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Effects of an Aqueous Extract of Vernonia Amygdalina Del. (Asteraceae) Leaves on Blood Glucose Levels in Rats

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Abstract | **Original Research Article**

Diabetes mellitus is a major public health problem, which has spurred research into plant-derived substances that may help control blood glucose levels. This study aimed to evaluate the hypoglycemic and antihyperglycemic activities of an aqueous extract of *Vernonia amygdalina* leaves in Rattus norvegicus rats. The leaves, harvested in Djébonoua (Côte d'Ivoire), were dried, ground, and then extracted with distilled water. A phytochemical screening was performed to identify the different groups of secondary metabolites. An acute toxicity study was conducted in female rats in accordance with OECD Test Guideline 423, using a single dose of 2,000 mg/kg body weight. Hypoglycemic and antihyperglycemic activities were evaluated in male rats, under normoglycemic conditions and after an oral glucose load, respectively, using glibenclamide as a reference drug. The aqueous extract of *V. amygdalina* leaves contained sterols, polyterpenes, polyphenols, flavonoids, alkaloids, and saponins, with polyphenols, flavonoids, and alkaloids being particularly abundant. At a dose of 2,000 mg/kg, no deaths, no clinical signs of toxicity, and no significant changes in body weight were observed after 14 days. Under normoglycemic conditions, the extract did not cause any significant reduction in blood glucose levels at doses ranging from 100 to 1,000 mg/kg. However, following a glucose overload, doses of 100, 200, and 500 mg/kg significantly reduced the induced hyperglycemia, with the maximum effect observed at 200 mg/kg. These results demonstrate the antihyperglycemic activity of the aqueous extract of *V. amygdalina* leaves, associated with good acute tolerance.

Keywords: *Vernonia amygdalina*, blood glucose, hypoglycemic activity, antihyperglycemic activity, diabetes.

1. INTRODUCTION

The overall goals of diabetes treatment are to restore blood glucose balance in order to prevent diabetes-related complications [1]. Indeed, in the absence of adequate insulin secretion and action, sugar remains in the blood, causing chronic hyperglycemia. This can lead to microvascular and macrovascular complications, some of which can be life-threatening if left untreated [2,3]. It is therefore in the patient's best interest to lower blood glucose levels in people with diabetes, regardless of the treatment used.

Currently, worldwide, drug therapy for diabetes mellitus consists primarily of insulin injections and/or oral antidiabetic and hypoglycemic agents [4,5]. Insulin therapy remains the cornerstone of blood glucose control in people with type 1 diabetes [6,7]. Oral antidiabetic and hypoglycemic agents form the basis of treatment for type 2 diabetes [8,9]. However, these various treatments must be tailored to the individual, taking into account treatment goals, lifestyle, age, overall health status, and the duration and severity of hyperglycemia. Consequently, the range of oral antidiabetic and hypoglycemic agents available on the market is constantly expanding [10,11]. Clinicians must make their choice by considering a number of factors, the most relevant of which are the adverse effects of these medications. The adverse effects and contraindications associated with the treatment of type 2 diabetes depend on the class of oral antidiabetic agents [12]. Data from the DCCT (Diabetes Control and Complications Trial) studies on type 1 diabetes have shown that hypoglycemia

is the most common adverse effect of insulin therapy in patients with this type of diabetes [13,14]. These adverse effects significantly compromise the pharmacotherapy of diabetes mellitus.

Today, the issue is no longer limited to diabetes mellitus alone, but encompasses all conditions that hyperglycemia can cause. In 2021, diabetes was directly responsible for approximately 1.6 million deaths worldwide, in addition to approximately 530,000 deaths due to diabetic nephropathy. Furthermore, hyperglycemia is estimated to account for approximately 11% of cardiovascular deaths worldwide [15]. This demonstrates that hyperglycemia is responsible for a significant burden of mortality beyond the deaths directly attributable to diabetes mellitus.

