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Effect of *Cassia sieberiana* DC (Fabaceae) on α-amylase and α-Glucosidase and Diabetes Treatment Trial in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author BOZMAS contributed to the literature search, data manipulation and wrote the manuscript. Authors KKAM and BKD contributed to analysis and interpretation of data and wrote the manuscript. Authors AMVH, AMVS, DAJ and NJD contributed to design and structuring of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Diabetes is a metabolic disease caused by a disturbance in the regulation of carbohydrate metabolism. It constitutes a public health problem whose major symptom is chronic hyperglycemia. The present study aims to assess the inhibitory effect of aqueous root extract of *Cassia sieberiana* (AECs) on two glycosidases, as well as its anti-diabetic potential in Wistar rats.
Study Design: Experimental Design.
Place and Duration of Study: Biology and Health Laboratory, Training and Research Unit Biosciences, Félix Houphouët-Boigny University of Abidjan, Côte d'Ivoire, is the place where this study was carried out. The work was conducted from November 2022 to February 2023.
Methodology: The inhibitory effect of AECs on α-amylase and α-glucosidase was determined *in vitro* by measuring its ability to block the activity of these enzymes in the presence of their substrate. As for anti-diabetic activity, this was assessed in Wistar rats during experimental alloxane-induced diabetes. Diabetic rats were then treated with AECs at doses of 25 and 50 mg/kg bw and glibenclamide at a dose of 10 mg/kg bw for three weeks.
Results: AECs exerts weak inhibitory effect on α-amylase activity (IC_{50} = $3.41x10^{4}$ mg/mL) compared to its effect on α-glucosidase activity (IC_{50} =$7.31x10^{-2}$ mg/mL). However, this inhibitory effect is lower than that of acarbose (IC_{50} = $3.13x10^{-4}$ mg/mL). In addition, treatment of diabetic rats reduced hyperglycemia by 38.75%, 55.48% and 63.55% in rats receiving AECs at doses of 25 and 50 mg/kg bw and glibenclamide respectively. In addition, changes in lipid, hepatic and renal profiles, and weight loss in diabetic rats were corrected by this extract.
Conclusion: AECs inhibits α-glucosidase and α-amylase activity, but this effect is better with α-glucosidase. This extract is also able to reduce glycemia in diabetic rats and regulate certain parameters linked to complications associated with diabetes.

Keywords: Cassia sieberiana; glycosidases activity inhibition; antidiabetic activity.

ABBREVIATIONS

AECs : Aqueous extract of Cassia sieberiana
IDF : International Diabetes Federation
WHO : World Health Organization
IC50 : 50% inhibitory concentrations
OECD : Organization for Economic Co-operation and Development
LD50 : Lethal dose 50
GOT : Glutamic-oxaloacetic transaminase
GPT : Glutamic-pyruvic transaminase
GLUT 2: Glucose transporter 2
PPARγ : Peroxisome proliferator-activated receptor gamma

1. INTRODUCTION

Diabetes is a metabolic disease caused by a disturbance in the regulation of carbohydrate metabolism. It is characterized by chronic hyperglycemia, i.e. fasting blood glucose levels in excess of 1.26 g/L, confirmed by at least two control tests [1]. It is one of the major global health emergencies of the 21st century. It ranks among the world's top 10 causes of death and, along with cardiovascular disease, cancer and chronic respiratory disease, accounts for over 80% of premature deaths due to non-communicable diseases [2]. According to the International Diabetes Federation, in 2022, 540 million people were living with diabetes [3], and this number is projected to reach 643 million by 2030, and 783 million by 2045 [4]. Africa had 24 million diabetics and 416,000 diabetes-related deaths [4].

The care of diabetics in developing countries is a major problem, as clinical treatment is extremely expensive and therefore out of the reach of

moderate-income populations. As a result, these populations turn to medicinal plants for treatment. According to the WHO, traditional medicine is used by more than 80% of people in developing countries to treat various pathologies [5], including diabetes. Medicinal plants represent a potential source of natural bioactive molecules, and are the focus of scientific studies aimed at discovering new therapeutic molecules effective against a wide range of diseases, including diabetes. It is in this context that we took an interest in *Cassia sieberiana* (Fabaceae), a plant in the Ivorian pharmacopoeia whose roots, made into powders, are prescribed by traditional health practitioners to diabetics to treat their ailment. Since hyperglycemia is due to a high level of simple sugars in the blood, inhibiting the catabolism of complex sugars into simple sugars could limit their absorption in the intestine, thereby lowering blood sugar levels.

The aim of the present study was to assess the *in vitro* inhibitory effect of aqueous root extract of *Cassia siberiana* on α-amylase and α-glucosidase activity. The anti-diabetic effect of this extract was also studied in Wistar rats during experimental diabetes induced by alloxane.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material consisted of *Cassia sieberiana* roots. Samples of this plant were transferred to the National Floristic Center of Félix Houphouët-Boigny University of Abidjan, where the species was identified.

