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Full Length Research Paper

Effect of aqueous extract of *Chrysophyllum cainito* leaves on the glycaemia of diabetic rabbits

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C*hrysophyllum cainito* is a plant recognized by the traditional healers of Aboudé-Mandéké, a village in the Department of Agboville (Côte-d'Ivoire), as having antidiabetic properties. The aim of this study is to evaluate, experimentally, the effect of an aqueous decoction of the plant's leaves that we called *C. cainito*, on rabbits induced with alloxane, a diabetogenic product. Different graded doses of this herbal medicine were applied on postprandial blood sugar levels of diabetic rabbits. At doses of 10 g/l, *C. cainito* does not induce the hypoglycaemic effect. A dose of 20 g/l reduced the hyperglycaemia from 5 g/l to 1.4 g/l. A dose of 30 g/l of *C. cainito* produced a graded decrease in hyperglycaemia from 6.3 g/l to 3.2 g/l. After 6 weeks of treatment, the induced diabetic rabbits stopped eating and succumbed between the 8th and the 9th weeks of experimentation. It was thus concluded that *C. cainito* leaves have glucose lowering effect at doses > 10 g/l and appears toxic and lethal at 30 g/l. *C. cainito* produces its hypoglycaemic effect mainly through alkaloids, sterols or triterpens, the antidiabetic active constituents.

Key words: Aboudé-Mandéké, alloxan, Côte-d'Ivoire, herbal medicine, traditional healer.

INTRODUCTION

Diabetes is a widespread metabolic disease that affects 6% of the world population. It is a major public health problem (Dièye et al., 2008). In Côte-d'Ivoire 3 to 7% of the population suffers from diabetes (Djédjé, 2002). This high prevalence rate makes diabetes appears like the most frequent of endocrinal diseases (Gentilini, 1993). Diabetes requires a lifelong treatment, which the patients have to put up with (Reichard et al., 1993).

In the search of fighting means, the medicinal proper-ties of many plants were recognized and used to combat this worrying disease. In this context, studies have been conducted. We mention, for examples, the works of Kadja (1998) that reported the antidiabetic properties of DIOCODA, a natural vegetable substance.

There are also those of Kwashié et al. (1998) that indicated the hypoglycaemic effect of aqueous extract prepared with the leaves of *Stereospermum kunthianum* (Bignoniaceae) . Kamtchouing et al. (2004) highlighted the hypoglycaemic effect of hexane extract of *Anacardium occidentale* (Anacardiaceae) on diabetic rats. Bhandari et al. (2005) reported that the ethanolic extract of *Zingiber officinale* (Zingiberaceae) produced significant antihyperglycaemic effect on diabetic rats. In their study, Dimo et al. (2007) reported the antidiabetic effect of *Sclerocarya birrea* (Anacardiaceae) on diabetic rats.

The ethnomedicinal survey conducted in Aboudé-Mandéké, in the Department of Agboville (Côte-d'Ivoire) made us discover that *Chrysophyllum cainito* (Sapotaceae) is used in traditional medicine to treat diabetes. Concerning the plant, some works reported its empirical antidiabetic effect (Aké- Assi, 1984; Ouattara, 2006). But no experimental study on its hypoglycaemic activity has been carried out. This study aims at finding new affordable therapies, non expensive, able to normalize and stabilize the glycaemia. Its objective is to

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Figure 1. *Chrysophyllum cainito* L. (Sapotaceae): a part of the stem with simple, entire, acuminated leaves, yellow flowers joined together in axillary fasciculs. Authenticated by Professor Aké-Assi Laurent, Laboratory of Botany, U.F.R. Biosciences, University of Cocody-Abidjan (Côte-d'Ivoire). Samples of the plant are preserved in the Herbarium of the National Center Flora (C.N.F): Man, 20 July 1965, AKÉ-ASSI 8145; Abidjan, Botanical Garden, 8 February 1991, AKÉ-ASSI EMMA 26; Agboville, 27 February 2006, N'GUESSAN KOFFI N°345.

experimentally study the effect of the total aqueous extract of the plant's leaves on the glycaemia of diabetic rabbits in order to provide scientific evidence of the effec - tiveness of the traditional use of *C. cainito*, as antidibetic.

