ACADEMIC JOURNALS
expand your knowledge

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Antihyperglycemic and pancreatic β-Cells protective effects of Cassia *siamea* in Alloxan-induced diabetic wistar rats

Camille KOFFI[1]*, N'goran Mathieu KOUAME[1], Kouassi Eugène KOFFI[4], Kanga Sita N'ZOUÉ[1], N'guessan Alain Roland YAO[2], Brahima DOUKOURE[3] and Mamadou KAMAGATE[1]

[1]Département de Pharmacologie Clinique, UFR Sciences Médicales Bouaké, Université Alassane Ouattara, Côte d'Ivoire.
[2]Laboratoire de Valorisation des Agroressources, UFR Sciences Biologiques, Université Péléforo Gon Coulibaly, Korhogo, Côte d'Ivoire.
[3]Laboratoire d'anatomie pathologique, UFR-SMA, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire
[4]Institut Pasteur de Côte d'Ivoire (IPCI), Abidjan, Côte d'Ivoire.

The study aimed to investigate the β-cell protective effects of ethanol extracts of *Cassia siamea* (Fabaceae) leaves (LECS). Initially, acute toxicity of LECS (2000 mg/kg/day/bw; po) was assessed in rats. In vitro, antioxidant activity was evaluated on HUVEC cultures. Subsequently, acute hypoglycemic and antihyperglycemic properties were examined for three doses of LECS (100, 200, and 400 mg/kg/bw; po) in Wistar rats, along with an assessment of its impact on intestinal glucose absorption. In the third phase, oral treatment with LECS (200 mg/kg/day/bw; po) was conducted for 4 weeks in alloxan-induced diabetic rats, with glibenclamide (10 mg/kg/day/bw; po) as the standard drug control. Various parameters, including fasting blood glucose, body weight, food intake, lipid profile, and biomarkers of liver and kidney functions, were determined. Additionally, histological analysis of pancreatic islets was performed. The data analysis revealed that *C. siamea* did not induce adverse effects or mortality in rats at a single dose of 2000 mg/kg. Furthermore, LECS significantly prevented oral glucose-induced hyperglycemia, reduced intestinal glucose absorption, and improved lipid profile in diabetic rats. Although the extracts did not significantly modify body weight, they effectively reversed hyperglycemia, enhanced pancreatic islet size and granulation, and exhibited antioxidant properties by reducing the production of reactive oxygen species (ROS) in HUVEC. Overall, these findings suggest that the ethanol extract of *C. siamea* leaves may have potential in managing diabetes through its antioxidant properties, improvement of β-cell function, and reduction of intestinal glucose absorption.

Key words: *Cassia siamea*, diabetes, HUVEC, rat wistar, antihyperglycemic, pancreatic β-Cells.

INTRODUCTION

Diabetes mellitus is considered a global epidemic health problem, imposing a high cost on national health services worldwide. The number of diabetic patients is expected to increase dramatically to over 300 million within the next 20 years (Khavandi et al., 2013). This endocrine disease is characterized by persistent hyperglycemia and metabolic disorders affecting carbohydrate, fat, and protein metabolism, resulting from a failure of insulin

secretion and/or action (American Diabetes Association, 2008). While several drugs are available to control and treat diabetic patients, complete recovery from diabetes has not yet been reported.

