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Effects of *Aloe barbadensis* and *Cymbopogon citratus* on Blood Glucose Levels of Alloxan–Induced Diabetic Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Diabetes is a heterogeneous complex metabolic disorder characterized by elevated blood glucose concentration, and glycosuria resulting from insulin deficiency. This project was embarked upon to investigate the effects of *Aloe barbadensis* and *Cymbopogon citratus* on blood glucose levels of alloxan – induced diabetic adult albino rats. The 24-hour acute toxicity test (LD_{50}) of the orally administered combined *Aloe vera* and lemon grass ethanolic extract was determined using the Lorke's method. The fasting blood glucose levels were determined using glucose strips and glucometer. The toxicity test revealed that LD_{50} of the combined ethanolic extract of *C. citratus* and *A. barbadensis* was >5.0g/kg body weight. The result showed a significant ($P<0.05$) increase in the fasting blood glucose levels of the diabetic untreated animals compared with the fasting blood

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glucose levels of the normal control animals after inducing diabetes. There was also a significant ($P<0.05$) decrease in the fasting blood glucose levels of the animals treated with ethanolic extract of *Aloe vera* gel, lemon grass leaves and its combination when compared with the diabetic untreated. It could be concluded that that *Aloe vera*, lemon grass or its combination can be used as an alternative medicine for the treatment and management of diabetes mellitus.

Keywords: Synergistic; diabetes; Aloe barbadensis; Cymbopogon citrates; blood glucose level.

1. INTRODUCTION

"Diabetes is a metabolic disorder, characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion and insulin action" [1]. "It can also be defined as a heterogeneous complex metabolic disorder characterized by elevated blood glucose concentration secondary to either resistance to the action of insulin, insufficient insulin concentration, or both (American Diabetes Association's Standards of Medical Care in Diabetes" [2]. "Diabetes is an epidemic disorder occurring in many parts of the world which could be generated as a result of predisposing factors such as obesity, aging, and antigen/antibody reaction and insufficient exercise, exposure to chemicals or infections affecting humans and animals in both developed and developing countries" [1]. "Long-term complications of diabetes include retinopathy with a potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot's joints; and autonomic neuropathy causing gastrointestinal, genitourinary, cardiovascular symptoms, sexual dysfunction, and even death" [1,3].

According to International Diabetes Federation [4], "537 million adults of the world population are living with diabetes which is predicted to rise to 643 million by 2030 and 783 million by 2045. Over 3 in 4 adults with diabetes live in low-and middle income countries while in Africa, 1 in 22 adults (24 million) are living with diabetes".

"The premise of Type II diabetes mellitus treatment and management includes patient education regarding the disease, physical exercise, dietary modulation, application of synthetic hypoglycemic agents such as glycosidase inhibitors, biguanides, and the use of plant extracts. These exert an anti-diabetic effect through different mechanisms either by stimulating insulin secretion or absorption of glucose. The main disadvantage of current drugs is their prolonged usage throughout one's life thereby losing their effectiveness and thus producing side effects that are detrimental to health" [5]. "For example; Metformin and Glucagon-like peptide-1agonists are associated with gastrointestinal distress. Sulfonylureas usually causes hypoglycemia and weight gain, while Pioglitazone may increase the possibility of developing bladder cancer and other disorders, such as edema, heart failure, weight gain, and distal bone fractures in postmenopausal women in addition to the high cost of these medications" [6].

"Due to this challenge, the search for alternative remedies such as medications from natural plants is essential and should be recommended" [7]. "*Aloe barbadensis* (*Aloe vera*) and *Cymbopogon citratus* (lemon grass) are well-documented herbs for the treatment of diabetes mellitus. The hypoglycemic effect of these plant extracts could be as a result of natural compounds such as flavonoids, saponins, chromium, alprogens, acemannan, anthraquinones, phytosterols that possess many health benefits. These chemical compounds improve the beta function of the pancreas in producing the hormone; insulin by regenerating and inhibiting damage to the islets of Langerhans cells so that insulin secretion returns to normal in the blood and restores the sensitivity of the insulin receptor cells" [8]. The aim of this study is to evaluate the effects of *Aloe barbadensis* and *Cymbopogon citratus* on the blood glucose levels of alloxan–induced diabetic albino rats.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in the Zoology Research and Development Center of the Department of Zoology located at Nnamdi Azikiwe University, Awka, Anambra State.