MATERIALS AND METHODS

Vegetable material

The fresh leaves of *C. cainito* (Figure 1) were collected within the village of Aboudé-Mandéké and rinsed then dried for 2 weeks in the shade, well-ventilated place. Then they were pulverized with an electric grinder (type RETSCH) and a powder was obtained and used in the experiment.

Extraction by decoction

The method by decoction was used in which a given amount of powder is left in water. Figure 2 summarizes the extraction method used.

Animal material

We used rabbits (*Oryctolagus cuniculus*, Leporidae) we bought in a farm located in Bingerville, suburbs of Abidjan (Côte-d'Ivoire). The rabbits were kept in the net house at the National Center Flora and fed with the granules produced by the Ivorian Compound Food Manufacturing Society (F. A. C. I.). We used tap water. There were 18 rabbits divided into 6 batches of 3, as follows:

-Batch 1: Sample rabbits with normal glycaemia.
-Batch 2: Sample diabetic rabbits induced with alloxan.
-Batch 3: Diabetic rabbits induced with alloxan and treated with insulin (40 IU/ml).
-Batch 4: Diabetic rabbits induced with alloxan and treated with *C. cainito* 10 g/l.
-Batch 5: Diabetic rabbits induced with alloxan and treated with *C. cainito* 20 g/l.
-Batch 6: Diabetic rabbits induced with alloxan and treated with *C. cainito* 30 g/l.

Technical material

"Epicrâniens" adapted to syringes were used to take blood samples we collected in hemolysis tubes. Electrical scales for weighing powders were needed. A digital camera allowed us to make shootings. The glycaemia level determination device included a spectrophotometer of KENZA type (Kadja, 1998).

Chemicals

Surgical spirit was necessary to treat injured rabbits. The alloxan, an agent known to cause diabetes, was used. For the treatment of induced diabetic rabbits of control group, we used insulin, the reference product having hypoglycaemic effect. To carry out the phytochemical screening, we used solvents (ether of oil, methanol and distilled water) and various classic reagents (N'Guessan, 2008). Classical methods described in the works of Ronchetti and Russo (1971), Hegnauer (1973), Wagner (1983), Békro et al. (2007) were used to characterize the chemical groups.

Induction of diabetes

We used 5.6-dioxyuracil, which marketing name is alloxan and whose diabetogenic property is established with dogs (Lukens, 1948; Siliart and André, 1987) . Instead of dogs, we used rabbits, easier to handle and in which the diabetogenic property was also observed (Kadja, 1998; Djédjé, 2002). The alloxan injection depends on the weight of the animal: 70 mg of alloxan dissolved in 3 ml of distilled water for 1 kg of rabbit weight. The alloxan solution is injected through the marginal vein of the ear. Therefore, the health status of rabbits is closely monitored until the hyperglycaemia appears and is detected weekly by determination of blood sugar quantity.

Treatment of diabetic rabbits with insulin

The rabbits of batch 3 are treated with insulin. Every day, the diabetic rabbits are injected 5 ml of insulin (40 IU/ml), intravenously, through the marginal vein of the ear.

Treatment of diabetic rabbits with the extract from leaves of *C. cainito*

The rabbits of batches 4, 5 and 6 are treated with *C. cainito*. To do things like the traditional healers, the rabbits are given to drink, each day; 100 ml of *C. cainito*, per kilogram of body weight; *C. cainito* was ready, each morning at 7 am in a secure container, in the cage. Five to seven hours later, when the whole herbal medicine available for the animals was drunk, we fill the empty container with tap water. This treatment was performed regularly until the end of manipulation, 91 days after the first injection of alloxan to the rabbits.

