Evaluation of the Toxicity of *Hemizygia bracteosa* (Benth) Plant Used in Traditional Medicine for the Treatment of Diabetes Mellitus in Benin

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Abstract This study aims to assess the toxicity of *Hemizygia bracteosa* (Benth), a medicinal plant used for the treatment of diabetes mellitus in Benin. Wistar rats were force-fed by using solutions obtained by dissolving the powder of this plant in distilled water. The assessment of biochemical parameters (blood glucose, triglycerides, cholesterol, HDL cholesterol, proteins) of Wistar rat serum was used to highlight the hypoglycaemic properties of this plant and its effects on cardiovascular risk factors. The results showed an increase in HDL-cholesterol levels (0.13 ± 0.004 vs 0.48 ± 0.15) and a decrease in total cholesterol levels (1.06 ± 0.40 vs 0.81 ± 0.12), triglycerides (1.2 ± 0.43 vs 1.18 ± 0.38), glucose (1.60 ± 0.25 vs 0.98 ± 0.24) and proteins (95. 33 ± 20.59 vs 58.30 ± 6.26) in rats serum after one month treatment. No significant differences were observed in control rats for all parameters. Our data also shows that at concentrations from 2 g/kg this plant appears to present significant toxicity on kidneys and liver. The use of this plant for diabetes treatment must be done with caution and doses must be controlled.

Keywords: diabetes mellitus, traditional medicine, toxicity, *Hemizygia bracteosa* (Benth), Benin


1. Introduction

The use of medicinal plants for the treatment of diseases or phytotherapy is the oldest therapy. Medicinal plants have a long history in the treatment of various diseases [1]. Their use has long been practiced by men and continues to be practiced today [2]. About 35,000 species of plants are used in the worldwide for medicinal purposes, which are the largest range of biodiversity used by humans [3]. 20 years ago, the WHO recognized the importance of traditional medicine and had proposed its incorporation in official health systems, especially in developing countries (ref). In 2000, WHO estimates that 80% of the world's inhabitants are using this medicine for primary health care needs [4]. Medicinal plants still play an important role despite the growing influence of the modern health system. Currently, the global market for herbal medicines is estimated at nearly 62 billion dollars and the demand continues to grow [5]. Medicinal plants have been used traditionally to treat many diseases including cardiovascular diseases (diabetes, hypertension). These diseases now represent real public health problem because of their increasing prevalence,. Indeed, the global prevalence of diabetes for all age groups was estimated at 2.8% in 2000 (171 million of people), with a projection of 4.4%; 366 million people in 2030 [6]. This disease is the sixth leading cause of death in under developed countries by an abnormality in the metabolism of sugars related to a blood insulin lower level resistance either organ receptors of this hormone [7]. According to author and al [8], the first generation of medicinal plants was used in crude form as therapeutic agents more or less on the basis of empirical studies. The use of medicinal plants is based on scientific processes that consisted to isolate their active ingredient for drugs manufacturing (ref). The high cost and inaccessibility of these drugs bring most patients use plants by self-medications or by consulting herbalists. These plants certainly relieve some patients but the
therapeutic doses and toxicity are not well defined what can cause other pathologies in the long term. Plants used in traditional medicine contain a variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids that have shown beneficial properties on the body. This is the case of *Hemizygia bracteosa* (Benth) [9] plant of Ocimeae Labiatae family [10,11]. Initially recognized as section Ocimum Bentham, *Hemizygia bracteosa* (Benth) was included in the West African heritage in the name of Orthosiphon bracteosus by authors of Hutchinson et al. [12] Because of its therapeutic properties, *Hemizygia bracteosa* (Benth) begins by being specifically used in the treatment of diabetes and certain cardiovascular diseases in Benin. However, its use as a therapeutic agent in the treatment of these diseases is based on empirical observations and is not supported by any scientific study. In addition, the promotion of endogenous values in the field of African herbal medicine in general and Benin in particular requires a scientific knowledge of the safety of plants and their biological effects. The present study have evaluated the effectiveness of *Hemizygia bracteosa* (Benth) in the treatment of diabetes and cardiovascular disease in order to promote its use.

2. Material and Methods

2.1. Preparation of Aqueous Extract of *H. bracteosa*.

Whole plant of *Hemizygia bracteosa* (Benth) was collected in Savalou (Central region of Benin, Latitude: 7.93333, Longitude: 1.96667) and identified by specialists in the National Herbarium of Benin. They were dried in the laboratory at 22°C before being sprayed. The aqueous extract of *H. bracteosa* was obtained by maceration. 100g of its powder were introduced in a 500 ml flask containing 500 ml of water (50/50). The bottle was capped and stirred continuously for 72 hours. After filtration, the extracts were evaporated to dryness at 60 °C using a Heidolph-type rotary evaporator before being dried in an oven at 50 °C until obtaining a dried extract.