2.2 Animal Material

The animal model consisted of albino rats (*Rattus norvegicus*) of Wistar strain supplied by the animal house of the Pharmaceutical and Biological Sciences Training and Research Unit of Félix Houphouët-Boigny University of Abidjan. These animals were 8 to 10 weeks old and weighed between 170 and 230 g.

2.3 Preparation of *Cassia sieberiana* Aqueous Extract

Cassia sieberiana aqueous root extract was prepared following the method recommended in traditional medicine [6]. One hundred (100) g of *C. seiberiana* root powder was dissolved in 2 L of distilled water in a suitable container. The mixture was boiled at 100°C for 15 min using an electric stove. After cooling, the resulting homogenate was filtered once through a square of white cloth, then through absorbent cotton and 3 times through filter paper. The filtrate was collected in crystallizers and dried at 60°C for 48 h in an oven (BUCHI ®, France). The dry extract obtained was stored at 4°C for future use.

2.4 Determination of the Effect of *Cassia sieberiana* Extract on α-amylase

The activity of *Cassia sieberiana* aqueous root extract on α-amylase was performed according to the method of Sudha et al. [7]. On a 96-well plate, a reaction mixture containing 50 µL of phosphate buffer (50 mM, pH = 6.8), 10 µL of α-amylase (10 IU/mL) and 20 µL of varying extract concentrations (10^{-6}, 10^{-4}, 10^{-2} and 1 mg/mL) was pre-incubated at 37°C for 10 min. Next, 20 µL of soluble starch (0.05%) was added as substrate and the mixture incubated again at 37°C for 15 min. The reaction was stopped by adding 20 µL HCl (1N), followed by the addition of 100 µL iodine reagent (5 mM I_2 and 5 mM KI). Absorbance was read at 620 nm using an ELISA plate reader (Micro Plate Read, Gentaur). Acarbose at various concentrations (0.01-0.1 mg/mL) was used as a reference. An extract-free control was run in parallel. The percentage of α-amylase inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

The concentration of extract inhibiting 50% of α-amylase activity (IC_{50}) was then determined and compared with that of acarbose.

2.5 Determination of the effect of *Cassia sieberiana* extract on α-glucosidase

The activity of *Cassia sieberiana* aqueous root extract on α-glucosidase was carried out according to the method of Bachhawat et al. [8]. On a 96-well plate, a reaction mixture containing 50 µL phosphate buffer (50 mM, pH = 6.8), 10 µL α-glucosidase (1 IU/mL) and 20 µL of varying extract concentrations was pre-incubated at 37°C for 15 min. A 20 µL volume of p-Nitrophenyl α-D-glucopyranoside (p-NPG) (1 mM) was then added as substrate and the mixture incubated at 37°C for 30 min. The reaction was stopped by adding 50 µL of sodium carbonate (0.1 M), then absorbance was read at 405 nm using an ELISA

plate reader (Micro Plate Read, Gentaur). Acarbose at various concentrations (0.2-1 mg/mL) was used as a reference, and an extract-free control was run in parallel. The percentage of α-glucosidase inhibition was calculated according to the following formula:

$$\% \text{ Inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

The concentration of extract inhibiting 50% of α-glucosidase activity (IC_{50}) was then determined and compared with that of acarbose.

2.6 Acute Toxicity

The acute toxicity of aqueous root extract of *Cassia sieberiana* (AECs) was performed according to Organization for Economic Cooperation and Development (OECD) guideline n° 423 [9]. A predetermined dose (2000 mg/kg bw) of AECs was administered orally to three female rats. These rats were then observed for 24 h and then daily for 14 days.

2.7 Assessment of Antidiabetic Activity

2.7.1 Induction of diabetes

Diabetes was induced in rats using the method of Sing et al. [10]. Normoglycemic rats were injected intraperitoneally with a single dose of 150 mg/kg bw of freshly prepared alloxane solution. Following alloxane injection, an anhydrous glucose solution 5% was made available overnight to overcome hypoglycemia. Seventy-two (72) hours after induction of diabetes, blood was collected by caudal amputation to determine blood glucose levels. Rats with blood glucose levels above 2 g/L were retained for further study.

2.7.2 Treatment of diabetic rats

Five (5) groups of 6 rats each were formed. Treatment was carried out over a 3-week period as follows:

- Group 1 (normal control): normal rats fed distilled water throughout the experiment;
- Group 2 (Diabetic control): diabetic rats given distilled water throughout the experiment;
- Group 3 (Diabetic + glibenclamide): diabetic rats treated with glibenclamide at 10 mg/kg bw;
- Group 4 (Diabetic + AECs25): diabetic rats treated with AECs at 25 mg/kg bw;
- Group 5 (Diabetic + AECs50): diabetic rats treated with AECs at 50 mg/kg bw.

At the end of each week, the animals were weighed, anaesthetized and blood sampled by caudal amputation.

