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Assessment of the Effects of an Aqueous Extract of the Leaves, Flowers, and Seeds of *Crotalaria retusa* L (Fabaceae) on the Glycemia in Rats

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ABSTRACT

The aim of this study was to evaluate the effects of an aqueous extract of *Crotalaria retusa* L (Fabaceae) (EACr) on glycemia in Wistar rats, to contribute to the valorization of plants used in traditional medicine for the improvement of people's health. A phytochemical screening and pharmacological study of the aqueous extract of *Crotalaria retusa* L (Fabaceae) on glycemia in rats, were carried out. The administration of the substances in animals was made orally. The qualitative phytochemical study carried out with the aqueous extract of the leaves, the flowers, and the pods of *Crotalaria retusa* L (Fabaceae) made to highlight the presence of polyphenols, flavonoids, catechic, and gallic tannins, quinonic compounds, alkaloids, sterols, and polyterpenes. This extract showed hypoglycemic activity in the normoglycemic animals, treated with the dose of 1000 mg/kg BW with a percentage of reduction of the glycemia of 21.04%. After an overload of glucose, EACr (1000 mg/kg BW) and the glibenclamide (10^{-2} g/kg BW) showed better antihyperglycemic activity in the rats post-treated, with respective percentages of reduction of 41.90 and 50.39%. EACr has hypoglycemic and antihyperglycemic properties which would be probably related to the presence of alkaloids, tannins, and flavonoids. These compounds, generally recognized for their hypoglycemic and antihyperglycemic effects, confer to this extract similar properties to those of certain insulin-secretors. These results support the use of this plant for the treatment of diabetes in traditional medicine and bring a scientific base.

Keywords: Aqueous extract, *Crotalaria retusa*, Hypoglycemic and Antihyperglycemic, phytochemical screening.

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I. Introduction

Diabetes mellitus is defined as an abnormal rise in the sugar rate in the blood. They are a metabolic disorder of multiple etiology characterized by chronic hyperglycemia due to a defect of secretion or action of insulin or both at the same time [1]. According to the International Federation of Diabetes (FID), the rate of prevalence of diabetes in sub-Saharan Africa will be increased by 98 % in 2030 if preventive urgent measures are not taken to slow down its incidence. Thus, according to FID, Côte d'Ivoire does not escape from this pandemic. Indeed, the prevalence of diabetes moved from 5.7 % before 2000 to 9.6% in 2010 [2]. Unfortunately, taking care of diabetics is very expensive, with the regular catch of glycemia and its adjustment by the injection of insulin. Thus, faced with these very expensive costs, the population of developing countries turns to traditional medicine for the treatment of diabetes [3]. It is known that more than 80% of Africans use medicinal plants to treat many pathologies [4]. It is in this perspective that we undertook to study the effects of the aqueous extract of *Crotalaria retusa* L (Fabaceae), a plant used in traditional medicine to treat diabetes mellitus [5].

Our present work has, as a general objective, to evaluate the pharmacological effects of an aqueous extract of the leaves, flowers, and pods of *Crotalaria retusa* L (Fabaceae) on the glycemia in rats to promote the use of the plants in traditional medicine for the improvement of people's health.

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II. Material and Methods

A. Plant Material

Plant material consists of leaves, flowers, and pods of *Crotalaria retusa* L (Fabaceae). The plant was collected in the month of September 2018 in Senoufla, (Vavoua), in the western center of Côte d'Ivoire. This plant is identified and authenticated at the National Floristic Center (CNF) of the Felix Houphouët-Boigny University (Abidjan, Côte d'Ivoire) by Mr. Assi Jean thanks to the herbaria numbers 15133 of the 22nd February 1980, 51 of the 15th February 1991, 4258 of the 18th November 1974 and 11022 of the 6th January 1970 of the known as the center.

B. Preparation of the Aqueous Extract of Crotalaria retusa L (Fabaceae)

The leaves, the flowers, and the seeds are dried at room temperature and are crushed in a mechanical crusher for at least one hour. Two hundred and fifty grams (250 g) of the crushed are put in five (5) liters of distilled water. The unit was carried to maceration for twenty-four hours (24 h) under magnetic agitation and macerated, it was filtered on hydrophilic cotton and Wattman paper n°2. The filtrate is dried in the drying oven at 50 °C for 72 hours. We obtain a powder of chestnut color (33 g) which constitutes the aqueous extract of *Crotalaria retusa* (EACr). Distilled water is used as solvent to prepare different doses of the extract.

C. Animal Material

The healthy male rats of Wistar strain, aged from 8 to 16 weeks and weights ranging between 120 and 150 g come from the animal house of Formation and Research Unit of Pharmacy of the Felix Houphouët-Boigny University (Abidjan, Côte d'Ivoire). The breeding was carried at room temperature. Animals were maintained on 12:12 light/night cycle, fed with a standard chow and had water ad libitum. All procedures were approved by the ethical committee Felix Houphouët-Boigny University, for the use of laboratory animals and care.

