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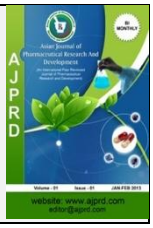

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Research Article

Vigna comosa Baker Aerial Part's Extracts Effects on Hyperglycemia and Hepatic Glucose's Liberation

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A B S T R A C T

Vigna comosa Baker (*V. comosa)* is a plant used by Beninese Oueme department's population for the treatment of diabetes without any scientific study showing its effectiveness. The objective of this study is to investigate the effects of the extracts of *V. comosa*on hyperglycemic rabbits by Oral Glucose Tolerance Test (OGTT) and on hepatic glucose liberation. Phytochemical screening revealed that the plant contains alkaloids, tannins, flavonoids, mucilage, triterpenoids, steroids, reducing compounds, saponins, oses and holosides. Cytotoxity test showed that those extracts were free of toxicity. The extracts showed anti-hyperglycemic activities doses and time depending. The effective doses are 500 mg/kg for the aqueous extract and 1000 mg/kg for the ethanolic extract. The extracts are effective as compared with glibenclamide (reference product). Moreover, the *ex vivo* test conducted on the liver revealed that *V. comosa* aqueous extract inhibits the hepatic glucose liberation and 500 mg/mL is the most effective dose. The results of this study account for the plant under consideration of its traditional usage in the treatment of diabetes.

Keywords: *Vigna comosa*, extracts, phytochemistry, cytotoxicity, hyperglycemia, hepatic glucose.

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INTRODUCTION

Diabetes is a serious metabolic disorder that increasingly threatens public health in the world [1].The world prevalence of diabetes in 2010 was 6.4% among adults, affecting 285 million people [2]. It is projected that this number would increase to 7.7% in 2030, affecting a total of 439 million by then. About 7 million Africans are mellitus diabetic patients, including 3.3 million in West Africa [3].

In Benin Republic, the prevalence of diabetes was 2.9 % in 2011 [4] and 5.1% in 2016 [5]. Given current soaring levels of diabetic patients, there is an urgent need to take suitable measures for a better approach to combating this disease.

Furthermore, due to the high cost of modern medicines and considering the fact that so many people worldwide cannot afford them, a great number of diabetic patients resort to traditional plant usage. *Vigna comosa* Baker (*V. comosa)* is one of those plants used by Beninese Oueme department's population for the treatment of diabetes without any scientific study showing its effectiveness. It is clear that many traditional healers base their diagnosis and treatment on purely empirical basis, often without any therapeutic efficacy [6]. To remedy this traditional practice, it is essential to conduct a scientific research in the field of herbal

medicine to ensure an efficient and effective treatment affordable to all social groups. It is in this light that our study was conducted to evaluate the biological properties of *V. comosa*. It is worth mentioning there was no previous research conducted on this plant.

Material and Methods

Material

Plant material

The material is composed of the aerial part (stem and leaves) of *V. comosa*, harvested at Djeregbe commune of Seme, Beninese Oueme State Department. The plant was identified and certified by the National Herbarium of Beninese University of Abomey-Calavi where voucher specimen was deposited and was given the following number: AA 6493/HNB.

Animal material

Albinos rabbits (*Oryctolagus cuniculus*) both sexes together (with non-pregnancy females) were used. Average weight was 1.5 ± 0.06 kg. They were housed in the Physiology Laboratory of effort of INJEPS under standard conditions (light and dark alternated cycle of twelve hours and room temperature was 25°C). The animals had free access to water and food.

Albinos' rabbit liver was used to conduct experiment on hepatic glucose liberation.

Methods

Aqueous and ethanolic extractions

The aqueous extract was done using 50 g of raw powder decoction in 500mL of boiling distilled water for 30 min and then filtered. The ethanolic extract was done by macerating 50 g of raw powder in 500mL of ethanol (96%) using a homogenizer for 48 h, then filtered. The product was evaporated using a Rotavapor which trademark is STUART/RE 300 then put into the Memmet oven at a temperature of 50 °C to perfect the extracts drying. Finally, the extracts were conserved at 4 °C in a refrigerator prior to the experiment.

Phytochemical analysis

Phytochemical analysis is based on precipitation reactions and differential coloring of the main chemical compounds in the plant [7].

