

Effect of Aqueous Extract of Vernonia Amygdalina (Bitter Leaf) and Ocimum Gratissimum (Scent Leaf) on the Blood Sugar Level Using Alloxan Diabetic Induced Wistar Rat

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ABSTRACT

This study aims to evaluate the antidiabetic potential of aqueous extracts of *Vernonia amygdalina* (bitter leaf) and *Ocimum gratissimum* (scent leaf) in alloxan-induced diabetic Wistar rats. Twenty (20) adult Wistar rats were used for this study. For the first week all the wistar rats were left to acclimatize, given no drugs, only water and their feed. After acclimatization they were weighed, labelled with marks and their cages were also labelled. They were injected with alloxan so as to induce diabetes mellitus, thereby raising blood glucose level. The wistar rats were divided into five (5) groups, with four (4) wistar rats in each group namely; Group 1 (positive control- which received no drug administration, just distilled water and feed, Group 2 (negative control)- Received no aqueous extract treatment but was administered with alloxan, so they are not expected to produce any positive result and can be used as the baseline. Group 3- *VernoniaAmygdalina* extract (bitter-leaf) only (10ml per kg of body weight). Group 4- *OcimumGratissimum* extract (scent-leaf) only (10ml per kg of body weight). Group 5- Administered both bitter leaf and scent leaf extract (10ml per kg of body weight). Extract of bitter leaf and scent leaf was also administered according to their body weight (10ml per kg), such that animal weighing 250g, 200g, 150g received 2.5ml, 2.00ml and 1.50ml per kilogram of body weight respectively. It was administered orally using oral cannula. Blood samples were collected from the wistar rats before and after the administration of alloxan and two days after every administration of the extract of bitter leaf and scent leaf so as to check the blood glucose level. Data obtained were analyzed using SPSS format, all data were collected and organized in a format that SPSS can read and process. One Way Analysis of Variance (ANOVA) were used for additional tests. difference between the means of two or more independent groups. the results with $p < 0.05$ Considered significantly. This present study showed that *Vernonia amygdalina*(bitter leaf) and *Ocimum gratissimum* (scent leaf) extracts exhibited a synergistic effect, producing a greater reduction in blood glucose levels compared to either extract alone.

Keywords: *Vernonia amygdalina* (bitter leaf), *ocimum gratissimum* (scent leaf), alloxan-induced diabetic, Wistar rats, glucose level

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, resulting from inadequate insulin production, improper insulin utilization, or both. It is a significant global health concern due to its associated complications, including cardiovascular diseases, kidney damage, neuropathy, and vision loss. According to the World Health Organization (WHO), the prevalence of diabetes has risen markedly, increasing from 108 million cases in 1980 to 422 million in 2014, with a higher burden observed in low- and middle-income countries. In 2019, diabetes and related kidney diseases accounted for an estimated 2 million deaths worldwide (WHO, 2023).[5] Alarming projections by the International Diabetes Federation indicate that by 2045, approximately 700 million individuals, or 10.9% of the global population, will be living with diabetes, underscoring the urgent need for effective and accessible interventions (Int J Gen Med, 2020).

Current management of diabetes involves lifestyle modifications, dietary regulation, and pharmacological interventions, including insulin therapy and oral hypoglycemic agents. However, synthetic antidiabetic drugs are often associated with adverse effects such as skin reactions, gastrointestinal disturbances, and organ toxicity, highlighting the necessity for alternative therapeutic options (Diabetes.co.uk, 2019). In response, there has been growing interest in exploring natural remedies, particularly medicinal plants, for their potential to regulate blood glucose levels with fewer side effects.

