Original Research Article

Evaluation of the pharmacological effects of an aqueous leaves extract of *Combretum micranthum* (Combretaceae) on the glycemia of normoglycaemic rats

Abstract

Aims: This study was to evaluate the effects of an aqueous leaves extract of *Combretum micranthum*(EAqCM) on glycemia on Wistar rats.

Methodology: A phytochemical screening study wasperformed. The pharmacological test on normoglycemic rats was carried out in 4 groups of 5 rats per group. Ratsin group 1 received 2 ml of distilled water, and rats in the test groups received doses ranging at 100 to 300 mg/kg B.W. of theEAqCM. Tests in pre-treated temporarily hyperglycemic rats were performed in 4 groups of 5 rats per group. The rats in groups 1 and 2 received 2ml of distilled water, the rats in test groups (3 and 4) received the dose of 10^{-2}g/kg B.W. and 100 mg/kg B.W. respectively glibenclamideand EAqCM. Thirty minutes later, all rats except those in group 1 received anhydrous glucose.The protocol for testing post-treated hyperglycaemic rats is the same as that for pretreated rats, except that in this experiment glucose is administered to the animals before the test substances. Blood glucose is measured in the rats at regular intervals of 30, 60, 90, 120, 150, and 180 minutes after administration of the substances.

Results: Qualitative phytochemical analysis of EAqCM revealed the presence of polyphenols, flavonoids, catechic, tannins, quinonic compounds, alkaloids, sterols, and polyterpenes. Pharmacological tests on the glycemia of normoglycemic rats showed that EAqCMhas hypoglycemicproperties at a dose of 100 mg/kgB.W. with a percentage reduction of 30%. In hyperglycemic rats,EAqCM and glibenclamide exhibited anti-hyperglycemic activity with respective percentages reduction of 57.47 % and 58.82 %.

Conclusion: Antihyperglycemic effect of EAqCMin post-treated rats would probably be due to the presence of polyphenols and flavonoids in this extract.These results support the use of this plant in traditional medicine for the treatment of diabetes.

Keywords:*Combretum micranthum*, diabetes, hypoglycemic and antihyperglycemic

1. INTRODUCTION

Diabetes mellitus is an endocrine and metabolic disease that has been a public health issue since ancient times [1]. Pathophysiology it is characterized by a chronic hyperglycaemia resulting from lack in insulin secretion (type 1), insulin action (type 2), or both anomalies [2]. Long considerered a disease of developed countries or affluent communities due to their lifestyles, diabetes now affects all social classes. The number of people with diabetes worldwide is approximately one hundred and sixty-three million out of a global adult population approximately eight billion [3]. According to the World Health Organization [4], if urgent action is not taken, this number will reach five hundred and seventy-eight million by 2030.

In sub-Saharan Africa, the number of people suffering from diabetes is estimated at two point eight million (2.8 million) [3]. In Côte d'Ivoire, the results of the survey conducted by the National Programme for the Fight Against Metabolic Diseases and the Prevention of Non-Communicable Diseases show that the prevalence of diabetes is 6.2% or more than seven hundred million people with diabetes. Diabetes is the second leading cause of hospitalization in Côte d'Ivoire with a mortality rate of 8.9% [5]. The proportion of undiagnosed diabetics is 51% worldwide and 59.70% in Africa [6]. This lack of awareness of diabetic status is the cause of the early onset of serious complications of the disease.

Diabetes management is a lifelong process. It is expensive and requires a combination of several therapies [7]. In Côte d'Ivoire, there are only two specializedcenters for the care of patients: the Antidiabetic Centre of Abidjan (CADA), which has become the Diabetology and Preventive Dietetics Service, and the Diabetology and Endocrinology Service of the University Hospital of Yopougon. These centers are all located in Abidjan, which means that there are few opportunities to treat patients from within the country, an additional economic problem for the population, and a major public health challenge in Côte d'Ivoire.

Face to the high cost of antidiabetic drugs and the considerable number of patients who do not have access to quality care, the search for new active, effective, and lessexpensive molecules against this pathology is essential. From this point of view, we undertook to study the effects of an aqueous leaves extract of *Combretum micranthum,* a plant included in the traditional African pharmacopoeia and considered to have anti-diabetic properties.

