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Synergistic Biochemical Effects of Combined Aqueous Extracts of *Vernonia Amygdalina* Leaf and *Irvingia Gabonensis* Seed in Alloxan-Induced Diabetic Rats

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ABSTRACT

With the increasing prevalence of diabetes and concerns over the side effects associated with conventional medications, there is a growing interest in exploring non-pharmacological interventions. This study assesses the combined effects of aqueous leaf extracts from Vernonia amygdalina and seed extracts from Irvingia gabonensis on selected biochemical parameters in alloxan-induced diabetic rats.

Diabetes was induced in male Wistar rats through intraperitoneal injection of alloxan (150 mg/kg). The rats were then randomly assigned to four groups: Group 1 - Normal rats, Group 2 - Diabetic control rats, Group 3 - Diabetic rats receiving both Irvingia gabonensis (200 mg/kg) and Vernonia amygdalina (80 mg/kg), and Group 4 - Diabetic rats receiving glibenclamide (5 mg/kg). The extracts were administered orally over 28 days. The combined treatment of Vernonia amygdalina and Irvingia gabonensis extracts resulted in a significant reduction in blood glucose and glycated hemoglobin levels in diabetic rats compared to the diabetic control group ($P < 0.001$). After 28 days of oral administration, the combined extracts significantly improved altered biochemical parameters compared to untreated controls ($P < 0.05$). Specifically, the combined extracts significantly decreased elevated levels of alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) compared with the untreated diabetic group ($P < 0.05$). Moreover, the combined administration of Vernonia amygdalina and Irvingia gabonensis extracts exhibited hepatoprotective and nephroprotective effects, as evidenced by the reduction in liver enzyme levels and improvement in kidney function markers.In conclusion, the synergistic effects of the aqueous leaf extract of Vernonia amygdalina and the seed extract of Irvingia gabonensis demonstrated significant improvements in selected biochemical parameters in alloxan-induced diabetic rats. These findings suggest the potential of combining these natural extracts as a promising therapeutic strategy for diabetes management.

Keyword: Vernomia amygdalina, Irvingia gabonensis, Alloxan Glibenclamide, kidney profile, Glycated hemoglobin.

1. INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder, poses a significant global health challenge, disrupting the body's ability to efficiently metabolize carbohydrates and leading to elevated blood glucose levels. The primary drivers include insulin deficiency and resistance, necessitating a comprehensive understanding of the disease for effective treatment strategies. Between 2018 and 2023, intensified research efforts have illuminated underlying mechanisms, potential interventions, and innovative approaches for diabetes management (Mahajan et al., 2018; Scott et al., 2019).

Recent studies have unveiled numerous susceptibility genes and genomic loci associated with diabetes, offering crucial insights into its hereditary component and potential for personalized treatments (Mahajan et al., 2018; Scott et al., 2019). Precision medicine in diabetes care, tailoring strategies based on individual characteristics, has shown promise in enhancing glycemic control and reducing complications, aided by technologies like continuous glucose monitoring and advanced insulin delivery systems (Tuttle et al., 2020; Cho et al., 2021).

Advancements in insulin delivery, including smart pens and closed-loop systems, have improved dosing precision and adherence (Garg et al., 2018; Forlenza et al., 2020). Emerging research emphasizes the role of inflammation and immune dysregulation in diabetes, leading to promising therapies targeting these pathways (Hotamisligil, 2019; Skyler & Bakris, 2020). Continuous glucose monitoring technology has evolved, allowing real-time tracking with improved accuracy and integration with mobile applications for enhanced patient empowerment and timely interventions (Beck et al., 2018; Wong et al., 2021).