As an alternative to synthetic agents, plants offer a potential source of hypoglycemic drugs and have been widely used in traditional medicine to prevent diabetes (Willcox et al., 2021). *Cassia siamea* (syn. Senna siamea) was selected based on a review of the literature on medicinal plants well-known and regularly used by Ivorians. This Fabaceae plant, native to Southeast Asia and found in most tropical countries, has a history in folklore medicine in Africa and Asia as a remedy for malaria, fever, constipation, hypertension, and diabetes. The phytochemistry of *C. siamea* leaves has been studied, revealing the presence of alkaloids, polyphenols, flavonoids, phenolic acids, triterpenes glycosides, carotenoids, tannins, and saponins. Several bioactive molecules, such as barakol, anhydrobarakol, cassiarin A-B, chrysophanol, emodin, D-pinitol, luteolin, lupeol, cassiamin A-B, sennoside A, coumarin, rutin, myricetin, quercetin, and kaempferol, have been identified (Kamagaté et al., 2014). In vitro studies demonstrated that the ethanol extract of C. siamea leaves can inhibit the enzyme α-glucosidase by approximately 19.10% (Tanty et al., 2018). Additionally, Cassiamin A exhibited pancreatic lipase inhibitory activity with an IC50 value of 41.8 µM, indicating the antilipidemic activities of *C. siamea* (Kumar et al., 2013). In vivo, the administration of ethanol extracts of *C. siamea* leaves at doses of 500 and 750 mg/kg body weight significantly reduced hyperglycemia by 50.32 and 47.29%, respectively, within 1-5 hours in glucose-induced hyperglycemia rats (Luangpirom and Saenbuaphan, 2006). Previous studies conducted by our team demonstrated that the administration of this extract at a dose of 200 mg/kg body weight for 4 weeks in Wistar rats and obese mice resulted in significant reductions in blood glucose and insulin levels, improvement in glucose tolerance and insulin sensitivity, and restoration of increased circulating AST and ALT levels, without modifying body weight and food intake (Koffi et al., 2016). These effects were associated with an increased activity of both the insulin (Akt) and AMPK pathways in the liver and skeletal muscles (Koffi et al., 2019). This suggests that *C. siamea* holds valuable potential as an alternative drug for managing diabetes. However, its effects on pancreatic tissue and its impact on oxidative stress in experimental cell models remain unknown. Therefore, the present study aimed to investigate the antidiabetic activities of the ethanol leaf extract of *C. siamea* by examining its potential beneficial effects on pancreas histopathology and intestinal glucose absorption in experimental models

of diabetic rats.

MATERIALS AND METHODS

Chemicals

The organic solvents employed included hexane (Quimicen®, Spain) and ethanol (Prolabo®, France). Additionally, Tween® 80, lidocaine gel (AstraZeneca®, UK), NaCl (Riedel-de Haen, Germany), (+) D-glucose (Riedel-de Haën®, Germany), metformin (Denk Pharma®, Germany), glibenclamide (Daonil®, Sanofi-adventis), and alloxan monohydrate (Sigma-Aldrich, Germany) were utilized in the study.

Plant material

C. siamea (Fabaceae) is an unprotected wild endemic plant, and its fresh leaves were collected from Adiopodoumé village, Côte d'Ivoire, in May 2020, adhering to national plant protection guidelines. The plant's authenticity was confirmed by a botany expert, Professor Ake-Assi Laurent, at the Department of Biosciences of the National Floristic Center (Félix Houphouët-Boigny University, Abidjan, Ivory Coast). Voucher specimens have been deposited in a public herbarium under No. 126/97. Its taxonomic serial number in the integrated taxonomic information system (ITIS) is 505177.

Extraction preparation

The dried leaves of Cassia siamea were pulverized using a Phillips® blender. Fifty grams (50g) of the powdered material were extracted with 800 mL of hexane and filtered. The residue was evaporated and subsequently used for ethanol extraction (LECS) using 80% ethanol (v/v) through cold maceration for 48 h with random mechanical shaking.

The filtrates of the extracts obtained from each solvent were distilled off at 40ºC in a circulating air oven (Memmert®, Germany) and stored at 4ºC until use.

HUVECs culture

Human umbilical vein endothelial cells (HUVECs) were cultured in 6-well plates containing EBM-2 medium (Endothelial Base Medium 2, Lonza), supplemented with 10% Fetal Bovine Serum, 5% antibiotics (penicillin/streptomycin), growth factors (Insulin-like Growth Factor, Vascular Endothelial Growth Factor), and glutamine at 37°C in a 5% CO2–95% air environment. The culture medium was renewed every 48 h. Cells were grown for 24 h in the absence or presence of hydrogen peroxide (H2O2, 0.1%) after preincubation with or without LECS (250 or, 500 µg/ml) for 1 h. Dimethylsulfoxide (0.1%) was used as a control. All experiments were performed in quadruplicate

In vitro, Measurement of Intracellular Reactive Oxygen Species (ROS)

The detection of Reactive Oxygen Species (ROS) production in

*Corresponding author. E-mail: koffi.camille@yahoo.fr. Tel: Tel: (+225) 0707194024.