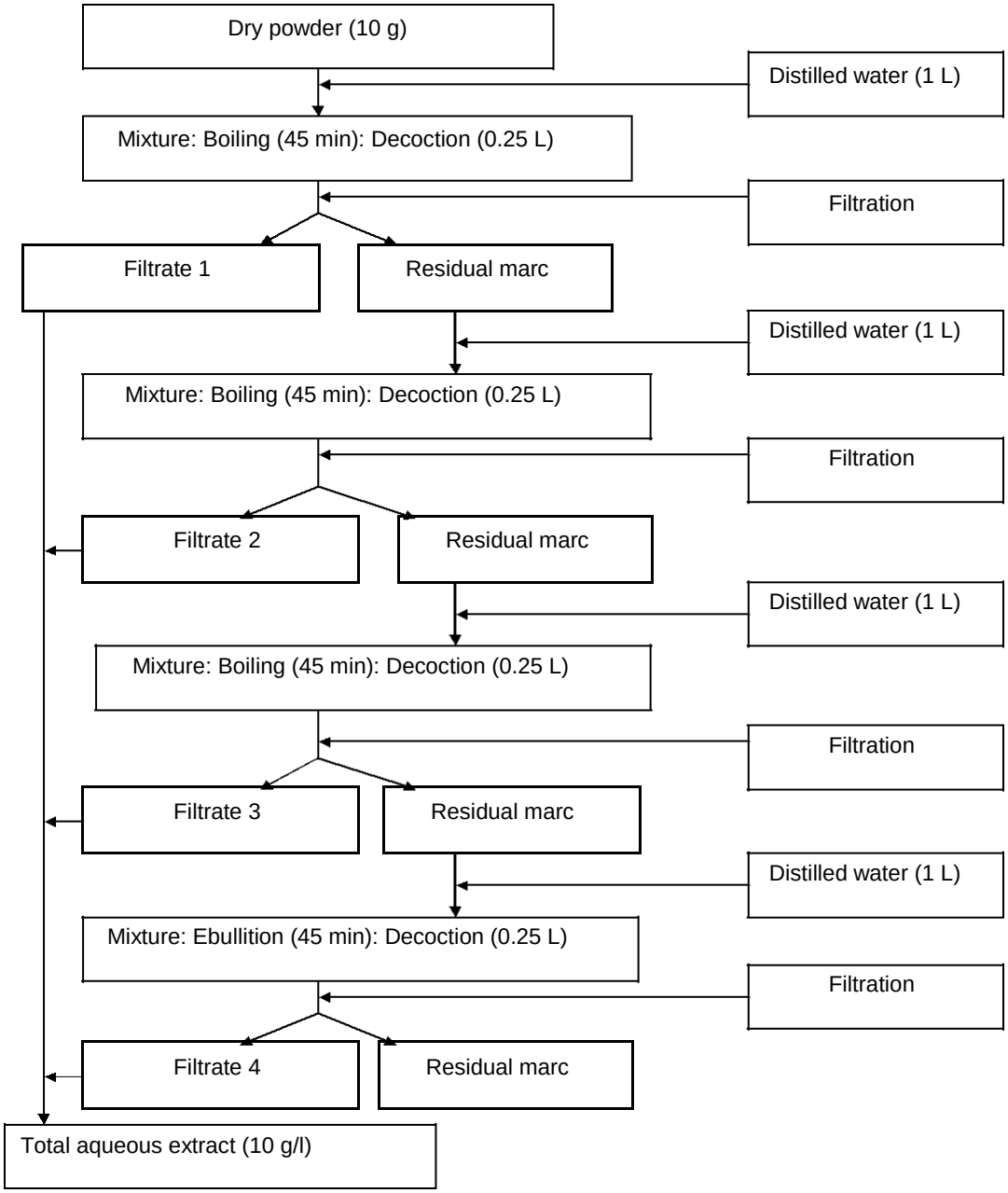

Figure 2. Process of preparation of herbal medicine from leaves of *C. cainito* (Sapotaceae), at 10 g/l.

Rabbits' blood sampling and glycaemia determination

The method used is that related to the enzyme (Djédjé, 2002).

Statistical 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 ($\alpha = 5\%$).

RESULTS

Effects of alloxan on rabbits with normal glycaemia level

A few moments after the injection of alloxan, the rabbits become calm, very agitated and refuse to eat and drink. We noticed an intense thirst and polyuria. Before the injection of alloxan, all rabbits had a postprandial glycaemia