The general aim of this study is to evaluate the pharmacological effects of an aqueous leaves extract of *Combretum micranthum* on the glycemia of rats.

2. MATERIAL AND METHODS

2.1. Material

2.1.1. Plant material

The plant material consists of leaves of *Combretum micranthum* (Combretaceae). The plant was collected in the month of August 2019 in Korhogo, in the north of Côte d'Ivoire. This plant has been identified and authenticated at the National Centre of Floristics (CNF) of the University Felix Houphouët-Boigny (Abidjan, Côte d'Ivoire) by Professor ZIRIHI Guédé Noel thanks to the herbaria numbers 10411 to 11/12/1968.

2.1.2. Animal material

The healthy male Wistar rats, aged between 8 and 16 weeks and weighing between 170 and 190 g, were obtained from the animal house of the Training and Research Unit of Pharmacy of the Felix Houphouët-Boigny University (Abidjan, Côte d'Ivoire). The animals were reared at room temperature. Animals were maintained on a 12:12 light/12:12 dark cycle, fed a standard chow diet, and given water ad libitum. All procedures were approved by the Felix Houphouët-Boigny University Ethics Committee for the use and care of laboratory animals.

2.2. Methods

2.2.1. Preparation of the aqueous leaves extract of *Combretum micranthum*

The leaves are dried at room temperature and crushed in a mechanical crusher for at least one hour. Two hundred and fifty grams (250 g) of the crushed leaves are placed in two (2) liters of distilled water. After boiling for 35 minutes, the decoction obtained is filtered on hydrophilic cotton and Wattman paper n°2. The filtrate is dried in a drying oven at 50°C for 72 hours. A chestnut-colored powder (28 g) is obtained, which represents the aqueous leaves extract of *Combretum micranthum*. The powder is dissolved in distilled water to prepare the aqueous solution of the extract at the different doses used.

2.2.2. Phytochemical study ofaqueous leaves extract of *Combretum micranthum*

This study was carried out at the Pharmacognosy Department of the Formation and Research Unit of Pharmaceutical and Biological Sciences of the Felix Houphouët-Boigny University in Abidjan. It has made it possible to highlight the major chemical groups of pharmacological interest, namely sterols, polyterpenes, flavonoids, tannins, quinone compounds, saponins and alkaloids. This was done by using a qualitative method described by Néné Bi et al. [8] and ABO [9].

2.2.3. Pharmacological study of an aqueous leaves extract of *Combretum micranthum*

This study consists of evaluating the effects of our extract on blood glucose levels in normoglycaemic and transiently hyperglycaemic rats. Blood glucose was measured using the Accu-Chek reactive strip glucometer.

2.2.3.1. Evaluation of the effects of an aqueous leaves extract of *Combretum micranthum*on glycaemia in normoglycemic rats

To evaluate the effects of the aqueous leaves extract of *Combretum micranthum* on blood glucose levels in normoglycaemic rats, different doses of the extract were administered by gavage and monitored for 3 hours.

The experiment was carried out on a total of 20 Wistar rats weighing between 170 and 190 g. These animals were divided into 4 groups of 5 rats and fasted for 18 hours. The average weight of each group was determined. Blood glucose is measured in all animals at T0 before the administration of the test substances. Rats in group 1 (control group) receive 2 ml of distilled water. Rats in groups 2, 3, and 4 (test groups) receive 2 ml of doses of 100, 200, and 300 mg/kg B.W of the aqueous extract, respectively. Blood glucose is measured in the rats at regular intervals of 30, 60, 90, 120, 150, and 180 min after administration of the test substances.