Research into the gut microbiome's impact on metabolic health and insulin sensitivity is growing, with interventions like dietary changes and probiotics holding potential for better glycemic control (Wu et al., 2020; Nieuwdorp et al., 2021). The development of novel therapeutics for diabetes, including

glucagon receptor antagonists and sodium-glucose cotransporter-2 (SGLT-2) inhibitors, diversifies treatment options (Mosenzon et al., 2019; Bhatt et al., 2020). Incretin-based therapies and glucokinase activators also show promise in enhancing insulin secretion and glucose regulation (Mosenzon et al., 2019; Bhatt et al., 2020).

Vernonia amygdalina, commonly known as bitter leaf, is a native African plant with a rich history of traditional medicinal use. Its diverse phytochemical composition, including sesquiterpenes, flavonoids, alkaloids, and saponins, has garnered scientific attention (Njoku et al., 2018; Onyedikachi et al., 2020; Omoregie & Pal, 2018). Recent studies have highlighted its anti-diabetic properties, influencing glucose metabolism and improving insulin sensitivity (Izevbuwa et al., 2021; Ngwoke et al., 2018; Ogbolu et al., 2018). Moreover, it shows promise in alleviating complications associated with diabetes, such as neuropathy and nephropathy (Amaechina et al., 2020).

Research also emphasizes its anti-inflammatory and antioxidant properties, with potential applications in chronic inflammatory conditions (Abosi & Raseroka, 2018; Oluborode et al., 2020). Bitter leaf's antioxidant activity may mitigate oxidative stress-related damage, reducing the risk of chronic diseases (Obi et al., 2019; Essien et al., 2019). Studies have explored its hepatoprotective and nephroprotective effects, indicating potential in protecting against organ injury (Omoregie et al., 2019; Essien et al., 2019). Despite promising findings, further research is necessary to determine optimal dosage, safety profiles, and potential interactions with medications. Clinical trials are crucial to validate preclinical results and assess its therapeutic potential in human populations.

Irvingia gabonensis, commonly known as African mango, is a tropical fruit native to Central and West Africa. With a rich phytochemical profile, including flavonoids, alkaloids, and glycosides, it has gained attention for its reported health benefits (Akubugwo et al., 2018; Oben et al., 2018). Research has focused on its potential as a natural anti-obesity agent, suggesting its influence on adipose tissue metabolism, reducing fat accumulation, and aiding in body weight management (Ngondi et al., 2018; Oben et al., 2018). *Irvingia gabonensis* demonstrates lipid-modulating properties, affecting key enzymes in cholesterol synthesis and fatty acid metabolism, potentially beneficial for managing dyslipidemia and cardiovascular risk (Ngondi et al., 2018). Studies indicate its impact on metabolic parameters, improving insulin sensitivity and regulating glucose metabolism, suggesting a role in managing diabetes and metabolic syndrome (Ngondi et al., 2018).

The fruit has also been explored for its anti-inflammatory and antioxidant effects, holding promise for mitigating chronic inflammatory conditions and protecting against oxidative stress-related damage (Abenavoli et al., 2018; Essien et al., 2019). Some constituents found in *I. gabonensis* seeds may have applications in medicinal, food, or cosmetic industries (Ezekwe et al., 2021). While promising, further research is essential to establish optimal dosage, safety profiles, and long-term efficacy. Clinical trials are warranted to validate findings from in vitro and animal studies and assess the fruit's potential as a therapeutic agent in human populations.

2. MATERIALS AND METHODS

2.1 Animals

Twenty-four (24) male Wistar rats weighing between 100g and 150g were purchased. The animals were housed in suitable plastic cages in a well-ventilated animal house of the Department of Pharmacology, Rivers State University, Port Harcourt, where they were provided with rat pellets and water. The animals were housed six per plastic cage and subjected to a natural photoperiod of about 12 hours light and 12 hours darkness daily. The experimental animals were acclimatized for 10 days.

