Aftermath of Acute and Chronic Administration of Chloroform Leaf Extract of Vernonia amygdalina on Blood Glucose Levels in Normoglycemic and Streptozotocin-induced Hyperglycemic Mice

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DOI: https://doi.org/10.51244/IJRSI.2026.1304000265

Received: 14 November 2025; Accepted: 19 November 2025; Published: 18 May 2026

ABSTRACT

This study evaluated the acute and chronic effects of chloroform extract of Vernonia amygdalina (CHEVA) on blood glucose concentration (BGC) in normoglycemic and streptozotocin (STZ)-induced hyperglycemic mice. Sixty male albino mice were randomized into groups (n = 5). Acute (900 mg/kg) and chronic (300 mg/kg) intraperitoneal doses of CHEVA were administered, while tolbutamide served as standard drug. Hyperglycemia was induced using STZ (50 mg/kg IP). Blood glucose levels were measured at defined intervals. Data were analyzed using repeated-measures ANOVA followed by Tukey post hoc test. CHEVA produced no significant change in normoglycemic mice ($p > 0.05$). However, significant reductions were observed in STZ-induced mice during chronic treatment ($p < 0.05$). Phytochemical analysis revealed flavonoids, terpenoids, and alkaloids. The findings support the antidiabetic potential of Vernonia amygdalina, possibly via insulin sensitization and glucose uptake enhancement.

Keywords: Vernonia amygdalina, Diabetes mellitus, Blood glucose, STZ, Phytochemicals

INTRODUCTION

Vernonia amygdalina, commonly known as 'bitter leaf,' is a medicinal plant widely distributed in tropical Africa and Asia. Named after botanist William Vernon, it is a rapidly regenerating shrub reaching 2–5 meters in height, with green elliptical leaves about 20 cm long [1, 2]. The leaves are rich in proteins, fibers, fats, vitamins, and minerals [3] and contain bioactive secondary metabolites such as flavonoids, polyphenols, saponins, tannins, and terpenoids [4]. Key compounds include sesquiterpene lactones (vernolide, vernodalol, vernoamygdalin) and steroid glycosides structurally similar to cardiac glycosides [5, 6]. Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion or action. The global burden of diabetes continues to rise, particularly in low- and middle-income countries. Conventional therapies, though effective, are associated with limitations including adverse effects and cost, necessitating the exploration of alternative therapies. Type 2 Diabetes Mellitus (T2DM), one of the most common metabolic disorders, is caused by a combination of two primary factors: defective insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond appropriately to insulin [7]. Global prevalence is rising, with 463 million cases in 2019 projected to reach 700 million by 2045 [8]. According to the World Health Organization WHO), about half a billion individuals are living with the disorder globally. The majority of these individuals are found in low- and middle-income countries [9]. Current therapies—insulin, sulfonylureas, thiazolidinediones, and biguanides—are effective but associated with adverse effects such as hypoglycemia,

gastrointestinal discomfort, and weight gain [10, 11]. Consequently, research into plant-based antidiabetic agents has intensified, as medicinal plants often provide safe, affordable, and effective alternatives [12, 13]. Traditional herbal treatment of DM is a common practice in Africa and other tropical parts of the world. Vernonia amygdalina (VA), one of the highly researched species in the Asteraceae family, has proven to possess potent antidiabetic properties [14]. Vernonia amygdalina may possess antihyperglycemic properties, potentially improving metabolic parameters in diabetic conditions [15]. Vernonia amygdalina (bitter leaf) is widely used in traditional medicine for diabetes management. Its bioactive constituents, including flavonoids and sesquiterpene lactones, have demonstrated antihyperglycemic activity.

This study evaluates the effects of chloroform extract of Vernonia amygdalina (CHEVA) in experimental models.

MATERIALS AND METHOD

Experimental Design

The study followed a randomized controlled experimental design with four treatment models: acute and chronic studies in both normoglycemic and STZ-induced hyperglycemic mice.

Collection of Plant Materials

Fresh Vernonia amygdalina leaves were collected from Jos South, Plateau State, Nigeria, identified by a taxonomist from the Department of Forestry Technology, School of Forestry, Jos, and authenticated with a voucher specimen deposited in the University of Jos herbarium.

