SYNERGISTIC EFFECT OF AQUEOUS EXTRACTS OF CROTON ZABENSICUS AND VERNONIA AMYGDALINA LEAVES AS AN ANTIHYPERGLYCEMIC AGENT IN AN ALLOXAN INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Aims: This study was aimed at investigating the antihyperglycemic effect of combined extract of *Vernonia amygdalina* and *croton zabensicus* compare with a hypoglycemic drug, glibenclamide.
Methodology: Twenty 20 experimental animals were used (albino rats); the rats were divided equally into four groups of five rats each; namely A (control), B (glibenclamide 10mg/kg body weight), C (synergetic treatment 1000mg/kg body weight), D (synergetic treatment 500mg/kg of body weight). Diabetes was induced intraperitoneal using Alloxan Monohydrate to all the animals and their blood glucose rise above 200mg/dl.
Results: It was observed that group B and group C treated with glibenclamide (10mg/kg body weight) and synergetic aqueous extract (1000mg/kg body weight) show significant decrease in the blood glucose level from 451.75mg/dl to 64.50mg/dl and 339.50mg/dl to 182.50mg/dl respectively compared with group D with 278.25mg/dl to 194.75mg/dl**.** However, a change was also observed in the body weight of the groups; Group A (Normal control) showed continuous increase in the body weight, Group B, C and D were observed to have decrease in body weight from induction period, but a steady increase were observed as treatment commences.
Conclusion: Hence this combined extract can be used as antihyperglycemic; only that it is slower in remediation compared with the glibenclamide; but without side effect as may be in the case of most standard drug.

Keyword: Synergistic, aqueous extract, *Croton zabensicus, Vernonia amygdalina* and hyperglycemic

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1. INTRODUCTION

Diabetes mellitus is a metabolic disorder found in all nations of the world. It is one of the most prevalent endemic diseases of the 21st century. Diabetes Mellitus is a syndrome of impaired carbohydrate, fat and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin characterized by hyperglycemia

Type 1 diabetes typically occurs in children and young adults, though it can appear at any age. In the past, type 1diabetes was called juvenile diabetes or insulin-dependent diabetes mellitus. Over time, high blood glucose damages nerves and blood vessels, leading to complications such as heart disease, stroke, kidney disease, blindness, dental disease, and amputations. No one is certain what starts the processes that cause diabetes, but scientists believe genes and environmental factors interact to cause diabetes in most cases. Insulin must be used in Type I, which must be injected; while diabetes mellitus Type 2 is a disease of insulin resistance by cells. (1) has defined Diabetes mellitus based on laboratory findings as a fasting venous plasma glucose concentration greater than7.8 mmol/l (140 mg/dl) or greater than 11.1 mmol/l (200 mg/dl) two hours after a carbohydrates meal or two hours after an oral ingestion of the equivalent of 75 g glucose. The beginning of diabetes in rat is judged as blood glucose being higher than the expanded normal upper level. The criteria for rats are close to that for human. Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species (2). Diabetes mellitus is further characterized by an inability to reabsorb water resulting in increased urination (polyuria), excessive thirst (poldipsia) and excessive eating (Polyphagia). Herbal products are of interest to many patients and health care practitioners since about 70% of population worldwide rely on herbal medicines for part of their primary health care. In different regions and cultures, herbal products are used as single herb, combination of herbs, or combination of herb(s) and drug(s) e.g China and India. The most desirable interactions are those which can result in additional therapeutic benefit. This is often the intended or expected outcome when using combination therapy. In African traditional medicine, several plants include *Anacadium occidental*, *Congronema latifolium*, *Croton zabensicus, Vernonia amygdalina* etc; have been used to lower hyperglycemia.

Croton zabesicus is commonly known in Nigeria as *KirobaIcen maser* in Hausa and *Ajeobale* or *Ajeofole* in Yoruba (3). The ethanolic extract of the leaf was reported to produce a significant reduction in blood glucose level of diabetic rat (4). *Vernonia amygdalina* popularly known as bitter leaf is a shrub of 2 – 5m tall. It is popularly called bitter leaf because of its abundant bitter principles. It is cultivated in Nigeria mainly for its nutritional value.

