

Aqueous extract of *Cassia siamea* Lam leaves exhibited antihyperglycemic effect and improved kidney function in diabetic Wistar rats

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Abstract

Background and Objective: The leaves of *Cassia siamea* Lam. (Fabaceae) have been widely used in folklore medicine for the treatment of diabetes and hypertension. This study aimed to investigate if aqueous extract of *C. siamea* leaves can protect against hyperglycemia in experimental diabetes.

Materials and methods: Preliminary, acute toxicity of aqueous extract of *C. siamea* (LACS) was evaluated. Then, acute hypoglycemic test and oral glucose tolerance test (OGTT) were performed for three doses of LACS (100, 200 and 400 mg/kg/bw; vo) in non-diabetic Wistar rats. Third, in alloxan-induced diabetic rats, oral treatment with LACS (200 mg/kg/day), vehicle (10 mL/kg/day) and glibenclamide (10 mg/kg/day) was performed for 4 weeks. Fasting blood glucose, changes in body weight and food intake were evaluated. Biomarkers of kidney functions (urea and creatinine) were determined.

Results: Dose of 2000 mg/kg bw of aqueous extracts of *C. siamea* was not toxic. In non-diabetic rats, LACS significantly prevented oral glucose-induced hyperglycemia. In diabetic rats, this extract significantly increased the body weight without modifying the food intake after four weeks of administration. Interestingly, it significantly reversed the hyperglycemia and improved kidney functions, i.e reduced serum urea and creatinine levels without affecting the serum concentrations of Na⁺, K⁺, Cl⁻ and Ca²⁺.

Conclusions: These findings proved that leaves of *C. siamea* owned a benefic effect on hyperglycemia and protected against kidney dysfunctions in diabetes. Hence, it could be used in the management of diabetes.

Keywords: *Cassia siamea*, Antihyperglycemia, Diabetes, Kidney function

1. Introduction

Diabetes mellitus is a complex multisystemic disorder characterized by alterations in the metabolism of carbohydrate, fat and protein, and is caused by a relative or absolute deficiency of insulin secretion [1]. Worldwide, the total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 with 10% of type 1 diabetes mellitus [2]. This endocrine disease is associated with damage of various organs, particularly to heart, kidney, eyes, nerves, liver, blood vessels, and immunological and gastrointestinal system [3]. For its control and treatment, various chemical agents were available, but total recovery from diabetes has not been reported. So, as alternative to conventional medicine, more than 800 plants are used as traditional remedies against diabetes mellitus due to their effectiveness, less side effects and low cost [4].

Cassia siamea (syn. *Senna siamea*) was a Fabaceae native of South-east of Asia and found in most tropical countries. Further studies had reported that it had been used as remedy for malaria, fever, constipation, hypertension, and diabetes mellitus. Phytochemistry studies have shown the presence of many bioactive compounds including alkaloids, polyphenols, flavonoids, phenolics acid, triterpenes glycosides, carotenoids, tanins and saponins. Several bioactive molecules have been identified as barakol, anhydrobarakol, cassiarin A-B, chrysophanol, emodin, D-pinitol, luteolin, lupeol, cassiamin A-B, sennoside A, coumarin, rutin, myricetin, quercetin, and kaempferol [5]. In literature, few studies have been conducted to determine antidiabetic activity of this medicinal plant. Hence, the present study assessed the efficacy of aqueous extract of leaves of *C. siamea* (LACS) on non-diabetic and alloxan-induced diabetes rats.

2. Materials and methods

2.1. Chemicals

Organic solvents such as hexane (Quimicen[®], Spain), ethanol (Prolabo[®], France), and methanol (Quimicen[®], Spain) were used. Also, lidocaine gel (AstraZeneca[®], UK), NaCl (Riedel-de Haen, Germany), (+) D-glucose (Riedel-de Haën[®], Germany), metformin (Denk Pharma[®], Germany), glibenclamide (Daonil[®], Sanofi-adventis) were used. Tween[®] 80 and alloxan monohydrate were purchased from Sigma–Aldrich (Germany).

