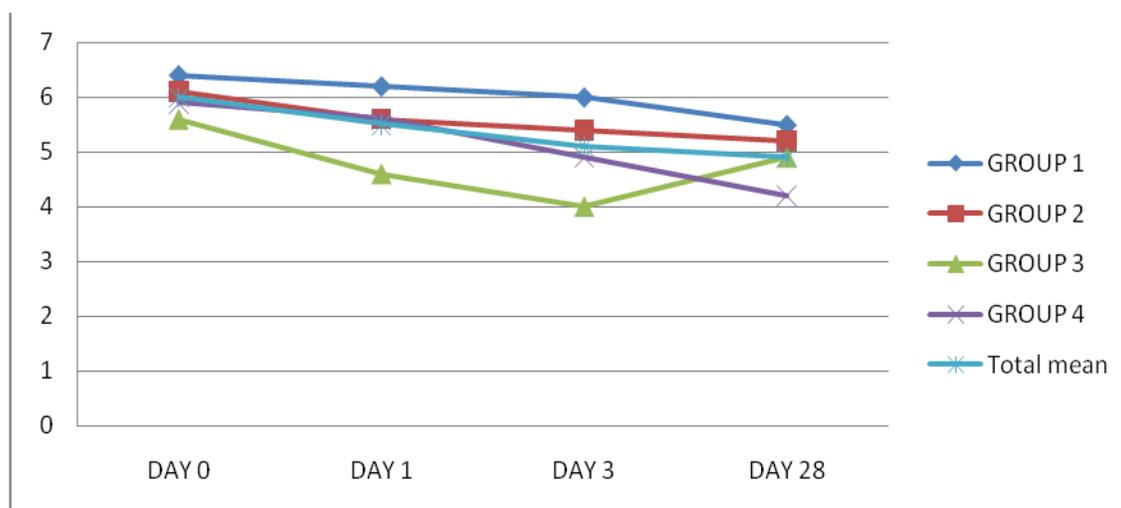


Figure 2: Graph showing the effect of combined extracts on fasting blood glucose concentration(mMol/L)

The hypoglycaemic effect of the combined extracts (Group 4) was gradual and consistent compared with OG and VA administered singly.

Table 2: Determination of Time-dependent Hypoglycaemic Efficacy of OG and VA compared with the 50:50 (OG+VA)

0.5g/Kg/ extract per rabbit in Groups 2-4	day	Mean effect after DAY 1 (%)	Mean effect after DAY 3 (%)	Mean effect after DAY 28 (%)
Group 1 (Baseline control)		0.00	0.00	0.00
Group 2(OG)		6.90	9.70	13.40
Group 3(VA)		23.10	32.60	18.40
Group 4(OG+VA)		6.40	18.40	30.10

(Hypoglycaemic efficacy (%) = $\frac{FBG_0 - FBG_x}{FBG_0} \times 100$)

Where FBG_0 = baseline concentration, FBG_x = time-dependent post treatment concentration)

In Table 2 and figure 3, Group 3 treated with VA had the highest hypoglycaemic efficacy within 72 hours followed by the combined extracts (OG+VA) on day 28.

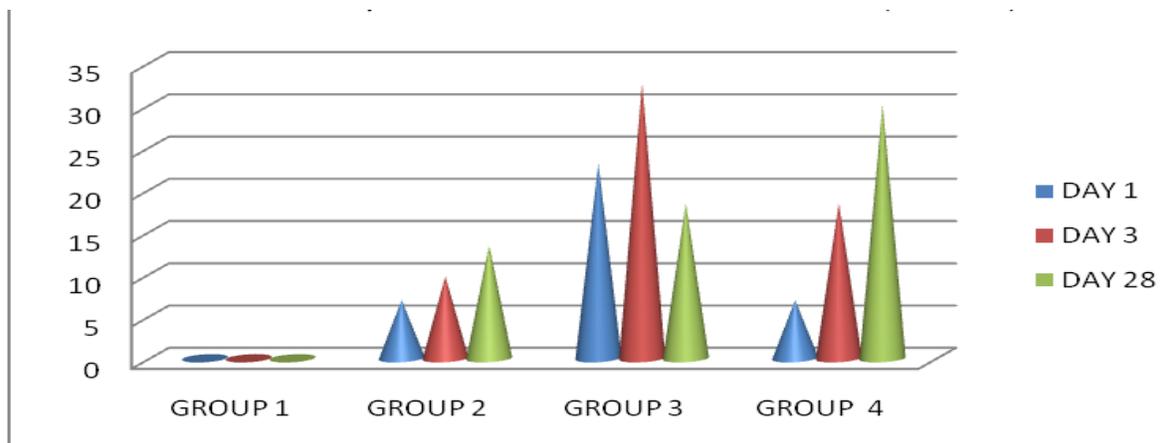


Figure 3: Time-depedent hypoglycaemic efficacy of OG and VA compared with the combined 50:50(OG+VA)

Group 1 baseline control (no extract); Group 2 (OG); Group 3 (VA) and Group 4 (OG+VA).

The vertical axis is the hypoglycaemic efficacy (%).

The hypoglycaemic potency of OG increased steadily with (OG+VA). VA potentiated OG after 72-hour treatment, as shown pictorially in Figure 3.

From the SPSS (version 15) multivariate analysis ($p=0.05$), VA significantly lowered normal fasting blood glucose during the 1st and 3rd day while (OG+V) gradually and consistently lowered normal fasting blood glucose throughout the duration of the study. Administration of only OG had insignificant hypoglycaemic effect in normal rabbits; hence VA potentiates the

hypoglycaemic effect of OG. The animals remained healthy and the growth rate was almost the same in all the groups during the period of study. The baseline mean fasting blood glucose concentrations obtained was $5.98 \pm 0.5 \text{ mMOLL}^{-1}$.

CONCLUSION

This study showed the time-dependent hypoglycaemic potencies of VA and combination of OG+VA in normal rabbits. VA potentiated OG and the combined (OG+VA) gradually and consistently lowered fasting blood glucose concentration for a longer time. Therefore, the consumption of VA or (OG+VA) could be therapeutic or prophylactic in diabetes mellitus (DM). Therefore, further researches are recommended on the efficacy of this herbal combination therapy.

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