2.2 Procurement of Experimental Animals

A total of 180 adult albino rats with an average weight of 150g were used for the experiment.

The test animals were purchased from Chris's Experimental Animal Farm and Research Laboratory in Awka, Anambra State. They were kept and maintained in cages in the Zoology Research and Development Center under normal temperature in which they were fed daily with water and vital growers' chick mash pellets purchased at Vital Feed Distributor, Awka for one (1) week to get acclimatized with the environment, before the commencement of the experiment.

2.3 Collection and Identification of Plant Materials

Fresh leaves of *Cymbopogon citratus* (lemon grass) and *Aloe barbadensis* (*Aloe vera*) were collected from Forestry farms, Amawbia, Awka South Local Government, Anambra State, and were identified by Mr. Iroka Finian of the Department of Botany, Faculty of Biosciences, Nnamdi Azikiwe University with herbarium number 187A and 207A respectively.

2.4 Preparation of Plant Materials

2.4.1 Ethanolic extraction of lemon grass

Fresh lemon grass leaves were washed to remove impurities, cut into smaller pieces, and air-dried in the Zoology laboratory. Thereafter, the dried leaves were blended into coarse powder with the aid of a grinding machine. The measured coarse powder (1500g) was mixed with 300ml of 70% ethanol, stirred vigorously every three (3) hours and allowed to stand for 48hrs. The mixture was sieved with a muslin cloth, filtered with Whatman's filter paper, and the filtrate was placed in a water bath at 60°C. The crude extract obtained was cooled, stored in an air-tight container and kept in the refrigerator for later use [9].

2.4.2 Ethanolic extraction of *Aloe vera*

Succulent and fresh *Aloe vera* leaves were collected from a matured healthy plant and washed thoroughly with fresh running tap water to remove unwanted particles. The leaves were dissected longitudinally using a sterile knife and the fleshy mucilaginous gel was carefully scrapped out from its thick epidermis layers. The scrapped gel was ground with an electric blender (Sonik Japan SB-735) to get a homogenous mixture. The weighed mixture (5,295g) was mixed with 70% ethanol and stirred thoroughly using a spatula in a plastic bowl. The ethanolic mixture was allowed to stand for 48 hours with continuous stirring every three hours to enable the complete extraction of active ingredients. After 48hours the ethanolic mixture was sieved using a muslin cloth and filtered with Whatman's filter paper no. 1 which was then placed on a large, flat steel pan and allowed to evaporate in a water bath to reduce the ethanolic content at 60°C. The crude extract was collected in an air-tight container and preserved in a refrigerator before the commencement of administration [10] with little modifications.

2.4.3 Lethal dosage test

The lethal dose of *Aloe vera* extract has been reported to be >5000mg/kg [11] while the lethal dosage of lemon grass has been reported to be >5000 mg/kg [12]. However, the lethal dosage of the combined ethanolic extracts was determined using the Lorke method [13]. This method involves two (2) phases. In the first phase, test animals were randomly divided into three (3) groups of 3 rats each and administered equal amount of graded doses of 10 mg/kg (5 mg of *Aloe vera* (AV); 5 mg of lemon grass (LG), 100 mg/kg (50 mg of AV; 50mg of LG), and 1000 mg/kg (500 mg of AV; 500mg of LG) of the combined ethanolic extracts. In the second phase, one (1) rat each was administered graded doses of 1600 mg/kg (800mg of AV; 800 mg of LG), 2900 mg/kg (1450mg of AV; 1450 mg of LG), and 5000 mg/kg (2500mg of AV; 2500 mg of LG). In both phases, behavioral observations and the mortality rate were observed.

2.5 Experimental Design

After one (1) week of acclimatization, the albino rats were weighed and randomly grouped into 3 replicates. Each of the replicates were divided and labeled into 12 groups with 15 albino rats in each group. Group A and Group B were positive and negative controls respectively while groups C, D, E, F, G, H, I, J, K, and L are the treatment groups. The details of groups are show in Chart 1.

2.6 Preparation of Glibenclamide Suspension

Glibenclamide was purchased from Ned King Pharmacy, Ifite-awka, Awka. Five (5) grams of glibenclamide tablet was crushed and mixed with 20 ml of distilled water. The mixture was kept in an ultrasonic water bath for 45 minutes until a homogenous mixture is obtained [14]. The

suspension was administered to the induced diabetic rats at 200mg/kg body weight.