2.7.3 Determination of biochemical parameters

Blood samples collected in tubes without anticoagulant were centrifuged at 3000 rpm for 15 min. The sera collected were used to assay biochemical parameters such as glycemia, glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), total cholesterol, triglycerides, creatinine, urea and total protein, using an automatic analyzer Cobas C311 (ROCHE).

2.8 Statistical Analysis

Statistical analysis of results was performed using Graph Pad Prism 7.0 software (Microsoft USA). Results were expressed as mean values with standard errors on the mean (Mean ± ESM). Differences between means were determined using a single-factor analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Differences are considered significant when P is less than 0.05 ($P < .05$).

3. RESULTS

3.1 Effect of *Cassia sieberiana* Extract on Glycosidases

The 50% inhibitory concentrations (IC_{50}) of aqueous root extract of *Cassia sieberiana* on α-amylase and α-glucosidase are 3.41×10^{4} mg/mL and 7.31×10^{-2} mg/mL respectively. That of acarbose on α-amylase and α-glucosidase are 2.57×10^{-4} mg/mL and 3.13×10^{-4} mg/mL respectively (Table 1). These results show that AECs exerts a more pronounced inhibitory activity on α-glucosidase than on α-amylase, but this activity is lower than that of acarbose.

3.2 Acute toxicity

Administration of aqueous root extract of *C. sieberiana* at a dose of 2000 mg/kg bw produced no signs of toxicity or mortality in rats during the observation period. The lethal dose 50 (LD_{50}) is therefore higher than 2000 mg/kg bw.

Table 1. Effect of *C. sieberiana* aqueous extract on α-amylase and α-glucosidase activity

Enzyme	IC_{50} (mg/mL)		Ratio of IC_{50}
	AECs	Acarbose	
α-amylase	$3.41x10^{4}$	$2.57x10^{-4}$	$1.72x10^{8}$
α-glucosidase	$7.31x10^{-2}$	$3.13x10^{-4}$	234

3.3 Effect of *Cassia sieberiana* aqueous extract on blood glucose levels in rats

The effect of aqueous root extract of *Cassia sieberiana* (AECs) on blood glucose levels in rats is shown in Fig. 1. Before induction of diabetes (D0), the initial blood glucose values of the different groups of rats were statistically equal ($P > .05$) and ranged from 0.73 ± 0.11 to 0.86 ± 0.05 g/L. Three days after alloxane administration (D3), blood glucose levels increased significantly in all intoxicated rats compared with normal control rats (0.84 ± 0.03 g/L). Indeed, blood glucose levels reached values of 3.56 ± 0.31; 3.32 ± 0.44; 3.20 ± 0.62 and 3.01 ± 0.14 g/L respectively in animals from the diabetic control, reference control (Glibenclamide), AECs25 and AECs50 groups, representing increases of 323.81%; 295.24%; 280.95% and 258.33% respectively. Daily administration of *C. sieberiana* extract and glibenclamide to diabetic rats reduced blood glucose levels after one week of treatment (D10). This reduction was significant only with *C. sieberiana* extract at a dose of 50 mg/kg bw. After two weeks of treatment (D17), blood glucose levels were significantly lower in all treated rats than in untreated diabetic rats. At the end of treatment (D24), blood glucose values recorded in all treated animals were 1.28 ± 0.17; 1.96 ± 0.27 and 1.18 ± 0.21 g/L respectively in the reference control, AECs25 and AECs50 groups, corresponding to respective reduction rates of 65.12%; 46.59% and 67.85%.

3.4 Effect of *Cassia sieberiana* Aqueous Extract on Cholesterolemia and Triglyceridemia in Rats

Table 2 shows the effect of aqueous root extract of *Cassia sieberiana* on total cholesterol and triglyceride levels in rats. The results show that the initial total cholesterol and triglyceride levels of all rats were statistically identical. Three days (D3) after induction of diabetes, a significant increase in cholesterol and triglyceride levels was observed in almost all intoxicated rats compared with normal control rats. Treatment of diabetic rats resulted in a decrease in the levels of these biochemical parameters. In the case of total cholesterol, this reduction was significant after one week of treatment (D10), and continued