Concerns about the morbidity caused by chronic hyperglycemia are a major issue for certain countries, such as Côte d'Ivoire, which is already struggling to cope with other diseases with high morbidity rates (malaria, AIDS, tuberculosis).

Independent national surveys in Africa indicate that 90% of Ethiopians and Burundians, 85% of South Africans, 75% of Malians, and 70% of Rwandans, Beninese, and Ghanaians use medicinal plants [16]. In Nigeria, approximately 80% of the population consults traditional healers for their health needs [17]. In the Democratic Republic of the Congo, 79.4% of the population of Lubumbashi has used traditional medicine, motivated by its perceived effectiveness, rapid healing, and low cost [18]. It would therefore be more appropriate and beneficial to direct scientific research on these plants in this direction in order to promote them and optimize their use.

This research study falls within this same framework. The objective was to evaluate the hypoglycemic and antihyperglycemic activities of the aqueous extract of *Vernonia amygdalina* leaves in Rattus norvegicus rats.

2. MATERIALS AND METHODS

2.1. Biological Material

The plant material consists primarily of leaves of *V. amygdalina* (Asteraceae). These leaves were collected fresh in the Djébonoua subprefecture, located 18 km south of Bouaké (Gbêkê region, Côte d'Ivoire). The plant was identified at the National Center for Floristics at Félix HOUPHOUËT-BOIGNY University (Abidjan, Côte d'Ivoire).

Female Wistar strain white rats, Rattus norvegicus (Muridae), aged 9 weeks and weighing between 148 and 153 g, were used for the toxicity test. The selection of females is in accordance with the recommendations of OECD Guideline 423, which generally recommends the use of females for acute oral toxicity tests, as they may exhibit slightly greater sensitivity than males [19]. These female rats are nulliparous and not pregnant. For the pharmacological study, male rats of the same species as those used in the toxicological study were used. Their age ranged from 9 to 10 weeks, and their body weight ranged from 150 to 166 g. Selecting males can help avoid metabolic changes caused by pregnancy or lactation. Sex hormones can significantly influence insulin sensitivity and how the body processes glucose. [20]. These animals, raised in the vivarium at the École Normale Supérieure d'Abidjan, were housed in a room maintained at a temperature of 28 ± 2°C with a photoperiod of 12 hours of natural light and 12 hours of darkness. Humidity ranged from 50 to 55%, and the animals had free access to water and food (56.9% carbohydrates, 16.6% protein, 3.9% fat, 13.2% fiber, and 9.4% minerals) provided by the Ivorian Compound Feed Manufacturing Company.

2.2. Extraction Method

The leaves of *V. amygdalina*, dried away from direct sunlight, were ground using a Moulinex-type electric grinder, and the resulting powder was used to prepare the total aqueous extract.

The aqueous extract of *V. amygdalina* leaves (AEVa) was prepared following the method described by Yapo *et al.,*[21]. 50 g of leaf powder were mixed with 1 liter of distilled water in a blender. After a series of three blending cycles, each lasting three minutes at room temperature, the resulting homogenate was filtered twice through a square of white cloth (poplin), then five times through cotton gauze. The resulting filtrate was evaporated in an oven at 50°C for 3 days until a dry extract was obtained.

2.3. Phytochemical Screening Study

The phytochemical analyses were conducted at the pharmacognosy laboratory of the Faculty of Pharmaceutical and Biological Sciences at Félix HOUPHOUËT-BOIGNY University. The various chemical groups in EAVA were characterized using the techniques described in the works of Wagner & Bladt [22], Békro *et al.,*[23], and Adiukwu *et al.,*[24].