2.2.3.2-Evaluation of the effects of an aqueous leaves extract of *Combretum micranthum*on the glycaemia on temporary hyperglycemicrats

2.2.3.2.1-Evaluation of the effects of an aqueous leaves extract of *Combretum micranthum*on the glycaemia of pretreated hyperglycemic rats

To evaluate the effects of EAqCM on transient hyperglycemia in pretreated rats, four (4) groups of six (6) normal rats were formed. Rat weights varied between 170 and 190 g. These animals were fasted eighteen (18) hours prior to the experiments and the average weight of each batch was determined. Blood glucose was measured in all rats at time T0 to determine the animals' baseline blood glucose levels. The following different tests were then carried out:

-Group 1 (R-T) is the control in which the rats received 2 ml of distilled water;

- Group 2 (R-T+) is the positive control. This is the group of hyperglycemic control rats. The rats in this group received distilled water followed 30 min later by 4 g/kg body weight of anhydrous glucose;

-Group 3 (R-Glib) consists of rats given glibenclamide (oral hypoglycemic sulfonylurea) at a dose of 10-2 g/kg BW followed 30 min later by 4 g/kg BW of anhydrous glucose.

- Group 4 (R-EAqCM) consists of rats given 200 mg/kg PC of EAqCM followed 30 min later by 4 g/kg BW of anhydrous glucose.

The blood glucose levels of these animals were measured every thirty (30) minutes for one hundred and eighty (180) minutes.

2.2.3.2.2-Evaluation of the effects of an aqueous leaves extract of *Combretum micranthum*on the glycaemia of post-treatedhyperglycemic rats

The protocol for testing post-treated hyperglycemic rats is the same as that for pre-treated rats, except that in this experiment glucose is administered to the animals before the test substances.

2.3. 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).

3. RESULTS

3.1. Phytochemical composition of the aqueous leaves extract of *Combretum micranthum*

The results of the phytochemical study showed that EAqCM contains polyphenols, flavonoids, catechin tannins, quinones, alkaloids, polyterpenes and saponosides. We notice the absence of gallic tannins (Table I).

3.2. Effects of an aqueous leaves extract of *Combretum micranthum* on the glycaemia on normoglycemic rats

Figure 1 shows the effect of increasing doses of EAqCM on the glycaemia of normoglycaemic rats.

Our results show that before the gavage of the different doses of EAqCM, the glycaemia of the rats in all groups was approximately equal to 83 ± 1.40 mg/dl. The administration of doses of 200 and 300 mg/kg BW did not cause any significant variation in the glycaemia of the treated rats after three (3) hours. As for the dose of 100 mg/kg BW, it causes a significant reduction ($P < 0.05$) in the glycaemia of the treated rats from the 150th minute. The glycaemia of the rats fell from 83 ± 1.40 mg/dl to 70 ± 0.97 mg/dl, a reduction of 18.57%. This drop in glycaemia becomes more pronounced after the 180th minute when the glycaemia drops from 83 ± 1.40 mg/dl to 64 ± 2.40 mg/dl, a decrease of 30%.

3.3. Anti-hyperglycemic effects of the aqueous leaves extract of *Combretum micranthum* on the glycaemiaof rats

3.3.1. Effects of an aqueous leaves extract of *Combretum micranthum*on the glycaemia of pretreated rats

Figure 2 shows the variation in glycaemia of transiently hyperglycaemic rats pretreated with the aqueous leaves extract of *Combretum micranthum*.

Before the administration of glucose, the glycaemia of the rats in all groups was approximately equal to 83 ± 1.40 mg/dl. Thirty minutes after the administration of anhydrous glucose, a peak of hyperglycaemia was observed in all animals that had received the glucose. This peak of hyperglycaemia is 147 ± 4.40 mg/dl, an increase of 77.11% in the positive control rats, 120 ± 3.40 mg/dl, an increase of 44.17% in the rats pretreated with glibenclamide and 119 ± 2.80 mg/dl, an increase of 43.37% in the rats pretreated with the aqueous extract.

