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Antidiabetic activity of aqueous extract of leaves of *Cnestis ferruginea* vahl ex DC. (Connaraceae) on alloxan-induced diabetic mice

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Abstract

Background: *Cnestis ferruginea* is a plant species widely used in the traditional african medicine to treat diabetes mellitus. The present study aimed to evaluate the antidiabetic activities of the aqueous extract of the leaves of *Cnestis ferruginea* (AECF) in diabetic mice.

Methods: The mice were grouped into five groups of five animals per group: Group A was not induced with alloxan, Group B animals were induced but not treated, Group C animals were treated with 500 mg / kg of BW of metformin, Group D and E animals were treated with 100, 200 mg/kg BW of AECF respectively. The extracts were administered to the animals orally for 14 days. Fasting blood sugar was measured by a glucometer. Serum concentrations of hematological and biochemical parameters were measured by standard methods.

Results: The animals administered with 100 and 200 mg/Kg B.W of extract showed highly significant decrease ($P < 0.0001$) in blood sugar level compared to the untreated animals. The 100 mg / kg BW dose of AECF produce low significant decrease ($P < 0.05$) of total cholesterol, HDL Cholesterol, AST, ALT, urea, levels of white blood cells, platelets, hematocrit. The 200 mg / kg BW dose of AECF produce in addition significant decrease ($P < 0.01$) of AST and urea.

Conclusion: The 200 mg / kg dose of BW of AECF had a greater antidiabetic activity than the dose of 100 mg / kg BW.

Keywords: *Cnestis ferruginea*; antidiabetic activity; alloxan; diabetes mellitus.

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Background

Plants are inexhaustible sources of various chemical compounds used in traditional medicine to treat various pathologies. The secondary metabolites secreted by plants frequently have biological activity and therefore constitute one of the pathways for the discovery of new drugs [1]. Several recipes from plants are used as alternative medications in the treatment of so-called "emerging" diseases such as diabetes mellitus. Diabetes mellitus is a metabolic disorder characterized of the presence of chronic hyperglycemia [2]. It is a condition that occurs when the body cannot produce insulin, does not make enough insulin, or cannot effectively use the insulin it does produce. This chronic hyperglycemia causes a metabolic disorder and an imbalance of biochemical and hematological parameters leading to cardiovascular and renal complications over time [2,3]. Indeed, according to figure given of the International Diabetes Federation, 463 million people are living with diabetes mellitus in 2019 and this number is expected to reach 578 million in 2030 and 700 million in 2045 [3]. An estimated 4.2 million adults between the ages of 20 and 79 died in 2019 from diabetes and its complications [3].

The latter, because of these alarming figures, is a priority in terms of research into bioactive natural substances. *Cnestis ferruginea* is a species of the Connaraceae family that is found in West Africa, from Senegal to western Cameroon [4]. It is a perennial sarmentose creeping shrub 3.0-3.6 m in height, up to 6 m, sometimes possessing woody tendrils [5,6]. This species is traditionally used to treat diarrhea, general asthenia, inflammation, bronchitis, conjunctivitis, syphilis, gum pain, wounds, dysentery, gonorrhea and diabetes mellitus [7–9]. Scientific studies have demonstrated its laxative, antioxidant, anti-inflammatory, analgesic, antimicrobial and aphrodisiac properties [10,11]. The chemical compounds of the leaves, fruits, stem and roots of *C. ferruginea* have been identified in previous work [11–14]. These different parts contained sterols, polyphenols, flavonoids, tannins, quinone substances, alkaloids and saponosides The anti-diabetic activities of ethyl acetate and methanol extracts from *C. ferruginea* leaves have been demonstrated in a previous study [15]. The aim of this study was to evaluate the antidiabetic activities of the aqueous extract of the leaves of *C. ferruginea* in diabetic mice.