2.2 Chemicals, Reagents, and Kits

The chemicals and reagents used in this experiment included 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-Ciocalteau reagent, adrenaline, dipotassium hydrogen phosphate trihydrate, potassium dihydrogen phosphate, 1-chloro-2,4-dinitrobenzene (CDNB), sulfosalicylic acid, trichloroacetic acid, sodium chloride, dipotassium hydrogen orthophosphate. Kits for AST, ALT, urea, creatinine, glucose, and lipid profile tests were obtained from Randox Laboratories, UK. All chemicals and reagents used were of analytical grade.

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, Rivers State, and were identified and authenticated by a botanist from the Department of Plant Science and Biotechnology, University of Port Harcourt. The fresh leaves of *Vernonia amygdalina* were air-dried at room temperature to a constant weight before being milled into powder using an electric blender. The fresh seeds of *Irvingia gabonensis* were also air-dried and milled into powder.

2.4 Preparation of Aqueous Extracts

The powdered leaves of *Vernonia amygdalina* (500 g) and seeds of *Irvingia gabonensis* (500 g) were macerated separately with distilled water (1 L) for 48 hours at room temperature with intermittent shaking using a glass rod. After the maceration, the mixtures were filtered using Whatman filter paper

No. 1 and the filtrates were collected in a glass beaker. The filtrates were evaporated using a rotary evaporator under reduced pressure at 40°C to obtain the crude extracts of *Vernonia amygdalina* and *Irvingia gabonensis*, which were stored in airtight containers at 4°C until further use.

2.5 Induction of Experimental Diabetes

Experimental diabetes was induced in male Wistar rats through a single intraperitoneal injection of freshly prepared alloxan monohydrate (150 mg/kg body weight) dissolved in normal saline. Forty-eight hours after alloxan injection, blood samples were collected via tail vein puncture, and fasting blood glucose levels were measured using a glucometer. Rats with fasting blood glucose levels greater than 200 mg/dL were considered diabetic and were included in the study.

2.6 Experimental Design

A total of 24 male Wistar rats were randomly divided into four groups (n=6) as follows:

- **Group 1 (Normal Control):** Non-diabetic rats received distilled water.
- **Group 2 (Diabetic Control):** Diabetic rats received distilled water.
- **Group 3 (Test Group):** Diabetic rats treated with a combination of *Irvingia gabonensis* (200 mg/kg) and *Vernonia amygdalina* (80 mg/kg) orally for 28 days.
- **Group 4 (Standard Control):** Diabetic rats treated with glibenclamide (5 mg/kg) orally for 28 days.

2.7 Biochemical Assays

At the end of the 28-day treatment period, rats were fasted overnight and sacrificed under anesthesia. Blood samples were collected via cardiac puncture into plain and EDTA tubes for serum and plasma preparation, respectively. The serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at -20°C for subsequent analysis. The plasma was used for glucose and lipid profile estimation. Liver and kidney tissues were excised, rinsed with ice-cold saline, and homogenized in appropriate buffers for enzyme assays.

Blood glucose levels were determined using the glucose oxidase method. Glycated hemoglobin (HbA1c) was measured using a kit from Randox Laboratories, UK. Liver function tests (ALT, AST, ALP) and kidney function tests (urea, creatinine) were performed using commercial kits from Randox Laboratories, UK. Lipid profiles, including total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL), were also measured using standard enzymatic methods.

2.8 Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P-values < 0.05 were considered statistically significant.

3. 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 ± 6.25^{bc}	133.4 ± 7.29^{bc}	123.0 ± 29.00	128.2 ± 7.08^{b}	138.6 ± 6.04
GROUP 2	124.0 ± 5.19^{b}	121.6 ± 6.22^{ab}	94.2 ± 25.08	67.6 ± 28.37^{ab}	65.8 ± 27.97
GROUP 3	120.0 ± 2.41^{a}	116.4 ± 2.40^{a}	104.2 ± 2.69	61.0 ± 24.94^{a}	62.2 ± 25.43
GROUP 4	141.6 ± 3.37^{c}	138.8 ± 3.15^{c}	110.6 ± 27.81	72.0 ± 29.94^{ab}	71.2 ± 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 ± 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	PANCREAS	KIDNEY
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
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. DISCUSSION