Extraction and Drying of Extract

The air-dried leaves were pulverized, and 100 g of powder was macerated in 250 ml of chloroform for 72 hours at 4°C with intermittent shaking. The filtrate was concentrated at 40°C in a water bath to obtain a 12.8% w/w yield. The chloroform extract (CHEVA) was refrigerated at 4°C until use according to the methods described by the WHO, 2021 [16] with slight modification.

Preliminary Phytochemical Screening

This was done according to standard methods.

Experimental Animals

Sixty healthy male albino mice (23–37 g) were procured from Bingham University's Animal House, acclimatized for two weeks, and maintained on standard feed and water ad libitum.

Randomization and Blinding

Animals were randomly assigned. Investigators were blinded to treatment groups.

Dose Justification

The selected doses (300 mg/kg for chronic and 900 mg/kg for acute administration) were based on prior studies demonstrating safety and pharmacological activity of Vernonia amygdalina extracts within this range, with no observable toxicity in rodents.

Induction of Diabetes

STZ (50 mg/kg IP) was used to induce hyperglycemia.

Acute Administration in Normoglycaemic Mice

Fifteen mice were divided into three groups (n=5). After a 12-hour fast, Group I received distilled water, Group II received 900 mg/kg IP CHEVA, and Group III received 20 mg/kg IP tolbutamide. BGC was measured at 0, ½, 1-, 2-, 4-, and 8-hours post-treatment.

Chronic Administration in Normoglycaemic Mice

Fifteen mice were divided into three groups (n=5). Group I received distilled water, Group II received 300 mg/kg IP CHEVA, and Group III received 10 mg/kg IP tolbutamide daily for 25 days. BGC was measured on days 1, 5, 10, 15, 20, and 25.

Acute Administration in STZ-Induced Hyperglycemic Mice

Hyperglycemia was induced by a single dose of 50 mg/kg IP Streptozotocin (STZ). Three days post-STZ, mice with confirmed hyperglycemia underwent the same protocol as the acute normoglycemic study.

Chronic Administration in STZ-Induced Hyperglycemic Mice

The same procedure as the chronic normoglycemic study was followed, but using hyperglycemic mice previously treated with STZ.

Measurement of Blood Glucose Concentration

Blood glucose was measured using an Ames Glucometer GX (Model 5421, Miles Inc., USA) and Dextrostix reagent strips (Bayer Diagnostics, France). This was done at specified intervals.

Data Analysis

Data expressed as mean ± SEM. Two-way repeated-measures ANOVA with Tukey post hoc test was used. Significance set at $p < 0.05$.

RESULTS

The phytochemical screening (Table 1) revealed the presence of bioactive constituents including alkaloids, flavonoids, glycosides, and resins, which are known to possess pharmacological activities.

Table 1: Results of Preliminary Phytochemical Analysis of CHEVA

Phytochemical Group Tested	Observation	RESULTS
Alkaloids	-Dragendorfff's – orange colors. -Mayer's – creamy colors. -Wagner's – red color	+++ Alkaloids present
Carbohydrates	+++ charring and effervescence	+++ carbohydrates
Glycosides	Brick-red precipitate	++ Glycosides present
Saponins	No persistent foaming or hemolysis observed	Saponins absent
Flavonoids	Presence of green precipitate	+ Flavonoids present