2.2. Collection of the plant material

Fresh younger leaves of *C. siamea* were collected from *Adiopodoumé* village, Ivory Coast, in June 2015. The taxonomic serial number in the integrated taxonomic information system (ITIS) was 505177. A specimen voucher was recorded at the National Herbarium of Floristic Center of Abidjan, Ivory Coast, with collection number 126 /97.

2.3. Preparation of extracts

After shade drying, the dried leaves of *C. siamea* were powdered in mechanical grinder (Phillips[®]). Fifty gram (50 g) of the powdered material were extracted in decoction within 30 min in 1.5 L of distilled water. The aqueous extract was filtered using Fisherbrand[®] paper, then lyophilized (Martin Christ-ALPHA 2-4 LD plus[®], Germany) and stored at 4°C until use.

2.4. Animals

Three-months-old healthy male and female Wistar rats (150 - 200 g) were procured from animal resources laboratory of the Pasteur Institute of Ivory Coast. The animals were provided with standard pellet diet and water ad libitum. They were kept at standard living conditions (room temperature of 25 ± 2°C and 12 h dark/light cycle) during all experiments according to the international standards of animal use and care.

2.5. Acute oral toxicity study

The study was conducted in strict accordance with the guidelines of organization for economic cooperation and development for testing of chemicals, acute toxic class method [6]. Female Wistar albino rats were grouped randomly into two groups (n = 5 / group) and fasted overnight. Thus, the animals in each group were treated 1-1 at intervals of 48 hours. Orally, group I received vehicle (2% Tween 80); group II received a single dose at 2000 mg/kg/b.w. (p.o.) of LACS. After administration of these substances, food was withheld for a further period of 3-4 hours. General clinical observations were made after dosing at least once during the first 30 minutes, with special attention given during the first 4 hours; periodically during the first 24 hours and daily thereafter, for a total of 14 days.

2.6. Study on non-diabetic animals

2.6.1. Acute hypoglycemic test

Overnight fasting normoglycemic rats were randomly divided into five groups (n = 5 / group). Fasting blood glucose (FBG) level from tail vein of each animal was determined at initial time (0 h) using glucometer (Accu-Chek[®] Active, Roche). After that, group I (negative control) received vehicle, i.e, 2% tween 80 (v/v); group II (positive control) received 10 mg/kg b.w. (p.o.) of glibenclamide, a standard hypoglycemic drug (Daonil[®], Sanofi-adventis); group III-V received LACS at the doses of 100, 200 and 400 mg/kg b.w. (p.o.), respectively. Glucose level was estimated at 0.5, 1, 2, 4 and 6 h.

2.6.2. Oral glucose tolerance test (OGTT)

To determine the effect of *C. siamea* on postprandial glycemia, health normoglycemic rats fasted overnight and randomly divided into five groups (n = 5 / group): group I served as a vehicle control; group II for the standard drug, 10 mg/kg/b.w. (p.o.) of glibenclamide; group III-V received LACS at 100, 200 and 400 mg/kg/b.w. (p.o.) respectively. Before administration of the different tested substances, the baseline blood glucose level from tail vein was estimated using glucometer. Thirty minutes (30 min) after dosing, all groups received an oral solution of (+) D-glucose (4 g/kg b.w.). Thus, blood glucose level was determined at 0, 0.5, 1, 1.5, 2, 4 and 6 h after oral glucose administration.

2.7. Sub-acute activities of LACS on diabetic animal model

2.7.1. Induction of experimental diabetes

Diabetes was induced in overnight-fasted rats by a single subcutaneous injection of freshly prepared alloxan monohydrate solution (100 mg/kg b.w., dissolved in NaCl 0.9%), according to Moradabadi *et al.* [7]. Rats were tested for successful induction of diabetes 3 days after alloxan injection by determining FBG levels from the tail vein of overnight fasted rats. Only rats with blood glucose levels ≥ 200 mg/dL were enrolled in the study.

2.7.2. Experimental design

In the experiment, 24 rats (6 non diabetic rats and 18 diabetic persisting rats) were used. All diabetic animals were randomly divided into three groups (n = 6/group). So, group I (NDC –non diabetic control rats) and group II (DC - diabetic control rats) both received vehicle (2% Tween 80); group III (D+Glib - diabetic rats treated with glibenclamide 10 mg/kg/day b.w.), and group IV (D+LACS - diabetic rats treated with LACS 200 mg/kg/day b.w.).