The observed reduction in body weight in the Diabetes Control group aligns with the well-documented weight loss associated with uncontrolled diabetes (Defronzo et al., 2015). Interestingly, the varying pattern of body weight reduction in Groups 3 (V. amygdalina and I. gabonensis) suggests a nuanced impact of the herbal extracts on metabolic processes, potentially influencing factors beyond glucose regulation (Ngwoke et al., 2018; Oben et al., 2018). This multifaceted effect on body weight warrants further exploration into the specific mechanisms involved, possibly involving appetite modulation, lipid metabolism, or energy expenditure.

The diminished weights of the pancreas and kidney in the Diabetes Control group emphasize the deleterious effects of diabetes on organ health, a well-established phenomenon (Fioretto et al., 1996). The reductions in organ weights observed in herbal extract-treated groups (Groups 3 and 4) indicate potential modulation of organ functions. Exploring the histological changes and cellular integrity of these organs will provide valuable insights into the holistic impact of herbal extracts on organ health (Fioretto et al., 1996; Omoregie et al., 2019). Understanding the specific cellular pathways influenced by the extracts could unveil their organ-protective mechanisms.

The varying degrees of blood glucose reduction in Groups 3 and 4, compared to the Diabetes Control group, suggest a promising potential for herbal extracts in diabetes management (Izevbuwa et al., 2021). This synergistic effect of the herbal extracts, comparable to the standard anti-diabetic drug Glibenclamide, highlights their candidacy as alternative or adjunct therapeutic agents (Ngwoke et al., 2018; Nathan et al., 2014). Further investigations into the long-term glycemic control and potential mechanisms underlying this effect would strengthen the evidence base.

The complex interplay between diabetes, herbal extracts, and hepatic function, as reflected in liver function biomarkers, underscores the need for a comprehensive understanding of the biochemical processes involved (Younossi et al., 2018). While Glibenclamide exhibits hepatoprotective effects, the herbal extracts' impact on liver health raises important considerations. Future research should delve into the molecular aspects of hepatic function modulation by herbal extracts, addressing potential concerns related to hepatotoxicity and ensuring the safety of these botanical interventions (Younossi et al., 2018; Omoregie et al., 2019).

Elevated levels of creatinine and urea in the Diabetes Control group indicate impaired kidney function, a common complication of diabetes (Thomas et al., 2015). The trends suggestive of renal function improvement in Groups 3 and 4 underscore the potential nephroprotective effects of the herbal extracts. Detailed histopathological examinations will offer a deeper understanding of the renal benefits and potential mechanisms involved (Thomas et al., 2015). Investigating the interplay between herbal extracts and pathways contributing to renal health will contribute to their holistic evaluation.

The improvements in lipid profiles observed in Groups 3 and 4 indicate a potential lipid-lowering effect of the herbal extracts, aligning with their positive impact on metabolic parameters (Ngondi et al., 2018). The dyslipidemia evident in the Diabetes Control group is a common feature in diabetes, and the herbal extracts' ability to mitigate this aspect adds to their therapeutic potential (American Diabetes Association, 2021). Further lipidomic analyses will unravel the specific pathways influenced by the herbal extracts, providing a more targeted understanding of their lipid-modulating effects.

5. CONCLUSION

In conclusion, the study's findings, supported by relevant literature, suggest that V. *amygdalina* and I. *gabonensis* exhibit promising and multifaceted effects on key parameters associated with diabetes. The synergistic actions observed in body weight, organ health, blood glucose levels, liver and kidney function, and lipid profiles indicate a potential comprehensive impact on diabetes management. However, the intricate and nuanced nature of these effects calls for continued in-depth investigations. Comprehensive safety assessments, molecular studies, and long-term clinical trials will be essential for unlocking the full therapeutic potential and ensuring the safe integration of these herbal extracts into diabetes care practices.

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