2.2. Processing of Biological Material

Wistar rats acclimated to ambient conditions were obtained at the pet of the Unit of Education and Human Biology Research of the Faculty of Health Sciences, University of Abomey-Calavi. Before first gavage by 100 mg and 250mg per Kg of body weight, rats were subjected on an empty stomach for 12 hours. The solution used for the feeding was obtained from the dissolution *Hemizygia bracteosa* (Benth) extract in sterile distilled water. After gavage, rats were observed immediately, during 45 minutes. Rat’s weight was then measured every three days (J1, J4, J7, J10, J14 and J30). After the 30th day of treatment rats were kept under observation for five days, and sacrificed. Blood sample was collected for biochemical assays. In the same, a second gavage was done to analysis toxicity effect on rats by feeding other groups with one dose of 2g and 3g per Kg of body weight and kept for 14 days. The 14th day, liver and kidneys were collected, and immediately placed in formalin for histological analysis.

All biochemical parameters were determined using ELITECH Clinical Systems SAS.

2.3. Biochemical Parameters

2.3.1. Blood Glucose Assay with Glucose Oxidase

Blood glucose was performed by colorimetric assay. From the stock glucose solution at 5.5 mmol / L, diluted solutions were prepared at 1/2, 1/4, 1/8, 1/16 and 1/32. Then, 30 mL of each solution of glucose were added. Distilled water was used as control. Tubes were kept in a water bath for 10 min at 37 °C before measuring the absorbance with spectrophotometer at 500 nm. The standard curve A = f ([glucose]) was plotted. The same procedure was performed with the serum to obtain blood glucose. Concentration was calculated with the formula:

$$C = \frac{(OD \text{ sample})}{(OD \text{ standard})} \times C \text{ standard}.$$

2.3.2. Triglycerides Assay

Triglycerides were assayed according method reported by Fossati et al. [13]. The glycerol released after hydrolysis of triglycerides by lipoprotein lipase was converted to glycerol-3-phosphate glycerokinase. Glycerol 3-phosphate was subjected to the action of the glycerol phosphate oxidase to form dehydroxyacetone phosphate and hydrogen peroxide. The latter in presence of peroxidase oxidizes a chromogen group 4-aminoantipyrine / phenol to form a colored compound in red. Absorbance was read at 500 nm.

2.3.3. Triglycerides Assay

Cholesterol esters were hydrolyzed by a cholesterol ester hydrolyase into fatty acid and cholesterol. Both were then oxidized by cholesterol oxidase to A4-cholestenone and hydrogen peroxide. The latter, in the presence of peroxidase, oxidizes the chromogen 4-amino-antipyrine in a red colored compound of maximum absorbance at 505 nm.

2.3.4. HDL-Cholesterol Assay

LDL-cholesterol was precipitated by a haemolysis tube in which 50 µL of reagent A (ELITECH Kit) and 500µL of serum were introduced. The sample was mixed by vortex and let for 10 minutes and then centrifuged at 4000 rpm/ min for 15 min. After centrifugation, the cholesterol associated with high density lipoprotein (HDL)-cholesterol was assayed in 50 µL of supernatant which was added to 1000µL of reagent A (ELITECH Kit). The optical density of the mixture was read with spectrophotometer at 500 nm after 5min of incubation at 37°C. LDL-cholesterol was estimated by the following formula: LDL-cholesterol (g / L) = total cholesterol (g / L) –HDL-cholesterol (g / L) – triglycerides (g / L) / 5 if triglycerides are less than 3.4 g /L.

2.3.5. Protein Assay

Protein assay was performed by the Biuret method as reported by [14]. In alkaline solution, the proteins form with the cupric ions a colored complex with absorbance measured at 540 nm. The determination of different concentrations was done using a calibration curve of human serum albumin.
2.4. Histological Study Removed Organs

Toxicity was investigated according to the Organization for Economic Cooperation and Development (OECD) recommendations guidelines for testing of chemicals products [15]. Histological study includes: the preparation of cassettes and tissue fixation, circulation, coating, cutting microtome, spreading, staining and mounting.

2.4.1. Preparation of Cassettes And Tissue Fixation

The organs from the dissection were cut into small pieces, placed in cassettes for their fixing. The choice of the fixator was focused on 10% formalin buffered morphic studies, easy to prepare and stable. In addition, it penetrates well the tissue. The duration of fixation was 12 days for whole organs and 3 days for the small cuts.