Chart 1. Details of groups

Group	Dosage
A	No induced diabetes, no treatment
B	Induced diabetes, no treatment
C	Induced diabetes, treated with glibenclamide
D	100 mg/kg of lemon grass extract
E	200 mg/kg of lemon grass extract
F	300 mg/kg of lemon grass extract
G	100 mg/kg of *Aloe vera* extract
H	200 mg/kg of *Aloe vera* extract
I	300 mg/kg of *Aloe vera* extract
J	50 mg/kg of *Aloe vera* : 50 mg/kg lemon grass extract
H	100 mg/kg of *Aloe vera* : and 100 mg/kg lemon grass extract
I	150 mg/kg of *Aloe vera* : 150 mg/kg lemon grass extract

2.7 Introduction of Alloxan in the Experimental Animals

Alloxan monohydrate was purchased from Sigma Aldrich Missouri, USA. The initial blood glucose levels of the albino rats were determined using glucose test strips and a glucometer. Intraperitoneal injection of alloxan suspension with distilled water was carried out using an insulin syringe to the overnight fasted normal glycemic experimental albino rats at 130mg/kgBW to induce diabetes mellitus. Fifty grams (50g) of Dextrose was dissolved in 1000 ml of clean water and given to the induced animals to reduce the risk of hypoglycemia due to sudden unavailability of blood glucose as a result of destruction of beta cells of the pancreas arising from inducing alloxan. After 3 days of induction, the animals were tested for diabetes. An animal was considered diabetic when their blood glucose level was above 200 mg/dl [4].

2.8 Treatment and Data Collection

The experimental animals in Group A and Group B were given normal feed (growers' chick mash) and clean water. Group C was given glibenclamide at 5mg/kg body weight. Groups D, E, and F were given lemon grass extract while Groups G, H, and I were given *Aloe vera* extract, and Groups J, K, and L were given a combination of *Aloe vera* and lemon grass at 100 mg/kg, 200 mg/kg and 300 mg/kg respectively once in 24 hours through oral gavages using canular for 3 weeks during the experimental period at a fixed time of 10:00 am. Fasting blood glucose levels were checked by collecting a blood sample from the tail vein every 3 days and determined using glucometer and glucose test strips (code 25) at 8:00 am before treatment and feeding while body weight was checked every week using G&G (model JJ300) weighing balance.

2.9 Statistical Analysis

Data on the fasting blood glucose levels were expressed as Mean ± SEM and compared among groups with ANOVA Tests at 5% significant level using the Statistical Package for Social Science software for windows version 25 (SPSS Inc., Chicago, Illinois, USA).

3. RESULTS

Table 1 shows the result of the 24-hour acute toxicity test of the orally administered combined ethanolic extract of both *Aloe vera* gel and lemon grass leaves in adult albino rats. In the first phase, the groups that were administered 10 mg/kg, 100 mg/kg, and 1000 mg/kg body weight showed no sign of toxicity. The behavioral pattern observed was normal and active with zero mortality recorded. Due to the fact that no mortality was recorded, the study required a second phase in which observations of behavioral pattern and the mortality rate was recorded after 24 hours. The behavioral pattern of the rat administered 1600mg/kg body weight was normal, active with zero mortality also recorded. The rat administered 2900mg/kg body weight of the combined ethanolic extracts was observed to be slightly weak with little activeness and zero mortality recorded while the rat administered 5000mg/kg body weight was observed to be very weak with no engaged activity and zero mortality recorded. The results obtained showed that the LD_{50} of the combined *Aloe vera* gel and lemon grass leaves ethanolic extract is >5000 mg/kg body weight.

The result of twenty-one (21) days of treatment of alloxan-induced diabetic rats with *Aloe vera* gel, lemon grass, and its combination is shown in Table 2. The group of rats administered different doses of the individual and combined extract showed a significant ($P<0.05$) reduction in the fasting blood glucose levels from the 3rd day

Table 1. Result of the acute toxicity study (LD_{50}) of the combined ethanolic extract

Phases	Groups	Dose (mg/kg)	Behavioral pattern	Mortality rate
Phase 1	A	10	Normal and active	None
	B	100	Normal and active	None
	C	1000	Normal and active	None
Phase 2	D	1600	Normal and active	None
	E	2900	Slightly weak and little activeness	None
	F	5000	Very weak with no activity	None

of treatment compared with the diabetic untreated group. The fasting blood glucose profiles of the group of rats treated with glibenclamide, individual and combined ethanolic extract showed a significant ($P<0.05$) reduction on days 3, 6, 15, 18, and 21 in comparison with the diabetic untreated group.