HUVEC cells was carried out using Electron Paramagnetic Resonance (EPR) spectroscopy, as previously described (Agouni et al., 2009). The Spectrometer MiniScope MS200 from Magnettech, Berlin, Germany, was utilized. A solution of Deferoxamine-chelated Krebs-HEPES containing 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH, 500 µmol/L, Noxygen, Mainz, Germany), deferoxamine (25 µmol/L, Sigma-Aldrich), and diethyldithiocarbamate (5 µmol/L, Sigma-Aldrich) served as a spin trap.

Quantitative measurements of the ROS-Spin trap signal amplitude are expressed in units per milligram per milliliter of endothelial cell proteins (A/mg/mL).

In vivo, pharmacology study

Animals

Male and female Wistar rats (3-month-old, 150 to 200 g) were obtained from the animal resources management unit of the Pasteur Institute of Ivory Coast. The animals were given a standard pellet diet and had access to water ad libitum. They were housed under standard living conditions, including a room temperature of 25 ± 2ºC and a 12-h dark/light cycle, throughout all experiments. All experiments were conducted in accordance with the Guidelines for experiments involving animals (McGrath et al., 2010).

Acute oral toxicity test

The study was conducted in strict accordance with the guidelines of the Organization for Economic Cooperation and Development for the testing of chemicals, specifically the acute toxic class method (OECD 423, 2001). Female Wistar rats were randomly grouped into two groups (n = 5/group) and fasted overnight. Animals in each group were treated at intervals of 48 h, with group I receiving the vehicle (2% Tween 80) orally, and group II receiving a single dose of LECS at 2000 mg/kg body weight. After the administration of the extract, the rats were continuously observed every hour. Food was withheld for an additional period of 3-4 h, and further observations were carried out every 24 h for 15 days to identify any changes, such as tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma, or a decrease in respiratory rate, as well as any lethality.

Acute hypoglycemic test

Overnight fasting normoglycemic rats were randomly divided into five groups (n=5/group). The fasting blood glucose (FBG) level of each animal was determined at the initial time (0h). Subsequently, group I (control) received the vehicle, i.e., 2% Tween 80 (v/v); group II received the standard hypoglycemic drug glibenclamide (Glib) at a dose of 10 mg/kg b.w. (Daonil®, Sanofi-adventis); groups III to V received LECS at doses of 100, 200, and 400 mg/kg b.w., respectively. Glucose levels in blood samples from the tail vein were estimated at 0.5, 1, 2, 4, and 6 hours using a glucometer (Accu-Chek® Active, Roche).

Oral Glucose Tolerance Test (OGTT)

To assess the impact of *C. siamea* on postprandial glycemia, healthy normoglycemic rats that had fasted overnight were randomly divided into five groups (n=5/group): group I received only the vehicle and served as a control group; group II received the standard drug glibenclamide at a dose of 10 mg/kg/b.w.; groups III to V received LECS at doses of 100, 200, and 400 mg/kg/b.w., respectively. Initially (T-0.5), the fasting blood glucose level from a blood sample taken from the tail vein was estimated using a

glucometer. Immediately after, each group of animals received the substances according to the experimental design described above. Thirty minutes later (T0), the blood glucose level for each animal was determined, followed by oral administration of a (+) D-glucose solution (4 g/kg, b.w.). Blood glucose levels were then determined at 0.5, 1, 1.5, 2, 4, and 6 hours after oral glucose administration.

Intestinal glucose absorption test

This study was conducted following the methodology described by Lima et al. (2012). Briefly, overnight fasting normoglycemic rats were randomly divided into three groups (5 rats/group). Orally, group I received the vehicle (2% Tween 80), group II received the standard drug (metformin 15 mg/kg, b.w.), and group III received LECS (200 mg/kg, b.w.). After 30 minutes, all groups received an oral solution of (+) D-glucose (2 g/kg, b.w.). After 1 hour, all animals were euthanized by cervical dislocation, and their small intestines were removed. The intestines were perfused with 40 mL of distilled water, and the contents were collected for centrifugation at 500 g for 10 min. Supernatants was used to determine the glucose level by spectrophotometry.