Table I: Chemical composition of the aqueous leaves extract of *Combretum micranthum*

Compounds		aqueous leaves extract
Sterols and polyterpenes		+
Polyphenols		+
Flavonoids		+
Gallic tannins		-
Catechics tannins		+
Quinones		+
Alkaloids	DRAGENDORFF	+
	BOUCHARDAT	+
Saponosides		+

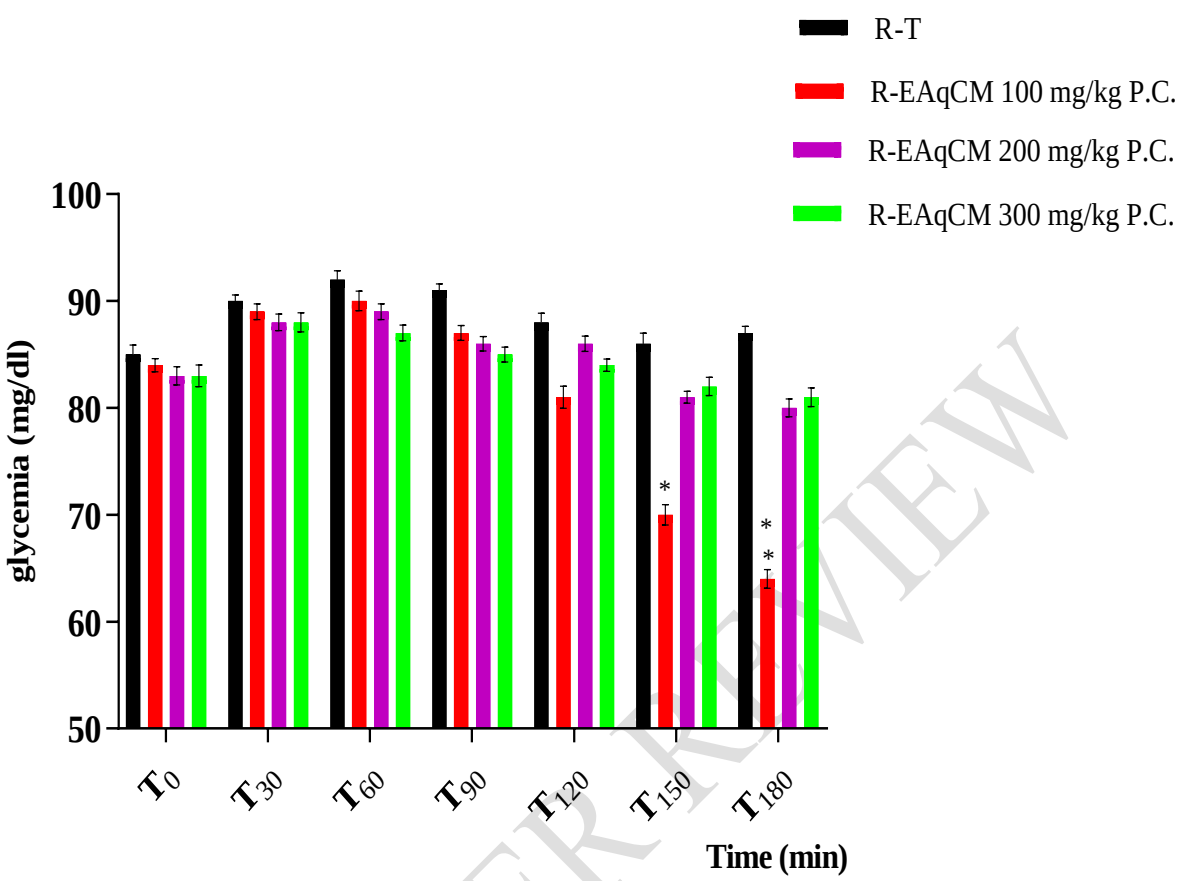

Figure 1 : Variation on glycaemialevels of normoglycemic rats after administration of doses of aqueousleavesextract of *Combretumicrantum*.

*EAqCM at a dose of 100 mg/kg BW induces hypoglycemic effects at 150 minutes (P<0.05). This effect is more pronounced at 180 minutes (P<0.01) (mean ± SEM, *P<0.05; **P<0.01; n=6).*

SEM: Standard Error on the mean;

R-T: Control rats;

EAqCM: Aqueous leave extract of *Combretum micranthum;*

R-EAqCM 100 mg/kg BW: Rat having received the dose of 100 mg/kg BW of EAqCM;

R-EAqCM 200 mg/kg BW: Rat having received the dose of 200 mg/kg BW of EAqCM;

R-EAqCM 300 mg/kg BW: Rat having received a dose of 300 mg/kg BW of EAqCM.