The decrease in glucose levels in groups treated with lemon grass extract in all doses (100mg, 200mg, and 300 mg/kgBW) after a period of 21 days was more significant in comparison with groups treated with *Aloe vera* and combined extract respectively. The fasting blood glucose levels of the diabetic untreated rats remained high during the course of the experiment while the normal non-diabetic rats remained within the normal glucose range.

4. DISCUSSION

The result of the 24 hour acute toxicity test of the orally administered combined ethanolic extract of both *Aloe vera* gel and lemon grass leaves in adult albino rats showed that the median lethal dose (LD_{50}) was >5000mg/kgBW as seen in Table 1. According to Lorke [13], LD_{50} of orally administered extract observed to be 5g and above simply denotes that the extract is not toxic.

The blood glucose profile of animals treated with the various doses of lemon grass, *Aloe vera*, and combined extracts (100mg/kgBW, 200mg/kgBW and 300mg/kgBW) for 3,6,9,12,18 and 21 days respectively as shown in Table 2. There appears to be no consistent pattern in the blood glucose levels as values appear to be fluctuating in all instances.

The result of the antidiabetic study carried out for 21 days showed that there was a significant ($P<0.05$) reduction in the fasting blood glucose levels of the animals treated with lemon grass and *Aloe vera* in comparison with the diabetic untreated group. This observation agrees with Saghir et al., Narsapuram et al., and Nayan; Temu et al., Tanveer et al., Oloyede et al. [15-20] that *Aloe vera* and lemon grass extract significantly reduces blood glucose levels respectively and can be used as an herbal remedy for diabetes. However, the reduction of blood glucose levels was better with those animals treated with lemon grass extract in comparison with *Aloe vera* and combined extract. The hypoglycemic effect of these extracts could be as a result of natural compounds such as flavonoids, saponins, chromium, alprogens, acemannan, anthraquinones, phytosterols that have many health benefits. These chemical compounds improve the beta function of the pancreas in producing the hormone insulin. Damage to the islets of Langerhans cells in the pancreas will be inhibited and regenerated so that insulin secretion returns to normal into the blood. This in turn restores the sensitivity of the insulin receptor cells by closing and inhibiting K^+ channels in beta cells which stimulate insulin secretion and this claim was supported by Arif et al. [8]. The observation in this study is in contrast with Khleef and Saeed [21] who reported a decrease in blood glucose levels in the diabetic rats when administered *Aloe vera* juice but the decrease was not statistically significant when compared with the diabetic untreated group. The difference could be as a result of the 70% ethanol used in the extraction of phytochemical components of *Aloe vera* gel and lemon grass leaves used in this study or its method of preparation of *Aloe vera* juice. The blood glucose levels of normal control animals were maintained throughout the study period. The diabetic control group also showed a significant increase in fasting blood glucose levels during the study period, this observation agrees with Narsapuram et al. [16] who stated that blood glucose level of normal control animals was maintained throughout the study period.

Table 2. **Effect of lemon grass, *Aloe vera* and its combination on FBG of alloxan-induced diabetic rats expressed as mean ± SEM**

