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Effect of Aqueous Extract of *Ageratum conyzoides* Leaves on the Glycaemia of Rabbits

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Diabetes is an alarming affection which affects 5% of the population in Côte-d'Ivoire. In the search of means of fighting, we evaluated, experimentally, the effect of *Ageratum conyzoides* leaves used by traditional healers to treat diabetes. The rabbits used received orally a solution of glucose (4 g/l) to cause hyperglycaemia. As treatment, the hyperglycaemic rabbits were given, glibenclamide (0.25 mg/ml) and herbal medicine to drink. The rabbits deprived of food overnight had basic glycaemia of 1.02 g/l. The untreated rabbits' glycaemia increased and reached 1.20 g/l. The glibenclamide lowers blood sugar at 0.7 g/l. The others hyperglycaemic rabbits received different graded doses of the herbal medicine. At 2 mg/ml, phytomedicine does not induce hypoglycaemic effect. At 20 mg/ml, its hypoglycaemic effect is light. At 200 mg/ml, the herbal medicine exerts a significant glucose-lowering hypoglycaemic effect that is comparable to those of glibenclamide, appears like an antidiabetic.

Keyword: Côte-d'Ivoire, Ethnopharmacology, Medicinal plants, Traditional Healers

1. Introduction

Diabetes, a chronic disease caused by absolute or relative lack of insulin [1], induces disability and death and appears like a major public health problem [2]. This endocrine disorder exists everywhere in the world [3] and interests approximately 6% of the world population [4]. Côte-d'Ivoire has 3 to 7% of diabetics [5]. In modern medicine, no satisfactory effective therapy is still available to cure diabetes which can be managed by exercise, diet and chemotherapy [6]. Moreover, the pharmaceutical drugs are either too expensive [7]. In the search of means of fighting, man recognized and used the medicinal properties of many cultivated or wild plants and many drugs to combat this worrying affection. We mention, for examples, the works of Kwashié *et al.* [8] which indicated the hypoglycaemic effect of aqueous extract prepared with leaves of *Stereospermum kunthianum* (Bignoniaceae). Kamtchouing *et al.* [9] highlighted the hypoglycaemic effect of hexane extract of *Anacardium occidentale* (Anacardiaceae) on diabetic rats. Bhandari *et al.* [10] related that ethanolic extract of *Zingiber officinale* (Zingiberaceae) produced significant antihyperglycaemic effect in diabetic rats. In their study, N'Guessan *et al.* [11] indicated the hypoglycaemic activity of aqueous extract of leaves of *Crescentia cujete* (Bignoniaceae). N'Guessan *et al.* [12] reported the antidiabetic properties of leaves of *Chrysophyllum cainito* (Sapotaceae) on rabbits. The ethnopharmacological survey led with Traditional Healers in Abidjan (Côte-d'Ivoire) made us discover that the herbalists advise various species of plants to develop medicamentous receipts to treat diabetes. Among these antidiabetic plants,

there is *Ageratum conyzoides* L. (Asteraceae). The present investigation was undertaken to experimentally study the antidiabetic effects of aqueous extracts of *Ageratum conyzoides* leaves on hyperglycaemic rabbits in order to provide scientific evidence of the effectiveness of the traditional use of the plant, as antidiabetic.

2. Methods

2.1. Plant material and preparation of extract

The leaves of *Ageratum conyzoides* L. (Asteraceae) were collected, freshly, within Yopougon municipality in the District of Abidjan. 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 (Moutcho, Côte-d'Ivoire, 11 May 1990, N'guessan Koffi n° 150) was deposited in National Floristic Center (Université Félix Houphouet-Boigny, Côte-d'Ivoire). One thousand (1000) grams of the drug (fresh leaves of *Ageratum conyzoides*) were collected and rinsed. The drug was introduced in 4000 ml of distilled water. The mixture, bulled 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 ACA, is then kept in sterilized glass bowls, hermetically closed, in a fridge.

2.2. Distribution in batches of animal used

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 tap water. 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 200 mg/ml
-batch 5: hyperglycaemic rabbits treated with herbal medicine at 20 mg/ml
-batch 6: hyperglycaemic rabbits treated with herbal medicine at 2 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 200 mg/ml.

2.3. Phytochemical screening

To carry out the phytochemical screening, we used water and various classic reagents. Classical methods described in the works of Ronchetti and Russo [13], Hegnauer [14], Wagner [15], Békro *et al*. [16] were used to characterize the chemical groups.

2.4. Induction of hyperglycaemia and treatment of rabbits

We used glucose, a glucidic agent whose hyperglycaemic property is established with rabbits [4, 5, 17, 18, 19]. 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 receive 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.

