

COMPARATIVE EFFECTS OF AQUEOUS LEAF EXTRACTS OF *Vernonia amygdalina* AND SEED EXTRACT OF *Irvingia gabonensis* ON SELECTED BIOCHEMICAL PARAMETERS IN ALLOXAN-INDUCED DIABETIC RATS

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Abstract

ABSTRACT

With the increasing prevalence of diabetes and the side effects associated with chemical medications, exploring non-pharmacological treatments is of significant interest. Vernonia amygdalina and Irvingia gabonensis are among the widely used medicinal herbs (Akah et al., 2004; Muhammad et al., 2016). This study compares the effects of aqueous leaf extracts of Vernonia amygdalina and seed extract of Irvingia gabonensis on selected biochemical parameters in alloxan-induced diabetic rats. Diabetes was induced in male Wistar rats by intraperitoneal injection of alloxan (150 mg/kg) (Oyedepo et al., 2013). The extracts were administered orally for 28 days (Akah et al., 2004). The rats were randomly allocated into six groups: Group 1: Normal control, Group 2: Diabetic control, Group 3: Diabetic rats treated with Vernonia amygdalina (80 mg/kg), Group 4: Diabetic rats treated with Irvingia gabonensis (200 mg/kg), and Group 5: Diabetic rats treated with glibenclamide (5 mg/kg). The extracts were administered orally for 28 days. Treatment with both extracts significantly reduced blood glucose and glycated hemoglobin levels in diabetic rats compared to the diabetic control group ($P < 0.001$) (Akubugwo et al., 2018). Both extracts also significantly decreased altered biochemical parameters in diabetic rats compared to untreated controls ($P < 0.05$) (Omoriegbe & Pal, 2018). Furthermore, Vernonia amygdalina demonstrated hepatoprotective effects as it significantly decreased elevated levels of alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) compared to the untreated diabetic group ($P < 0.05$) (Onyedikachi et al., 2020; Oben et al., 2018). Irvingia gabonensis supplementation significantly decreased resulted in a significant decrease in liver enzymes, excluding except ALP, compared to the diabetic control group ($P < 0.05$) (Ngondi et al., 2018). Furthermore, both extracts demonstrated hepatoprotective and nephroprotective effects, as evidenced by the reduction in liver enzyme levels and improvement in kidney function markers. In conclusion, the aqueous leaf extract of Vernonia amygdalina and seed extract of Irvingia gabonensis exhibited beneficial effects on selected biochemical parameters in alloxan-induced diabetic rats. Despite the comparable therapeutic efficacy, Vernonia amygdalina may be superior to Irvingia gabonensis seeds.

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Glibenclamide, kidney profile, Glycated hemoglobin.

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Introduction

1.0 INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder, poses a global health challenge by disrupting carbohydrate metabolism and elevating blood glucose levels (Mahajan et al., 2018; Scott et al., 2019). Insulin deficiency and resistance are key contributing factors, necessitating a comprehensive understanding for effective management (Tuttle et al., 2020; Cho et al., 2021). Between 2018 and 2023, research efforts intensified, shedding light on diabetes mechanisms, interventions, and treatment approaches (Mahajan et al., 2018; Scott et al., 2019).

Studies have identified susceptibility genes and genomic loci linked to diabetes, offering insights into its hereditary aspects (Mahajan et al., 2018; Scott et al., 2019). Precision medicine in diabetes care, which tailors strategies based on individual traits, holds promise, especially with the advent of technologies like continuous glucose monitoring (Tuttle et al., 2020; Cho et al., 2021).

Advancements in insulin delivery systems, such as smart pens and closed-loop systems, have improved dosing precision and adherence (Garg et al., 2018; Forlenza et al., 2020). Research into the inflammatory and immune dysregulation aspects of diabetes has led to promising therapeutic developments (Hotamisligil, 2019; Skyler & Bakris, 2020). The role of the gut microbiome in metabolic health and insulin sensitivity is also a growing area of interest (Wu et al., 2020; Nieuwdorp et al., 2021).

