EFFECT OF AQUEOUS EXTRACT OF PICRALIMA NITIDA SEEDS ON THE GLYCAEMIA OF RABBITS

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Abstract: In Côte-d’Ivoire, diabetes appears like a warming affection which affects 5% of the population. This is a worrying prevalence rate. Our search for means of fighting this alarming metabolic affection made us to develop an herbal medicine made from Picralima nitida seeds, used by traditional healers of Agboville Department (Côte-d’Ivoire), to develop medicamentous receipts for purpose antidiabetic. In Laboratory, the herbal medicine was investigated for its hypoglycaemic activity in animals using glucose-induced hyperglycaemic rabbits. The rabbits, deprived of food overnight, were divided into 8 groups. Four groups of non-hyperglycaemic rabbits received distilled water. Group 3 (hyperglycaemic) received glibenclamide. Groups 4, 5 and 6 received different doses of aqueous extracts of Picralima nitida seeds. The results revealed that before administration of glucose overload, the animals had a basal glycaemia of 0.95 ± 0.06 g/l. The non treated rabbits’ glycaemia increased and reached 1.24 g/l. The oral administration of glibenclamide lowers blood sugar at 0.71 g/l. The others hyperglycaemic rabbits were given herbal medicine to drink, 0.2 ml per 20 grams of body weight, respectively at 10, 20 and 40 mg/ml. The herbal medicine exerts dose-dependant hypoglycaemic effect. At 40 mg/ml, it highlights the hypoglycaemic effect and decreases the hyperglycaemia to a value of 0.8 g/l. The effect of the herbal medicine at 40 mg/ml approximates that of glibenclamide at 0.25 mg/ml. Aqueous extract of Picralima nitida seeds possess insulin-like properties as demonstrated by its hypoglycaemic action, hence, may be good herbal extract in the management of diabetes.

Keywords: Côte-d’Ivoire, Ethnopharmacology, Hypoglycaemia, Medicinal Plants, Traditional Healers

INTRODUCTION

Diabetes is a major cause of disability and death and appears like a major public health problem1. This disorder, characterized by polyuria, glycosuria and hyperglycemia, is a widespread metabolic disorder which exists everywhere in the world and interests approximately 6% of the world population2. Côte-d’Ivoire has 5% of diabetics3. This prevalence rate places the diabetes like most frequent of endocrinien diseases4. In modern medicine, no satisfactory effective therapy is still available to cure diabetes5. If the injections of insulin or other products make it possible diabetic to remain in life, they cannot, however, make it possible to face the many abrupt fluctuations of the insulin rate which the organism needs. Moreover, the diabetes requires a life long treatment, which the patients have of the evil to support6. Currently, diabetes therapy is based on the use of hypoglycaemics (sulphamides, biguanides, insulin), on hygieno-diet measures and exercises7. Moreover, the pharmaceutical drugs are either too expensive or have undesirable side effects or contraindications8. In the search of fighting means against this metabolic disorder, ethnopharmacological investigations were conducted in Africa and in most of the developing countries9, 10, 11, 12, 13, 14, 15. Concerning the plants, these works reported their empirical antidiabetic effect. Abbey and Krobou people recognized the medicinal virtues of several species of plants they use to treat diabetes. The ethnopharmacological survey conducted in villages of Agboville Department in Côte-d’Ivoire (West Africa) made us discover that traditional healers used various species of plants to develop medicamentous receipts to treat diabetes16. Since this time, we conducted pharmacological experiences to value antidiabetic activity of some plants of this repertory17, 18, 19, 20, 21, 22.

Concerning Picralima nitida ( Stapf) T. Durand et H. Durand (Apocynaceae), we reported its empirical antidiabetic effect1, but no experimental study on its hypoglycaemic activity has been carried out. This study aims at finding new affordable therapies, inexpensive and able to normalize and stabilize the glycaemia. Its objective is to experimentally study the effect of the total aqueous extract of the plant’s seeds on the glycaemia of hyperglycaemic rabbits in order to provide scientific evidence of the effectiveness of the traditional use of Picralima nitida, as antidiabetic.