The vehicle, tested extracts or standard drug were administered by gavage once daily during 28 days under oral lidocaine gel anaesthesia (Astra Zeneca®, UK). Body weight, food intake, and FBG levels were determined weekly during the four weeks treatment period. After sacrifice, blood samples were left to stand for 15 min and centrifuged at 500g for 10 min at 2°C, then serum was collected and kept at -20°C for subsequent biochemical determination.

2.7.3. Biochemical analysis

The serum levels of glucose, urea, creatinine, Na⁺, K⁺, Cl⁻ and Ca²⁺ were analyzed using HITACHI 704R® with Biolabo® biochemical kits and ISE 3000® automatics electrolytes analyzers with compatibles reagents packs.

2.8. Statistical Analysis

The results were expressed as mean ± standard error of mean (S.E.M.). Statistical analysis was performed using Graph Pad Prism 5.0® software. The difference between groups was assessed by analysis of variance (ANOVA), followed, when necessary, by the Turkey's test or Bonferroni post-tests. Differences were considered statistically significant for p < 0.05.

3. Results

3.1. Acute oral toxicity

Single administration of LACS (2000 mg/kg b.w., vo) did not produce abnormal behavior for initial 4 h. Mortality was not recorded during 14 days after treatment. The body weight of LACS administered rats was also similar to those of vehicle treated rats (data not shown). According to OCDE analysis, the acute oral LD₅₀ of LACS in Wistar rats were estimated > 2000 mg/kg. Hence, doses of 100, 200 and 400 mg/kg were chosen for further experiments.

3.2. Acute effect of LACS on fasting glucose in non-diabetic rats

Acute administration of LACS in fasting rats at the dose of 100, 200 and 400 mg/kg did not induce significantly hypoglycemic effect compared to the control group after 6h. Indeed, initial blood glucose level was reduced by 30.25 %, 22.71 %, and 19.11 % for LACS at the respective three doses compared to control group (13.13%). As expected, glibenclamide reduced significantly the blood glucose level by 50.99, p < 0.01 (Table 1).

Table 1: Hypoglycemic test in non-diabetic rats

Groups	Blood glucose level (mg/dL)					
	Mean ±SEM (% change)					
	T0	T30 min	T1H	T2H	T4H	T6H
CTR	80.80±5.52	87.00±9.56	86.60±10.04	80.20±9.08	76.20±11.21	70.60±6.97
(2%Tween80)	(0.00)	(6.78)	(6.34)	(-1.64)	(-7.24)	(-13.13)
Glib 10 mg/kg	87.20±5.51	91.40±5.96	65.80±1.83	47.20±2.27***	48.20±4.26**	41.80±4.26**
	(0.00)	(5.11)	(-23.19)	(-44.92)	(-44.15)	(-50.99)
LACS	91.40±10.49	84.00±5.94	82.80±8.78	73.00±10.37	68.20±12.89	64.60±10.08
100 mg/kg	(0.00)	(-5.76)	(-8.88)	(-20.93)	(-27.70)	(-30.25)
	94.20±2.58	94.80±3.31	95.80±5.92	87.20±3.89	78.00±4.64	73.00±4.44
200 mg/kg	(0.00)	(0.70)	(1.83)	(-7.28)	(-17.22)	(-22.71)
	88.80±4.40	93.40±2.54	86.00±5.37	79.00±4.53	73.20±8.75	71.80±4.78
400 mg/kg	(0.00)	(5.74)	(-3.26)	(-10.82)	(-17.96)	(-19.11)

Results are express as mean ± SEM, (n=5). The data was analyzed using Two-way ANOVA followed by Bofferroni posttests (p < 0.05), *statistically different to CTR. LACS did not exhibit hypoglycemic effect.

3.3. Effect of LACS on postprandial hyperglycemia in non-diabetic rats

Blood glucose level reaches progressively a peak 30 min after glucose oral administration. As expected, standard drug, glibenclamide (10 mg/kg b.w, vo) significantly exhibited inhibitory effect on increasing of blood glucose level (p < 0.001) compared to control. At the dose of 400 mg/kg, LACS significantly reduced the peak of hyperglycemia, p < 0.05 (Table 2).