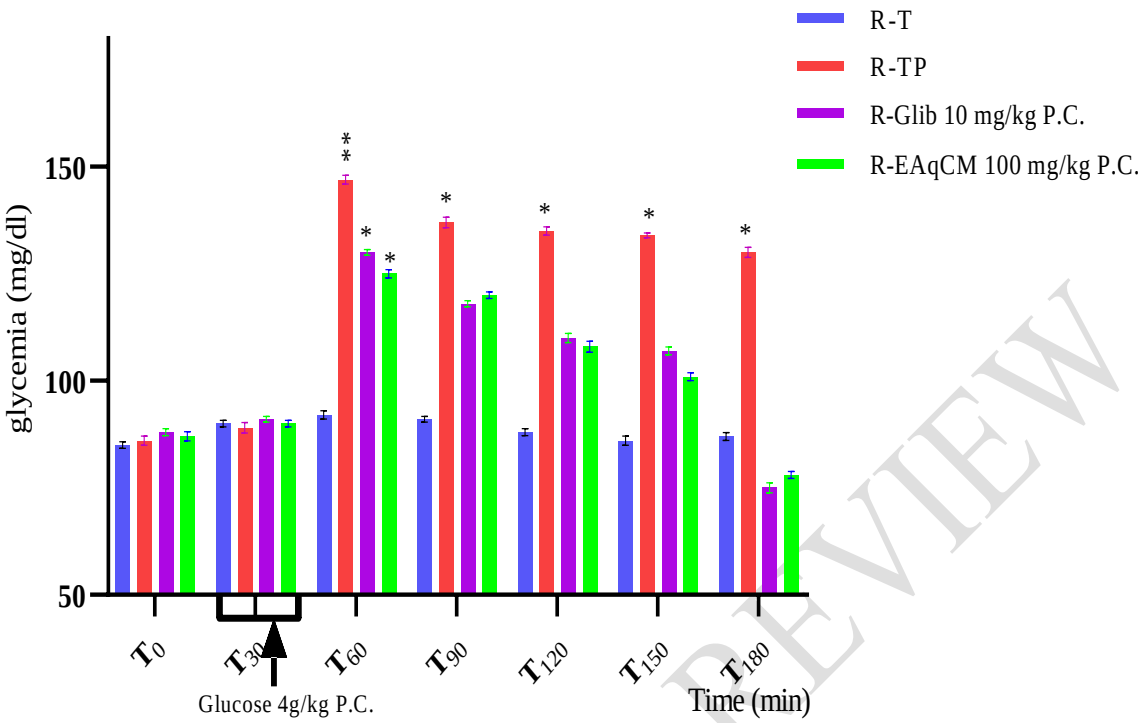

Figure 2: Variation on glycaemia levels of temporarily hyperglycemic rats pretreated with an aqueous leaves extract of *Combretum micranthum* and glibenclamid

*Rats pretreated with EAqCM showed peaks of hyperglycemia at 60 minutes. Ninety (90) minutes after the peak of hyperglycaemia, the glycaemia decreased without reaching baseline blood sugar in all treated animals,(mean ± SEM, *P<0.05; **P<0.01; n=6).*

SEM: Standard Error on the mean;

EAqCM: Aqueous leaves extract of *Combretum micranthum*;

R-T: Control rats.

R-TP: Positive control rat;

R-Glib: Rat having received a dose of 10 mg/kg BW of glibenclamid;

R-EAqCM 100 mg/kg BW: Rat having received the dose of 100 mg/kg BW of EAqCM.

From the 90th minute, a progressive decrease in hyperglycaemia was observed in all pretreated animals until the 180th minute. The glycaemia of rats treated with glibenclamide then fell from 130 ± 1.80 mg/dl to 118 ± 1.39 mg/dl, a reduction of 9.23%. Glycaemia in rats treated with the aqueous extract decreased from 125±2.39 mg/dl to 120±1.98 mg/dl, a decrease of 4%. At the 180th minute, the reduction in glycaemia in the treated rats is more significant, being 73.33% in the rats treated with glibenclamide and 53.84% in the rats treated with the aqueous extract.