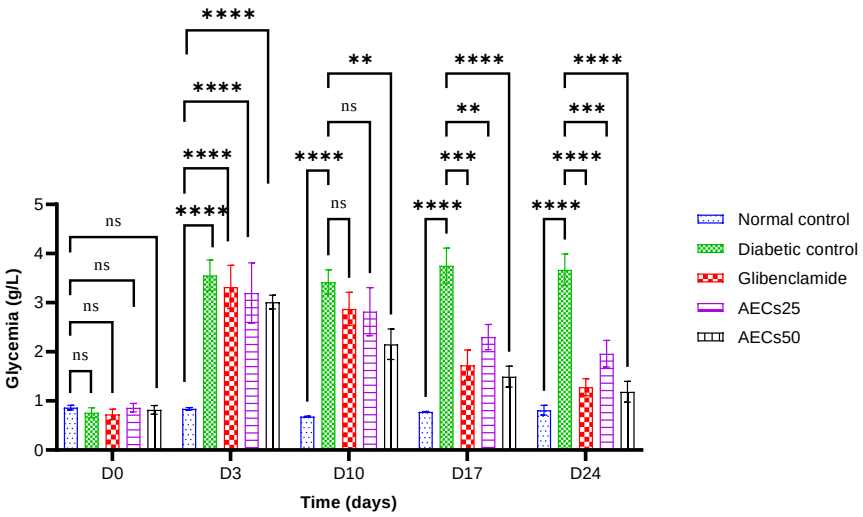

Fig. 1. Effect of *C. sieberiana* aqueous extract on blood glucose levels in diabetic rats

*AECs25: group of rats treated with aqueous extract of C. sieberiana at 25 mg/kg bw; AECs50: group of rats treated with aqueous extract of C. sieberiana at 50 mg/kg bw. Values are expressed as mean ± SEM, n= 5. Symbol (*) represent statistical significance. From D0 to D3, the values of the normal control group were compared with those of the other groups. From D10 to D24, the values of the diabetic control group were compared with those of the other groups. ns: not significant. **P < .01; ***P < .001; ****P < .0001: significant difference between diabetic control group and other groups*

Table 2. Effect of *Cassia sieberiana* aqueous extract on total cholesterol and triglyceride levels in rats

Parameters	Groups	D0	D3	D10	D17	D24
Total cholesterol (g/L)	Normal	1.44±0.01	1.54±0.05	1.60±0.03$^{###}$	1.60±0.3$^{##}$	1.53±0.03$^{###}$
	Diabetic	1.45±0.09^{ns}	2.02±0.03**	2.05±0.01	2.04±0.06	2.11±0.05
	Glibenclamide	1.58±0.22^{ns}	1.95±0.09*	1.69±0.04$^{###}$	1.54±0.18$^{###}$	1.64±0.13$^{###}$
	AECs25	1.63±0.18^{ns}	1.96±0.14*	1.94±0.06$^{#}$	1.83±0.10^{ns}	1.75±0.06$^{##}$
	AECs50	1.51±0.14^{ns}	1.84±0.24^{ns}	1.85±0.04$^{##}$	1.72±0.08$^{#}$	1.70±0.06$^{##}$
Triglycerides (g/L)	Normal	0.83±0.06	0.86±0.05	0.90±0.01$^{#}$	0.84±0.03$^{##}$	0.85±0.03$^{###}$
	Diabetic	0.97±0.10^{ns}	1.10±0.07**	1.12±0.10	1.10±0.07	1.11±0.05
	Glibenclamide	0.88±0.11^{ns}	1.02±0.08*	1.08±0.07^{ns}	0.89±0.09$^{##}$	0.82±0.10$^{###}$
	AECs25	0.92±0.04^{ns}	1.05±0.05**	1.12±0.10^{ns}	0.98±0.05^{ns}	0.89±0.02$^{##}$
	AECs50	0.97±0.13^{ns}	1.00±0.04^{ns}	0.96±0.05^{ns}	0.89±0.04$^{##}$	0.84±0.02$^{###}$

D: day; AECs25: group of rats treated with aqueous extract of C. sieberiana at 25 mg/kg bw; AECs50: group of rats treated with aqueous extract of C. sieberiana at 50 mg/kg bw. Values are expressed as mean ± SEM, n= 5. Symbols (and #) represent statistical significance. For each parameter, comparisons were made by column. From D0 to D3, the values of the normal control group were compared with those of the other groups. From D10 to D24, the values of the diabetic control group were compared with those of the other groups. *P < .05; **P < .01: significant difference between the normal control group and the other groups. #P < .05; ##P < .01; ###P < .001: significant difference between diabetic control group and other groups*

until the end of treatment (D24). The percentage reductions were 22.27% for the reference control group (glibenclamide), 17.06% for the AECs25 group and 19.43% for the AECs50 group at D24. With regard to triglyceridemia, the reduction was significant ($P < .05$) in all treated rat groups only after three weeks of treatment (D24). The percentages of reduction were 26.13% for the reference control group, 19.82% for the AECs25 group and 24.32% for the AECs50 group at D24.

3.5 Effect of *Cassia sieberiana* Aqueous Extract on Urea and Creatinine Levels in Rats

Urea and creatinine levels in different groups of rats were significantly identical ($P > .05$) prior to diabetes induction (D0) (Table 3). Three days after alloxane administration (D3), a non-significant increase ($P > .05$) in the level of these parameters was observed in all intoxicated rats compared with that of normal control rats. During treatment of diabetic rats, decreases in urea levels were recorded from the second week (D17), and at the end of treatment (D24), the values of all treated rats were significantly lower than those of diabetic control rats. The percentage reductions were 35.15%, 15.85% and 30.96% respectively for rats in the reference control (glibenclamide), AECs25 and AECs50 groups. With regard to creatinine levels, the decreases recorded were only significant for all treated groups from the second week of treatment (D17) onwards. The percentages of

Table 3. Effect of *C. sieberiana* aqueous extract on urea and creatinine levels in rats.