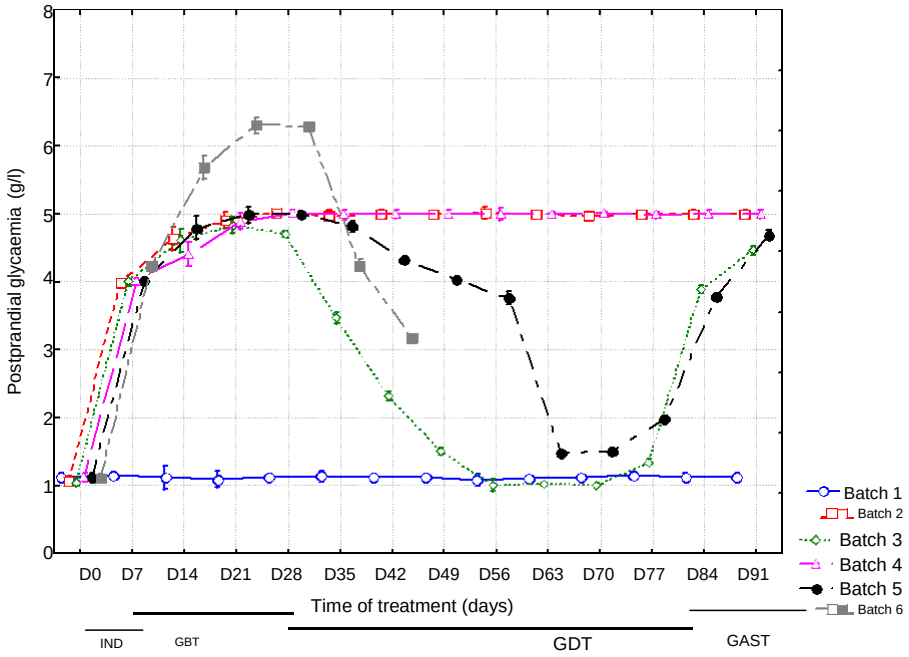

Figure 3. Glycaemia variation curves for the different batches of rabbits. Mean ± SEM, n = 3, P < 0.05. Significance of the symbols: IND: Induction; GBT: glycaemia before treatment; GDT: glycaemia during treatment; GAST: glycaemia after stop of treatment. Batch 1: Sample rabbits with normal glycaemia level; Batch 2: Diabetic sample rabbits Batch 3: Diabetic rabbits treated with insulin; Batch 4: Diabetic rabbits treated with *C. cainito* at 10 g/l; Batch 5: Diabetic rabbits treated with *C. cainito* at 20 g/l; Batch 6: Diabetic rabbits treated with C cainito at 30 g/l.

glycaemia of 1.1 g/l ± 0.10. The postprandial glycaemia of rabbits of batch 1 (sample rabbits not induced by alloxan) remains basically stable, during the experiment. After injection of alloxan, it appears an increase of glycaemia which rises gradually to reach 5 or 6.3 g/l ± 0.10. In rabbits of batch 2 (sample rabbits induced with alloxan but not treated), we noticed an increasing phase during which glycaemia goes from 1.07 to 5.02 g/l ± 0.10 and a phase that remains stationary throughout the experimental period, after the peak of 5.02 g/l.

Effects of insulin on rabbits of batch 3 induced with alloxan

The insulin lowers hyperglycaemia (Figure 3) from 4.90 to 1 g/l ± 0.10. After a stop of treatment, there is a resumption of hyperglycaemia, from 1 to 4.98 g/l ± 0.10.

Effects of herbal medicine (*C. cainito*) on the glycaemia of diabetic rabbits

The decoction of *C. cainito* leaves has glycaemic properties which vary from one dose to another (Figure 3). The glycaemia of rabbits of batch 4, treated with *C. cainito* at 10 g/l, experienced a similar evolution to that of the sample induced rabbits non treated (rabbits of batch 2); despite treatment, the glycaemia rate which rose to 5.05 g/l ± 0.10, after injection of alloxan, practically does not vary: *C. cainito* does not exert an hypoglycaemic effect at dose of 10 g/l. The glycaemia of the rabbits of batch 5, treated with *C. cainito* at 20 g/l, lowers from 5 to 1.4 g/l ± 0.10. After treatment is stopped, there is a resumption of hyperglycaemia: at a rate of 20 g/l, *C. cainito* has a glucose-lowering effect. The rabbits of batch 6, treated with *C. cainito* at 30 g/l, present a beginning of evolution comparable with those of batches 3 and 5. Unfortunately, when we attended a fall of the hyper-glycaemia which passed from 6.30 to 3.20 g/l ± 0.10, after 6 weeks of treatment, the rabbits stopped eating; they drank less water; become calm, apathetic, they died, between the 8^{th} and the 9^{th} weeks of experimentation. Thus the experiment, with this batch, could not continue. The herbal medicine (*C. cainito*) seems to be toxic, at higher dose (30 g/l).

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 *C. cainito*. Thus *C. cainito* leaves were chemically screened and yielded alkaloids, flavonoids, phenols, sterols and triterpenes (N'Guessan, 2008).