Vernonia amygdalina, commonly known as bitter leaf, is a medicinal plant widely recognized for its bitter taste and extensive therapeutic properties. Indigenous to Africa, it has been traditionally employed to treat conditions such as fever, stomach

ailments, and diabetes. Bitter leaf is rich in bioactive compounds, including flavonoids, triterpenoids, and glycosides, which have demonstrated antidiabetic properties by modulating blood glucose levels (Times of India, 2019). Similarly, *Ocimum gratissimum*, or scent leaf, is another medicinal plant known for its aromatic properties and traditional use in managing headaches, gastrointestinal disorders, and diabetes. It contains phytochemicals such as tannins, alkaloids, and phenolic compounds, which have been linked to its antidiabetic effects.[3]

Research suggests that alloxan and streptozotocin (STZ) are commonly used diabetogenic agents that induce Type 1 diabetes-like hyperglycemia by targeting pancreatic β -cells[3]. Comparative studies have demonstrated the efficacy of bitter leaf and scent leaf extracts in lowering blood glucose levels, with a synergistic effect observed when both extracts are administered together.[7] These findings provide a compelling rationale for further investigation into the combined use of these plants as a potential therapeutic strategy for diabetes management.

This study aims to evaluate the antidiabetic potential of aqueous extracts of *Vernonia amygdalina* and *Ocimum gratissimum* in alloxan-induced diabetic albino Wistar rats. The specific objectives include assessing their individual and combined effects on blood glucose levels and determining the therapeutic efficacy of these plant extracts over a two-week treatment period. Given the high prevalence of diabetes in Nigeria, where approximately 11.2 million individuals (5.77% of the population) are affected, identifying low-cost, accessible, and less toxic alternatives for blood glucose regulation is of paramount importance (WHO, 2023; Cleveland Clinic, 2023).[12] This research holds promise for addressing a critical public health challenge by

exploring the potential of locally available medicinal plants to improve diabetes management.[8]

MATERIALS AND METHOD

Research Materials

Aqueous extract of Vernonia Amygdalina (bitter leaf) and Ocimum Gratissimum (scent leaf) were obtained with permission. Alloxan was also obtained. Other materials used include: cages with iron netting, litter (saw dust), animal feed, water, laboratory coat and gloves, syringes, sample bottles, weighing balance, glucometer, glucometer

strip, all other equipment used were standard laboratory equipment.

POPULATION OF THE STUDY

Twenty adult Wistar rats weighing 150–300g each were purchased from the Department of Physiology's Animal House at Ebonyi State University in Abakaliki. They were then split up into five groups, with four rats in each group. A week before the experiment began, the wistar rats were allowed to acclimate in wire mesh cages with standard diet and ambient conditions.

Table 1: Frequency and Distribution of Population.

Population of the Study	Frequency	Percentage
Group 1	3	15%
Group 2	4	20%
Group 3	4	20%
Group 4	5	25%
Group 5	4	20%
Total	20	100%

SAMPLE COLLECTION AND COLLECTION TECHNIQUE

Collection of Blood Sample

Blood samples were collected from the wistar rats before and after the administration of alloxan and two days after every administration of the extract of bitter leaf and scent leaf so as to check the blood glucose level. This was done by using a 23-gauge needle to collect the blood from the lateral tail vein. The tail may need to be washed with diluted Hibiscrub (1%) to see the blood vessel. Blood flow is stopped by applying pressure with sterile gauze to achieve hemostasis.

Collection of Plants

The bitter leaf and scent leaf were gotten freshly from the market.

Aqueous Extraction and Preparation of Extract

The extraction of the bitter leaf and scent leaf samples were done by maceration method. The freshly obtained leaves were

dried at room temperature after which it was grinded and the medicinal plants was soaked with water and covered for 3 days (72 hours). After 3 days, the extract was filtered and obtained. The extract was measured and administered to the rats per gram of body weight.

SAMPLE SIZE

The extract dosage was administered to the rats 10ml per kilogram of their body weight. E.g; If the weight of the rat is 200g the moderate dose to be administered is 2.0ml

The Sloven's formula was used in the computation of the sample of the study to determine the sample size.

Computation for the sample size:

Sloven's Formula:

Sample Size; $n =$

where n = sample size

N = population of the study

e = tolerable error of 5%

1 = constant figure

Therefore, $n =$

Sample Size= 19.05

RESEARCH DESIGN

For the first week all the wistar rats were left to acclimatize, given no drugs, only water and their feed. After acclimatization they were weighed, labelled with marks and their cages were also labelled. They were injected with alloxan so as to induce diabetes mellitus, thereby raising blood glucose level.