D. Phytochemical Study of Crotalaria retusa L (Fabaceae)

This study was carried out at the Pharmacognosy department of Formation and Research Unit of Pharmaceutical and Biological Sciences of the University Felix Houphouët-Boigny, Abidjan. It makes it possible to highlight the great chemical groups of pharmacological interest, namely sterols, polyterpenes, flavonoids, tannins, quinone compounds, saponins, and alkaloids. It has been done by a qualitative method described by [6] and [7].

E. Pharmacological Study

This study consists in evaluating the effects of our extract on glycemia in rats normoglycemic and temporary hyperglycemic rats. The glycemia is measured using the Accu-Chek glucometer with reactive strips.

F. Normoglycemic Rats

For this study, 20 Wistar rats, weighing between 120 and 150 g, are used. These animals, randomly assigned into groups of 4 rats each, are fasted for, 18 hours and classified as follows: the control group (1) received distilled water and test groups (2, 3, 4, and 5) were taken the aqueous extract of *Crotalaria retusa* L, respectively at the doses of 500, 1000, 2000, and 2500 mg/kg BW. Glycemia is measured in all animals before the start of the experiment (T0). Blood glucose in rats is measured at regular time intervals of 30, 60, 90, 120, 150, and 180 minutes after the administration of substances.

G. Temporary Hyperglycemic Rats

Normal rats, weighing between 120 g and 150 g, were fasted 18 hours before the beginning of the experiment. Four groups of four (4) rats are made and randomly as follows, for the experiments on the pretreated hyperglycemic rats: control group (1) was taken distilled water, positive control (2) received distilled water and, 30 min afterward, 4 g/kg BW of anhydrous glucose, glibenclamide group (3) received the glibenclamide orally at the dose of 10-2 g/kg BW and 30 min afterward, 4 g/kg BW of anhydrous glucose, *Crotalaria retusa* L aqueous extract group received 1000 mg/kg BW of EACr and, 30 min afterward, 4 g/kg BW of anhydrous glucose.

The effects of substances in rats in this series of experiments are followed for 180 minutes and blood glucose is measured at regular time intervals of 0, 30, 60, 90, 120, and 180 minutes.

For the test of post-treated hyperglycemic rats, the protocol is the same one as that of the pretreated rats except that in this experiment, glucose is administered to animals before the test substances.

H. Statistical Analysis

The statistical analysis of data was carried out thanks to the software and Graphpad Prism 7 (San Diégo, California, the USA). The statistical difference between the results was obtained by using the analysis of variances (ANOVA), followed by multiple comparison tests of means (Newman-Keuls), and a threshold of $P<0.05$ significance. All the values are presented in the Mean ± SEM (Standard error of the mean).

III. Results

A. Phytochemical Study of Crotalaria retusa

The phytochemical screening (Table I) of the aqueous extract of the leaves, the flowers, and the pods of *Crotalaria retusa*, revealed the presence of sterols, polyterpenes, polyphenols, catechic and gallic tannins, flavonoids, alkaloids, and quinonic compounds.

B. Effects of the Aqueous Extract of the Leaves, the Flowers, and Seeds of Crotalaria retusa (EACr) on the Glycemia in Normoglycemic Rats

Fig. 1 shows the effects of the increasing doses of EACr on the glycemia of the rats. The values of the basic glycemia in the fasting rats, measured before the various treatments, are statistically the same in all groups; they are about 80.21 ± 13.41 mg/dl. This extract, with doses of 500 and 2500 mg/kg BW, does not modify to a significant level ($P > 0.05$) the values of the glycemia in the treated rats, compared to the normal control, after 180 minutes. The dose of 1000 mg/kg BW of EACr, involves a more important decrease in glycemia in treated rats compared to the control. To this dose,

the glycemia moves from 80.2 ±5.39 to 65±2.75 mg/dl (P < 0.01), with 18.96% of reduction to the 120th minute. This reduction of glycemia is accentuated a bit at the 150th minute, with a reduction of 21.04% compared to the normal control until the end of the experimentation. The aqueous extract of *Crotalaria retusa*, with the dose of 2000 mg/kg BW, involves a significant fall (P < 0.05) of the glycemia to the 120th minute, (80.21± 13.41-72±3.04 mg/dl), with a reduction of 10.24%.