For the treatment of induced hyperglycaemic rabbits of control group (rabbits of batch 3), we used glibenclamide (DAONIL tablet, 5 mg/20 ml distilled water, that to say 0.25 mg/ml), the reference product with hypoglycaemic effect. The rabbits of batches 4, 5 and 6 are treated with the herbal medicine at different doses. All the hyperglycaemic rabbits received, orally, 0.2 ml of glibenclamide and herbal medicine for 20 g of body weight, one hour, after the cramming by glucose.

2.5. Rabbits' Blood Sampling and Glycaemia Determination

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. One hour 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.

The method used for glycaemia determination is that related to the enzyme [5]. It consists in oxidizing glucose by the glucose oxydase, enzyme with production of gluconic acid and hydrogen pyroxyde (H_2O_2). The hydrogen pyroxyde reacts with phenol and 4-amino-antipyrine in the presence of peroxydase 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.

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose-oxydase}} \text{Gluconic Acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{amino-4-antipyrin} \xrightarrow{\text{Peroxydas}} \text{Quinoneimine} + 4H_2O$$

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:

$$\text{Glucose rate (g/l)} = \frac{D_0 \text{ sample}}{D_0 \text{ standard}} \text{ X n, with n = standard value.}$$

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 (Abidjan, Côte-d'Ivoire).

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

3. Results and Discussion

3.1. Evolution of glycaemia after glucose overload

The level of blood glucose in normal, hyperglycaemic control and experimental groups of rabbits is reported in figures 1-8. Before treatment, all the animals had a basic glycaemia of about 1.02 g ± 0.08. This result on the basic glycaemia in rabbits deprived of food overnight confirms work of Diatewa *et al.* [20]. After administration of glucose to all the animals, the glycaemia rise gradually to reach 1.20 g/l ± 0.10. The rabbits showed a significant level of blood glucose. The glucose overload induces on rabbits a hyperglycaemia, after its administration. That confirms the property of glucose as a product able to create hyperglycaemia. This result confirms that of Gharras *et al.* [21] on rats' glycaemia.

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). Their glycaemia fluctuates between 0.90 and 1.00 g/l: glycaemia remains basically stable, during the experiment. We notice two stages in the evolution of glycaemia with the rabbits of batch 2 (sample rabbits induced with glucose overload but not treated): an increase phase from basic glycaemia (0.90 g/l) to the peak (1.20 g/l) during which the rabbits showed a significant level of blood

glucose and a decreasing phase during which glycaemia goes from 1.20 to 1.10 g/l. A fall of 8.3% is noted, after the glycaemic peak. At the end of the experimentation, the blood glucose value (1.05 g/l) is near normal level. The induced hyperglycaemia is transitory: the organism is able to restore normal glycaemia, after a glycaemic overload.

3.2. Effects of Glibenclamide and Herbal Medicine 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 the peak 1.18 g/l to 0.7 g/l. A fall of 40% is observed, 4 hours later (fig. 2): glibenclamide exerts a hypoglycemic effect, in accordance with the results of Gharras *et al*. [21]. 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, two hours, after glucose overload. This result tallies with that of N'Guessan *et al*. [18, 19] on rabbits. Four (4) hours after administration, the glycaemia of treated rabbits with the reference product decreases significantly but its normal value is not restored.

The figure 3 shows the level of blood glucose in hyperglycaemic control and experiment groups. The herbal medicine has a significant hypoglycaemic effect. The glycaemia of the rabbits of batch 4, treated with the herbal medicine at 200 mg/ml, decreases but does not go back to a normal level. The fall (20%) is lower to that of glibenclamide (40%); at a rate of 200 mg/ml, the herbal medicine has a glucose-lowering effect. Herbal medicine and glibenclamide treatment to hyperglycaemic rabbits significantly reversed the level of blood glucose. However, the effect of herbal medicine is not more prominent when compared with glibenclamide (fig. 4). The use of the aqueous decoction from leaves of *Ageratum conyzoides* revealed that it has glycaemic properties, which vary from one dose to another (Fig. 5). The glycaemia of rabbits of batch 5, treated with the herbal medicine at 20 mg/ml, goes down from the peak of 1.12 g/l to 0.98 g/l, three hours later. A fall of 11% is obtained. From the peak, the glycaemia increases gradually and at the end of the experiment, its value of 0.95 g/l: the normal value of approximately 1.00 g/l is not restored. The administration of the herbal medicine at lower doses (2 mg/ml) induces a light hypoglycemic effect; at lower doses (subliminal doses < 2 mg/ml), the herbal medicine would have normoglycemic activity. The administration of the herbal medicine at 200 mg/ml highlights the hypoglycaemic effect but there is no restoration of normal glycaemia, after 4 hours of treatment.