Groups	Mean Fasting Blood glucose (mg/dl) at various days after treatment ± SEM								
	Before induction	**24 hrs after induction**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Day 21**
A	77.00±0.58	99.8±0.58	88.60±0.58	87.40±0.00	93.40±0.58	90.80±0.58	85.8±0.58	86.60±0.580	86.00±0.58
B	82.11±6.33	501.11±15.91*	479.11±15.85$^{\#\#}$	478.67±18.26$^{\#\#}$	488.44±18.19$^{\#}$	473.33±25.34$^{\#}$	482.78±29.43$^{\#}$	482.67±29.43$^{\#}$	511.89±23.61$^{\#}$
C	81.00±0.50	489.44±21.29*	81.33±2.59$^{\#\#}$	314.5 ±11.54*	281.89±130.44$^{\#\#}$	104.72±29.83$^{\#\#}$	313.83±141.31*	190.39±63.57$^{\#\#}$	236.72±71.69$^{\#\#}$
D	79.22±4.75	488.72±18.66*	110.06±19.60$^{\#\#}$	257.06±87.51$^{\#\#}$	279.22±107.40	293.72±108.46*	183.56±58.21$^{\#\#}$	260.89±100.70$^{\#\#}$	111.72±25.64$^{\#\#}$
E	82.89±3.60	430.28 ± 2.06*	204.00±69.55$^{\#\#}$	384.78±34.00*	328.56 ± 88.61*	366.95 ±83.91*	204.28±85.88$^{\#\#}$	294.50±124.43$^{\#\#}$	75.11 ± 5.22$^{\#\#}$
F	82.06±0.20	467.50±11.31*	81.61 ± 8.90$^{\#\#}$	200.83±53.26$^{\#\#}$	301.61±126.10*	206.61±71.26$^{\#\#}$	193.7±64.90$^{\#\#}$	183.06 ± 73.47$^{\#\#}$	122.72±19.33$^{\#\#}$
G	89.64±0.22	498.75±55.66*	88.00 ± 7.37$^{\#\#}$	135.47±30.44$^{\#\#}$	249.69±88.07*$^{\#\#}$	95.33±0.88$^{\#\#}$	139.53±41.25$^{\#\#}$	206.53 ±60.02$^{\#\#}$	118.69±20.36$^{\#\#}$
H	97.83±3.32	385.33±9.67*	95.83±13.71$^{\#\#}$	277.50±52.62*	265.67±115.04	227.17±60.33	151.33±67.79$^{\#\#}$	178.67±64.43$^{\#\#}$	137.33±55.16$^{\#\#}$
I	88.67±1.86	574.45±8.92*	117.50±11.82$^{\#\#}$	306.00±109.31*	274.6 ±139.98$^{\#\#}$	250.06±18.40$^{\#\#}$	289.28±58.65$^{\#\#}$	314.11± 98.52*$^{\#\#}$	152.78±51.65
J	77.67±5.87	449.17±79.35*	144.33±38.59$^{\#\#}$	233.33±74.40	348.39±149.13*	169.33±57.16$^{\#\#}$	169.22±58.84$^{\#\#}$	257.17±71.51	142.78 ±33.91
K	91.22±0.67	511.67 ± 1.54*	93.11 ± 4.23$^{\#\#}$	226.11±90.47$^{\#\#}$	320.78±150.50*	172.78±68.51$^{\#\#}$	208.56±80.73$^{\#\#}$	311.10±59.73*	125.11±21.70$^{\#\#}$
L	95.89±6.98	359.72±35.78*	133.89±29.44$^{\#\#}$	274.06 ±94.49	261.22±100.58	89.22±17.35$^{\#\#}$	233.37±108.18	191.72±46.65	148.22±40.06$^{\#\#}$

Values without superscripts were not significantly different from the initial FBG and FBG values at 24 hrs after induction
**Significant increase with respect to initial fasting blood glucose;*
***Significant decrease with respect to initial fasting blood glucose;*
$^{\#}$*significant increase with respect to fasting blood glucose 24hrs after induction;*
$^{\#\#}$*Significant decrease with respect to fasting blood glucose 24hrs after induction.*
The mean difference is significant at the 0.05 level (P< 0.05)

The result of this study also revealed fluctuating blood glucose levels in rats administered with combined ethanolic extract of the plants. These fluctuations could be attributed to chemical interactions amongst the constituent phytochemical of the individual extract, which could either be harmonious or self-subsistent in exhibiting their various potentials and capacity to decrease elevated blood glucose level of diabetic rats. This observation is in line with Okorie et al. [22, 23] who studied the effect of mixed aqueous extracts of *Allium sativum*, *Annona muricata* and *Cymbopogon citratus* leaves on the blood glucose of hyperglycemic rats.

5. CONCLUSION

This study on the effect of lemon grass, *Aloe vera*, and combined ethanolic extracts orally administered at varying doses on alloxan-induced diabetes rats revealed that the extracts are potent in lowering blood glucose levels. This implies that consistent administration of lemon grass, *Aloe vera*, and combined extract within a considerable time span reduces the extremity of diabetes.

CONSENT

It's not applicable.

ETHICAL APPROVAL

Ethical approval was obtained from the Nnamdi Azikiwe University-Animal Research Ethics Committee and was assigned the reference number; NAU/AREC/2022/00011.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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