Table 2: Oral glucose tolerance test in non-diabetic rats

Groups	Blood glucose level (mg/dL)						
	Mean \pm SEM (% change)						
	T -30min	T0	T30min	T1H	T2H	T4H	T6H
CTR (2%Tween80)	85.60 \pm 3.16 (0.00)	88.80 \pm 3.93 (3.89)	195.80 \pm 16.33 (127.91)	169.80 \pm 11.45 (98.56)	135.80 \pm 11.38 (60.24)	100.00 \pm 10.68 (17.20)	84.60 \pm 8.38 (-1.14)
Glib 10 mg/kg	85.20 \pm 5.29 (0.00)	72.60 \pm 6.14 (-14.99)	89.20 \pm 3.35 ^{***} (6.32)	88.60 \pm 4.13 ^{***} (5.46)	67.60 \pm 2.54 ^{***} (-20.02)	58.60 \pm 2.40 ^{**} (-30.47)	58.40 \pm 0.93 (-30.45)
LACS 100 mg/kg	89.20 \pm 4.99 (0.00)	93.80 \pm 1.69 (6.32)	164.20 \pm 12.47 (85.42)	140.60 \pm 10.36 (59.20)	114.80 \pm 10.69 (32.24)	78.80 \pm 2.96 (-10.70)	75.40 \pm 2.38 (-14.43)
200 mg/kg	90.20 \pm 6.36 (0.00)	94.20 \pm 60.09 (4.96)	170.80 \pm 20.17 (87.32)	142.40 \pm 15.64 (57.62)	120.00 \pm 7.20 (36.14)	73.60 \pm 3.6 (-17.27)	71.80 \pm 1.50 (-18.42)
400 mg/kg	92.20 \pm 7.10 (0.00)	95.40 \pm 6.98 (4.00)	159.00 \pm 11.90 [*] (75.09)	150.00 \pm 9.04 (65.50)	131.20 \pm 3.26 (45.98)	78.60 \pm 3.37 (-13.44)	76.40 \pm 3.23 (-15.93)

Results are express as mean \pm SEM, (n=5). The data was analyzed using Two-way ANOVA followed by Bofferroni posttests (p < 0.05), *statistically different to CTR. LACS significantly reduced the peak of hyperglycemia at the dose of 400 mg/kg, p<0.05.

3.4. Effect of LACS on body weight and food intake in alloxan-induced diabetic rats

Figures 1a and 1b present the body weight gain and food intake of experimental rats within the period of treatment, respectively. So, LACS increased the body weight gain compared to non-diabetic control (p<0.001) and diabetic control (p<0.05) groups. Food intake was significantly high in diabetic control rats compared to non-diabetic control rats (p<0.001). The supplementation of LACS did not reduce significantly the increase of food intake observed in diabetic control animals. This effect was similar to that of glibenclamide.