DISCUSSION

Effects of insulin on rabbits treated with Alloxan

The insulin injection reduced hyperglycaemia and restores normal glycaemia at 1 g/l. Insulin exerts a hypoglycaemic effect, in accordance with the results of Djédjé (2002).

We administered by oral way the extract (*C. cainito*) in order to do like the traditional method. Consequently, as reference products, we should have used oral hypoglycaemic agents such as Sulfonamides (DAONIL, Dianicron or Armel) and Biguanides (Glucophage, Stagid or Metforal), instead of insulin that is administered by intravenous way. The administration of oral hypoglycaemic agents was not possible for four reasons (Djédjé, 2002). These hypoglycaemic products currently used are more expensive than the insulin treatment. It's useful to take these drugs when the hyperglycaemia is moderated, that is not the case here because we recorded hyperglycaemia rate being able to reach 6.3 g/l. Sulfonamides and biguanides expose diabetics people to serious accidents related to the occurrence of hypoglycaemia. They are unusable in the case of insulin-dependent diabetes, as it is the case in this study.

Effects of herbal medicine (*C. cainito*) on the glycaemia of diabetic rabbits

At dose of 10 g/l, *C. cainito* does not induce the hypoglycaemic effect. At 20 g/l, *C. cainito* decreases the hyperglycaemia and brings back to the glycaemia to a value close to the normal (1.40 g/l) . The administration of *C. cainito* at 20 g/l highlights the hypoglycaemic effect, comparable to that of insulin and this effect approximates that of *Jatropha gossypiifolia* at 25 g/l (N'Guessan et al., 2008a). *C. cainito* exerts a hypoglycaemic effect, in accordance with the results of N'Guessan et al. (2008b); the authors reported that the aqueous extract of the leaves of *Crescentia cujete* (Bignoniaceae) lowers the hyperglycaemia at 20 g/l. The hypoglycaemic effect of *C. cainito* is similar to that of the aqueous extract of *Persea Americana* seeds on the glycaemia of diabetic rabbits (N'Guessan et al., 2009). The herbal medicine lowers the hyperglycaemia level without bringing back the glycaemia to its normal value of 1 g/l. At 20 g/l, *C. cainito* exerts a hypoglycaemic effect that can not quite be compared to that of DIACODA, a vegetable natural substance used in the treatment of diabetes mellitus, which lowers the hyperglycaemia, restores normal glycaemia and stabilizes it to 1 g/l when a dose of 25 g/l is administered (Kadja, 1998). DIACODA appears as an extract that can permanently cure diabetes; it can be a good substitute for the insulin, which is not the case for *C. cainito*.

The diabetic rabbits succumbed, after a few weeks of treatment by *C. cainito* at 30 g/l. The prospecting of the aqueous extract of *C. cainito* leaves, as prepared by the traditional healers, requires a supervision of posology in order to avoid intoxication events. There is no risk of toxicity if posology is respected. Unfortunately the traditional posology which consists to drink decoctate, in the morning and evening, out of the mealtimes, twice a day, for adult and teenager and half a glass, for child, until recovery, lacks of precision. This imprecision constitutes a true problem of the traditional medicine. This is why Adjoungoua et al. (2006) recommend a pharmaceutical monitoring to call the traditional healers' attention on the risks they put the patient at, in the event of massive taking of herbal medicine.

C. cainito was chemically screened and yielded alkaloids, flavonoids, phenols, sterols and triterpens (N'Guessan, 2008). Among these compounds, the alkaloids, sterols and triterpens can be incriminated in the antidiabetic activity of the plant. Alkaloids would be used as stimulatives of the hepatic glycogenogenesis (Neuwinger, 1996) . Sterols and triterpens are recognized for their properties to decrease the rate of blood glucose (Nacoulma, 1996). Alkaloids, Sterols or triterpens highlighted in the leaves of the plant would be responsible for the observed antidiabetic effect.

Conclusion

The aqueous decoction of *C. cainito* leaves has no influence on glycaemia of induced diabetic rabbits at doses 10 g/l. The herbal medicine (*C. cainito*) exerts hypoglycaemic activity, at doses 20 g/l. The dose of 20 g/l is the most effective among the administered doses. From 30 g/l, the plant would exert a toxic effect. Its use, as antidiabetic plant, would require supervision of posology.

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