Induction of experimental diabetes

Diabetes was induced in overnight-fasted rats by a single subcutaneous injection of a freshly prepared alloxan monohydrate solution (100 mg/kg b.w., dissolved in NaCl 0.9%), following the method previously described by Moradabadi et al. (2013). The success of diabetes induction was assessed in rats 3 days after alloxan injection by determining fasting blood glucose (FBG) levels from the tail vein in overnight-fasted rats. Only rats with blood glucose levels ≥ 200 mg/dL were enrolled in the study.

Experimental design

In the experiment, a total of 24 rats were utilized, consisting of 6 non-diabetic rats (group I, ND) and 18 persistently diabetic rats (group II, D). The diabetic animals were randomly divided into four groups (n = 6/group). Therefore, group I (ND, non-diabetic rats) and group II (D, diabetic rats) both received the vehicle (2% Tween 80). Group III (D+Glib 10 mg/kg) consisted of diabetic rats treated with the standard drug glibenclamide at a dose of 10 mg/kg b.w., and group IV (D+LECS 200 mg/kg) included diabetic rats treated with LECS at a dose of 200 mg/kg b.w. The vehicle, plant extracts, or standard drug were administered by gavage once daily for 28 days under oral anesthesia with lidocaine gel (AstraZeneca®, UK). Food intake, body weight, and fasting blood glucose (FBG) levels were determined weekly during the four-week treatment period. After sacrifice, blood samples were left to stand for 15 min and then centrifuged at 500 g for 10 min at 2ºC. Serum samples were collected and kept at -20ºC for subsequent biochemical determination.

Biochemical analysis

The serum levels of glucose, cholesterol (TC), triacylglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were analyzed using the HITACHI 704R® analyzer with Biolabo® biochemical kits.

Histopathology of pancreas

After sacrificing the animals, the pancreas from all experimental
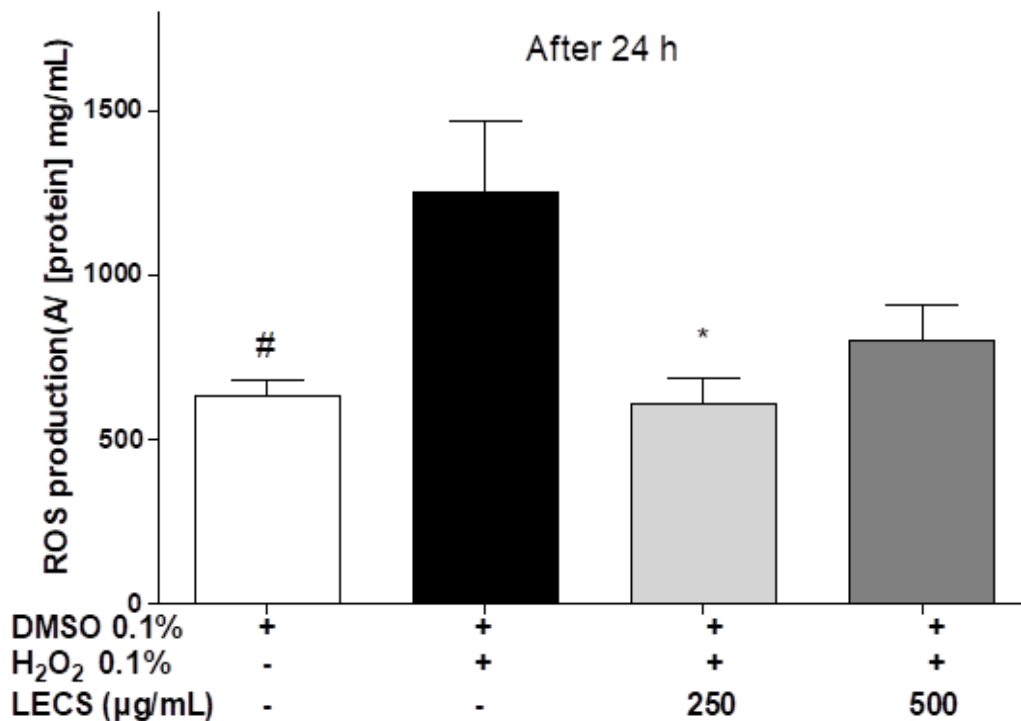
Figure 1. Effect of *Cassia siamea* on ROS production in HUVEC cells.
ROS production in HUVEC pre-treated with LECS for 24h was determined by spectroscopy assay. LECS promotes reduction of H_2O_2-induced ROS production in HUVEC cells. Values are expressed as Mean ± SEM (n=5), ANOVA, Turkey's Test, *P<0.05 compared with the positive H_2O_2 control group. LECS: Leaves Ethanol extract of *C. siamea*, ROS: Reactive Oxygen Species, H_2O_2: Hydrogen peroxide.