Methods

Animals

Male mice of the Swiss strain *Mus musculus*, were used in this study. Age 9-10 weeks old and weighed an average of 25,12 ± 1,53 g. They were housed in a room with a 12/12 hours' light / dark cycle and an ambient temperature of 28 ± 2 ° C. The animals were fed with a standard diet.

Plant material

The plant material consisted of the leaves of *C. ferruginea*. They were collected in May 2019 in the region of Buyo in Ivory Coast. A sample was authenticated at the National Floristic Center of the University of Félix HOUPHOUËT-BOIGNY based on taxonomic characters and by direct comparison with the herbarium specimens' N ° 3974, 4327 and 15116. The fresh leaves were dried in a room at room temperature for one month. They were then crushed to obtain a fine powder.

Preparation of the extract

In other to prepare the extract, 50 g of *C. ferruginea* leaves powder was introduced into 1000 mL of distilled water (m / V). The mixture was stirred with the mixer for 9 minutes. The homogenate was filtered through Whatman n° 1 paper. The filtrate obtained was evaporated in an oven at 50 ° C until a dry extract was obtained [16]. The weight of the dry extract of *Cnestis ferruginea* leaves (AECF) obtained was 6.96 g, which corresponds to a yield of 13.92%.

Induction of diabetes

The animals fasted for 14 hours and diabetes was induced by intraperitoneal injection of a single dose of alloxan (220 mg / kg BW) dissolved in isotonic solution (0.9% NaCl) at D- 7. The animals developed diabetes after 3 days. Mice with blood glucose greater than or equal to 3 g / L (clinical diabetes ≥1.26 g / L) were selected for the study. The treatment of the animals with the different test substances started on the seventh day after the injection of alloxan which means D0, considered to be the first day of the 14 days of treatment [17].

Animal treatments

The test samples were administered to mice by intragastric gavage at 0.5 mL. Five groups of five mice were formed. Control groups 1 and 2, composed of healthy and diabetic animals respectively, received distilled water while groups 3, 4, and 5 (diabetic groups) received respectively 500 mg/kg of metformin BW, 100 and 200 mg/kg BW of AECF. Fasting blood sugar and body weight were measured on D0, D6, D13. The blood glucose estimate was performed with an On Call® Extra (USA) test strip glucometer. On the 14th day of treatment, caudal blood samples were collected in dry and EDTA tubes for biochemical and hematological testing, respectively.

Biochemical and hematological assay

Kidney function was assessed using serum creatinine and urea. Liver function was evaluated by the enzymatic activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and serum total protein. A lipid profile was used to evaluate the risk of developing cardiovascular disease. The assay of these various biochemical parameters as well as the hematological parameters were carried out using an automatic analyzer (Mindray BS-200E, China). Total cholesterol, HDL-cholesterol and the triglycerides were measured through enzymatic method [18,19]. The AST level are measured through colorimetric method of Karmen [20]; the ALT are measured using colorimetric method as recommended by International Federation of Clinical Chemistry (IFCC) [21]. The total protein concentration was measured according to the method as described by Biuret [22]. The creatinine concentration was measured by the colorimetric method described by Henry [23]. Urea concentration are measured by a colorimetric method [24].

The determination of white blood cell, red blood cell, blood platelets, hemoglobin and hematocrit were made immediately after the blood was collected inside tubes containing anticoagulant EDTA. The analyses are made according to the standard method as described by Baker [25].

Statistical analysis

The various values obtained were expressed as the mean followed by the standard error of the mean (M ± SEM). The significance of the differences observed between the different groups examined is assessed by the analysis of variance (ANOVA) of the Turkey-Kramer multiple comparison test using the GraphPad Prism 7.03 software (San Diego California, USA).

Results

The serum concentration of different parameters in the control group corresponds to the normal standard value.