Larval toxicity of extracts

The experiment was done on *Artemia salina* Leach larvae brine shrimps harvested after the incubation of shrimps eggs in sea water [8].

Anti-hyperglycemic activities of *Vigna Comosa* extracts

The experimental model used is that of glucose overloading [9-12].It consisted of administering orally to rabbits two grams per kg of body weight (2 g/kg) of α-(D) + glucose diluted in seven milliliters of distilled water after a prior fasting for eighteen (18) hours before the gavage. To conduct the experiment, forty (40) rabbits were divided into eight (8) groups of five (5) rabbits each (2 females and 3 males).

- Group 1:control group in the hyperglycemic condition caused by oral-dose of glucose with 2 g/kg body weight (D) + glucose in 7 mL of distilled water.
- Group 2: standard experiment group received 10 mg per kg of body weight of glibenclamide two (2) hours before the OGTT.
- Group 3, 4 and 5: received oral-dose of 500 mg/kg, 1000 mg/kg and 1500 mg/kg body weight of aqueous extracts of *V. comosa* (VCBA) respectively.
- Group 6, 7 and 8: received oral-dose of 500 mg/kg, 1000 mg/kg, and 1500 mg/kg body weight of the ethanolic extracts of *V. comosa* (VCBE) respectively.
- The glycaemia is observed for five (5) hours. Just after the gavage (t = 0), glucose level was measured and at 30, 60, 120, 180, 240, and 300 min after the OGTT.

A SD CHECK GOLD Blood glucose monitoring system glucometer (Standard Diagnostics, Inc. Corea) was used for the measurement. Blood samples were collected by puncture from the marginal vein of the rabbit's left ear.

Ex vivo study of the effect of *V. comosa* aqueous extract on Hepatic glucose liberation

The hepatic glucose liberation test was done on aqueous extract of *V. comosa* because only given its effectiveness on OGTT and its usage by the population.

The washed liver method of Claude Bernard (1955) was used and was implemented and improved by other researchers [13-15]. Actually, an anesthetic albinos' rabbit was sacrificed and its liver was immediately removed, weighted and put into a flask containing Mac Ewen physiologic solution. The liver was then cut into pieces of averagely 300 mg, washed in Mac Ewen solution again. The pieces were divided into different solutions:

- Solution A (control solution): 1 mL of Mac Ewen solution;
- Solution B: 1mL Mac Ewen solution + 2.5 UI/mL insulin solution to 100 UI/mL;
- Solution C: 1mL Mac Ewen solution +62.5 mg/mL of aqueous extract (VCBA 62.5 mg/mL);
- Solution D: 1mL Mac Ewen solution +125 mg/mL of aqueous extract (VCBA 125 mg/mL);
- Solution E: 1mL Mac Ewen solution +250 mg/mL of aqueous extract (VCBA 250 mg/mL);
- Solution F: 1mL Mac Ewen solution +500 mg/mL of aqueous extract (VCBA 500 mg/mL).

All the solutions were incubated in Bain-Marie Thermostat 37 °C throughout the experiment. After 20 min of incubation, the glucose level was measured by enzymatic method based on the oxidation of glucose for each time t = 0, 10, 20, 30, 40, 50 and 60 min with Biomate spectrophotometer Thermo Spectonic Genesys 6

(Rochester NY USA) at 500 nm. The glucose concentration for the sample was calculated using the following formula:

Glucose concentration = (DO sample/DO glucose standard) x 1 g/L.

The experimental protocols have been approved by Benin Institute of Applied Biomedical Science Ethical Committee.

Statistical analysis

Data from the experiments are presented as mean ± standard error of the mean (X ± SEM). The statistical analysis of results is done using the STATISTICA software 5.5 STAT Soft Inc. A statistical analysis of mean is conducted following One-Way ANOVA "t- tests for paired samples "and" t-tests for independent samples." A level of $p < 0.05$ was considered significant.

RESULTS

Extraction yields

Extraction yield was 6.22 and 15.10% respectively for the ethanolic and aqueous extracts.

Phytochemical analysis

Phytochemical analysis of *V. comosa* raw powder showed the presence of various chemical compounds as presented in the following table I.