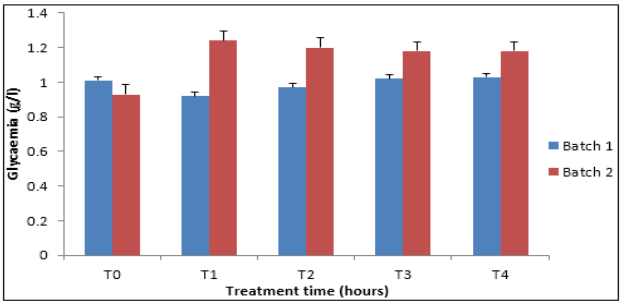

Fig 1: Glycaemia variation histogram for sample untreated rabbits with normal glycaemia and sample hyperglycaemic rabbits treated with glucose (4 g/l);
Mean ± SEM, n =3, P < 0.05.
Batch 1: sample untreated rabbits with normal glycaemia

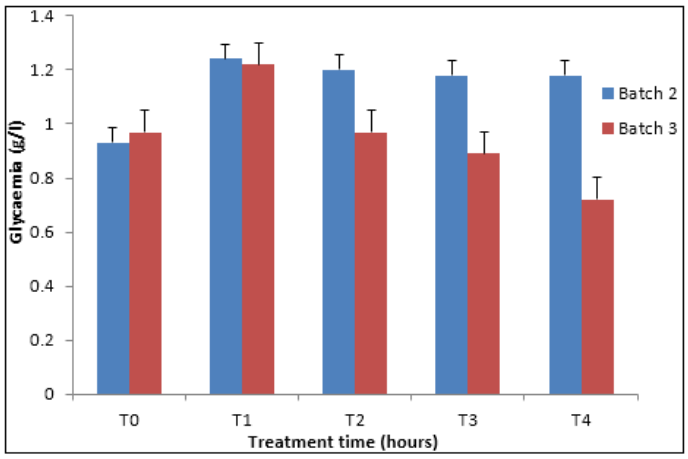

Fig 2: Glycaemia variation histogram for sample hyperglycaemic rabbits treated with glucose and sample hyperglycaemic rabbits treated with glibenclamide; Mean ± SEM, n =3, P < 0.05.

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

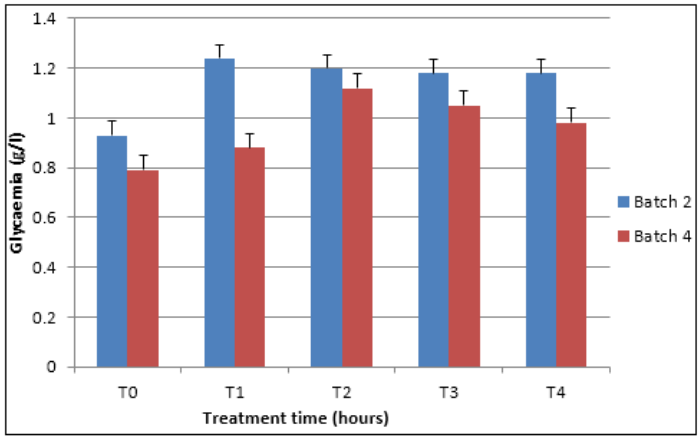

Fig 3: Glycaemia variation histogram for sample hyperglycaemic rats treated with glucose and hyperglycaemic rabbits treated with herbal medicine at 200 mg/ml; Mean ± SEM, n =3,
P < 0.05.

Batch 2: sample hyperglycaemic rabbits treated with glucose (4 g/l)
Batch 4: hyperglycaemic rabbits treated with herbal medicine at 200 mg/ml

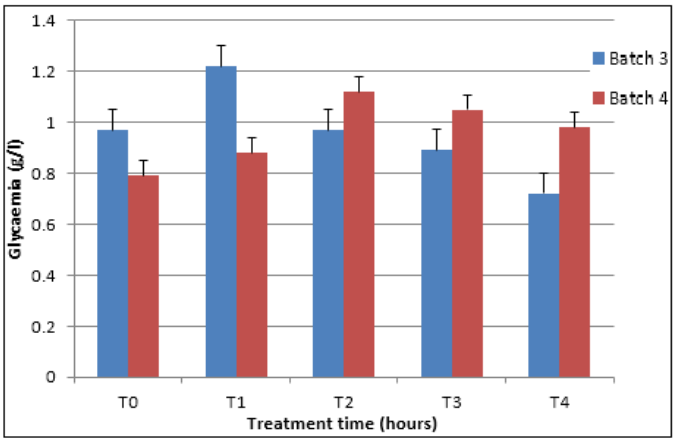

Fig 4: Glycaemia variation histogram for hyperglycaemic rabbits treated with glibenclamide and with herbal medicine; Mean ± SEM, n =3, P < 0.05.
Batch 3: hyperglycaemic rabbits treated with glibenclamide (0.25 mg/ml)
Batch 4: hyperglycaemic rabbits treated with herbal medicine at 200 mg/ml

The effect of the herbal medicine at 200 mg/ml does not approximate that of glibenclamide at 0.25 mg/ml. In all the treated groups of rabbits, until the end of the experiment, there is no stabilization of glycaemia at its normal value, after 4 hours of experimentation. The glycaemia of rabbits treated with herbal medicine experienced an evolution which is not similar to that of hyperglycaemic treated rabbits, comparably to different others studies [18, 19].