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Diabetes mellitus is a metabolic disorder found in both young and old, rich and poor. It can be very expensive to manage; however, the use of conventional medical approach of simply using insulin and oral drugs to control diabetes Mellitus is not only costly but inadequate, boring and lack compliance and yet they are rarely available. This give rise to the increase in counterfeit thus the patient's exposure to long term complication remains a risk. Nigeria government has failed to recognize this as a challenge and proffer a lasting solution. This research work intend to encourage the use of a synergistic herbal medicine as a competitive antihyperglycemia for an effective management of type 1 diabetes which is readily available, cheap and with no record of toxicity. The specific objectives of this work are to study the synergistic effect of aqueous extract of *croton zabensicus and vernonia amygdalina as an* antihyperglycemic agent in alloxan induced diabetic rats; the percentage yield of the aqueous extraction of *croton zabensicus and vernonia amygdalina;* the phytochemical analysis of aqueous extracts of *croton zabensicus and vernonia amygdalina* singly and in combination and investigate the acute toxicity of aqueous extracts of *croton zabensicus and vernonia amygdalina.*

2. MATERIAL AND METHODS

2.1 Materials

The fresh leaves of *Vernonia amygdalina and Croton zabensicus* were got from Lagos state polytechnic; Ikorodu bush at early hour of the morning at about 7a.m and identified by a botanist in the Environmental Biology unit, Biological sciences department of Lagos state polytechnic; Ikorodu With reference number: lpbh: 016/001, 002

The animals used for this study include six albino mice of both sex (14 – 22g) and twenty male albino rats (80g - 200 g) were bought from Lagos State Teaching Hospital LASUTH in Ikeja. They were acclimatized for two weeks and fed with standard rat feed obtained from ZM veterinary store at Odogunyan, Ikorodu; Lagos and given clean and sterile potable water.

2.2 Phytochemical Screening

The phytochemical analysis was carried out using the method described by (5). The plant extracts were screened for the presence of Tannins, Saponin, Flavonoid, Glycosides and Phenol.

Test for Tannins: 5ml of extract with the addition of 0.1% $FeCl_3$ reagent solution was made. The formation of greenish black precipitate was observed and regarded as positive for the presence of alkaloids in all the extracts.

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Test for Saponin: 2ml of each of the extracts was added into 5ml of distilled water and both were vigorously shaken with the application of heat. It was observed that saponin was present in all the extracts.

Test for Flavonoid: 2ml of sample extract was diluted with sodium hydroxide and hydrochloric acid solutions respectively and filtered. The filtrate, zinc dust and concentrated hydrogen chloride were added together and red colour formation as observed, showing that flavonoids was present in all the extracts.

Test for Glycosides: 2ml of extract with the addition of hydrochloric acid solution (HCl) was neutralized with sodium hydroxide (NaOH) solution, then few drops of ferric chloride solution ($FeCl_3$) was added as well with 1ml of concentrated H_2SO_4 sulphuric acid underlaid. A reddish brown ring at the interface was observed, indicating the presence of Cardiac Glycosides in all the extracts.

Test for Phenol: 2ml of extract was added to 5.0ml of 95% ethanol; they were boiled in waterbath for five minutes and filtered hot. 5.0ml of distilled water was added and the ethanol was evaporated at a reduced pressure in the waterbath. The resultant concentrate with the addition of five drops of 1% of Ferric Chloride and 1% Potassium Ferric cyanide solution were added. A violet, wine, red, purple colour was developed, indicating a positive test for phenolic compounds.

2.3 Preparation of the Extract

The *Croton zabensicus and Vernonia amygdalina* leaves were sorted out to obtain only fresh leaves and washed with distilled water without squeezing to remove debris and dust particle. They were air dried separately and ground into powder; 100g each of the powered leaves were soaked with distilled water and ethanol respectively. The solutions were stirred at 1 hour interval for 72hours and filtered using a filter paper to obtain aqueous extract of *Croton zabensicus (*A1*),* and aqueous extract *Vernonia amygdalina (*B1). The filtrates were centrifuged at 1000 revolution per minute for 5minutes to obtain a pure and clear supernatant; the extract was then concentrated using a rotary evaporator and weighed, the

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weight was used to calculate the percentage yield of extract. The concentrated extracts were kept in a refrigerator below 4^0C for preservation till use.

Calculations

$$\% \text{ yield of extract} = \frac{\text{weight of concentrated extracts} \times 100}{\text{Weight of powered extract}}$$

1g each of the extracts A1 and B1 was weight and mixed together with 0.5ml of tween 80 and stirred with glass rod until they dissolved. The mixture was making up to 10ml with normal saline.