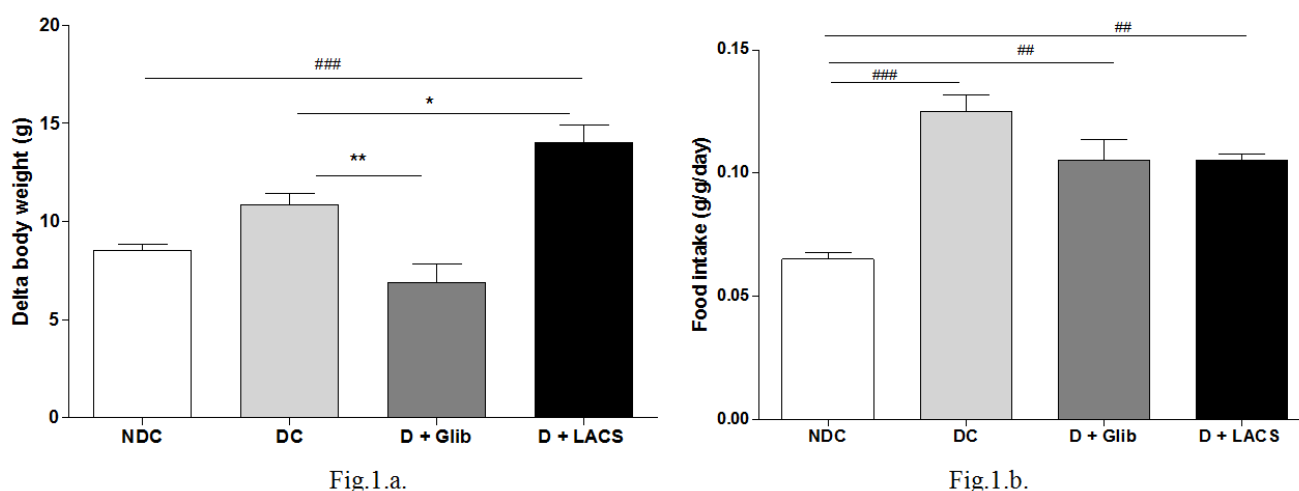


Figure 1: Body weight gain and food intake in alloxan-induced diabetic rats

Results are express as mean \pm SEM, (n=6). The data was analyzed using One-way ANOVA followed by Turkey's tests (p < 0.05), #statistically different to NDC, *statistically different to DC. Oral sub-acute treatment with LACS significantly increased body weight gain without modify food intake.

3.5. Effect of LACS on glucose level in alloxan-induced diabetic rats

Initially, all diabetic groups showed high FBG levels compared to normal control group (p < 0.001). After 4 weeks treatment with LACS (200 mg/kg/day, vo), the FBG level of diabetic rats was decreased by 39.38% (328.00 \pm 22.62 to 198.83 \pm 17.36 mg/dL) compared to non-treated animals with an increase of FBG by 10.10% (364.67 \pm 23.37 to 394.17 \pm 15.54 mg/dL). As expected, glibenclamide reduced FBG by 65.09% (330.83 \pm 24.21 to 115.50 \pm 12.83 mg/dL) (Table 3).

Table 3: Blood glucose level in alloxan-induced diabetic rats

Groups	Blood glucose level (mg/dL)				
	T0	S1	S2	S3	S4
NDC (Tween 2%)	94.17 ±3.74	91.00±2.31	89.33±2.48	103.33±3.51	97.67±2.28
DC (Tween 2%)	364.67 ±23.37 ^{###}	376.67±16.96 ^{###}	383.00±17.61 ^{###}	386.17±17.34 ^{###}	394.17±15.54 ^{###}
D+Gib 10 mg/kg	330.83 ±24.21	286.50±15.54 ^{***}	213.33±15.36 ^{***}	173.33±14.67 ^{***}	115.50±12.83 ^{***}
D+LACS 200 mg/kg	328.00 ±22.62	312.17±18.81 [*]	295.33±15.81 ^{***}	244.33±21.28 ^{***}	198.83±17.36 ^{***}

Results are express as mean ± SEM, (n=6). The data was analyzed using Two-way ANOVA followed by Bofferroni posttests ($p < 0.05$), [#]statistically different to NDC, ^{*}statistically different to DC. LACS significantly reduced FBG level in diabetic rats after 4 weeks of administration, $p < 0.001$.

Like FBG, the serum glucose level increased significantly in diabetic control rats compared with normal control rats ($p < 0.001$). This increase of serum glucose level in diabetic control rats was reduced by oral administration of LACS and glibenclamide ($p < 0.001$), respectively (Fig.2). However, the effect of LACS was significantly less compared to that of glibenclamide ($p < 0.001$).