Sterols and polyterpenes were detected using the Liebermann reaction. The ferric chloride ($FeCl_3$) reaction was used to characterize polyphenols. Flavonoids were detected using the so-called "cyanidin" reaction. Catechin tannins were identified using Stiasny's reagent, while gallic tannins were detected using $FeCl_3$. Borntraegen's reagent was used to detect free and bound quinone substances. Alkaloids were characterized using Dragendorff's reagent (potassium iodobismuthate) and Bouchardat's reagent (iodo-iodide). To test for saponosides, 15 mL of AEVa was poured into a test tube 15 cm long and 15 mm in diameter, then the tube was shaken vigorously for 10 seconds and allowed to stand for 10 minutes. If the foam remains at a height of more than 3 cm, then saponins are present.

2.4. Toxicological Study

Toxicology studies the harmful effects of chemical substances on living organisms [25]. That said, any biologically active substance is capable of producing adverse or even harmful effects at high or low doses and with prolonged administration [26].

This study was conducted in accordance with OECD Chemical Testing Guideline 423 [19]. The limit test using a dose of 2000 mg/kg body weight was conducted. Six nulliparous, non-pregnant female rats were divided into two groups of three animals each: one group treated with the extract and one control group. They were individually marked, fasted overnight (12 hours), and weighed immediately before the experiment. Subsequently, a single dose of 2,000 mg/kg was administered orally to each rat in the treated group using an esophageal tube. After treatment, the female rats were again deprived of food for 4 hours. They were observed individually during the first 30 minutes and once daily for 14 days. The observation of the animals consisted of noting symptoms of disease or behavioral changes.

2.5. Pharmacological Study

In this study, the rats were fasted for 12 hours prior to the experiments. The substances were administered orally.

2.5.1. Hypoglycemic Activities

For this study, 25 rats were used. They were divided into five groups of five rats each. Group 1 (control) received distilled water. Group 2 received 10 mg/kg of glibenclamide. Groups 3, 4, 5, and 6 were treated with AEVa at doses of 100, 200, 500, and 1,000 mg/kg, respectively. Baseline blood glucose levels were first determined immediately before treatment. Blood glucose levels were then measured every 30 minutes for 150 minutes, and the percentage reduction in blood glucose was calculated.

2.5.2. Antihyperglycemic Effects

Hyperglycemia is induced in animals by oral administration of 4 g/kg of glucose. Blood glucose levels in rats from each group are measured immediately before glucose administration and 30 minutes later, just before the rats are treated with different doses of AEVa or glibenclamide. Blood glucose levels are then measured at 30-minute intervals for 150 minutes. In this test, 30 rats divided into six groups of five rats are used. Group 1 receives 4 g/kg of glucose, followed by distilled water 30 minutes later. This is the control group. Group 2 receives 4 g/kg of glucose, followed by 10 mg/kg of glibenclamide 30 minutes later. Groups 3, 4, 5, and 6 receive 4 g/kg of glucose, followed by 100, 200, 500, and 1000 mg/kg of EAVa, respectively, 30 minutes later.

2.6. Statistical Analysis of the Results

Statistical analysis of the results was performed using GraphPad Uninst_Prism 7 software. The results are presented as the mean followed by the standard error of the mean (mean ± SEM). Analysis of variance (ANOVA) was used to compare the obtained means. This software was also used to generate the graphs.

3. RESULTS

3.1. Phytochemical Screening

Phytochemical analysis of AEVa revealed the presence of sterols and polyterpenes, polyphenols, flavonoids, alkaloids, and saponosides (Table 1). However, polyphenols, flavonoids, and alkaloids were found to be the most abundant compounds in the extract. In contrast, AEVa does not contain tannins or quinone compounds.

Table 1: Chemical composition of the aqueous extract of *V. amygdalina* leaves

Chemical Groups	Observations
Sterols and Polyterpenes	+
Polyphenols	++
Flavonoids	++
Tannins	-
Quinones	-
Alkaloids	++
Saponins	+

(++): high levels; (+): low levels; (-): absent

3.2. Acute Toxicity Study

A dose of 2,000 mg/kg of AEVa did not result in death 14 days after administration in rats. No clinical signs of toxicity were observed. The body weight of the treated animals (174 g) was statistically identical ($p > 0.05$) to that of the control animals (176 g).