Grouping of Animals

The wistar rats were divided into five (5) groups, with four (4) wistar rats in each group namely;

- weight)

- Group 1 (positive control- which received no drug administration, just distilled water and feed
- Group 2 (negative control)- Received no aqueous extract treatment but was administered with alloxan, so they are not expected to produce any positive result and can be used as the baseline.
- Group 3- Vernonia Amygdalina extract (bitter-leaf) only (10ml per kg of body weight)
- Group 4- Ocimum Gratissimum extract (scent-leaf) only (10ml per kg of body weight)
- Group 5- Administered both bitter leaf and scent leaf extract (10ml per kg of body

Administration of Alloxan, Bitter Leaf and Scent Leaf Extract

Intravenous injection of 150mg/kg dose of Alloxan was administered to the experimental animals. Example:

For G1R1 with body weight of 300.41= $\frac{150 \times 300.41}{1000} = 45.06\text{mg}$

Converting 45.06 to ml at a dosage of 10ml per bodyweight; $\frac{45.06 \times 10}{1000} = 0.45\text{ml}$

Extract of bitter leaf and scent leaf was also administered according to their body weight (10ml per kg), such that animal weighing 250g, 200g, 150g received 2.5ml, 2.00ml and 1.50ml per kilogram of body weight respectively. It was administered orally using oral cannula.

Data obtained were analyzed using SPSS format, all data were collected and organized in a format that SPSS can read and process. One Way Analysis of Variance (ANOVA) were used for additional tests. difference between the means of two or more independent groups. the results with $p < 0.05$ Considered significantly.

STATISTICAL ANALYSIS

RESULTS

Table 1: Statistical analysis of results showing groups, initial blood glucose and the final blood glucose.

Groups	Initial Blood Glucose (mg/dl)	Final Blood Glucose (mg/dl)
Positive control	94.00±4.16	124.50±5.02
Negative control	88.75±2.93	300.50±7.50
Diabetic+ Vernonia amygdalina	81.25±2.17	467.33±10.02*
Diabetic+ Ocimum gratissimum	93.8±3.18	516±13.11*
Diabetic+ Vernonia amygdalina+ Ocimum gratissimum	84.25±1.49	422.67±11.34*