TABLE I: PHYTOCHEMICAL STUDY OF THE AQUEOUS EXTRACT OF CROTALARIA RETUSA

Chemical compounds		Results
Polyphenols		+
Sterols and polyterpenes		+
Flavonoids		+
Saponosides		-
Quinones		+
Alkaloids		+
Tannins	Catechic	+
	Gallic	+

(+) positive tests, (-) negative tests

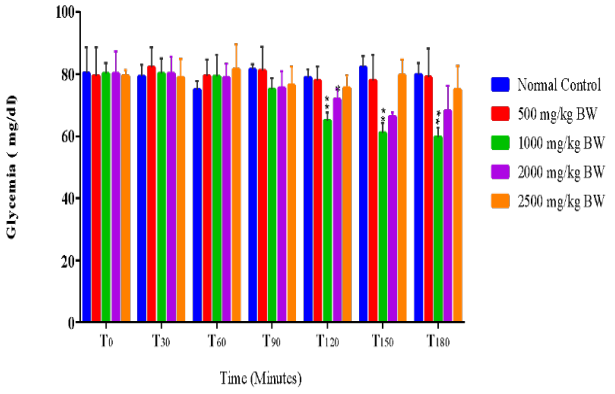

Fig. 1. Effects of the aqueous extract of *Crotalaria retusa* on the glycemia in normoglycemic rats (Mean ± SEM, *P < 0.05, ** P < 0.01, n=3). SEM: Standard error of the mean.

C. Effects of the Aqueous Extract of the Leaves, the Flowers, and Seeds of Crotalaria retusa on the Glycemia in Temporary Rats' Hyperglycemic

Fig. 2 presents the variations of the glycemia of the various pretreated rats' groups. Thirty (30) minutes after the pretreatment of rats with the test substances, the glucose solution (4 g/kg BW) was administered. It is observed a peak of hyperglycemia of 120.95± 8.76, 122.28±5.67, and 118.56 ±10.87 mg/dl, respectively in the positive control (R-T +) and in rats treated with 1000 mg/kg BW of EACr (R-EACr) and 10^{-2} g/kg BW of glibenclamide (R-Glib). After the hyperglycemic peaks were observed, the glycemia decreases gradually until the end of the experimentation (180 minutes) with reductions of 4.92%, 24.32%, and 44.23%, respectively in the positive control, and in rats pretreated with EACr, and glibenclamide compared to the negative control.

Fig. 3 presents the variations of the glycemia of the various groups in the post-treated rats. In this experiment, the glucose solution was administered thirty (30) minutes before the testing substances to rats. The peaks of hyperglycemia were 131.01± 9.57 mg/dl for the rats which received EACr (1000 mg/kg BW) and 127.75± 7.98 mg/dl for those treated with the glibenclamide (10^{-2} g/kg BW). After 180 minutes, EACr with 1000 mg/kg BW and the glibenclamide reduced the hyperglycemia, respectively of 41.90% and 50.39%, compared to the negative control.

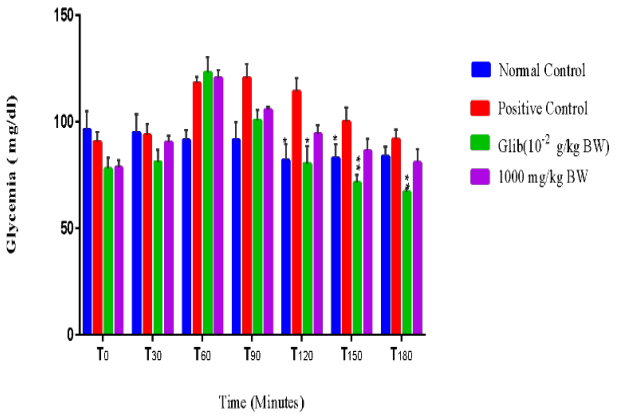

Fig. 2. Effects of EACr and the glibenclamide on the glycemia in pretreated hyperglycemic rats (Mean ± SEM, *P < 0,05; ** P < 0,01; n=3). SEM: Standard error of the mean.

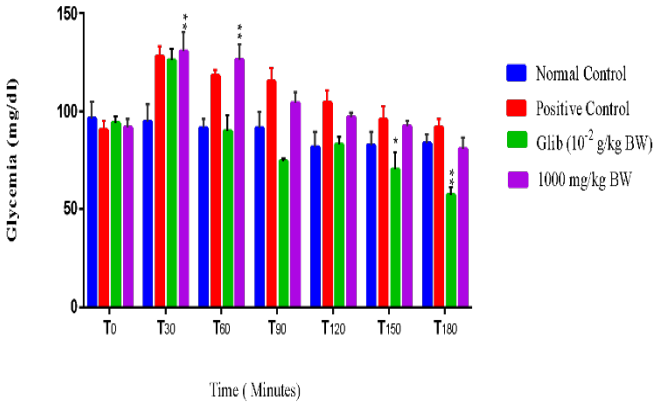

Fig. 3. Effects of EACr and the glibenclamide on the glycemia in hyperglycemic post-treated rats, Mean ± SEM, *P< 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001, n=5). SEM: Standard error of the mean.