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Vernonia amygdalina, commonly known as bitter leaf, is an indigenous African plant widely used in traditional medicine. It is renowned for its diverse phytochemical composition, including sesquiterpenes, flavonoids, alkaloids, and saponins, which have attracted significant scientific attention (Njoku et al., 2018; Onyedikachi et al., 2020; Omoregie & Pal, 2018). Research highlights its potential in diabetes management, inflammation, oxidative stress, and organ protection (Njoku et al., 2018; Akah et al., 2004). However, further studies are needed to fully unlock its therapeutic potential and ensure safe integration into healthcare practices (Omoregie & Pal, 2018; Onyedikachi et al., 2020).

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Irvingia gabonensis, known as African mango, is a tropical fruit native to Central and West Africa, noted for its rich phytochemical profile, including flavonoids, alkaloids, and glycosides (Akubugwo et al., 2018; Oben et al., 2018). It has gained attention for its potential as a natural anti-obesity agent, influencing adipose tissue metabolism and aiding in body weight management (Ngondi et al., 2018). Additionally, *Irvingia gabonensis* demonstrates lipid-modulating properties, affecting key enzymes in cholesterol synthesis and fatty acid metabolism, which may be beneficial for managing dyslipidemia and cardiovascular risk (Ngondi et al., 2018). Ongoing

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research is crucial to fully understand its therapeutic potential and to validate its efficacy in clinical settings (Oben et al., 2018; Muhammad et al., 2016).

2.0 MATERIALS AND METHODS

2.1 Chemicals, Reagents, and Kits

The chemicals and reagents used in this experiment include hydrochloric acid, Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB), hydrogen peroxide, potassium chloride, Tris buffer, sodium hydroxide, sodium carbonate, potassium sodium tartrate, copper sulfate pentahydrate, Folin-Ciocalteu reagent, adrenaline, dipotassium hydrogen phosphate trihydrate, potassium dihydrogen phosphate, 1-chloro-2,4-dinitrobenzene (CDNB), sulfosalicylic acid, trichloroacetic acid, sodium azide, dipotassium hydrogen orthophosphate. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, glucose test strips, and lipid profile test kits were obtained from Randox Laboratories, UK. All chemicals and reagents used were of analytical grade and of the highest purity available.

2.2 Drugs

The drugs used in this experiment include alloxan and glibenclamide.

2.3 Plant Materials

Fresh leaves of *Vernonia amygdalina* and seeds of *Irvingia gabonensis* were purchased from the Port Harcourt fruit market in Port Harcourt, Nigeria. The plants were identified and authenticated at the Department of Botany, Rivers State University, Port Harcourt.

2.31 Preparation of *Vernonia amygdalina* Leaves

After washing, *Vernonia amygdalina* leaves were sun-dried for seven days and milled to a coarse powder using a mortar and pestle. The powder (250 g) was soaked in 500 ml of distilled water, allowed to stand for 24 hours with intermittent shaking, and then filtered. The filtrate was freeze-dried to obtain a solid residue (48.7 g; 19.5% yield). The extract was reconstituted in distilled water at the appropriate concentration before administration (Akah et al., 2004).

2.32 Preparation of *Irvingia gabonensis* Seeds

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Irvingia gabonensis seeds were shade-dried and ground into powder. A portion (100 g) of the powder was soaked in 500 ml of distilled water for 24 hours, followed by filtration. The filtrate was evaporated to dryness at 40°C, yielding a dark brown residue. The residue was weighed, and the concentration was determined as 200 mg/ml. The extract was stored in a refrigerator for subsequent use (Muhammad et al., 2016).

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2.4 Induction of Diabetes

Diabetes was induced by intraperitoneal injection of alloxan (150 mg/kg body weight) dissolved in 0.9% physiological saline into overnight-fasted rats (Oyedepo et al., 2013). After 48 hours, blood glucose levels were measured using an Accu-Chek glucose meter. Rats with baseline blood glucose levels of 200 mg/dL and above were considered diabetic. Blood glucose levels were monitored weekly for four weeks, and body weights were recorded before induction, after induction, and during the treatment period.

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2.5 Experimental Animals

Thirty male Wistar rats weighing between 100 g and 150 g were purchased and housed in plastic cages in a well-ventilated animal house at the Department of Pharmacology, Rivers State University, Port Harcourt. The rats were provided with rat pellets and water ad libitum and were subjected to a natural 12-hour light-dark cycle. The animals were acclimatized for ten days before the experiment.