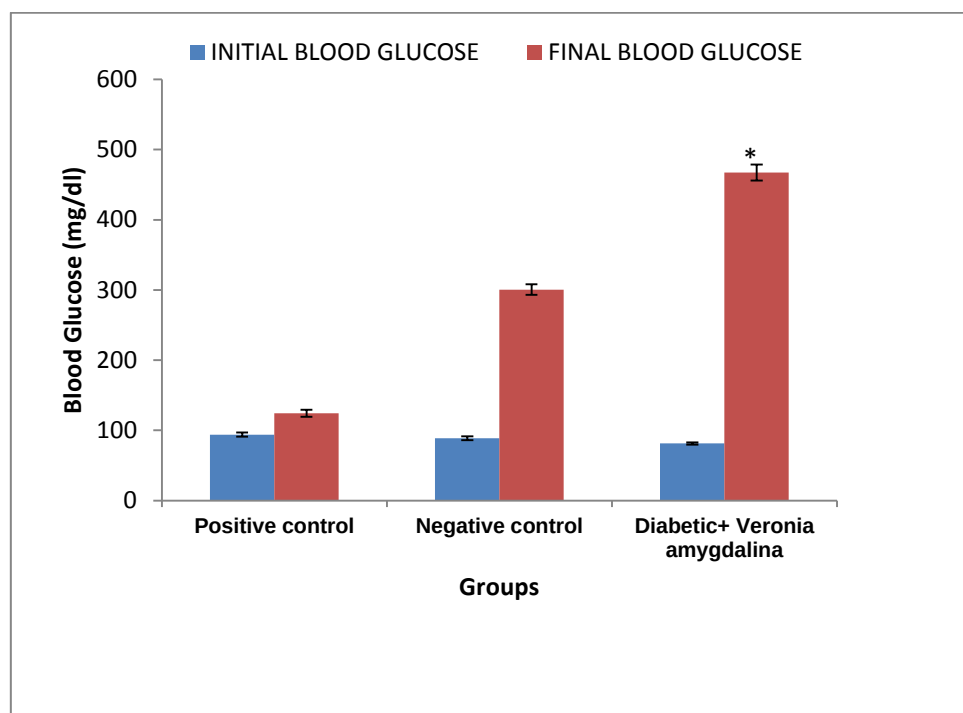


Fig. 1: Effect of Bitter Leaf on Blood Glucose in Alloxan Induced Diabetic Wistar Rats.

The wistar rats treated with Bitter leaf extract showed significant reductions in blood glucose levels compared to the untreated diabetic group

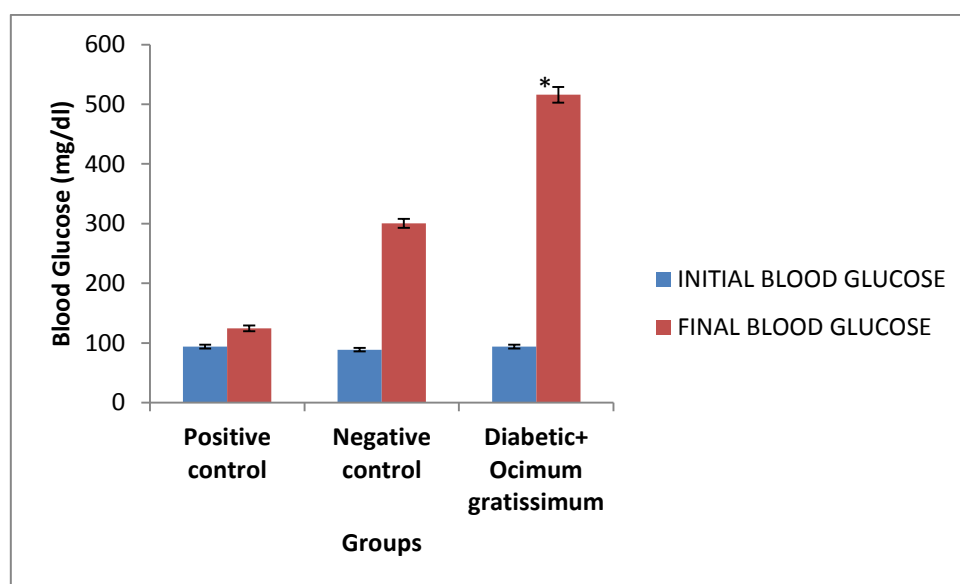


Fig. 2: Effect of scent leaf on blood glucose level in Alloxan induced diabetic wistar rats.

The wistar rats treated with Scent leaf extract showed significant reductions in blood glucose levels compared to the untreated diabetic group