2.4.2. The Circulation

It consisted in staying pieces of tissue in a series of liquids to give them a favourable rigidity thin section. It takes place in three (03) phases: dehydration, clarification and impregnation.

- Dehydration was done gradually by introducing tissues parts in increasing concentrations of alcohol baths (70 °, 80 °, 100 °, 100 °, 100 °, 100 ° and 100 °). Tissues have stayed for an hour in each bathroom.

- Three xylene baths were used to enlightenment in a chemical fume hood. Once the first xylene bath begins to become cloudy (reflecting an incomplete dehydration of tissues), the tissue was removed and immediately incubated 45 minutes in each bathroom.

- The impregnation was carried out with three (03) paraffin bath preheated to facilitate its penetration into the tissue. Tissue stayed about one (01) hours in each bathroom.

2.4.3. Coating

Impregnated tissues were paraffin-coated for obtaining blocks. For this, they were deposited and oriented in metal mussel so as to facilitate further study. Then the melted wax is poured into the mussel used as support for blocks. For this, they were deposited and oriented in metal mussel so as to facilitate further study. Then the melted wax is poured into the mussel used as support for blocks. After solidification, paraffin block was separated from the mussel and was ready to be cut with a microtome.

2.4.5. The Microtome

The paraffin blocks containing the coated pieces are cut into slices of 5 mm thickness on a microtome brand AO Scientific Instruments 820. For this, the block inserted into the clamps and immobilized by the locking screw is oriented using the adjustment screw making sure that the lower and upper faces of the block are parallel with the edge of the razor. After checking the parallelism between the block and the razor we turn the driving wheel of the instrument with the crank. The strips obtained at the cutting were recovered using brushes and spread on a black background plate.

2.4.6. Spreading

Spreading the cuts on microscope slides was done with a warm plate set at 45 °C. Thus, clean, etched plates are covered with water with glyc erine albumin. Highly successful ribbon segments were then selected using two lancets and arranged on the slides. The whole was placed on the plate and ribbon was allowed to stretch. Using two (02) lancets the edges of the tissue were pulled to eliminate persistent folds. Excess fluid was absorbed with an absorbent paper and allowed to adhere the ribbons to the blades. The latter were then placed in an oven at 40°C for one (01) hour after drying before cooling 15 minutes at least. The preparation was now ready for staining.

2.4.7. Staining

Staining was performed with haematoxylin-eosin which enables to identify the nucleus, the cytoplasm and the collagen. Haematin, basic dye binds to nuclear structures while eosin is an acid dye having an affinity for the cytoplasm and the collagen fibers.

2.4.8. Microscopic Observation and Photomicrography

Microscopic observations were made on a brand light microscope Olympus BX-41 with a JVC camera ½ inches. Pictures were transferred to image processing software (Adobe Photoshop Image) by a scanning device. The sections were observed at magnifications of 100 and 400; image are captured by the X40 lens and converted to JPEG data format.

2.5. Processing and Analysis of Data

The results were organized using Microsoft Excel 2007 and treated with Minitab 16 for the comparison of means and the analysis of variance (ANOVA). The test was considered statistically significant if p <0.05.

| Table 1. Effect of aqueous extract of H. bracteosa on biochemical parameters of rats |
|----------------------------------|-------------------|------------------|-------------------|-------------------|
| Samples Parameters              | Controls          | 100mg/Kg of weight | 250/Kg of weight  |
| Blood sugar                      |                   |                   |                   |
| Day 0                            | 1.42±0.25a        | 1.96±0.20a        | 1.60±0.25a        |
| Day 30                           | 1.60±0.25a        | 1.60±0.25a        | 1.61±0.15a        |
| p-v                              | 0.699             | 0.003             | 0.001             |
| Protein                          |                   |                   |                   |
| Day 0                            | 82.84±16.13a      | 83.02±16.25a      | 95.33±20.59a      |
| Day 30                           | 95.33±20.59a      | 83.02±16.25a      | 58.30±6.26b       |
| p-v                              | 0.986             | 0.005             | 0.001             |
| Total cholesterol                |                   |                   |                   |
| Day 0                            | 1.28±0.33a        | 1.29±0.33a        | 1.06±0.40a        |
| Day 30                           | 1.06±0.40a        | 1.06±0.40a        | 0.81±0.12a        |
| p-v                              | 0.994             | 0.223             | 0.946             |
| HDL -Cholesterol                 |                   |                   |                   |
| Day 0                            | 0.40±0.16a        | 0.47±0.15a        | 0.13±0.004a       |
| Day 30                           | 0.13±0.004a       | 0.13±0.004a       | 0.48±0.15b        |
| p-v                              | 0.991             | 0.001             | 0.43±0.09b        |
| Triglyceride                     |                   |                   |                   |
| Day 0                            | 1.10±0.43a        | 1.17±0.48a        | 1.2±0.43a         |
| Day 30                           | 1.2±0.43a         | 1.2±0.43a         | 1.18±0.38a        |
| p-v                              | 0.785             | 0.718             | 0.033             |