This experience was conducted to study the antidiabetic activity of *Ageratum conyzoides* 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 200 mg/ml can be 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 and appears like an antidiabetic.

3.3. Effect of glibenclamide and herbal medicine 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), at 200 mg/ml. The reference product exerts a significant basal glucose-lowering effect unlike herbal medicine. The two substances would not exert the same effect on the insulinosecretion. The noninsulinic treatment of diabetes utilizes oral hypoglycaemiants type of sulphamides and type of biguanides. Sulphamides to which belonged glibenclamide act by stimulating the secretion of insulin [8]. The biguanides reinforce the peripheral use of glucose and appear to inhibit the gluconeogenesis. The herbal medicine would have an extra-pancreatic action by stimulating peripheral use of glucose, similar to that observed with the biguanides. The antidiabetic effect of the herbal medicine would be due to an increase in the membrane permeability to glucose blood similar to that observed after biguanides administration. Administration of herbal medicine significantly increased the activities of membrane enzymes for glucose utilization in hyperglycaemic rabbits.

3.4. 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 *Ageratum conyzoides* leaves were chemically screened and yielded alkaloids, polyphenols, flavonoids, tannins, sterols and triterpens. 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 [22].

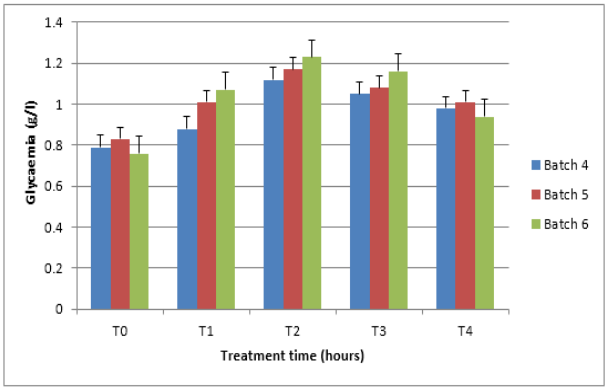

Fig 5: Glycaemia variation histogram for hyperglycaemic rabbits treated with herbal medicine (200 mg/ml, 20 mg/ml, 2 mg/ml); Mean ± SEM, n =3, P < 0.05.

Batch 4: hyperglycaemic rabbits treated with herbal medicine at 200 mg/ml

Batch 5: hyperglycaemic rabbits treated with herbal medicine at 20 mg/ml

Batch 6: hyperglycaemic rabbits treated with herbal medicine at 2 mg/m

Sterols and triterpens are recognized for their properties to decrease the rate of blood glucose by stimulating insulin production [23]. Alkaloids, Sterols or triterpens highlighted in the leaves of the plant would be responsible for the observed antidiabetic effect.

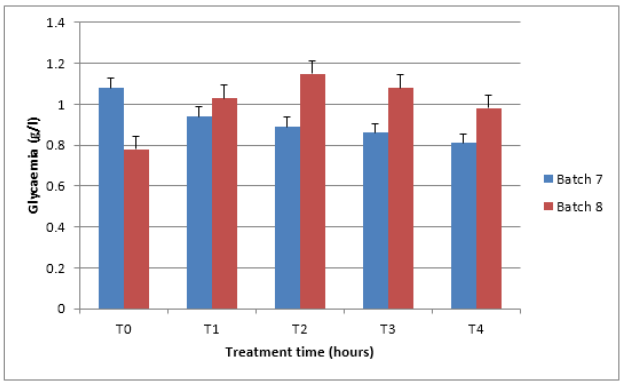

Fig 6: Glycaemia variation histogram for normal glycaemic rabbitts treated with glibenclamide and herbal medicine; Mean ± SEM, n =3, P < 0.05.

Batch 7: normal glycaemic rabbits treated with glibenclamide (0.25 mg/ml)

4. Conclusion

The data generated from this study show that hyperglycaemic rabbits were obtained using glucose. The extracts (*Ageratum conyzoides* leaves) 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 its antidiabetic activity due to chemical components as alkaloids, sterols and triterpens.

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