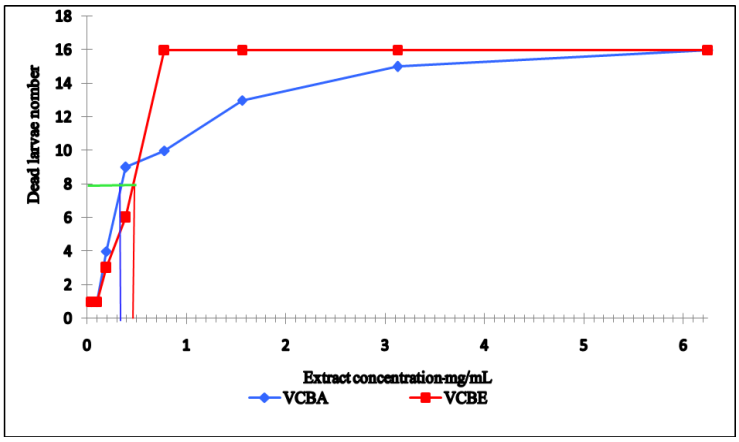
Table 1: Results of phytochemical screening

Chemical compounds	Results
Alkaloïds	+
Tannins	+
Catechiques Tannins	+
Galliques Tannins	+
Flavonoids	+
Anthocyanin	-
Leuco-Anthocyanin	-
Saponosids	+
Steroids	+
Triterpenoids	+
Quinonics compounds	-
Cyanogenics compounds	-
Mucilages	+
Coumarin	-
Reducing compounds	+
Oses and Holosides	+
HeterosideCardiotonics	-
Cardenolids	-
Free Anthracenes	-
Combined Anthracenes (C-Heterosids)	+
Combined Anthracenes (O-Heterosids)	-
Narcotics	-
Opium	-
Water content	11,50 %

- : Absent, +÷present

Larval toxicity

Toxicity test conducted using *Artemia salina* enabled us to have lethal concentrations in which half of the larvae were dead (LC_{50}): 0.35mg/mL for aqueous extract and 0.47 mg/mL for ethanolic extract of *V. comosa* (figure 1).



VCBA: V. comosa aqueous extract;*VCBE: V. comosa ethanolic extract; LC_{50}: lethal concentration of half of the larvae*

Figure 1: Larval mortality variation based on the concentration of *Vigna comosa* aqueous and ethanolic extracts(N = 3), LC_{50} (mg/mL): VCBA = 0.35 ; VCBE = 0.47

Anti-hyperglycemic activities of *Vigna comosa* extracts

The changes caused by OGTT and the effects of various extracts over five hour's bloc are showed in figures 2 and 3.The administration through oral route of glucose to the control group of rabbits caused a significant increase of glucose level on the 60th min as compared to the initial data (1.61 ± 0.05 compared with 0.90 ± 0.02 g/L; p = 0.004). From the 120th min, the glucose level decreased significantly (1.28 g/L à p = 0.005) as compared to the one of the 60th min. This decrease remained consistent up to the 300th min (p = 0.004) (figures 2 and 3).

The group of rabbits to which we administered glibenclamide reached its highest level of glucose on the 30th min (1.53 ± 0.04 g/L; p = 0.00003). Compared with the control group, anti-hyperglycemic effect started on the 60th min and reached its highest level on the 120th min (0.98 g/L à; p = 0.0007). The decrease continued gradually up to the 300th min (0.78 g/L with p = 0.02) (figures 2 and 3).

For the *V. comosa* aqueous extract, the three doses (500, 1000 and 1500 mg/kg) caused a significant decrease of the glucose level after the OGTT (figure 2). When comparing the anti-hyperglycemic effects of the three doses, it appears that the lowest effective dose was 500 mg/kg body weight. Compared to the control group, the dose of 500 mg/kg showed highly significant differences from the 60th min to the 300th min ($0.0000001 \leq p \leq 0.01$). Anti-hyperglycemic effect of the same dose of the extract compared to glibenclamide showed a significant difference only on the 240th min.