p-v = p-value.
3. Result

3.1. Effect of *Hemizygia bracteosa* (Benth) Powder on Biochemical Parameters of Rats

Results showed an increase in HDL-cholesterol and a decrease in total cholesterol contents, triglycerides, glucose and proteins in fed rats. No significant difference was observed in controlled rats for all parameters as shown in Table 1.

3.2. Effect of *Hemizygia bracteosa* (Benth) Powder on Liver and Kidney

![Figure 1](image1.png)

**Figure 1.** Normal liver lobule of Wistar rat (HE × 400) showing liver bays separated by sinusoids and arranged around a central vein (V)

![Figure 2](image2.png)

**Figure 2.** Hepatic lobules of Wistar rats treated with various doses of *H. bracteosa* (HE x 400)

Although the liver architecture was generally preserved at 2 g / kg (A), it should be noted the appearance at 3 g / kg (B) an hepatocyte necrosis signs marked by homogenizing and acidophilia of the cytoplasm and nuclei disappearance.

![Figure 3](image3.png)

**Figure 3.** Normal Renal Cortex of Wistar rat (HE × 400) showing renal glomeruli containing the glomerular capillaries and renal tubules

Cortical necrosis was observed with hyalinization of glomeruli giving an image of "glomerulus in sealing wafers "and a loss of light kidney channels.

![Figure 4](image4.png)

**Figure 4.** Renal Cortex Wistar rats treated with 2 g / kg (A) and 3 g / kg (B) *H. bracteosa* (HE x 400).

4. Discussion

Very few studies have been reported on the *H. bracteosa* [9,16]; so that this plant could be considered as a neglected and underutilized species. The administration of this plant caused a significant decrease (p <0.05) in blood glucose and triglycerides in mice while no significant difference was observed in the control mice. At 100 mg /
Kg of body weight, an increase in HDL cholesterol was observed while the total cholesterol decreases. These results prove that this plant actually has a hypoglycaemic activity; which will justify its use in the treatment of diabetes. It is not possible to identify exact mechanism of the hypoglycaemic effect [17]. However, studies on the hypoglycaemic effect of other species showed that this effect is due to an extra pancreatic mechanism, namely on sensitization of peripheral tissues in the use of glucose [18,19]. The phytochemical analysis of H. bracteosa (Benth) powder during our previous work [9] has revealed the presence of flavonoids that may be responsible for the hypoglycaemic effect observed [20,21]. Recent research has demonstrated that flavonoid glycosides were responsible [22]. But this plant could act through other mechanisms including direct activation of the insulin receptor, or stimulation of insulin secretion by the beta cells of the islets of Langerhans. Results from histological tests showed a toxicity of this plant on the kidneys at all tested concentrations and liver to 3 g / kg. The side effects of herbal medicine were often mentioned in recent years: nephrotoxicity and hepatotoxicity of Kava kava or Teucrium chamedrys, drug interactions with Hypericum perforatum etc. Traditionally, plants deemed safe and can be toxic when used on a large scale. Several factors can be incriminated: improper storage, contamination of various toxicological (heavy metals, microorganisms), presence of allopathic substance in the preparation, extraction of toxic substances or presence of excipients that could cause unexpected toxicity, spoilage during packaging, introduction of new indications, modification of the absorbed doses [23].

H. bracteosa (Benth) is commonly called “tea Savalou in Benin” as it is widely consumed as tea in this town located center of the country. The interest of patients for this plant due to the fact that the Pharmacopoeia is substantially cheaper. Furthermore, the treatment is less restrictive with respect to that of modern medicine. However, the present study demonstrates that this practice is not safe for the consumer. It will therefore accurately determine doses to ingest in order to limit the adverse effects of this practice.

5. Conclusion

In the present study, we showed the hypoglycaemic activity of Hemizygia bracteosa (Benth) on Wistar rats with hyperglycaemic condition. This study also demonstrated that certain doses of this plant are toxic for liver and kidneys. The use of this plant for the treatment of this disease should be made with caution.

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References