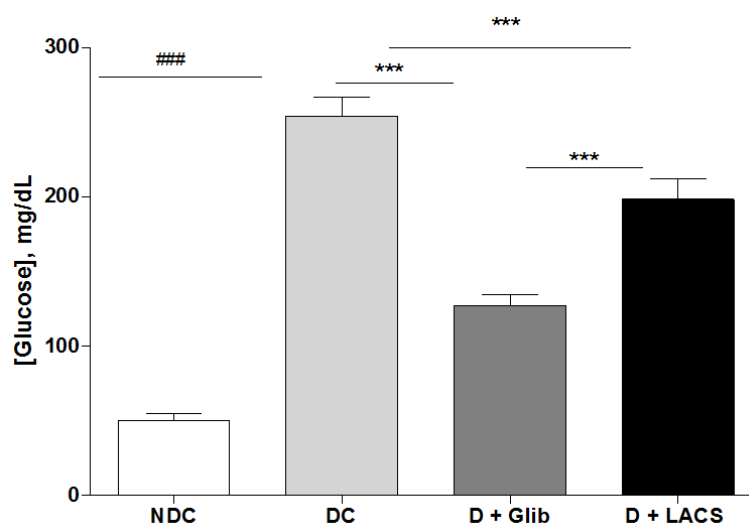


Figure 2: Serum glucose level after 28 days of treatment

Results are express as mean ± SEM, (n=6). The data was analyzed using One-way ANOVA followed by Turkey's tests ($p < 0.05$), [#]statistically different to NDC, ^{*}statistically different to DC. LACS significantly reduced serum glucose level in diabetic rats after 4 weeks of administration, $p < 0.001$.

3.6. Effect of LACS on kidney biomarkers and electrolytes in alloxan-induced diabetic rats

Serum levels of urea (Fig.3a) and creatinine (Fig.3b), biomarkers of kidney damage, increased significantly in alloxan-induced diabetic rats as compared with normal rats, $p < 0.001$ and $p < 0.05$, respectively. The treatment of diabetic rats with LACS reversed serum urea and creatinine levels near to normal values compared to control diabetic rats, respectively. No significant change in serum electrolytes (Na^+ , K^+ , Cl^- , Ca^{2+}) levels was observed in all experimental groups (Table 4).

Table 4: Effect on serum electrolytes concentrations after 28 days of treatment

Groups	Serum electrolyte concentrations (mg/dL)			
	Ca^{2+}	K^+	Na^+	Cl^-
NDC	10.34±0.30	122.40±4.00	2479.80±121.28	1890.90±19.62
DC	10.89±0.33	113.52±6.19	2543.10±17.79	1825.8±14.51
D+Glib10 mg/kg	10.30±0.47	119.76±6.01	2526.00±24.38	1832.10±16.57
D+LACS 200 mg/kg	10.32±0.37	128.94±8.52	2558.40±30.30	1854.30±19.93

Results are express as mean ± SEM, (n=6). The data was analysed using Two-way ANOVA followed by Bofferroni posttests ($p < 0.05$). No difference was observed between serum electrolytes levels in all experimental rats.

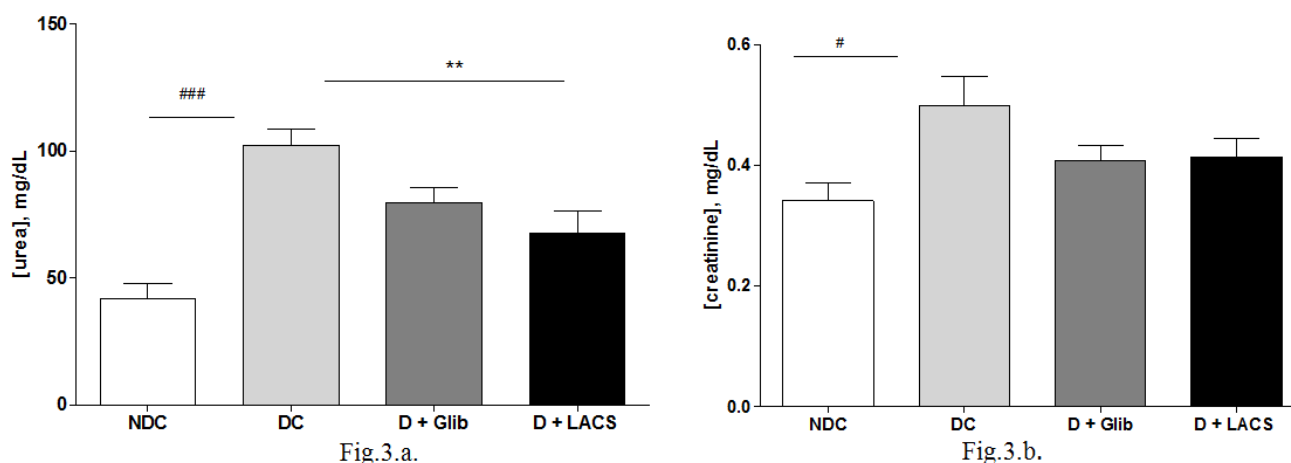


Figure 3: Effect on kidney enzymes activities markers after 28 days of treatment

Results are expressed as mean \pm SEM, (n=6). The data was analyzed using One-way ANOVA followed by Turkey's tests ($p < 0.05$), # statistically different to NDC, *statistically different to DC. LACS improved serum urea level in diabetic rats.