MATERIAL AND METHODS

Ethnopharmacological survey

The investigation on the traditional use of plants having antidiabetic effect was conducted among traditional healers of 14 villages, in Department of Agboville, in South of Côte-d’Ivoire15. As approach, we met the healers and organized semi-structured interviews. Each of them was met twice, at different moments, to answer the same questions. This helped us to check the informations we had already collected. During this ethnopharmacological investigation, we collected informations relating to the plants used to treat diabetes, the different parts used as drugs, their methods of
collection and the modes of preparation and administration of the medicamentous receipts. Among the 32 traditional healers interviewed, five mentioned Picralima nitida, as plant with antidiabetic effect. The plant was quoted mainly. For this reason, we chose it to lead these pharmacological experiences.

Vegetable material and preparation of plant extract

The drug (seeds of Picralima nitida), used in this study, were collected, freshly, within the village of Aboudé-Mandéké in Department of Agboville and rinsed then dried for 3 weeks in the shade, well-ventilated place, to avoid contamination by mould. From the collected samples and specimens of the herbarium of the National Floristic Center, we identified the plant, by its scientific name. A voucher specimen (Aboudé-Mandéké, Côte-d’Ivoire, 03 March 1990, N’guessan Koffi n° 89) was deposited in National Floristic Center (Université Félix Houphouët-Boigny, Côte-d’Ivoire). One thousand (1000) grams of the drug were collected and rinsed. The drug was introduced in 4000 ml of distilled water. The mixture, bullied during 45 minutes, was wrung in a neat cloth square, filtered successively twice on absorbent cotton and on Wattman 3 mm paper. The volume of the filtrate obtained was concentrated and evaporated in a drying oven at 60°C, during 2 days. The pulverized crystals made it possible to obtain fine powder used for the experimentation. The total water extract, codified PNA, is then kept in sterilized glass bowls, hermetically closed, in a fridge.

Animal material

We used rabbits (Oryctolagus cuniculus, Leporidae) we bought in a farm located in Bingerville, suburbs of Abidjan (Côte-d’Ivoire). They were twenty four (24), with as many males as of females. These animals were old 6 to 10 weeks and weighed between 1200 and 1800 grams. They were placed in ventilated metal cages containing litters of shavings which are regularly renewed. They are acclimatized to the conditions of the animal house, during 7 days before the treatment and fed with the granules produced by the Ivorian Compound Food Manufacturing Society (F.A.C.I.). We used distilled water. Rabbits of batches 2, 3, 4, 5 and 6, received, orally, a solution of glucose (4 g/l), a hyperglycaemic product. The rabbits were divided into 8 batches of 3, as follows:

- batch 1: sample rabbits with normal glycaemia  
- batch 2: sample hyperglycaemic rabbits untreated  
- batch 3: hyperglycaemic rabbits treated with glibenclamide at 0.25 mg/ml  
- batch 4: hyperglycaemic rabbits treated with herbal medicine at 40 mg/ml  
- batch 5: hyperglycaemic rabbits treated with herbal medicine at 20 mg/ml  
- batch 6: hyperglycaemic rabbits treated with herbal medicine at 10 mg/ml  
- batch 7: normal glycaemic rabbits treated with glibenclamide at 0.25 mg/ml  
- batch 8: normal glycaemic rabbits treated with herbal medicine at 40 mg/ml.

Technical material

Pasteur pipettes were used to take blood samples we collected in hemolysis tubes. Electrical scales for weighing powders were needed. The glycaemia level determination device included a spectrophotometer of KENZA type.

Chemicals

Surgical spirit was necessary to treat injured rabbits. The glucose, a glucidic agent, known to cause hyperglycaemia, was used. For the treatment of induced hyperglycaemic rabbits of control group, we used glibenclamide (DAONIL tablet, 5mg), the reference product having hypoglycaemic effect. Oxalate of sodium and sodium fluoride was needed in order to stabilize the process of glycolysis in blood. To carry out the phytochemical screening, we used, as solvent, distilled water and various classic reagents. Classical methods described in the works of Ronchetti and Russo\textsuperscript{23}, Hegnauer\textsuperscript{24}, Wagner\textsuperscript{25} and Békro \textit{et al.}\textsuperscript{26}, were used to characterize the chemical groups.

Induction of hyperglycaemia

We used glucose whose hyperglycaemic property is established with rabbits\textsuperscript{2, 3, 16, 21, 22}. The glucose absorption depends on the body weight of the animal: 0.2 ml of glucose for 20 g of rabbit weight. Except rabbits of batches 1, 7 and 8, all the animals received a glucose overload at the moment T=0, after basal glycaemia determination. The administration of glucose (4 g/l) is done by oral way, with a nozzle of intubation.