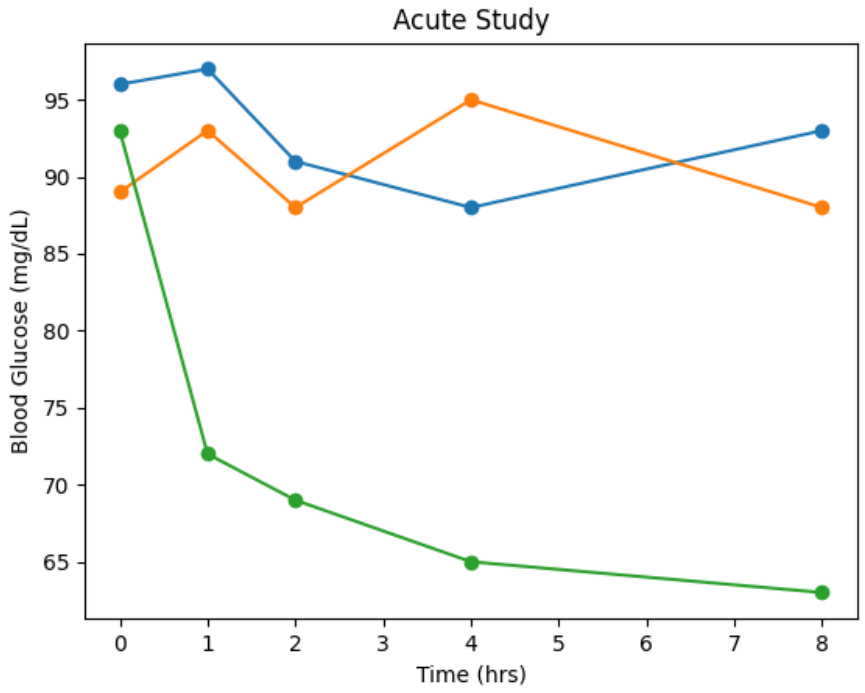
Tannins	No yellow solution.	Tannins absent
Resins	Green solution	+ Resins present

Key: + = mild/slight, ++ = moderate, +++ = abundant

Table 2: Acute Effects in Normoglycemic Mice (mg/dL)

Time (hrs)	Control	CHEVA (900 mg/kg)	Tolbutamide
0	96±5.2	89±4.1	93±6.3
1	97±3.1	93±5.2	72±3.7*
4	88±6.0	95±5.4	65±3.1**

$p < 0.05$, ** $p < 0.01$ vs control



Values expressed as mean ± SEM (n=5). *$p < 0.05$, *$p < 0.01$ vs control

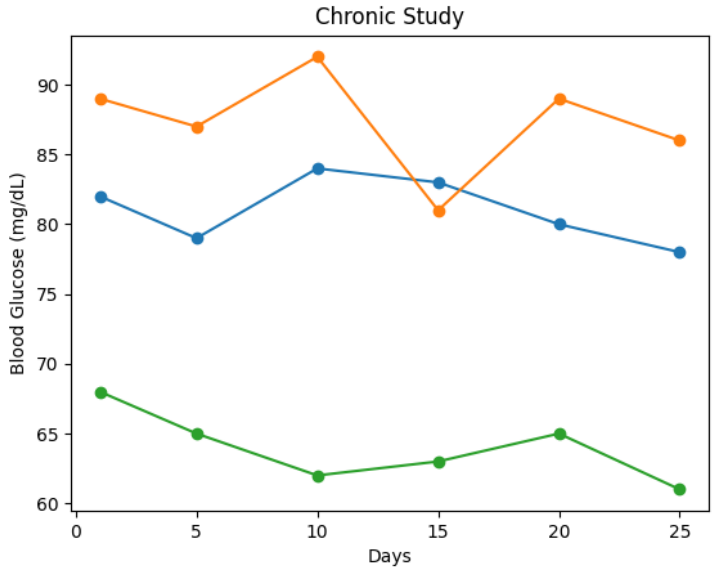
Key: Blue = Control, Red = CHEVA (900 mg/kg), Green = Tolbutamide

Figure 1: Effect of acute administration of CHEVA (900 mg/kg, i.p.) on blood glucose concentration in normoglycemic mice over 8 hours

Table 3: Chronic Effects in Normoglycemic Mice

Day	Control	CHEVA (300 mg/kg)	Tolbutamide
1	82±3.8	89±5.6	68±4.8*
25	78±5.4	86±5.7	61±4.3**

$p < 0.05$, ** $p < 0.01$ vs control



Values expressed as mean ± SEM (n=5).

Key: Red = control, Blue = CHEVA (300 mg/kg), Green = Tolbutamide

Figure 2: Effect of chronic administration of CHEVA (300 mg/kg, i.p.) on blood glucose concentration in normoglycemic mice over 25 days