2.6 Experimental Design and Treatments

The rats were randomly assigned to five groups of six animals each:

- **Group 1 (Normal Control):** Received only feed and distilled water.
- **Group 2 (Diabetic Control):** Received a single intraperitoneal dose of alloxan (150 mg/kg).
- **Group 3 (Diabetic + *Vernonia amygdalina*):** Received *Vernonia amygdalina* extract (80 mg/kg) orally.
- **Group 4 (Diabetic + *Irvingia gabonensis*):** Received *Irvingia gabonensis* extract (200 mg/kg) orally.
- **Group 5 (Diabetic + Glibenclamide):** Received glibenclamide (5 mg/kg) orally.

All treatments were administered once daily for four weeks. At the end of the treatment period, animals were sacrificed, and blood was collected by cardiac puncture into EDTA tubes for plasma separation. The liver and kidney were excised, rinsed in ice-cold saline, and preserved in 10% formalin for histopathological analysis.

2.7 Biochemical Assays

Plasma glucose was determined by the glucose oxidase method (Trinder, 1969). Plasma levels of AST, ALT, ALP, total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), urea, and creatinine were measured using Randox test kits according to the manufacturer's instructions. Glycated hemoglobin (HbA1c) was estimated using a commercial ELISA kit.

2.8 Histopathological Examination

Liver and kidney tissues were processed for histopathological examination following standard protocols. Sections were stained with hematoxylin and eosin and examined under a light microscope.

2.9 Statistical Analysis

All data were expressed as mean \pm standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

3.0 RESULTS

Table 3.1: Effects of Selected Herbal Extracts On Body Weight Of Treated Rats.

GROUPS	INITIAL (g)	WEEK 1	WEEK 2	WEEK 3	WEEK 4
GROUP 1	138.0 \pm 6.25 ^{bc}	133.4 \pm 7.29 ^{bc}	123.0 \pm 29.00	128.2 \pm 7.08 ^b	138.6 \pm 6.04
GROUP 2	124.0 \pm 5.19 ^b	121.6 \pm 6.22 ^{ab}	94.2 \pm 25.08	67.6 \pm 28.37 ^{ab}	65.8 \pm 27.97
GROUP 3	120.0 \pm 2.41 ^a	116.4 \pm 2.40 ^a	104.2 \pm 2.69	61.0 \pm 24.94 ^a	62.2 \pm 25.43
GROUP 4	141.6 \pm 3.37 ^c	138.8 \pm 3.15 ^c	110.6 \pm 27.81	72.0 \pm 29.94 ^{ab}	71.2 \pm 29.52

Table 3.1: showing the effect of different herbal extract on body weight of treated rats for 4 weeks (28 days). Values are expressed as Mean \pm SD (n=4), *P<0.05 versus control. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Duncan post hoc test.

Group 1 = Normal Control, Group 2 = Diabetes Control, 3 = *V. amygdalina* and *I. gabonensis* Group 4 = Glibenclamide.

Table 3.2: Effects of Selected Herbal Extracts on Organ Weight of Treated Rats

GROUPS	PANCREA	KIDNEY
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GROUP 1	2.90 ± 0.32	1.29 ± 0.12
GROUP 2	0.27 ± 0.14	0.75 ± 0.67
GROUP 3	0.74 ± 0.32	0.36 ± 0.14
GROUP 4	0.58 ± 0.25	0.33 ± 0.13

Table 3.2: showing the effect of different herbal extract on organ weight of treated rats. Values are expressed as Mean ± SD (n=8), *P<0.05 versus control. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Duncan *post hoc test*.

Table 3.3: Effects of Selected Herbal Extracts on Blood Glucose Level Of Treated Rats.

GROUPS	INITIAL (mg/dl)	WEEK 1	WEEK 2	WEEK 3	WEEK 4
GROUP 1	98.6 ± 3.83	115.0 ± 4.82	98.6 ± 3.82	80.4 ± 4.04	108.2 ± 5.17
GROUP 2	579.0 ± 11.02	425.4 ± 60.55 ^b	578.0 ± 10.02	312.4 ± 72.26	268.8 ± 62.88
GROUP 3	296.52 ± 78.32	152.1 ± 62.12	109.5 ± 47.52	75.0 ± 26.83	73.2 ± 31.92
GROUP 4	288.0 ± 78.73	264.4 ± 115.19 ^b	189.0 ± 78.73	176.4 ± 82.51	125.4 ± 50.34

Table 3.3: showing the effect of different herbal extracts on blood glucose level of treated rats for 4 weeks (28 days). Values are expressed as Mean ± SD (n=6), *P<0.05 versus control. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Duncan *post hoc test*.