2.4 Acute Toxicity Studies

The median lethal dose (LD50) of the aqueous extract was estimated using Lorke's modified method. Six albino mice were weighed and divided into two groups H and L of three mice each and followed by administration of different doses of the extracts to two groups of three albino mice each; H and L . In this phase, each mouse in group L was administered with different low dose (500, 1000 and 2000) mg/kg of the combined extracts respectively; whereas group H was administered with high dose of 3000, 4000 and 5000mg/kg of the combined extracts respectively. The animals were observed for signs of toxicity; such as respiration, activeness and death within 21 days.

Calculation

i. volume of extracts administered = $\frac{\text{weight of mice (g)} \times \text{dosage (mg/kg)}}{1000 \times \text{concentration of extracts (mg/ml)}}$

2.5 Induction of Alloxan (Diabetes)

Twenty (20) albino rats were used. The rats were divided into four groups of five rats each; A – D. At the first day before induction; all the rats in the groups were tested for diabetes and they were all negative. A (control), B – D groups were induced with diabetes intraperitoneally using Alloxan Monohydrate sigma Uk with a standard dose of 150 mg/kg body weight and a concentration of 50mg/ml. 1g of alloxan monohydrate was suspended in 20ml of 0.9% normal saline everyday for four days; however; 48hours later; At sixth day Diabetes was confirmed using digital glucometer . Animals with blood glucose level ≥ 200 mg/dl were considered diabetic and included in the study. Body weights of all animals in each group were monitored using a digital weighing balance throughout the period of the experiment; so as to calculate the volume of extracts to be administered each day.

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2.6 Combined Extract and Drug Administration

At the end of the nine days, the animals that tested positive for diabetes were fasted for 12 hours, and then the blood glucose level was checked and recorded and administered the following drug and extracts orally as treatment once daily in a 24 hour cycle at 8: am for nine days; B received (glibenclamide 10mg/kg body weight); C received (combined extract 1000mg/kg) and D receives (combined extract 500mg/kg of body weight).

2.7 Statistical analysis

Data collected were expressed as mean, standard error of mean (SEM). Statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 17.0 with Duncan's Multiple Range Test (DMRT) option. A value of $P < .05$ was considered to indicate significant difference between groups.

3. RESULTS

Table 3.1 percentage yield of extracts

Plant	Weight of powdered plant	Weight of concentrated extracts	% yield of extracts
A1	100g	9.26g	9.26%
B1	100g	9.99g	9.99%

Table 3.2 Phytochemical analysis

Sample	Tannin	Phenol	Saponin	Flavonoid	Glycosides
A1	+	++	++	++	+
B1	+	+	+++	+	+
A1+B1	++	++	+++	++	+

Table 3.3 Acute Toxicity Studies.

Mice	Body weight	Dosage	Volume	Respiration	Activeness	Death

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	(g)	(mg/kg)	(ml)			
L1	14.05	500	**0.035**	**Normal**	**Very active**	**No death**
L2	16.02	1000	**0.080**	**Normal**	**Very active**	**No death**
L3	20.04	2000	**0.200**	**Normal**	**Very active**	**No death**
H1	21.70	3000	**0.326**	**Fast**	**Active**	**No death**
H2	15.49	4000	**0.309**	**Very fast**	**Weak**	**No death**
H3	**18.89**	**5000**	**0.472**	**Rapid pulse**	**Very weak**	**No death**

Key

LI : low dose (500mg/kg) H1: high dose (3000mg/kg)

H2: high dose (4000mg/kg) L2; low dose (1000mg/kg)

H3: high dose (5000mg/kg) L3: low dose (2000mg/kg)

Table 3.4 Effect of treatment blood glucose

GROUP	FBG0 (mg/dl)	FBG1 (mg/dl)	FBGL5 (mg/dl)	FBGL7 (mg/dl)	FBGL9 (mg/dl)
A	118.00**±19.79**[a]	120.50**±19.33**[a]	119.50**±2.24**[a]	117.00**±19.44**[a]	**117.00±20.28**[a]
B	128**±5.88**[a]	451.75**±54.43**[c]	191.25**±8.95**[a]	106.00**±16.63**[a]	**64.50±11.90**[a]
C	124.75**±13.53**[a]	339.50**±124.73**[bc]	204.25**±131.77**[a]	194.00**±134.95**[a]	**182.50±139.72**[a]
D	**122.75±16.35**[a]	**278.25±136.80**[b]	**214.5±139.23**[a]	**204.25±139.39**[a]	**194.75±141.89**[a]

Significant difference ($p < .05$) appears between groups with different superscript while significant difference ($p < .05$) does not appear between groups with the same superscript.