As far as the ethanolic extract of *V. comosa* is concerned, the results of the experiment showed that the dose of 1000 mg/kg was the most active (figure3). Compared to the control group, the highest differences ($0.00006 \leq p \leq 0.02$) appeared from the 60th min to the 300th min. When comparing, the anti-hyperglycemic effect of this dose of 1000 mg/kg to the glibenclamide, the significant difference (p = 0.02) appeared only on the 240th min. A comparative analysis of the anti-hyperglycemic effects of the aqueous (500 mg/kg) and ethanolic (1000 mg/kg) extract of *V. comosa* showed no significant difference.

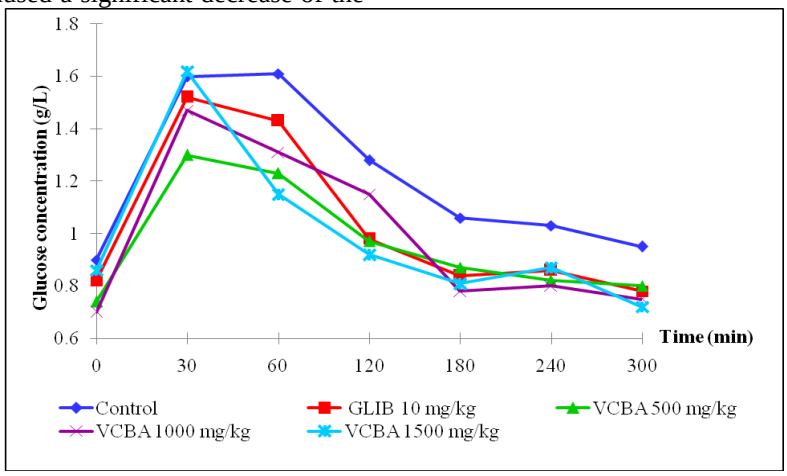


Figure 2: Effect of *Vigna comosa* aqueous extract on glycaemia evolution, Data are presented as mean ± standard error of the mean (X ± SEM). p< 0.05 as compared to the control group (ANOVA) N = 5 per group,

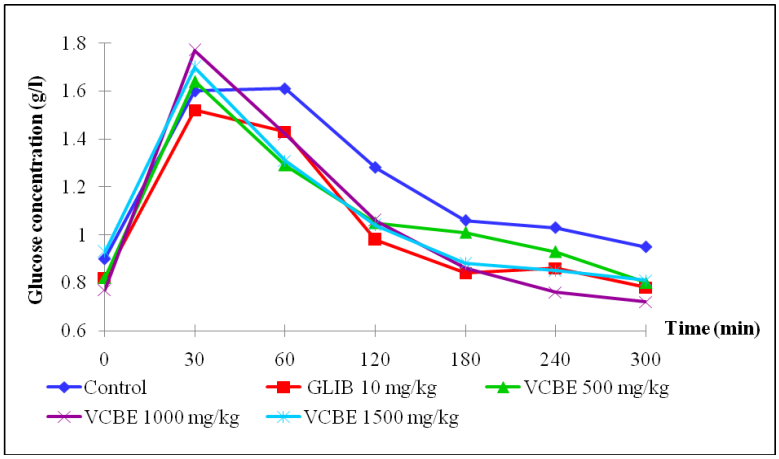


Figure 3: Effect of *Vigna comosa* ethanolic extract on glycaemia evolution, Data are presented as mean ± standard error of the mean (X ± SEM). P < 0.05 as compared with the control group (ANOVA) N = 5 per group.

The effect of *Vigna Comosa* aqueous extract on the hepatic glucose liberation.

Average values of the liberated glucose in extract solutions of different concentrations of *V. comosa* are presented in the following concentration-time curves (figure 4):

While observing the curves, it appeared that the control solution curve was virtually linear for the glucose concentration and rose throughout the experiment time. The variation rate compared with the initial glucose value increased from 136.98% in the 10th min to 298.48% in the 60th min for the control solution. As for insulin 2.5 IU, lower rates of variation occurred with a non-significant decrease 0.78% as compared with the control solution in the 10th min. As for the aqueous extract of *V. comosa*, all doses significantly inhibited the release of glucose from the liver ($0.000 \leq p \leq 0.009$) as compared with the control solution and insulin. Except for the dose of 62.5 mg/mL which showed its highest percentage (109.20%) of decrease in the 10th min, all the other doses: 125; 250 and 500 mg / mL showed the highest percentage respectively 164.52; 311.37 and 349.19% inhibition at 0. The 500 mg/mL dose was the most effective. The effect of aqueous extract of *V. comosa* is dose dependent.