4. Discussion

Cassia siamea has been reported as non-toxic medicinal plant [5]. The present study was conducted to determine the beneficial effect of the aqueous extract of leaves of this plant on changes in experimental diabetes. Alloxan-induced diabetic model was used because it is one of the important animal diabetes models like human diabetes type 1 with clinical conditions such as hyperglycemia, polyphagia, polydipsia, polyuria and weight loss [8-9].

Our results have confirmed the non-toxic effect of *C. siamea*, and then it has shown that this plant exhibited interesting activities on hyperglycemia and kidney functions. Indeed, acute administration of LACS (400 mg/kg bw, vo) has shown remarkable reduction of oral glucose-induced hyperglycemia suggesting that *C. siamea* could prevent the postprandial hyperglycemia. The mechanisms involved were unknown. But, this effect could be due to the presence of flavonoids such as quercetin [10], which were involved in inhibition of intestinal glucose absorption by inhibiting GLUT2 [11]. This plant could also exhibit an inhibitory effect on α -glucosidase enzyme [12]. Further study might sign up such mechanism. Moreover, *C. siamea* did not induce hypoglycemia in normal rats as biguanides. So, it could improve the sensitivity of peripheral and liver cells to glucose utilization.

Interestingly, we found that 4 weeks treatment with 200 mg/kg/day of LACS reduced significantly hyperglycemia in alloxan-induced diabetic rats. Similar antidiabetic effects of ethanol extracts of leaves of *C. siamea* were reported in STZ-induced diabetic rats [13] and in alloxan-induced diabetic rats [14]. The mechanisms of action and the bioactive compounds involved in these antidiabetic effects were not identified. In literature, it has been reported that *C. siamea* contains flavonoids such as luteolin and D-pinitol which exhibited antidiabetic effects [15-17].

Also, LACS significantly increased body weight without modifying food intake in diabetic rats. These results showed that this extract of *C. siamea* could contain nutrients which could justify the body weight gain. Indeed, it has been reported that *C. siamea* is widely used as food and is a good source of essential nutrients [18-19]. These nutritional potentials of *C. siamea* would be an advantage for improving the massive weight loss associated with disruption of lipid metabolism and protein in type 1 diabetes patients.

In this study, the serum urea and creatinine levels of diabetic control rats were significantly high when compared with non-diabetic control rats. Indeed, it is known that diabetes increases oxidative stress, inflammation and kidney dysfunctions causing abnormally high serum levels of creatinine and urea, two biomarkers of kidney damage [3]. Interestingly, the serum urea and creatinine levels were reduced significantly in the diabetic rats treated with the LACS which show that the treatment reduced the oxidative damage to the kidney as well as the diabetic conditions. This protective effect could be due to the high potential of antioxidant and anti-inflammatory of *C. siamea* [5, 20].

The serum electrolytes (Na^+ , K^+ , Cl^- , Ca^{2+}) levels in this experiment indicated that chronic LACS administration after 28 days did not modify these biochemical parameters.

In short, the present study clearly indicates that the aqueous extract of the leaves of *C. siamea* (200 mg/kg bw, vo) exhibited significant antihyperglycemic effect and improved kidney functions in diabetic rats, thereby confirming its ethnomedicinal use against diabetes. Therefore, further investigations are necessary to determine the exact bioactive compounds and mechanisms of action involved in antidiabetic activities.

Acknowledgments

This work was supported by the French-Ivory Coast cooperation project AMRUGE-CI (Campus France N° 854039K) and by a scholarship from the Ministry of Superior Education and Scientific Research of Ivory Coast (Decision N° 459/MERS/DB/SD-BHCI du 12/10/2015).

Conflict of Interest

The authors declare that they have no conflict of interest.

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