Group 1 = Normal Control, Group 2 = Diabetes Control, Group 3 = *V. amygdalina* and *I. gabonensis* Group 4 = Glibenclamide

Table 3.4: Effects of Selected Herbal Extracts on Liver Function Biomarkers in Treated Rats.

GROUPS	AST	ALT	ALP	TP	ALB
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GR 1	35.00 ± 2.00	13.50 ± 0.50	53.50 ± 2.50	70.30 ± 0.45	50.00 ± 0.20
GR 2	54.50 ± 4.50	60.00 ± 2.00	112.50 ± 12.50	54.45 ± 0.10	34.30 ± 1.20
GR 3	35.00 ± 1.00	10.80 ± 0.30	28.50 ± 1.50	66.50 ± 1.50	42.00 ± 1.00
GR 4	22.50 ± 1.50	11.45 ± 0.35	36.50 ± 1.50	68.50 ± 1.50	41.40 ± 0.50 ^c

Table 3.4: showing the effect of different herbal extracts on liver function biomarkers of treated rats. Values are expressed as Mean ± SD (n=6), *P<0.05 versus control. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Duncan *post hoc test*.

Group 1 = Normal Control, Group 2 = Diabetes Control, Group 3 = *V. amygdalina*
 Group 4 = *I. gabonensis* Group 5 = *V. amygdalina* and *I. gabonensis* Group 6 =
 Glibenclamide.

Table 3.5: Effects of Selected Herbal Extracts on Kidney Function Biomarkers in Treated Rats.

GROUPS	CREATININE 65-120umol	UREA 1.9- 8.4mmol/l
GROUP 1	92.95 ± 7.05	4.85 ± .05
GROUP 2	236.00 ± 6.00	17.35 ± .45
GROUP 3	161.50 ± 6.50	8.10 ± .60
GROUP 4	133.00 ± 2.00	5.75 ± .05

Table 3.5: showing the effect of different herbal teas on kidney function biomarkers of treated rats. Values are expressed as Mean ± SD (n=6), *P<0.05 versus control. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Duncan *post hoc test*.

Group 1 = Normal Control, Group 2 = Diabetes Control 3 = *V. amygdalina* and *I. gabonensis* Group 4 = Glibenclamide.

3.6: EFFECTS OF SELECTED HERBAL EXTRACTS ON LIPID PROFILE OF TREATED RATS.

Table 3.6: Effects of Selected Herbal Extracts on Lipid Profile of Treated Rats.

GROUPS	TC	TG	HDL	LDL	VLDL
GROUP 1	4.35 ± 0.55	1.50 ± 0.10 ^a	1.65 ± 0.15 ^a	1.50 ± 0.10 ^{ab}	0.45 ± 0.02
GROUP 2	7.30 ± 0.20	3.55 ± 0.15 ^b	0.50 ± 0.10 ^a	5.25 ± 0.50	2.27 ± 0.01
GROUP 3	2.35 ± 0.05	1.01 ± 0.03 ^b	1.19 ± 0.02 ^c	1.73 ± 0.05 ^b	0.46 ± 0.01
GROUP 4	2.85 ± 0.05	1.63 ± 0.03	1.69 ± 0.03	1.80 ± 0.04 ^b	0.74 ± 0.01

Table 3.6 showing the effect of different herbal extracts on lipid profile of treated rats. Values are expressed as Mean ± SD (n=8), *P<0.05 versus control. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Duncan *post hoc test*.

Group 1 = Normal Control, Group 2 = Diabetes Control 3 = *V. amygdalina* and *I. gabonensis* Group 4 = Glibenclamide.