3.3.2- Effects of an aqueous leaves extract of *Combretum micranthum*on the glycaemia of post-treated rats

Figure 3 shows the variation in blood glucose levels of transiently hyperglycaemic rats after treatment with an aqueous leaves extract of *Combretum micranthum*.

Thirty (30) minutes after glucose administration, peaks of hyperglycemia were observed in all groups of animals that had received glucose at a dose of 4 g/kg BW. These hyperglycemia peaks were 139 ± 3.6 mg/dl in positive control rats, 135 ± 2.67 mg/dl in glibenclamide-treated rats, and 137 ± 4.78 mg/dl in EAqCM-treated rats.

Thirty (30) minutes after the observation of the hyperglycaemic peaks, the aqueous extract and glibenclamide caused a decrease in the glycaemia of the post-treated rats up to 180 minutes. This reduction was 58.82% in the glibenclamide-treated rats and 57.47% in the EAqCM-treated rats.

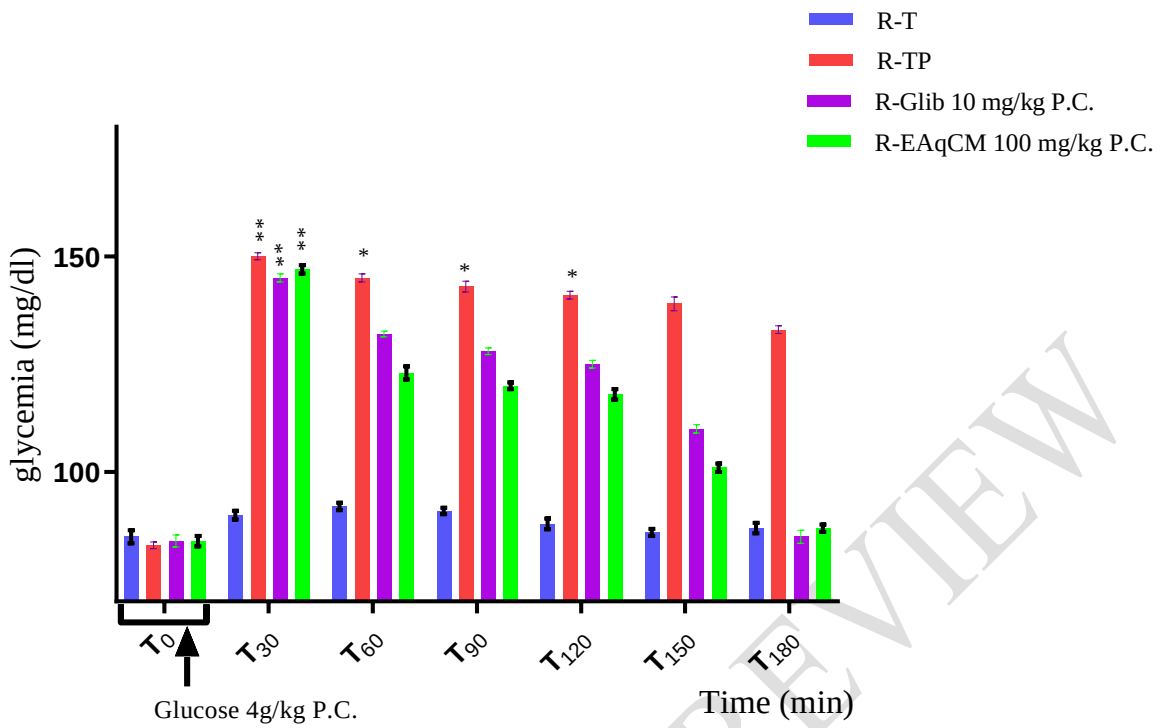

Figure 3: Variation on glycaemia levels of temporarily hyperglycemic rats post-treated with an aqueous leaves extract of *Combretum micranthum* and glibenclamid

*All batches of animals having received glucose (4 g/kg BW) all present peaks of hyperglycaemia thirty (30) minutes after administration. Ninety (90) minutes after the peak of hyperglycaemia, the level of hyperglycaemia decreases without however reaching the baseline serum glucose in the animals of the R-TP, R-Glib batches and R-EAqCM. (mean ± SEM, *P<0.05; **P<0.01; n=6).*

SEM: Standard Error on the mean;

EAqCM: Aqueous leaves extract of *Combretum micranthum*;

R-T: Control rats.