Change in blood sugar and body weight

Alloxan induces persistently elevated basal glucose in mice three days after administration. The daily oral administration of 100 and 200 mg/kg of BW of AECF causes the same effect on the blood sugar and weight of mice like the dose of 500 mg/kg of BW metformin. The three doses administered produce a very significant decrease (P < 0,001) in hyperglycemia level about 60 %, 68 % et 65 % respectively at the end of 7 days and at the end of our study the decrease rate was highly significant about 69 %, 75 % et 77 % (P < 0,0001) compared to diabetic control (Figure 1). Normal animals gained 21% body weight while a 39% weight loss was observed in the diabetic control at the end of the study. The mice treated with the test substances showed a 21% weight loss following the injection of the diabetogenic agent before their treatments. However, these animals regained their initial weight after two weeks of treatment (Figure 2).

Variation in biochemical parameters

No significant change in the serum concentration of total protein and the creatinine was observed in the groups treated with 100, 200 mg/kg of BW of AECF and 500 mg/kg of BW metformin compared to diabetic control (Table 1). The three doses administered cause a non-significant change in the Total cholesterol and the HDL cholesterol. The dose of 100 and 200 mg/kg BW of AECF cause no effect on the concentration of TG and the ratio Total cholesterol /HDL cholesterol of diabetic mice, contrary to metformin that cause a non-significant reduction (P <0.05) (Table 2). For the other parameters, 100 mg/kg of BW of AECF causes a very little significant reduction (P <0.05) in serum concentration of AST, ALT and urea. The 200 mg/kg of BW of AECF causes a little significant reduction (P <0.05) in serum concentration of AST and significant (P <0.01) in the concentration of urea and ALT. Metformin causes a significant reduction (P <0.01) in the serum concentration of ALT and very significant (P <0.001) in the level of urea and AST (Table 1).

Variation of hematological parameters

The doses of 200 mg/kg BW of AECF and 500 mg/kg of BW cause little significant reduction (P <0.05) in all hematological parameters compare to diabetic control. The 100 mg/kg of BW dose of AECF cause no significant difference in the level of red blood cell and hemoglobin compared to diabetic control (Table 3).

Discussion

Alloxan induces persistent elevation of blood glucose in mice, general elevation of blood concentration in biochemical and hematological parameters, and subsequent acute weight loss. These results are in agreement with those of the literature [16,17]. The significant increase in the concentrations of lipid parameters was due to an increase in gluconeogenesis, the main cause of hyperglycemia, caused by insulinopenia and facilitated by the increase in counter regulating hormones (glucagon, catecholamines, cortisol and growth hormone) [26]. Therefore, the activation of certain enzymes due to insulin deficiency results in an increase in lipolysis associated with an increased breakdown of endogenous proteins, leading to an increase in the serum concentration of the precursors of gluconeogenesis. The early degradation of these primary metabolites would justify the acute weight loss that was observed in diabetic control mice. In addition, the significant increase in the ratio T-Chol / HDL-Chol in these diabetic animals, compared to the normal control would increase the risk of developing cardiovascular disease [27]. An increase in transaminases was also observed in this group. This is a predictive index of liver damage [28]. Although the causes of this elevation can be multiple [29]. However, in the context of persistent hyperglycemia and hypertriglyceridemia, non-alcoholic steatohepatitis could be the cause. This non-alcoholic steatohepatitis results from lipid infiltration into hepatocytes [30]. In addition, the significant increase in the plasma concentration of urea in the diabetic control was due to increased protein degradation under the influence of the counter-regulatory hormones. Animals in this group would therefore be at risk of renal failure. Boeri et al have shown that the significant increase in the plasma concentrations of urea, uric acid and creatinine exposes a sick population to the risk of renal failure which would be due to the complications generated by the process of macroangiopathy [31]. In addition, the risk of infection is considerably high in a hyperglycemic environment. This attests to the increase in the number of leukocytes which was observed in the diabetic control. Indeed, hyperleukocytosis is a classic marker of infection [32]. Diabetes mellitus significantly increases the risk of pulmonary, digestive and urogenital infections [33]. In contrast, significant decreases in the number of red blood cells, hemoglobins and hematocrit were observed in this same group. This suggests that the mice in this group are at risk of anemia. Indeed, it was shown that anemia is a common complication in people with diabetes, especially in those with declared kidney disease [34]. Regarding red blood cells, it was shown that chronic hyperglycemia causes an increase in the level of sorbitol in red blood cells, which interferes with the Na + / K + -ATPase pump, and consequently, an osmotic imbalance occurs, installs and leads to cell death [35].