Parameters	Groups	D0	D3	D10	D17	D24
Urea (g/L)	Normal	0.29±0.04	0.31±0.03	0.33±0.02$^{#}$	0.29±0.02$^{##}$	0.28±0.02$^{###}$
	Diabetic	0.33±0.04^{ns}	0.34±0.01^{ns}	0.38±0.00	0.40±0.01	0.43±0.01
	Glibenclamide	0.29±0.03^{ns}	0.31±0.01^{ns}	0.38±0.03^{ns}	0.32±0.02$^{##}$	0.28±0.01$^{###}$
	AECs25	0.29±0.03^{ns}	0.37±0.02^{ns}	0.39±0.01^{ns}	0.39±0.03	0.36±0.01$^{#}$
	AECs50	0.31±0.03^{ns}	0.35±0.02^{ns}	0.39±0.04^{ns}	0.31±0.03$^{##}$	0.30±0.02$^{###}$
Creatinine (mg/L)	Normal	6.24±0.25	6.79±0.46	6.82±0.36 $^{##}$	6.74±0.20$^{##}$	6.30±0.24$^{###}$
	Diabetic	6.71±0.20^{ns}	7.41±0.32^{ns}	9.54±0.44	9.86±0.24	9.92±0.61
	Glibenclamide	7.03±0.32^{ns}	7.06±0.27^{ns}	7.17±0.58$^{#}$	7.03±0.46$^{##}$	6.40±0.28$^{###}$
	AECs25	6.85±0.61^{ns}	7.24±0.48^{ns}	9.14±0.76^{ns}	8.07 ±0.30$^{#}$	7.95±0.24$^{#}$
	AECs50	6.94±0.45^{ns}	7.76±0.54^{ns}	8.59±0.67^{ns}	7.61±0.17$^{#}$	7.14±0.38$^{##}$

D: day; AECs25: group of rats treated with aqueous extract of C. sieberiana at 25 mg/kg bw; AECs50: group of rats treated with aqueous extract of C. sieberiana at 50 mg/kg bw. Values are expressed as mean ± SEM, n= 5. Symbols (and #) represent statistical significance. For each parameter, comparisons were made by column. From D0 to D3, the values of the normal control group were compared with those of the other groups. From D10 to D24, the values of the diabetic control group were compared with those of the other groups. *P < .05; **P < .01: significant difference between the normal control group and the other groups. #P < .05; ##P < .01; ###P < .001: significant difference between diabetic control group and other groups*

reduction at the end of treatment (D24) were 35.57% for the reference control group, 19.82% for the AECs25 group and 28.02% for the AECs50 group.

3.6 Effect of *Cassia sieberiana* Aqueous Extract on Rat Transaminase Activity

The effect of aqueous root extract of *Cassia sieberiana* on rat transaminase activity is summarized in Table 4. Induction of diabetes resulted in a non-significant (P >.05) increase in glutamo-oxaloacetic transaminase (GOT) and glutamo-pyruvic transaminase (GPT) activities in all intoxicated rats compared with non-diabetic control rats. During treatment, GOT activity was reduced in all treated diabetic rats compared with diabetic control rats. This decrease was significant in all groups of treated animals from the second week (D17) to the end of treatment (D24). The percentages of reduction recorded at the end of treatment were 16.86% for the reference control group (glibenclamide), 15.22% for the AECs25 group and 16.80% for the AECs50 group. GPT activity was significantly reduced in all treated rat groups after three weeks of treatment (D24). The percentage reductions recorded were 13.79, 8.91 and 15.34% respectively for the reference control, AECs25 and AECs50 groups.

3.7 Effect of *Cassia sieberiana* Aqueous Extract on Rat Total Protein Levels

Induction of diabetes resulted in a non-significant (P > .05) decrease in total protein level in all intoxicated rats compared with normal control rats (Table 5). During the treatment, total protein level increased in all treated diabetic rats compared with untreated diabetic rats. This increase was significant for the group of diabetic rats treated with the reference product (glibenclamide) after one week of treatment (D10), and for the groups treated with AECs, after two weeks of treatment (D17). The percentage increases in protidemia recorded at the end of treatment (D24) were 53.52, 37.55 and 45.47% respectively for the reference control, AECs25 and AECs50 groups.