IV. DISCUSSION

The qualitative phytochemical test carried out with the aqueous extract of the air part of *Crotalaria retusa* L (Fabaceae), revealed the presence of sterols, polyterpenes, polyphenols, catechic and gallic tannins, flavonoids, alkaloids, and quinonic compounds in this extract. These results are similar to those obtained by [8], with a small difference. Indeed, the results of the phytochemical screening of the same plant, carried out by this author, revealed the presence of saponoside. This difference could be explained by the natural ecological mediums of the harvest of the plant, and also the material used for the test. According to [9], a variation of the secondary metabolites could be observed on the level of the same plant because of the various mediums of life of this plant. The results of *Crotalaria retusa* L in the normoglycemic rats showed that this extract presents a better hypoglycemic activity at the dose of 1000 mg/kg BW with a 21.04% of reduction of glycemia. Similar results were obtained with the aqueous extracts of the leaves of *Rauvolfia vomitoria* [10] and of *Gnetum bulchozzianum* [11] which presented hypoglycemic activities in the rats. Moreover, after

an overload of glucose, EACr as the glibenclamide, presents an antihyperglycemic activity in the postreated, and pretreated rats. However, the glibenclamide presents a better antihyperglycemic activity than EACr with respective percentages of reduction of 44.23% compared with 16.48% (in the pretreated rats) and 50.39% against 41.90% (in the pos-treated rats). These results indicate that EACr, just like the glibenclamide, in addition to being hypoglycemic, is an antihyperglycemic substance; that would justify its use on a purely curative basis in the treatment of diabetes. These results are similar to those obtained with extracts aqueous of *Zizyphus mucronata* (Rhamnaceae), *Pseudarthria hookeri* (Fabaceae), of Annona senegalensis (Annonaceae), and *Hallea senegalensis* (Rubiacaceae) and *Parkia biglobosa* (Mimosaceae) on rats respectively by [12], [13], and [14] and [15]. The total aqueous extract of the leaves, the flowers, and seeds of *Crotalaria retusa* L (Fabaceae) would be a potential antidiabetic in the diabetics of the type 2. This antidiabetic effect would be conferred to him by the presence of certain secondary metabolites highlighted by phytochemical screening. Indeed, the authors such as [16] and [17] highlighted the hypoglycemic and antihyperglycemic properties of the flavonoids, which act by improving the sensitivity of the cells of the organization to insulin [18]. According to [19], the polyphenols would be at the base of the hypoglycemic and antihyperglycemic effects, which would justify the use of *Crotalaria retusa* in the treatment of diabetes. Moreover, the antidiabetic effect of tannins is attributed to their action on diabetes itself at the cellular level. They support the action of insulin (by decreasing resistance to insulin) and prevent the complications of diabetes by their antioxygenic and anti-enzymatic capacity, also neutralize the effect of the free radicals and limit the inflammatory reaction in various fabrics [15]. The work of [20] highlighted the inhibition of the α-glucosidase by alkaloids. Reference [21] reveals that the alkaloids contained in the aqueous extract of *Stachytarpheta indica* (Verbanaceae) inhibit glycogenolysis and stimulate glycogenesis at the origin of the reduction in glycemia.

The presence of alkaloids in *Crotalaria retusa* confirms its hypoglycemic and antihyperglycemic effects in the rats and suggests a probable inhibition of the α-glucosidase by this substance. The similar effects of EACr with those of the glibenclamide, hypoglycemic, and antihyperglycemic substance of the family of sulphamides on the glycemia suggest that our extract could act by the same mechanism as the substance of reference used.

V. Conclusion

The qualitative phytochemical study carried out with the aqueous extract of *Crotalaria retusa* revealed the presence of sterols and polyterpenes, alkaloids, polyphenols, catechic and gallic tannins, and flavonoids and quinones. These secondary metabolites would be responsible for the pharmacological effects of this extract. The study of the pharmacological effects of EACr on the glycemia of rats showed that this extract has hypoglycemic and antihyperglycemic effects respectively in the normoglycemic rats and the temporary hyperglycemic rats. The aqueous extract of *Crotalaria retusa* presents better antihyperglycemic activity in the post-treated rats. This property is similar to that of certain insulin-secretors. The hypoglycemic and antihyperglycemic properties of EACr make this extract a potentially active substance in the treatment of diabetes mellitus. These results are thus favorable to the exploitation of this plant for the treatment of diabetes in traditional medicine and bring a scientific base to its use.

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Conflict of Interest

Authors declare that they do not have any conflict of interest.

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