3.3. Hypoglycemic Effects

Blood glucose levels in the control rats remained statistically constant ($p > 0.05$) throughout the duration of the test. It remained at 0.77 ± 0.03 g/L. Doses of 100, 200, 500, and 1,000 mg/kg of AEVa did not cause a decrease in blood glucose levels in the rats ($p > 0.05$) during the 150-minute test. In contrast, glibenclamide caused a decrease in blood glucose levels in the rats. This decrease was significant ($p < 0.01$), and the percentage reduction in blood glucose was 23 ± 0.85% at the end of the test (Figure 1).

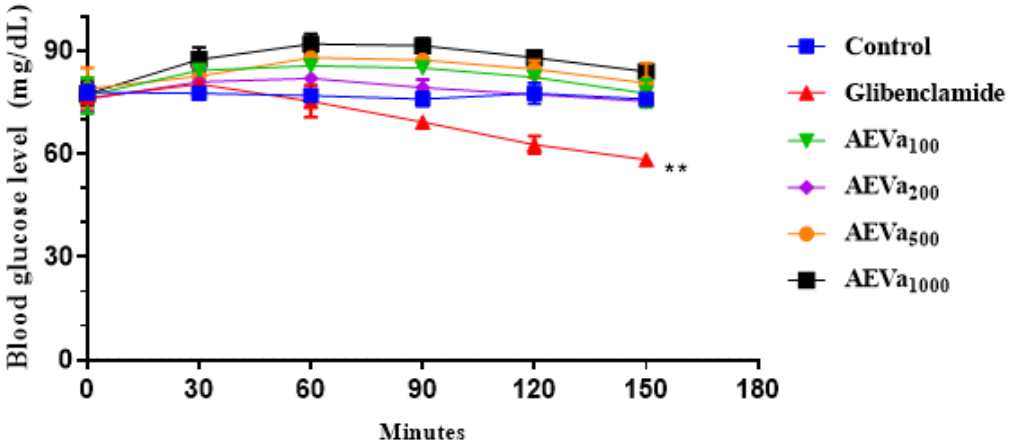

Figure 1: Changes in blood glucose levels in rats during the hypoglycemic test

Values are expressed as mean ± standard error of the mean (n=5) (*) P<0.05; (**): P<0.01; (***): P<0.001.
$AEVa_{100}$: 100 mg/kg of the aqueous extract of *V. amygdalina* leaves;
$AEVa_{200}$: 200 mg/kg of the aqueous extract of *V. amygdalina* leaves;
$AEVa_{500}$: 500 mg/kg of the aqueous extract of *V. amygdalina* leaves;
$AEVa_{1000}$: 1000 mg/kg of the aqueous extract of *V. amygdalina* leaves.

3.2. Antihyperglycemic Effects

Administration of glucose at the start of the test causes a highly significant increase ($p < 0.001$) in blood glucose levels in rats. The mean blood glucose level for all rats rises from 79 ± 8 mg/dL to 153 ± 7 mg/dL, representing a glucose load of 73 ± 8 mg/dL. Oral administration of EAVa, glibenclamide, or distilled water 30 minutes after glucose administration resulted in a relative reduction in the induced hyperglycemia in the animals. At the end of the test, blood glucose levels in the control group rats were reduced by 41 ± 10%. However, blood glucose levels in the groups treated with glibenclamide ($p < 0.001$), 100 mg/kg of AEVa ($p < 0.05$), 200 mg/kg of EAVa ($p < 0.01$), and 500 mg/kg of AEVa ($p < 0.05$) showed a significantly greater reduction of 141 ± 16%, 71 ± 13%, 97 ± 15%, and 67 ± 10%, respectively, compared to the control group. No significant difference ($p > 0.05$) was observed between the rate of blood glucose reduction in the group treated with 1,000 mg/kg of AEVa (48 ± 10%) and that of the control group (41 ± 10%) (Table 2).