3.8 Effect of *Cassia sieberiana* Aqueous Extract on Rat Body Weight

Fig. 2 shows the effect of aqueous root extract of *C. sieberiana* on rat body weight. Prior to diabetes induction (D0), rat body weights in all groups were statistically identical (P > .05), ranging from 200.80 ± 1.77 to 223 ± 6.71 g. Three days after alloxane injection (D3), weight values decreased significantly in all intoxicated rats compared with normal control rats (271.8 ± 1.8 g). The values recorded were 173.5 ± 19.56, 173.8 ± 1.93, 182.4 ± 12.47 and 181.4 ± 2.06 g respectively for the diabetic control, reference control (glibenclamide), AECs25 and AECs50 groups. A progressive increase in this parameter was observed during treatment of the animals with glibenclamide and AECs. This increase was significant (P = .0011; P < .001; P < .0001) after two weeks of treatment (D17). The growth rates recorded after three weeks of treatment (D24) were 33.11% for the reference control group, 31.75% for the AECs25 group and 33.84% for the AECs50 group.

Table 4. Effect of *C. sieberiana* aqueous extract on rat transaminase activity

Parameters	Groups	D0	D3	D10	D17	D24
GOT	Normal	77.36±2.50	78.67±1.77	78.14±3.6^{ns}	77.13±2.22$^{###}$	76.60±0.74$^{###}$
(IU/L)	Diabetic	77.37±2.20^{ns}	85.26±2.62^{ns}	85.43±0.36	92.52±0.94	97.53±1.79
	Glibenclamide	76.77±1.96^{ns}	80.40±2.62^{ns}	79.61±2.38^{ns}	83.18±1.46$^{##}$	81.09±1.17$^{###}$
	AECs25	75.71±0.88^{ns}	78.98±2.19$_{ns}$	85.33±1.06^{ns}	84.09±2.09$^{#}$	82.69±2.34$^{###}$
	AECs50	77.99±2.15^{ns}	82.64±2.93^{ns}	83.34±1.05^{ns}	84.68±2.10$^{#}$	81.15±2.41$^{###}$
GPT	Normal	35.30±0.53	36.20±0.51	35.26±0.81^{ns}	36.05±0.71$^{#}$	34.23±0.45$^{###}$
(IU/L)	Diabetic	33.72±0.90^{ns}	38.24±1.23^{ns}	38.94±0.94	41.26±1.03	43.35±1.03
	Glibenclamide	33.21±0.72^{ns}	37.88±0.89^{ns}	40.35±1.66^{ns}	37.97±1.53^{ns}	37.38±1.37$^{##}$
	AECs25	34.64±0.67^{ns}	38.21±0.77^{ns}	39.10±0.54^{ns}	40.44±0.79^{ns}	39.49±0.42$^{#}$
	AECs50	32.84±0.32^{ns}	39.51±0.38^{ns}	40.06±1.11^{ns}	38.50±0.91^{ns}	36.70±0.54$^{##}$

D: day; AECs25: group of rats treated with aqueous extract of C. sieberiana at 25 mg/kg bw; AECs50: group of rats treated with aqueous extract of C. sieberiana at 50 mg/kg bw. Values are expressed as mean ± SEM, n= 5. Symbols (and #) represent statistical significance. For each parameter, comparisons were made by column. From D0 to D3, the values of the normal control group were compared with those of the other groups. From D10 to D24, the values of the diabetic control group were compared with those of the other groups. *P < .05; **P < .01: significant difference between the normal control group and the other groups. $^{#}$P < .05; $^{##}$P < .01; $^{###}$P < .001: significant difference between diabetic control group and other groups.*

Table 5. Effect of *C. sieberiana* aqueous extract on rat total protein levels

Parameters	Groups	D0	D3	D10	D17	D24
Total protein (g/L)	Normal	70.51±2.23	70.63±1.32	67.60±1.32$^{\#}$	70.50±2.23$^{\#\#\#}$	71.60±0.96$^{\#\#\#}$
	Diabetic	74.57±1.66^{ns}	69.07±1.96^{ns}	58.04±2.01	51.59±2.67	48.19±2.86
	Glibenclamide	72.02±2.16^{ns}	69.05±1.60^{ns}	66.24±2.48$^{\#}$	69.69±2.51$^{\#\#\#}$	73.98±2.85$^{\#\#\#}$
	AECs25	73.40±3.94^{ns}	68.31±2.19^{ns}	62.32±1.41	63.39±3.00$^{\#\#}$	66.28±1.72$^{\#\#\#}$
	AECs50	70.34±1.11^{ns}	64.35±2.57^{ns}	59.85±1.21	64.68±0.85$^{\#\#}$	70.10±0.61$^{\#\#\#}$

D: day; AECs25: group of rats treated with aqueous extract of C. sieberiana at 25 mg/kg bw; AECs50: group of rats treated with aqueous extract of C. sieberiana at 50 mg/kg bw. Values are expressed as mean ± SEM, n= 5. The symbol (#) represents statistical significance. For each parameter, comparisons were made by column. From D0 to D3, the values of the normal control group were compared with those of the other groups. From D10 to D24, the values of the diabetic control group were compared with those of the other groups. $^{\#}P < .05$; $^{\#\#}P < .01$; $^{\#\#\#}P < .001$: significant difference between diabetic control group and other groups