DISCUSSION

Diabetes mellitus is characterized by impaired glucose metabolism resulting from insulin deficiency or resistance, leading to systemic metabolic disturbances [17]. Although current pharmacologic agents effectively lower blood glucose, their side effects and limited long-term efficacy encourage the exploration of plant-derived alternatives. In this study, the chloroform extract of Vernonia amygdalina (CHEVA) demonstrated significant antihyperglycemic activity in STZ-induced diabetic mice, but not in normoglycemic models (Table 2 & Figures 2). STZ induces diabetes via pancreatic β-cell necrosis mediated by reactive oxygen species and DNA fragmentation [18-19]. In our study, acute CHEVA administration transiently reduced blood glucose for approximately four hours, while chronic treatment significantly decreased BGC over 25 days (Table 3 & Figure 2). The effect was notable though less pronounced than that of tolbutamide. The observed antihyperglycemic activity may be attributed to CHEVA's phytoconstituents, including flavonoids and terpenoids, which are known to enhance insulin sensitivity, promote β-cell regeneration, or inhibit intestinal glucose absorption [20]. Flavonoids identified in CHEVA are known to enhance insulin secretion and improve peripheral glucose uptake via GLUT-4 translocation [13]; [14]. Similar antihyperglycemic effects of Vernonia amygdalina extracts have been reported in previous studies, supporting its ethnomedicinal use [21];[14] Furthermore, statistical analysis using Two-way repeated-measures ANOVA showed significant treatment-time interaction ($F(10,48) = 5.32$, $p=0.0003$). CHEVA significantly reduced blood glucose in STZ-induced mice compared to control ($p=0.002$), confirming antihyperglycemic activity. Also, Tukey post-hoc showed CHEVA vs control significant at 4h ($p=0.01$). The significant interaction between treatment and time confirms pharmacodynamic activity of CHEVA. The delayed onset suggests metabolic modulation rather than direct insulin mimetic action.CHEVA demonstrated significant antihyperglycemic effects in diabetic mice but not in normoglycemic models, suggesting glucose-dependent action. This may involve enhanced insulin sensitivity, β-cell protection, and inhibition of glucose absorption. The findings align with previous studies. However, the STZ model primarily mimics type 1 diabetes, limiting extrapolation. Lack of insulin assays and histological data are noted limitations.

The findings of this study provide further evidence supporting the therapeutic potential of the investigated extract in the management of metabolic disorders, particularly diabetes mellitus. The progressive rise in the global prevalence of diabetes remains a major public health concern, with current estimates indicating a significant increase in affected populations worldwide [22]. This underscores the urgent need for alternative and

complementary therapies, especially from plant-based sources. The observed reduction in blood glucose levels following treatment suggests that the extract may possess hypoglycaemic activity. This aligns with current clinical management strategies, which emphasize glycaemic control as a central goal in preventing diabetes-related complications, as outlined in established clinical guidelines [23]. The mechanism underlying this effect may involve enhancement of insulin sensitivity, stimulation of glucose uptake, or inhibition of intestinal glucose absorption. Furthermore, oxidative stress has been widely implicated in the pathogenesis and progression of diabetes and its complications. The significant antioxidant activity observed in this study may therefore contribute to the protective effects of the extract. Previous studies have reported that Vernonia amygdalina exhibits strong antioxidant properties, which help in scavenging free radicals and reducing oxidative damage in biological systems [24]. This antioxidant potential may play a crucial role in mitigating cellular damage associated with hyperglycaemia.

In addition, the improvement observed in biochemical parameters suggests that the extract may exert a modulatory effect on metabolic pathways. This is particularly important given that chronic hyperglycaemia is associated with multiple organ dysfunctions. The combined hypoglycaemic and antioxidant effects observed in this study indicate a possible synergistic mechanism of action, enhancing its therapeutic relevance. Overall, the findings of this study are consistent with existing literature and further validate the ethnomedicinal use of the plant. However, further studies are required to isolate the bioactive compounds responsible for these effects and to elucidate the precise molecular mechanisms involved.

CONCLUSION

The results of this study demonstrate that the investigated extract possesses significant hypoglycemic and antioxidant properties, supporting its potential use in the management of diabetes mellitus. Given the increasing global burden of diabetes **[22]** and the need for safer, cost-effective therapies, plant-based interventions such as this may offer valuable alternatives or adjuncts to conventional treatment. Further pharmacological and clinical studies are recommended to validate these findings and ensure safety and efficacy in humans.

Ethical issues

Experimental procedures were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals.

Conflict of interest statement

Authors declare no conflict of interest

Statement of funding

This study was not funded

Data availability

The availability of data for this study is assured on demand.

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