The daily treatment of diabetic mice with metformin for 14 days at a dose of 500 mg / kg BW induced a highly significant drop in their basal glycemia. As a result, there was a re-establishment of the balance of biochemical and hematological parameters and an initial weight recovery. According to *Foretz et al.,* the main action of metformin is to improve hyperglycemia by inhibiting hepatic gluconeogenesis [36]. Its mechanism of action had been partly elucidated. Metformin inhibits the flow of gluconeogenesis by reducing the synthesis of ATP by inhibiting complex I of the mitochondrial respiratory chain which leads to an increase in the ratio AMP / ATP, admittedly low, but sufficient to reduce this flow of gluconeogenesis. Additionally, increased intracellular AMP concentrations allosterically inhibit fructose-1,6-biphosphatase, a

key enzyme in gluconeogenesis, contributing to the inhibition of glucose production [37].

Treatment of diabetic mice with AECF caused a significant drop in their basal blood sugar and a significant increase in their body weight. In addition, the 200 mg / kg BW dose of AECF had more significant effects on the levels of biochemical and hematological parameters compared to the diabetic control than the 100 mg / kg BW dose, but less significant than metformin. Thus, the 200 mg / kg dose of PC offers better protection of the renal and hepatic function and an improvement of the hematological parameters. However, even though this dose leads to a decrease in total cholesterol and HDL cholesterol, it does not decrease the triglyceride level and the ratio total cholesterol / HDL cholesterol and therefore does not constitute protection against cardiovascular disease. *C. ferruginea* leaves are therefore endowed with antidiabetic properties but do not prevent cardiovascular complications. These results corroborate those of *Adisa et al.* who reported a significant reduction in basal blood sugar in diabetic rats after 10 days of treatment with 250 mg / kg BW of methanolic and ethyl acetate extracts from *C. ferruginea* leaves [15]. These authors found in animals treated with these extracts significant decreases in the levels of transaminases, urea and creatinine and, contrary to our results, a decrease in triglycerides.

Results of previous work showed that the leaves, fruits, stem and roots of *C. ferruginea* contained sterols, polyphenols, flavonoids, tannins, quinonics, alkaloids and saponosides [11–14]. All parts of this plant had high levels of sterols, polyphenols, flavonoids, alkaloids and saponosides while tannins and quinone substances were present in low amounts. The anti-diabetic activities of polyphenols, flavonoids and alkaloids have been widely demonstrated. Indeed, many experimental studies showed that the treatment of animals with these compounds or a diet rich in phenolic compounds and alkaloids prevented and remedied diabetes mellitus [38–40]. The antidiabetic effects of these compounds may be related to the inhibition of carbohydrate digestion by inhibition of salivary and pancreatic ά-amylase and ά-glucosidase in the brush border of the small intestine, to inhibition of glucose uptake, stimulation of insulin secretion and protection of pancreatic β cells against glucotoxicity. They can also suppress the release of glucose from the liver and improve glucose uptake in peripheral tissues by modulating intracellular signaling [41]. Polyphenols have antioxidant activity and can inhibit the formation of advanced glycation products [42]. Indeed, it is well established that antioxidant activity is positively correlated with the structure of polyphenols [43]. Moreover, such as biguanides (metformin) which derive their origin from an alkaloid (galégine) obtained from galéga officinal (Galega officinalis), the alkaloids contained in the leaves of *C. ferruginea* are thought to inhibit hepatic gluconeogenesis, the main cause of the disease. hyperglycemia in diabetes mellitus [44].