Table 2: Blood glucose levels in rats in the antihyperglycemic test

	Blood glucose level (mg/dL)		
Minutes	**0**	**30**	**180**
Control	78 ± 6,1	153 ± 9,2	108 ± 3,2
Glibenclamide	83 ± 4,3	154 ± 2,5	64 ± 5,2 ***
$AEVa_{100}$	83 ± 8,3	148 ± 2,8	87 ± 4,9 *
$AEVa_{200}$	78 ± 7,5	153 ± 2,8	78 ± 6,7 **
AEV_{500}	75 ± 8,9	157 ± 2,5	92 ± 3,5 *
$AEVa_{1000}$	84 ± 4,2	154 ± 4,9	103 ± 2.1

Values are expressed as mean ± standard error of the mean (n=5) (*) P<0.05; (**): P<0.01; (***): P<0.001.
$AEVa_{100}$: 100 mg/kg of the aqueous extract of *V. amygdalina* leaves;
$AEVa_{200}$: 200 mg/kg of the aqueous extract of *V. amygdalina* leaves;
$AEVa_{500}$: 500 mg/kg of the aqueous extract of *V. amygdalina* leaves;
$AEVa_{1000}$: 1000 mg/kg of the aqueous extract of *V. amygdalina* leaves.

4. DISCUSSION

The objective of this study was to evaluate the hypoglycemic and antihyperglycemic activities of the aqueous leaf extract of *V. amygdalina* (AEVa) in Rattus norvegicus rats. Phytochemical analysis of EAVa revealed the presence of sterols and polyterpenes, polyphenols, flavonoids, alkaloids, and saponins, with polyphenols, flavonoids, and alkaloids being particularly abundant. In contrast, no tannins or quinone compounds were detected. This profile confirms the richness of V. amygdalina in bioactive secondary metabolites. Previous studies using aqueous extracts of the leaves of this species have also reported the presence of alkaloids, flavonoids, phenolic compounds, saponins, and steroids, although differences were observed depending on the extraction method and experimental conditions [27,28]. The phytochemical composition can indeed vary depending on the extraction solvent, the origin of the

leaves, their stage of development, and the analytical methods used. In this regard, the potential role of polyphenols and flavonoids in the antidiabetic activity of *V. amygdalina* is of particular interest. Ong *et al.,* demonstrated, in particular, that a polyphenol-rich extract from this plant contained, among other compounds, derivatives of caffeoylquinic acid, chlorogenic acid, and luteolin-molecules that may contribute to the observed metabolic effects [29].

The acute toxicity study also provides favorable evidence regarding the tolerability of AEVa. Indeed, a single oral dose of 2,000 mg/kg resulted in no deaths or clinical signs of toxicity during the 14-day observation period. Furthermore, the average weight of the treated animals did not differ significantly from that of the controls. The absence of any significant weight changes suggests that this single administration did not significantly disrupt the animals' general condition during the observation period. These results are consistent with several previous studies on the toxicity of AEVa. In particular, acute toxicity studies conducted using an approach based on OECD guidelines revealed no cases of mortality or clinical signs of toxicity following administration of an aqueous extract, even at doses exceeding 2,000 mg/kg [30,31,32]. However, these results should be considered only as evidence of good acute tolerance, and not as evidence of absolute long-term safety.