R-TP: Positive control rat;

R-Glib: Rat having received a dose of 10 mg/kg BW of glibenclamid;

R-EAqCM 100 mg/kg BW: Rat having received the dose of 100 mg/kg BW of EAqCM

4. DISCUSSION

Qualitative phytochemical tests carried out on the aqueous leaves extract of *Combretum micranthum* revealed the presence of polyphenols, flavonoids, catechic tannins, quinones, alkaloids of sterols and polyterpenes and saponosides in this extract. We notice the absence of gallic tannins. These results are in agreement with those obtained by Zahoui [10] in the phytochemical study of the aqueous extract of the same plant.

The effects of the aqueous leaves extract of *Combretum micranthum* on glycaemia in normoglycaemic rats showed that the aqueous extract had a good hypoglycaemic activity in animals treated at a dose of 100 mg/kg B.W. These results are similar to those of Aminu and Shaibu [11], who in their work showed that the dose of 100 mg/kg b.w. caused a maximum reduction in glycaemia, unlike the doses of 200 and 400 mg/kg B.W

In rats pre-treated with glibenclamide, the percentage reduction was 73.33% compared to 53.84% in rats pre-treated with the aqueous extract. In rats post-treated with glibenclamide, the percentage reduction was 58.82% compared to 57.47% in rats post-treated with the aqueous extract. Glibenclamide therefore showed the best antihyperglycaemic activity.

These results are similar to those obtained with many medicinal plants in the African pharmacopoeia, such as *Crotalaria retusa* L (Fabaceae) [12], *Parkia biglobosa* (Mimosaceae), [13] *Annona senegalensis* (Annonaceae) and *Hallea senegalensis* (Rubiacaceae) [14].

The effects of the aqueous leaves extract of *Combretum micranthum* are similar to those of glibenclamide, a reference antihyperglycaemic agent. Glibenclamide works by stimulating insulin secretion by binding to and activating the sulfonylurea receptor 1, a regulatory subunit of the ATP-sensitive potassium (K^+-ATP) channel in pancreatic β-cells [15]. The resulting membrane depolarization leads to the opening of calcium-dependent calcium channels, resulting in the influx of calcium into β-cells and the subsequent release of insulin.

The results of our phytochemical screening of *Combretum micranthum* extracts revealed the presence of certain chemical compounds such as flavonoids, tannins, alkaloids and saponins. As several studies have shown, these substances are responsible for the pharmacological activities of the plant extracts. Substances such as polyphenols and flavonoids are generally recognized as having hypoglycaemic effects [16]. Thus, the pronounced antihyperglycaemic effects of the aqueous leaves extract of *Combretum micranthum* in post-treated transiently hyperglycaemic rats would probably be due to the presence of these chemical compounds.

5. CONCLUSION

Phytochemical studies carried out on an aqueous extract of *Combretum micranthum* revealed the presence of polyphenols, flavonoids, catechin tannins, quinones, alkaloids, sterols, and saponoside. We notice the absence of catechic tannins. This aqueous extract induces hypoglycaemic and antihyperglycaemic effects in normoglycaemic and after-treated rats. These effects are similar to those of certain insulin secretors. The results are favorable for the use of this plant in traditional medicine for the treatment of diabetes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All procedures were approved by the Felix Houphouët-Boigny University Ethics Committee for the use and care of laboratory animals

REFERENCES

1. Zhou I, Zhou S, Tang J, Zhang K, Guang I, Huang Y, Xu Y, Ying Y, Zhang I. Protective effect of berberine on beta cells in streptozotocin- and high-carbohydrate/high-fat diet-induced diabetic rats. Eur. J. Pharmacol. 2009; 606:262-268.

2. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas. Diabetes Res. Clin. Pract. 2019; 157:107-843.

3. Yao A, Lokrou A, Koffi-Dago P, Kouassi F, Hué A, Danho J, Abodo J. Epidemiological-clinical profile and mortality of diabetics hospitalized in the endocrinology-diabetology department of Yopougon University Hospital, Abidjan, Côte of ivory. Med. Wrong. Metab 2020; 8(14): 754-760.

4. World Health Organization (WHO) Global Diabetes Report Cataloguing in Source: WHO Library, 2016; 88p.

5. Mangambu MJD, Mushagalusa KF, Kadima NJ. Contribution to the phytochemical study of some antidiabetic medicinal plants from the city of Bukavu and its surroundings (South Kivu, DR Congo). J.Appl. Biosci. 2014; 75:6211-6220.

6. Kolling M, Winkley K, Von D.M. For someone who's rich, it's not a problem. Insights from Tanzania on diabetes health-seeking and medical pluralism among Dares Salam's urban poor. Globalization and Health. 2010, 6(1): 1-9.
7. Goh Bi La, Toto NK, Zahoui OS, Kassi Y, Nene Bi SA, Traore F. Acute and subacute toxicity assessment of an aqueous extract of Crotalaria retusa (Fabaceae) in Swiss mice and Wistar rats. JDDT. 2021; 11(6):94-100.
8. Nanti Goore GCG, Nene Bi SA, Touré A, Traore F. Assessment of antioxidant and anti-diabetic activity of Annona sanegalensis and Hallea ledermanni in alloxan-induced diabetic rats. Phol. 2019, 1:319-336.
9. Aminu C, Shaibu OB. Antihyperglycaemic activity of aqueous leaf extract of Combretum micranthum (Combretaceae) in normal and alloxan-induced diabetic rats. J. Ethnopharmacol. 2010. 129:34–37.
10. Kassi Y, Toto KN, Nanti GGCG, Nene Bi SA, Traoré F. Assessment of the effects of an aqueous trunk bark extract of Parkia biglobosa (mimosaceae) on blood sugar levels in rats. World J. Pharm Pharm Sci. 2018; 7:174-186.
11. Nene Bi SA, Traore F, Zahoui OS, Soro TY. Chemical composition of an aqueous extract of Brideliaferruginea, benth. (Euphorbiaceae) and studies of its toxicological and pharmacological effects in mammals. Afr. Sci. 2008; 4(2): 287-305.
12. Abo KJC. From plant to molecule: toxicity, pharmacological effects and mechanism of action of Justicia secunda (Acanthaceae), antihypertensive plant, on the cardiovascular system of mammals. Ph.D. Thesis. Félix HouphouëtBoigny University; 2013.
13. International Diabetes Federation (IDF). IDF response to the WHO consultation on the Global Action Plan 2013-2020 for the prevention and control of NCDs. Brussels; 2013.
14. Zahoui OS. Phytochemical, toxicological and pharmacological studies of aqueous extracts of four medicinal plants known to be antihypertensive: Heliotropium indicum Linn. (Boraginace), Anacardiumouest Linn. (Anacardiaceae), Combretum micranthum G. Don (Combretaceae) and Zanthoxylum zanthoxyloides Lam. (Rutaceae). Unique thesis in animal physiology and pharmacology. University of Cocody, Abidjan, 2009.
15. Serrano XM, Payares G, Mendoza AL. Glibenclamide, a Blocker of KATP Channels, Shows Antileishmanial Activity in Experimental Murine Cutaneous Leishmaniasis. 2006; Antimicrob. Ag. Chemother, 50 (12): 4214–4216.
16. Mangambu MJD, Mushagalusa KF, Kadima NJ. Contribution to the phytochemical study of some antidiabetic medicinal plants from the city of Bukavu and its surroundings (South Kivu, DR Congo). J.Appl. Biosci, 2014; 75:6211-6220.