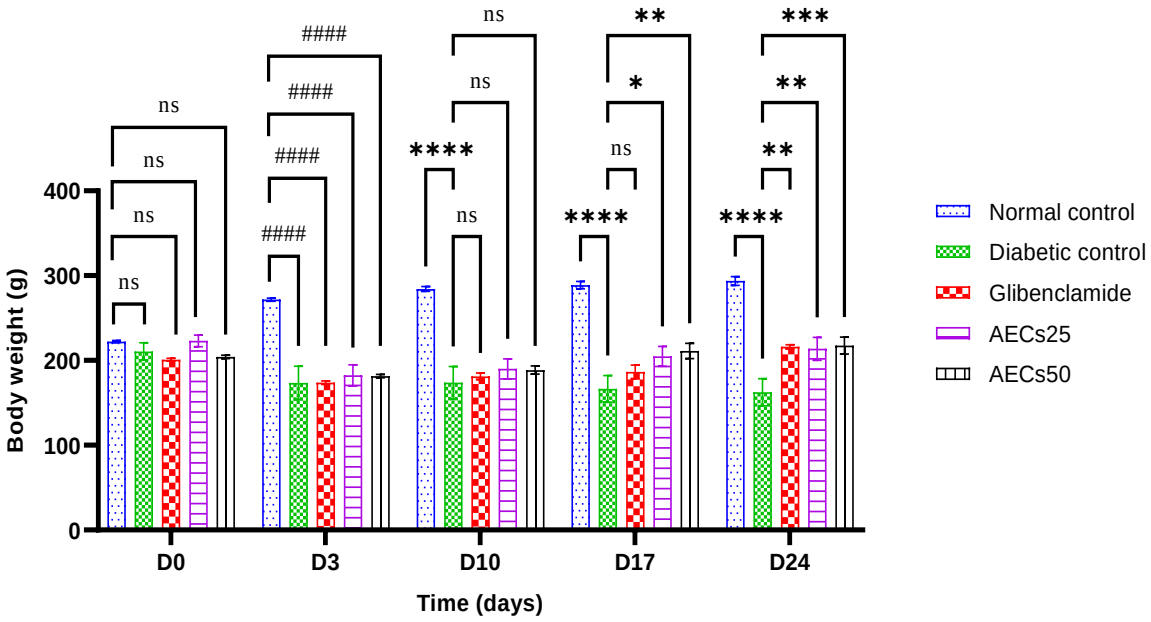

Fig. 2. Effect of *C. sieberiana* aqueous extract on rat body weight

*AECs25: group of rats treated with aqueous extract of C. sieberiana at 25 mg/kg bw; AECs50: group of rats treated with aqueous extract of C. sieberiana at 50 mg/kg bw. Values are expressed as mean ± SEM, n= 5. Symbols (*and #) represents statistical significance. From D0 to D3, the values of the normal control group were compared with those of the other groups. From D10 to D24, the values of the diabetic control group were compared with those of the other groups. ns: not significant. $^{\#\#\#\#}p < .0001$: significant difference between normal control group and other groups. $^{**}p < .01$; $^{***}p < .001$; $^{****}p < .0001$: significant difference between diabetic control group and other groups*

4. DISCUSSION

In this study, the inhibitory effect of aqueous root extract of *Cassia sieberiana* (AECs) on α-amylase and α-glucosidase activity was assessed. The anti-diabetic potential of this plant was also evaluated during experimental alloxane-induced diabetes in rats. The results obtained indicate that AECs weakly inhibits the activity of α-amylase compared with that of α-glucosidase. These enzymes are involved in carbohydrate digestion. Alpha-amylase hydrolyzes complex starches into oligosaccharides, while α-glucosidase hydrolyzes oligosaccharides into glucose and other monosaccharides. Their inhibition significantly reduces the post-prandial rise in blood glucose and may therefore be an important strategy in blood glucose management in diabetics [11,12]. The inhibitory effect of this extract, although better on α-glucosidase than on α-amylase, is however inferior to that of acarbose, the reference inhibitory substance. This effect is probably due to polyphenols, flavonoids and

catechic tannins present in this plant extract. According to Hanhineva et al. [13], flavonoids, phenolic acids and tannins inhibit the activity of α-amylase and α-glucosidase, key enzymes in carbohydrate metabolism.