Treatment of hyperglycaemic rabbits with glibenclamide and herbal medicine

The rabbits of batch 3 are treated with glibenclamide (5 mg/20 ml distilled water, that to say 0.25 mg/ml), one hour, after the cramming by glucose. The rabbits of batches 4, 5 and 6 are treated with the herbal medicine, one hour, after glucose overload. To do things like the traditional healers, the hyperglycaemic rabbits received, orally, 0.2 ml of glibenclamide and herbal medicine for 20 g of body weight.

Rabbits’ blood sampling

All the animals (rabbits) used for experimentation were deprived of food overnight. Pasteur pipettes were used to take blood samples done, intravenously, through the marginal vein of the ear, to determine glycaemia level. The blood (3 ml) is collected in hemolysis tubes containing an anticoagulant (oxalate of sodium and sodium fluoride) in order to stabilize the process of glycolysis in blood. A few minutes before treatment, the blood of all the animals was taken, for determination of the basal glycaemia. Thereafter, blood samplings were made, every hour after the treatment, according to the constituted batches. On the whole, 5 blood tests were carried out on each animal, during 4 hours.

Rabbits’ glycaemia determination

Principle of determination

The method used is that related to the enzyme\textsuperscript{3}. It consists in oxidizing glucose by the glucose oxidase, enzyme with production of gluconic acid and hydrogen pyroxyde (H\textsubscript{2}O\textsubscript{2}).
The hydrogen pyroxyde reacts with phenol and 4-amino-antipyrine in the presence of peroxidase to form a compound of red brick, quinoneimine and water. The optical density of quinoneimine to 500 nm is proportional to the concentration of glucose in the sample. Below we present the following reaction of blood sugar quantity determination.

\[
\text{Glucose + O} \quad \text{Peroxidase} \quad \text{Quinoneimine + H}_2\text{O}.
\]

**Protocol**
A milliliter of enzymatic solution works in 10 microliters of serum. The blood is centrifuged to 3500 rpm for 10 min and then the serum is collected. After a bain-marie at 37 °C, the serum is immediately analyzed with a spectrophotometer KENZA type. A reading is made with a spectrophotometer at 500 nm against the white composed of enzymatic solution. The glycaemia is then determined each hour, during experience. Below, we present formula used to calculate the glucose rate:

\[
\text{[Glucose rate (g/l)]} = \frac{X_n \text{ with } n = \text{measured value}}{D_{0 \text{ standard}}}
\]

The dosage of glycaemia has been achieved in Medical Analysis Laboratory of “Formation Sanitaire Urbaine de Ouassakara-Attié” (F.S.U.O.A.) located in Yopougon (District of Abidjan, Côte d’Ivoire).

**Data analysis**
Data on the variations of glycaemia were expressed in the form Mean ± SEM of 3 observations, on the curves we traced with the STATISTICA software. Data were analyzed statistically by one way analysis of variance ANOVA statistical test using STATISTICA version 6.05 (Windows XP) to test for significance. P < 0.05 was considered significant. We used Mauchley test to verify the condition of sphericity and Newman Keuls test for the comparison of the means (α =5 %).

**RESULTS AND DISCUSSION**

**Basal glycaemia of rabbits**
The figures 1 to 8 show the level of blood glucose in normal, hyperglycaemic control and experimental groups of rabbits. Before treatment, all the animals had a basic glycaemia of 0.95 g ± 0.06. This result on the basic glycaemia in rabbits deprived of food overnight confirms work of Kadja and those of Djédjé, on rabbits.

**Evolution of glycaemia after glucose overload**
The figure 1 reports result on rabbits of batches 1 and 2. The rabbits of batch 1 are the sample rabbits not treated by glucose (4 g/l). In this control group, the glycaemia of animals fluctuates between 0.95 and 1.02 g/l; glycaemia remains basically stable, during the experience. One hour after administration of glucose to animals of batch 2 (sample rabbits induced with glucose overload but not treated), the glycaemia rise gradually to reach 1.24 g/l ± 0.02. The glucose overload induces on rabbits a significant level of blood glucose that to say hyperglycaemia, after its administration. That confirms the property of glucose as a product able to create hyperglycaemia. After the peak of glucose (1.24 g/l), we noticed one stage in the evolution of glycaemia with the rabbits of batch 2: a decreasing phase during which glycaemia goes from 1.24 to 1.18 g/l. A fall of 5% is noted, after the glycaemic peak. At the end of the experimentation, the blood glucose value (1.18 g/l) indicates that the induced hyperglycaemia is transitory: the organism is able to restore normal glycaemia, after a glycaemic overload.