In addition, the antioxidant, hepato-protective and antimicrobial activities of the leaves of *C. ferruginea* constitute an asset for the protection of certain organs such as the pancreas, liver, kidneys, heart, spleen subject to tissue damage in an environment of chronic hyperglycemia [45–47].

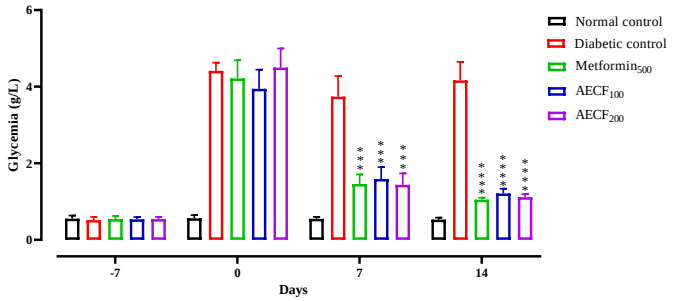

Figure 1. Effect of different doses of *C. ferruginea* leaves extract administration on the fasting blood sugar of animals after 14 days treatment.

*Data are presented as means ± SE (n = 5). *p< 0.05, ** p<0.01, *** p<0.001, **** p<0.0001 compared to Diabetic control*

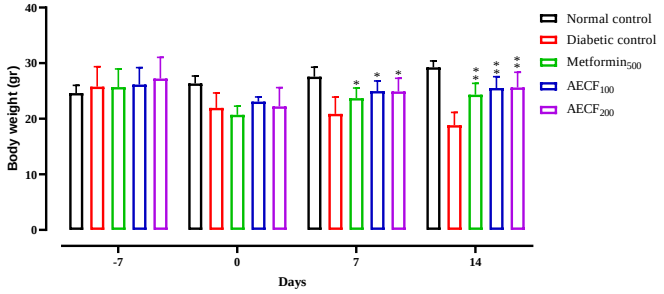

Figure 2. Effect of different doses of *C. ferruginea* leaves extract administration on the body weight of animals after 14 days treatment.

*Data are presented as means ± SE (n = 5). *p< 0.05, ** p<0.01, *** p<0.001, **** p<0.0001 compared to Diabetic control*

Table 1. Effect of different doses of *C. ferruginea* leaves extract administration on serum concentrations of urea, creatinin, AST, ALT and total protein of animals after 14 days treatment.

Treatments	Urea (g/L)	Creatinin (mg/L)	AST (UI/L)	ALT (UI/L)	TP (g/L
Normal control	0.41 ± 0.01	3.00 ± 0.40	196 ± 2	90 ± 2	69 ± 3
Diabetic control	0.54 ± 0.01	3.25 ± 0.48	326 ± 12	140 ± 11	72 ± 4
AECF$_{100}$	0.47 ± 0.01 *	3.50 ± 0.29	272 ± 11 *	110 ± 5 *	74 ± 4
AECF$_{200}$	0.45 ± 0.02 **	3.25 ± 0.25	268 ± 70 *	97 ± 8 **	69 ± 3
Metformin$_{500}$	0.43 ± 0.02 ***	3.25 ± 0.25	239 ± 18 ***	95 ± 2 **	70 ± 6

AST: Aspartate Amino-transferase, ALT: Alanine Amino-transferase, TP: Total Protein

*Data are presented as means ± SE (n = 5). *p< 0.05, ** p<0.01, *** p<0.001, **** p<0.0001 compared to Diabetic control*

Table 2. Effect of different doses of *C. ferruginea* leaves extract administration on serum concentrations of total cholesterol, HDL-cholesterol, ratio total cholesterol/HDL-cholesterol and triglyceride of animals after 14 days treatment.