In the anti-diabetic activity study, intraperitoneal administration of alloxane to rats resulted in a significant increase in blood glucose levels three days after intoxication. Alloxane is a glucose analogue which, at high doses, exerts a selective cytotoxic effect on pancreatic β-cells. It penetrates β-cells via the GLUT 2 transporter and leads to their destruction, generating a deficit in insulin secretion [14]. This reduced secretion of insulin, a hypoglycemic hormone, results in hyperglycemia. In the present study, untreated alloxane-treated rats (diabetic control rats) showed significantly higher blood glucose levels than that of normal control rats, and remained more or less stable throughout the experiment. On the other hand, as with glibenclamide at 10 mg/kg bw, treatment of diabetic rats for three weeks with aqueous root extract of *C. sieberiana* at doses of 25 and 50 mg/kg bw resulted in a very significant reduction in blood glucose levels. The reduction in blood glucose was better with the dose of 50 mg/kg bw of *C. sieberiana* extract. According to Vessal et al. [15] and Coskun et al. [16], the reduction in hyperglycemia observed in this study is linked to the action of certain hypoglycemic compounds present in the aqueous roots extract of this plant. In fact, saponins, tannins, flavonoids, alkaloids and terpenes found in this extract are anti-diabetic agents that act synergistically, as indicated by Maithili et al. [17].

Concerning lipid balance, analysis of the results revealed a non-significant increase in total cholesterol and triglyceride levels in the untreated diabetic group compared with the other experimental rats from the 1st to the 21st day of treatment. Elevated triglyceride and total cholesterol levels in diabetic rats are characteristic of dyslipidemia according to Girard [18]. This dyslipidemia is thought to be the result of both deficit in insulin secretion and insulin resistance characteristic of type 2 diabetes. After 21 days' treatment of rats with aqueous root extract of *C. sieberiana*, triglyceridemia and total cholesterol levels were significantly lower than in untreated diabetic rats. These hypolipidemic effects are thought to be linked to the presence of polyphenolic compounds in this extract. Indeed, according to Richard [19], flavonoids inhibit adipocyte differentiation and lipid accumulation by inhibiting the expression of peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ plays a major role in adipocyte differentiation, adipogenesis and lipid accumulation. As a result, *C. sieberiana* aqueous extract improves triglyceridemia and total cholesterol levels in diabetic rats. The reduction in hypercholesterolemia in rats treated with glibenclamide and those treated with *C. sieberiana* extract may be due to the lowering of key cholesterol transporters [20]. Several studies on the *Crataegus* genus have suggested that the antihypercholesterolemic effect may be due to flavonoids, triterpenes and saponins [21]. A clear reduction in total lipids was observed in animals treated with this plant, which could be due to lower triglyceride and total cholesterol levels.

The kidneys produce urine, which contains metabolic waste products such as urea and creatinine. They also play a vital role in maintaining the body's water and electrolyte balance. Any phenomenon or substance capable of altering these different renal functions (glomerular filtration, tubular reabsorption and secretion) inevitably leads to changes in plasma urea and creatinine concentrations at the metabolite level [22,23]. Urea and creatinine are common semiological parameters for the diagnosis of renal function [24]. They are significant markers of renal function [25]. Analysis of the results obtained in this study reveals that urea and creatinine levels in untreated alloxane-treated rats (diabetic control rats) were increased compared with those in normal control rats. This increase implies alloxane-induced renal dysfunction in these animals. Treatment of diabetic rats for 21 days with *C. sieberiana* extract of and glibenclamide significantly reduced uremia and creatinemia, resulting in a normal recovery of renal function.

The results also showed that glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activities were not significantly increased in diabetic control rats. Transaminases are the most commonly indexed indicators of liver damage. Any increase in their serum activity indicates destruction of liver parenchyma or increased membrane permeability of hepatocytes [26, 27]. A significant decrease in the activity of these enzymes was observed in all diabetic rats treated with *C. sieberiana* extract and glibenclamide after three weeks of treatment.

As regards total protein, the results revealed a significant decrease in levels in diabetic control rats. The same applied to body weight, which was significantly reduced in these rats. However, after 21 days of treatment, a significant increase in total protein levels was observed in all treated diabetic rats compared with untreated rats. Weight gain was also observed in all treated animals. This increase in total protein levels and body weight could be explained by the lower blood glucose levels in these rats. According to Babu and stanely [28], lower blood sugar levels in diabetic rats are accompanied by weight gain. These results may also suggest that aqueous root extract of *C. sieberiana* contains compounds capable of inhibiting proteolysis or restoring insulin activity. According to the work of Daisy et al. [29], insulin plays an important role in maintaining protein balance, as it not only stimulates amino acid absorption and protein synthesis, but also inhibits protein degradation. Aqueous roots extract of *C. sieberiana* would therefore improve protidemia in diabetic rats.

5. CONCLUSION

At the end of this work, it emerged that aqueous root extract of *Cassia sieberiana* (AECs) inhibits α-amylase and α-glucosidase activity *in vitro*, and this effect is more pronounced on α-glucosidase. Furthermore, AECs have good antihyperglycemic potential during experimental alloxane-induced diabetes in rats and may prevent diabetes-related complications. This justifies its use in traditional medicine for the treatment of diabetes.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experimental procedures have been examined and approved by the Ethical Committee of Health Sciences, Félix Houphouët-Boigny University of Abidjan.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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