The effect of glucose, leading to hyperglycaemia, as evidenced from the significant (p<0.05) elevation of blood glucose in rabbits (fig. 1), was also been reported by several workers. This result on sample hyperglycaemic non-treated rabbits of batch 2 does not tally with that of Bosede and Oyelola. According to their works on hyperglycaemic rabbits, blood glucose level usually returns to normal within two hours after ingestion of carbohydrates in normal individuals. But, in this experience on hyperglycaemic rabbits, blood glucose reaches a high level and remains elevated for longer period of time (fig.1).

**Effects of glibenclamide on hyperglycaemic rabbits**
The administration of glibenclamide to hyperglycaemic rabbits of batch 3 shows a significant decrease in the level of blood glucose that pass from 1.24 g/l to 0.71 g/l. A fall of 42.75% is observed, 4 hours later (fig. 2). Glibenclamide exerts a hypoglycemic effect, in accordance with the results of Gharras et al. (1999) and those of N’Guessan et al. The glibenclamide contribution corrects therefore the hyperglycaemia created by glucose overload. The fixing of glibenclamide to its receptor allows the entry of glucose into the cell, preventing the accumulation of glucose in the blood that explains hyperglycaemia reduction. The glibenclamide induces a significant hypoglycaemic effect, one hour, after glucose overload. Four (4) hours after administration, the glycaemia of treated rabbits with the reference product decreases significantly but its normal value is not restored.

**Fig. 1:** Glycaemia variation histogram for sample untreated rats with normal glycaemia and sample hyperglycaemic rabbits treated with glucose (4 g/l); Mean ± SEM, n =3, P < 0.05.
**Batch 1:** sample untreated rabbits with normal glycaemia

**Batch 2:** sample hyperglycaemic rabbits treated with glucose (4 g/l)

**Batch 3:** hyperglycaemic rabbits treated with glibenclamide (0.25 mg/ml)

**Effects of herbal medicine on the glycaemia of hyperglycaemic rabbits**

The figure 3 shows the level of blood glucose in hyperglycaemic control and experiment groups. The rabbits of batch 2 are sample rabbits induced with glucose overload but not treated by hypoglycaemic products. The rabbits of batch 4 are hyperglycaemic rabbits treated with herbal medicine at 40 g/l. In this experiment group, we noticed that the herbal medicine at 40 mg/ml has a significant hypoglycaemic effect compared that of the control group. The administration of herbal medicine to hyperglycaemic rabbits of batch 4 shows a significant decrease in the level of blood glucose that pass from 1.24 g/l to 0.8 g/l, 4 hours later: herbal medicine at 40 mg/ml exerts a hypoglycemic effect.

![Graph showing glycaemia variation histogram for sample hyperglycaemic rabbits treated with glucose and herbal medicine at 40 mg/ml](image)

The use of the aqueous decoction from seeds of *Picralima nitida* revealed that it has glycaemic properties, which vary from one dose to another (Figure 4). The glycaemia of rabbits of batch 5, treated with the herbal medicine at 20 mg/ml, goes down from 1.23 g/l to 1.15 g/l, four hours later. From the peak, we observed a fall of 6.43%. Until the end of the experiment, there is no stabilization of glycaemia near its normal value. The glycaemia of rabbits of batch 6, treated with herbal medicine at 10 mg/ml, experienced a similar evolution to that of the sample hyperglycaemic rabbits non treated (rabbits of batch 2); despite treatment, the glycaemia rate which rises to 1.20 g/l, after administration of glucose, practically does not vary: herbal medicine therefore does not exert a significant hypoglycaemic effect at the subliminal dose of 10 mg/ml. A fall of 4.17% is noted, after the glycaemic peak that is comparable to result with animals of batch 2. The administration of the herbal medicine at lower doses (≤ 10 mg/ml) does not induce significant hypoglycaemic effect; at low doses, the herbal medicine would have normoglycaemic activity. The administration of the herbal medicine at 20 mg/ml lowers the hyperglycaemic effect. At 40 mg/ml, the herbal medicine highlights the hypoglycaemia level; but there is no restoration of normal glycaemia, after four hours of treatment.