Treatments	T-Chol (g/L)	HDL-Chol (g/L)	Ratio T-Chol/HDL-Chol	Triglycerid (g/L)
Normal control	0.76 ± 0.08	0.17 ± 0.01	4.47	0.77 ± 0.02
Diabetic control	1.57 ± 0.17	0.27 ± 0.04	5.81	0.86 ± 0.02
$AECF_{100}$	1.12 ± 0.02 *	0.20 ± 0.01 *	5.60	0.82 ± 0.03
$AECF_{200}$	1.10 ± 0.06 *	0.21 ± 0.03 *	5.23	0.83 ± 0.05
$Metformin_{500}$	1.08 ± 0.03 *	0.21 ± 0.01 *	5.14 *	0.81 ± 0.02 *

T-Chol **:** *Total cholesterol, HDL : High Density Lipoprotein*
*Data are presented as means ± SE (n = 5). *p< 0.05, ** p<0.01, *** p<0.001, **** p<0.0001 compared to Diabetic control*

Table 3. Effect of different doses of *C. ferruginea* leaves extract administration on the white blood cell, red blood cell, hemoglobin, hematocrit, blood platelet of animals after 14 days treatment.

Treatments	WBC ($\times 10^3$ μL^{-1})	RBC ($\times 10^6$ μL^{-1})	HGB (g/dL)	HCT (%)	PLT ($\times 10^3$ μL^{-1})
Normal control	2.48 ± 0.22	8.90 ± 0.27	14.60 ± 0.41	49.15 ± 0.98	546 ± 6
Diabetic control	6.21 ± 0.49	7.80 ± 0.21	10.88 ± 0.52	36.43 ± 0.71	376 ± 31
$AECF_{100}$	3.99 ± 0.34 *	7.64 ± 0.26	11.83 ± 0.33	41.55 ± 2.78 *	449 ± 5 *
$AECF_{200}$	3.86 ± 0.15 *	8.11 ± 0.21 *	13.05 ± 0.61 *	39.05 ± 1.55	479 ± 34 *
$Metformin_{500}$	2.75 ±0.40 *	8.44 ± 0.12 *	13.05 ± 0.39 *	46.33 ± 0.62 *	494 ± 26 *

WBC: *White blood cell*, *RBC*: *Red blood cell*, *HGB*: *Hemoglobin*, *HCT*: *Hematocrit*, *PLT*: *Blood platelet*
*Data are presented as means ± SE (n = 5). *p< 0.05, ** p<0.01, *** p<0.001, **** p<0.0001 compared to Diabetic control*

Conclusion

This study enabled us to assess the anti-diabetic activity of the aqueous extract of *C. ferruginea* leaves from Ivory Coast. Ultimately, the leaves of *C. ferruginea* show anti-diabetic activities. The 200 mg / kg BW dose of AECF was found to be the most active. Its antidiabetic activities are like those of metformin. This plant can now be counted among the anti-diabetic plants in the Ivorian pharmacopoeia. However, studies on its toxicity must be undertaken to optimize its use and to achieve the aim of improved Traditional Medicines for the treatment of diabetes mellitus.

Abbreviations

ALT: Alanine Amino-transferase
AST: Aspartate Amino-transferase
BW: Body weight
EDTA: Ethylene Diamine Tetra-acetic Acid
HCT: Hematocrit
HDL: High Density Lipoprotein
HGB: Hemoglobin
IFCC: International Federation of Clinical Chemistry
PLT: Blood platelet
RBC: Red blood cell
T-Chol: Total cholesterol
TP: Total Protein
WBC: White blood cell

Authors' Contribution

Conceived and designed the experiments: EKB, EAO, ENZ, LCAK, DKB, KK. Performed the experiments: EKB, EAO, ENZ. Analyzed the data: EKB, EAO, ENZ, LCAK. Contributed reagents/materials/analysis tools: EKB, EAO, ENZ, LCAK, DKB, KK. Wrote the paper: EKB, EAO, ENZ, LCAK.

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Conflict of interest

The authors declare that there is no conflict of interest.

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