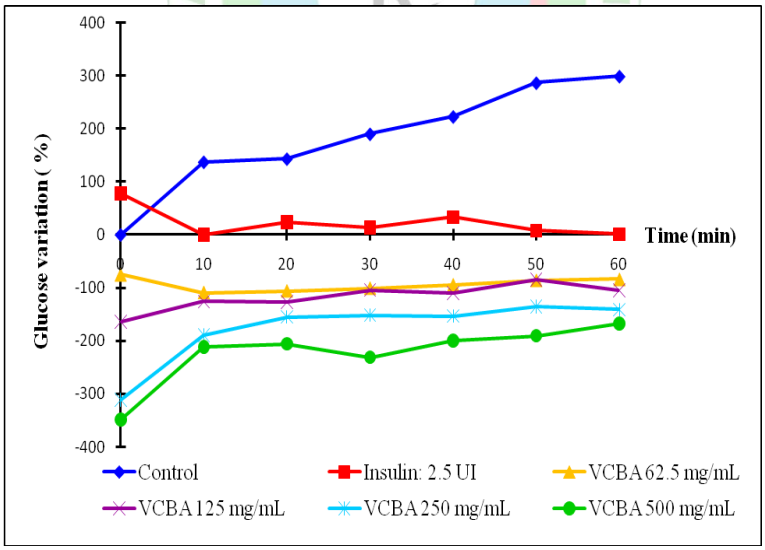


Figure 4: Effect of *Vigna comosa* aqueous extract on the hepatic glucose liberation (mean of triplicate)

4. DISCUSSION

The objective of the treatment for the diabetic patients is to reduce the glucose level as quickly as possible and to avoid or delay the complications of the disease as well. The present study aimed to examine the effects of *V. comosa* extracts on OGTT and on hepatic glucose liberation**.** The results showed that aqueous extraction yielded better than ethanolic one. This could be due to the fact that water extracted more chemical compounds than ethanol. Phytochemical screening showed the presence of several chemical compounds in *V. comosa* raw powder: alkaloids, tannins, flavonoids, mucilages, triterpenoids, steroids, reducing compounds, saponosids, anthracenes compounds (C-Heterosids) oses and holosides. Based on the results of the experiment, *V. comosa* contained most of the chemical compounds found in other plants that had been scientifically proven to have anti-hyperglycaemic or

antidiabetic activities [10-13]. Furthermore, considering the biological properties of these chemical compounds, we can conclude that *V. comosa*is indicated for the treatment of hyperglycaemia and to prevent or cure some of the chronic complications of diabetes. Actually, the alkaloids have sympatholytic actions on autonomic nervous system, and are a central nervous system stimulant etc[16]. Diabetes complications such as diabetic retinopathy, and diabetic foot can be prevented or corrected by the anti-oedematous, anti- oxidant and anti-inflammatory action of the flavonoids, saponoids, steroids and triterpenoids[16]. Mucilages have laxative actions [16].

As far as the toxicity test is concerned, there is proven evidence of correlation between larval toxicity and human cell cytotoxicity, especially the ones of colon carcinoma (HT-29), lung carcinoma (A-549), and human nasopharyngeal carcinoma (9PS and 9kB) [17]. The results of larval toxicity test of this study showed that the LC_{50} values are superior to 0.1 mg/mL, the value above which the extract is not toxic [18]. Based on these results, we can say that *V. comosa* aqueous and ethanolic extracts are not toxic and can be used as decoction or maceration.