n = 4;

Key

FBG0; Blood Glucose before alloxan induction

FBG1; Blood Glucose 48 hours after alloxan induction

FBG5 ; Blood Glucose after 5days Treatment

FBG7: Blood Glucose after 7days Treatment

FBG9: Blood Glucose after 9days Treatment

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Table 3.5: Effect of treatment on body weight

GROUPS	BW0 (g)	BW1(g)	BW9 (g)	CBW(g)	%WG
A	109.50±12.81[a]	112.00±12.67[b]	135.25±12.20[b]	25.75±1.25[b]	**23.80±3.21**
B	147.75±19.98[b]	144.00±5.35[c]	159.50±6.24[c]	**12.25**±0.95[ab]	**8.22±0.40**
C	103.75±5.56[a]	101.75±5.73[ab]	118.00±16.91[ab]	**14.25**±15.90[ab]	**13.65±15.15**
D	**97.75±5.73[a]**	**95.25±6.55[a]**	**104.50±14.27[a]**	**6.75±13.76[a]**	**6.97±14.39**

Significant difference ($p < .05$) appear between groups with different superscript while significant difference ($p < .05$) does not appear between groups with the same superscript, n = 4.

Key

BW0: body weight before induction

BW1: body weight after 48hour of induction

BW9: body weight after nine days treatment

CBW: change in body weight (BW9-BW0)

%WG: percentage weight gain

DISCUSSION

The result confirms that synergistic treatment of C. zabensicus and *V. amygdalinaI* produced more antihyperglycemia properties with the high dose of 1000mg/kg of body weight when compared with a lower dose of 500mg/kg of body weight while the conventional antihyperglycemic drug like glybenclamide produced a hypoglycemic condition after ninth day of treatment. The rats were considered treated when their fasting blood glucose level returned to almost their basal blood glucose levels. The increase in blood glucose level of diabetic rats was found to have reduced after oral administration of synergistic aqueous leaf extract of *C. zabensicus* and *V. amgydalina* and glibenclamide respectively and shows significant ($p<.05$) decrease in the blood glucose level. It was also observed that the rats in group B and C got treated quickly as compared to group D probably because the rats in group B and C were administered with an antidiabetic drug of a higher dose of the combined extract (1000mg/kg) respectively as compared with the rats in group D with a low dose of 500mg/kg. The efficacy of the extract on hyperglycemic rats corroborates the result of other researchers who had systematically demonstrated that the extract from the plant *C. zabensicus* and *V. amgydalina* have antidiabetic properties (4). Polyphenolics such as tannins and Saponins from several plant extracts had been shown to reduce blood glucose levels through inhibition of α- amylase and sucrose from the intestine. Flavonoids were

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reported to regenerate the damaged pancreatic β-cells in diabetic animal (6). Diabetes is also characterized by weight loss, alloxan administration brought about marked reduction in body weights of experimental rats. These reduced body weights were found to have increased significantly after the nine days treatment in group B, C and D. The percentage weigh gain in group C (13.65%) seems to be prominent when compared with group A, group B (8.22) and D (6.77). After nine days of treatment, this is an indication that the healing process was slow in the plant extracts and faster in the glibenclamide (standard drug). The facts that hypoglycemic drugs have side effects which can further complicate the health of patients; however, the natural herbs can serve as compliments to hypoglycemic drugs (7).

4. CONCLUSION

It can be concluded that synergetic treatment of aqueous extracts of *croton zabensicus* and *vernonia amygdalina* leaves as an antihyperglycemic agent in an alloxan induced diabetic albino rats produced more competitive result with a dose of 1000mg/kg body weight.

This investigation has demonstrated that the use of combination of aqueous extract of the two leaves of *C. zabemsicus and V. amygdalina* is safe, effective, cheap and more comfortable for the management of diabetes mellitus. Further research needs to be carried out to ascertain the appropriate dosage in relation with the duration of the extracts administration for treatment of hyperglycemia in order to prevent hypoglycemia; which is also a concern. Considering the efficacy of the synergistic treatment of combined extracts; awareness should be created to promote the medicinal advantage of the plants over a standard drugs and a careful selection should be made following a thorough toxicity investigation; when combining two or more medicinal plants for treatment; so as to prevent complications; bearing in mind that some could be contradicting or antagonizing each other.

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Ethical Approval:
As per international standard or university standard written ethical approval has been collected and preserved by the authors.

Consent: NA

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