4.0 Discussion

The study ~~evaluated~~ aimed to evaluate and compare the effects of the aqueous leaf extract of *Vernonia amygdalina* (bitter leaf) and the seed extract of *Irvingia gabonensis* (African mango) on various biochemical parameters in alloxan-induced diabetic rats, highlighting their potential in diabetes management. The significant findings from this research provide insights into the therapeutic potentials of these medicinal herbs, particularly in the context of managing diabetes mellitus and its associated complications.

Blood Glucose and Glycated Hemoglobin Levels:

Both ~~extracts~~ *Vernonia amygdalina* and *Irvingia gabonensis* significantly reduced blood glucose and HbA1c levels and glycated hemoglobin (HbA1c) in diabetic rats compared to the untreated diabetic control group. This reduction underscored is indicative of the hypoglycemic properties effects of these plants, consistent with herbal extracts, supporting previous studies findings on their efficacy in managing blood sugar levels (Garg et al., 2015; Ngondi et al., 2009). The The ability of these extracts to modulate glucose levels aligns with the known mechanisms of action of their bioactive compounds in these herbs, such as, including flavonoids and alkaloids, likely play a role by enhancing which have been documented to enhance insulin sensitivity and secretion (Akubugwo et al., 2018; Oben et al., 2007). These findings support the inclusion of these herbs in diabetes-related therapies aimed at maintaining glycemic control.

Liver Function Biomarkers:

Vernonia amygdalina was especially particularly effective in reducing liver enzyme levels (ALT, AST, ALP), indicating elevated levels of liver enzymes such as alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP). This suggests its

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hepatoprotective effects. This hepatoprotection may stem from properties, potentially due to its antioxidant and anti-inflammatory properties/effects, which alleviate/help in mitigating liver damage commonly/often associated with diabetes (Amaechina et al., 2020; Izevbuwa et al., 2021). While *Irvingia gabonensis* also contributed to/demonstrated a reduction in liver health by reducing ALT and AST levels, its effects on enzymes, albeit to a lesser extent, with ALP were less significant, suggesting levels not significantly reduced. This indicates that while *Irvingia gabonensis* possesses some hepatoprotective effects, *Vernonia amygdalina* may be more beneficial/effective in protecting liver function in diabetic conditions (Ngondi et al., 2009; Oben et al., 2008).

Kidney Function Biomarkers:

Both/The study also revealed that both herbal extracts improved/contributed to the improvement of kidney function, as evidenced by/reduced in diabetic rats. The decrease in serum creatinine and urea levels in the treated rats. This groups suggests that both *Vernonia amygdalina* and *Irvingia gabonensis* may exert nephroprotective effects/effects. This is significant/crucial given that diabetic nephropathy, is a common diabetes complication of diabetes, often leads/leading to chronic kidney disease (Oluborode et al., 2020; Ramachandran & Saravanan, 2018). The nephroprotective properties of these extracts could provide a complementary approach to prevent or manage kidney complications associated with diabetes.

Lipid Profile:

The study also found/improvements in extracts positively influenced the lipid profiles, including decreases/profile of diabetic rats, with significant reductions in total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL_c), alongside an increase in high-density lipoprotein (HDL_c). This lipid-modulating effect could be instrumental in addressing/is essential in managing diabetes-related dyslipidemia, which is a known risk factor for cardiovascular diseases (Mooradian, 2009; Wu et al., 2020). Such improvements may help reduce/The improvement in lipid parameters further supports the potential use of these herbs in reducing cardiovascular risks in diabetic patients, further supporting the therapeutic potential of these herbs/associated with diabetes (Ngondi et al., 2005; Ngondi et al., 2009).

Conclusion

The findings from this study highlights/underscore the potential therapeutic benefits of *Vernonia amygdalina* and *Irvingia gabonensis* in managing diabetes mellitus and its complications. Both extracts demonstrated significant hypoglycemic, hepatoprotective, nephroprotective, and lipid-modulating effects in alloxan-induced diabetic rats, with. While both herbs showed comparable efficacy, *Vernonia amygdalina* showing slightly appeared to offer superior benefits, especially for liver health, particularly in terms of liver function and overall biochemical regulation. These findings advocate for/results support the continued exploration and potential integration of these medicinal herbs into complementary therapies for diabetes therapies/management. However, further research/studies, including clinical trials, is crucial/are necessary to confirm/validate these findings and establish safe, effective/determine the optimal dosages for human/therapeutic use in humans.

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