For the anti-hyperglycaemic effect test, the control group (having hyperglycaemia) showed a significant decrease of glycaemia on the 120th min. We can say that rabbit organism naturally resorbs the extra level of glucose. This result is similar to the ones of other studies conducted by several researchers who investigated on other plants using rabbit on one hand [10-12] and Wistar rats on the other hand [19]. *V. comosa* extracts showed highly significant differences as compared to the control group throughout the experiment. However, the anti-hyperglycaemic activity of the effective dose of *V.comosa* aqueous extract ((500 mg/kg per body weight) compared to glibenclamide activity showed significant difference only on the two-hundred and fortieth minute. Furthermore, ethanolic extract at the dose of 1000 mg/kg showed no significant difference as compared to glibenclamide action. Taking into account all these results, we can say that aqueous extract is more effective at lower dose than ethanolic extract. The effectiveness of the aqueous extract could be due to the capacity of water to extract chemical compounds responsible for the anti-hyperglycaemic activity. As a matter of fact, most of the chemical compounds that *V. comosa* contains are water soluble and could be responsible for the observed activities and this is the reason why tradipractitioners use aqueous extract [10-20]. The best yield achieved for the aqueous extraction could be due to the water solubility of the chemical compounds as well. However, the low activity observed with the ethanolic extract could be due to differences between active ingredients extracted by this solvent. These results are in concordance with the findings of researches conducted using the mixture of leaves and bark of *Casuarinaequisetifolia* which showed that hydroethanolic extract has a low anti-hyperglycaemic activity compared to aqueous extract [10]. Chronic hyperglycaemia that diabetic patients suffer from is due to insulin secretion deficit exposing them to long-term complications, affecting several organs of their body. Glibenclamide is a hypoglycaemic sulphonamide (used by diabetic patients) that causes an increase in insulin secretion by pancreas cell β. Furthermore, *V. comosa* extracts had therapeutic effects similar to the one of glibenclamide. Consequently, because these extracts are rich in biological active chemical compounds which could increase insulin secretion. In the same way, phenolic extracts of four *Vigna* species (*Vigna radiata*, *Vignaa conitifoia*, black and red seeds of *Vigna Angularis*) also showed distinct variations in enzyme inhibition related to hyperglycaemia and hyperlipidaemia[21]. A study conducted in South Korea revealed that *Vigna nakasshimae* extract has hypoglycaemic and hypolipidemic effects. These effects occur through inhibition of glucosidase activity and endoplasmic reticulum stress[22]. Another research study in China on azuki beans (*Vigna angularis*) suggested that this specific species is a good source of phenolic compounds, and when the plant is grown at high altitude it may be an effective way to enhance the antioxidant and antidiabetic potential of the beans [23]. Moreover, in Japan another study reported that 40% ethanol fraction of hot-water extracts of azuki (*Vigna angularis*) suppressed the post prandial blood glucose level and serum insulin level in normal mice and streptozotocin -induced type 1 diabetic rat [24]. In Benin Republic, a study conducted on *Eleaisguineensis Jacq (arecaceae*) has been proven that flavonoid extract was the most active in OGTT [25].

The hepatic glucose liberation test showed that the control solution (incubated pieces of liver in Mac Ewen solution) presented a linear curb expressing a continued liberation of glucose on time basis. There has then been a movement of glucose from the inside towards the outside of hepatocytes. This result is similar to the ones of Claude Bernard (1955) and other researchers [13-15].And confirms that the liver has the capacity to store glucose in the form of glycogen at postprandial and to liberate it in case of deficit. However, in the experimental groups, all dose (62.5; 125; 250 and 500 mg/mL) of the aqueous extract of *V. comosa*, inhibited significantly the hepatic glucose liberation as compared with the control group and insulin. This inhibition could be due to the decrease of glycogen phosphorylase or glucose-6-phosphatase enzyme-cleavable by aqueous extract. The presence of several chemical compounds, especially of flavonoids could account for the observed reaction. It has been proven that flavonoid extract of *Eleaisguineensis Jacq (arecaceae*) inhibits significantly the hepatic glucose liberation at 135.45 % [25]. The findings of this study support the traditional use of *V. comosa* by the population to treat diabetes.

5. CONCLUSION

The phytochemical screening revealed that the raw powder of *V. comosa* contains a lot of chemical compounds which give to the plant therapeutic virtues. The results of the larval toxicity test support the safety of aqueous and ethanolic extracts.*V. comosa* extracts inhibited significantly hyperglycaemia and hepatic glucose liberation. Future researches will be carried out in order to study other therapeutic virtues and put more emphasis on the plant to relieve diabetic patients.

6. ACKNOWLEDGMENTS

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