In normoglycemic rats, administration of AEVa at doses of 100, 200, 500, and 1,000 mg/kg did not result in a significant decrease in blood glucose levels during the 150-minute follow-up period. In contrast, glibenclamide caused a significant decrease in blood glucose levels, reaching 23 ± 0.85% at the end of the experiment. The absence of an acute hypoglycemic effect of EAVa in normoglycemic rats suggests that, under the experimental conditions of our study, the extract does not induce a significant decrease in baseline blood glucose levels. This result is of pharmacological interest, as it indicates that the extract's activity may be more pronounced under hyperglycemic conditions than under normoglycemic conditions. This observation is consistent with the results of a study evaluating an aqueous extract of *V. amygdalina* in normoglycemic rats: no significant decrease in blood glucose levels was observed at the doses studied, despite a slight maximum reduction reported at certain doses [28]. Similarly, a study combining an aqueous extract of *V. amygdalina* with metformin showed only a slight effect of the extract alone on blood glucose levels in non-diabetic rats, unlike in diabetic animals, in which the effect was much more pronounced [33]. Glucose administration, on the other hand, led to a highly significant increase in blood glucose levels. This increase confirms the actual onset of induced hyperglycemia and thus validates the experimental model used to evaluate antihyperglycemic activity. In the control animals, however, blood glucose levels decreased spontaneously by 41 ± 10% at the end of the test. This decrease is attributable to the normal physiological response to a glucose load, involving, in particular, insulin secretion and the gradual uptake of glucose by peripheral tissues.

In this context, administration of AEVa accentuated the reduction in induced hyperglycemia compared with the control group. The reductions observed were 71 ± 13% at the 100 mg/kg dose, 97 ± 15% at the 200 mg/kg dose, and 67 ± 10% at the 500 mg/kg dose, with these differences being statistically significant. In contrast, the 1,000 mg/kg dose did not result in a significantly different reduction compared to the control group, with 48 ± 10% versus 41 ± 10%. These results therefore demonstrate the acute antihyperglycemic activity of AEVa, but without a strictly increasing dose-response relationship. The maximum activity observed at 200 mg/kg, followed by a decrease at 500 and 1,000 mg/kg, could indicate a nonlinear dose-response relationship. This response is not unusual with complex plant extracts, in which multiple constituents may act simultaneously, giving rise to synergistic effects, antagonism, saturation, or dose-dependent variations in bioavailability.

The antihyperglycemic effect of AEVa is particularly noteworthy when compared to the available data on V. amygdalina. Experimental studies have shown that an extract of this plant can improve glucose tolerance in diabetic animals and reduce fasting blood glucose levels after repeated administration [29]. Furthermore, studies specifically using aqueous extracts have also reported a decrease in blood glucose levels in diabetic rats. Goje *et al.,* observed, after two weeks of treatment with an aqueous extract of V. amygdalina, a significant reduction in fasting blood glucose levels in alloxan-induced diabetic rats [34].

The magnitude of the reduction observed with glibenclamide also serves as a relevant positive control in this trial. Glibenclamide is a sulfonylurea known to stimulate insulin secretion by pancreatic β-cells. The fact that AEVa caused a greater decrease in glucose levels in the glucose overload model than the control group, but less than that achieved with glibenclamide, suggests that the extract possesses measurable pharmacological activity, although it did not match the efficacy of the reference drug under the experimental conditions. The effect observed specifically at the 200 mg/kg dose is nevertheless worth noting.

The mechanisms responsible for this activity cannot be definitively established based solely on the results of this study. However, the high content of polyphenols, flavonoids, and alkaloids in AEVa suggests several complementary mechanisms. The available experimental data on *V. amygdalina* suggest, in particular, improved glucose utilization by muscles, increased GLUT4 translocation, and a reduction in certain hepatic glucose production pathways [29].

Recent studies also continue to highlight the importance of the phenolic and flavonoid components of V. amygdalina in its metabolic effects [35].

6. CONCLUSION

Overall, our results indicate that EAVA does not exhibit significant acute hypoglycemic activity in normoglycemic rats, but that it exerts significant antihyperglycemic activity following a glucose load, particularly at doses of 100, 200, and 500 mg/kg. This distinction is important because it suggests that the extract may act more on the regulation of postprandial blood glucose elevation than on the reduction of basal blood glucose levels. These results are generally consistent with several previous studies demonstrating an antidiabetic effect of *V. amygdalina*, while revealing a complex, dose-dependent response that warrants further investigation. Further studies on long-term blood glucose levels, insulin levels, glucose tolerance, and the cellular and molecular mechanisms involved would be necessary to elucidate the extract's mechanism of action.

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