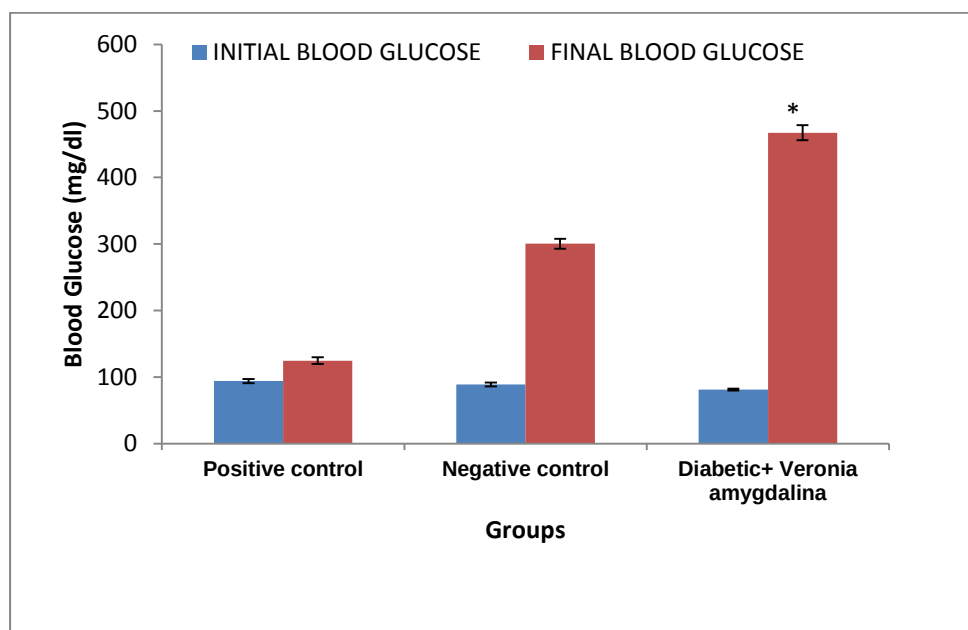


Fig. 3: Effect of bitter leaf and scent leaf on blood glucose level in Alloxan induced diabetic wistar rats.

The combination of Vernonia amygdalina and Ocimum gratissimum extracts exhibited a synergistic effect, producing a greater reduction in blood glucose levels compared to either extract alone

DISCUSSION

The findings of this study demonstrate the significant antidiabetic potential of Vernonia amygdalina (bitter leaf) and Ocimum gratissimum (scent leaf) in managing blood glucose levels in alloxan-induced diabetic Wistar rats. Diabetes was successfully induced in the rats through alloxan administration, a compound known to selectively destroy pancreatic β -cells by inhibiting glucokinase, a key glucose sensor[9]. The subsequent hyperglycemia observed in the untreated group confirmed the diabetogenic efficacy of alloxan, providing a robust model for assessing the hypoglycemic effects of the plant extracts.[10]

Phytochemical analysis revealed the presence of bioactive compounds such as flavonoids, alkaloids, tannins, saponins, and phenolic acids in both Vernonia amygdalina and Ocimum gratissimum.

These compounds are known to regulate glucose metabolism and mitigate diabetes-related complications[1]. The extracts demonstrated notable antidiabetic properties, with rats treated with either extract showing significant reductions in blood glucose levels compared to the untreated diabetic group. This aligns with previous findings highlighting the hypoglycemic potential of bitter leaf and scent leaf in diabetes management[2,4]

Notably, the combination of Vernonia amygdalina and Ocimum gratissimum extracts exhibited a synergistic effect, producing a greater reduction in blood glucose levels compared to either extract alone. This corroborates the findings of Udeh and Alexander (2019),[11] who reported enhanced glycemic control in diabetic rats treated with combined extracts. The results suggest that the combined use of these plants could offer an effective therapeutic strategy for managing diabetes. Statistical analysis further validated these findings, with p-values less than 0.05 indicating statistically significant differences between treatment groups and the positive control

group. The rejection of the null hypothesis confirms the efficacy of the extracts in reducing blood glucose levels. Additionally, fluctuations in blood glucose levels during treatment, ranging from hyperglycemia to normoglycemia and occasional hypoglycemia, underscore the potent hypoglycemic effects of the extracts. This highlights the importance of careful blood glucose monitoring during treatment to avoid adverse effects.

The study's findings also emphasize the potential cardioprotective properties of *Vernonia amygdalina* and *Ocimum gratissimum*, which may safeguard against diabetes-induced cardiovascular complications. Previous research has noted their ability to protect against oxidative stress and tissue damage, which are common in diabetic states (Asquo et al., 2010).

Overall, the results support the therapeutic potential of *Vernonia amygdalina* and *Ocimum gratissimum* as natural remedies for diabetes. Their accessibility, low cost, and bioactive compound profiles make them promising candidates for developing adjunctive therapies in diabetes management. However, further research is needed to elucidate their mechanisms of action, optimize dosing strategies, and evaluate their long-term safety and efficacy.

CONCLUSION

This present study showed that *Vernonia amygdalina* (bitter leaf) and *Ocimum gratissimum* (scent leaf) extracts exhibited a synergistic effect, producing a greater reduction in blood glucose levels compared to either extract alone.

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