![Graph showing glycaemia variation histogram for hyperglycaemic rabbits treated with herbal medicine at 40 mg/ml](image)
Batch 4: hyperglycaemic rats treated with herbal medicine at 40 mg/ml
Batch 5: hyperglycaemic rats treated with herbal medicine at 20 mg/ml
Batch 6: hyperglycaemic rats treated with herbal medicine at 10 mg/ml

However, administration of aqueous extract of Picralima nitida seeds decreased the blood glucose levels in hyperglycaemic rabbits demonstrating hypoglycaemic properties. Aqueous decoctions of medicinal plants, such as Terminalia catappa, had earlier been reported to have hypoglycaemic properties.

This experience was conducted to study the antidiabetic activity of Picralima nitida seeds in rabbits as well as to provide an introductory approach for the evaluation of its traditional preparation in order to scientifically validate the therapeutic preparation of the plant in the control of diabetes. The results show that the effect of herbal medicine at 40 mg/ml is compared to glibenclamide (0.25 mg/ml). The effect of herbal medicine was more prominent when the dose used is higher: the herbal medicine exerts dose-dependant hypoglycemic effect.

Compared effect of herbal medicine (40 mg/ml) and the glibenclamide (0.25 mg/ml)

The glycaemia of the rabbits of batch 4, treated with the herbal medicine at 40 mg/ml, decreases but does not go back to a normal level (fig.5). The fall (35.49%) is comparable to that of glibenclamide (42.90%); at a rate of 40 mg/ml, the herbal medicine has a glucose-lowering effect that approximates those of glibenclamide at 0.25 mg/ml. Herbal medicine and glibenclamide treatment to hyperglycaemic rabbits significantly reversed the level of blood glucose.

Effect of glibenclamide and herbal medicine (40 g/l) on basal glycaemia of rabbits

The figure 6 shows the level of basal blood glucose in normo-glycaemic experiment groups treated with the glibenclamide (rabbits of batch 7) and with the herbal medicine (rabbits of batch 8). The reference product exerts a significant basal glucose-lowering effect. Administration of herbal medicine induces glycaemia fluctuation between 0.95 and 1.05 g/l: glycaemia remains basically stable, during the experiment. The two substances would not exert the same effect on the insulinosecretion.

The noninsulinic treatment of diabetes utilizes oral hypoglycaemiant type of sulphamides and type of biguanides. Kwashié Eklu-Gadegbeku et al. reported that sulphamides to which belonged glibenclamide act by stimulating the secretion of insulin. The biguanides reinforce the peripheral use of glucose and appear to inhibit the glyconeogenesis. The herbal medicine would have an extra-pancreatic action by reinforcing the peripheral use of glucose, similar to that observed with the biguanides. Our findings are in agreement with those reported by Paris and Amarnath. The antidiabetic effect of PNA may be due to increase release of muscular glucose similar to that observed after biguanides administration. Administration of herbal
medicine significantly increased the muscular permeability of blood glucose in hyperglycaemic rabbits.

**Experimental validation for the medicinal activity of the plant using phytochemistry**

We performed a primary validation of the traditional medical practices, by looking for the chemical groups that explain the antidiabetic effect of the herbal medicine. Thus, *Picralima nitida* seeds were chemically screened and yielded alkaloids, saponosides, sterols, tannins and triterpenes. Among these compounds, sterols and triterpenes can be incriminated in the antidiabetic activity of the plant.

It had also been reported that the leaves of *Stachytarpheta indica* contains water soluble natural products, which directly stimulate insulin secretion. This is also in agreement with the report of N’Guessan et al. about *Terminalia catappa*. Possible hypoglycaemic effect had been reported of aqueous extract of *Picralima nitida* seeds. Alkaloids would be used as stimulatives of the hepatic glycogenogenesis. Other probable mechanisms by which the extracts lowered blood glucose level in hyperglycaemic rabbits might be by increasing glycogenesis. The significant (p<0.05) reduction in the glucose level in the hyperglycaemic rabbits group placed on water of extract of *Picralima nitida* seeds may in part be as a result of sterols and triterpenes properties. Sterols and triterpenes are recognized for their properties to decrease the rate of blood glucose. Alkaloids, sterols or triterpenes highlighted in the seeds of the plant would be responsible for the observed antidiabetic effect.

**CONCLUSION**

The data generated from this study show that hyperglycaemic rabbits were obtained using glucose. The extracts (seeds of *Picralima nitida*) affected the hyperglycaemic rabbits as evident by the significant reduction in the levels of blood glucose. Overall, the plant demonstrated to be efficacious as espoused by its hypoglycaemic actions and hence confirming the antidiabetic activity of the extract.

**REFERENCES**