groups was weighed and fixed in 10% formal saline. The tissues were cleaned and embedded in paraffin. Following paraffin embedding, serial sections of 5 μm thickness were prepared and stained with hematoxylin and eosin for microscopic examination of Langerhans islets.

Statistical analysis

The data are expressed as mean ± SEM. Statistical analysis was conducted using GraphPad Prism 5.0® software, involving analysis of variance (ANOVA), and Tukey's test or Bonferroni post-tests. Statistical significance was considered at p < 0.05.

RESULTS

Antioxidant effect of LECS in H_2O_2-induced ROS production in HUVECs

As depicted in Figure 1, EPR spectroscopy revealed an increase in ROS production in HUVECs exposed to H2O2 compared to the control group (DMSO 0.1%). Pre-treatment with LECS at 250 μg/ml significantly attenuated the ROS production induced by H2O2. However, an increase in LECS concentration (500 μg/ml) in the cell culture medium diminished its beneficial effects on ROS

levels in HUVECs (Figure 1).

Acute oral toxicity of LECS

A single administration of LECS at 2000 mg/kg b.w. did not induce abnormal behavior during the initial 4 hours, and no mortality was recorded during the 14 days following treatment. The body weight of rats administered with LECS was also comparable to that of vehicle-treated rats (data not shown). According to OECD analysis, the acute oral LD50 for LECS in Wistar rats was estimated to be greater than 2000 mg/kg. Therefore, doses of 100, 200, and 400 mg/kg were selected for further experiments.

Acute effect of LECS on fasting glucose

As expected, glibenclamide significantly reduced the fasting blood glucose level by 44.9 to 51.0% between 2 and 6 hours (p < 0.01). Acute administration of LECS at doses of 100, 200, and 400 mg/kg did not induce a hypoglycemic effect compared to the control group (Data not shown).
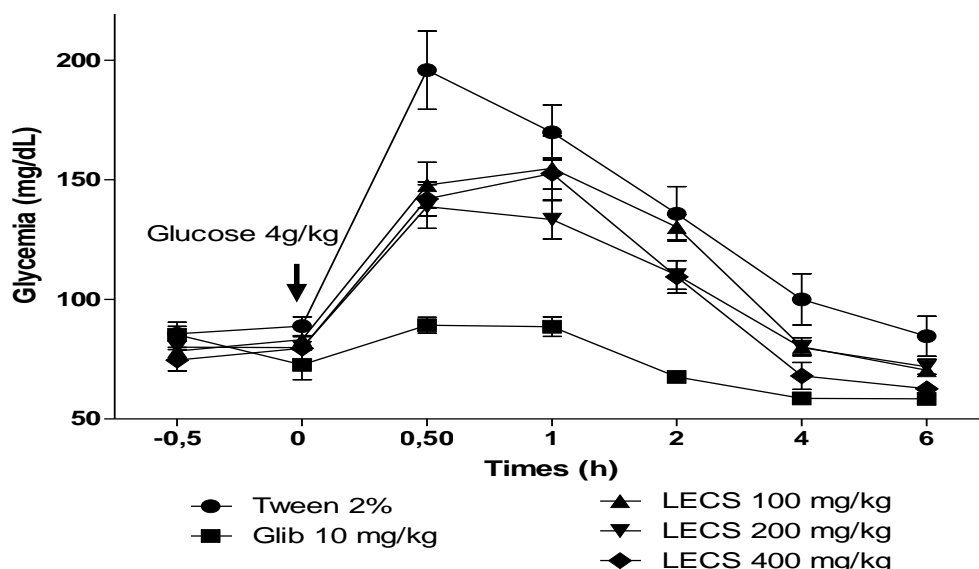
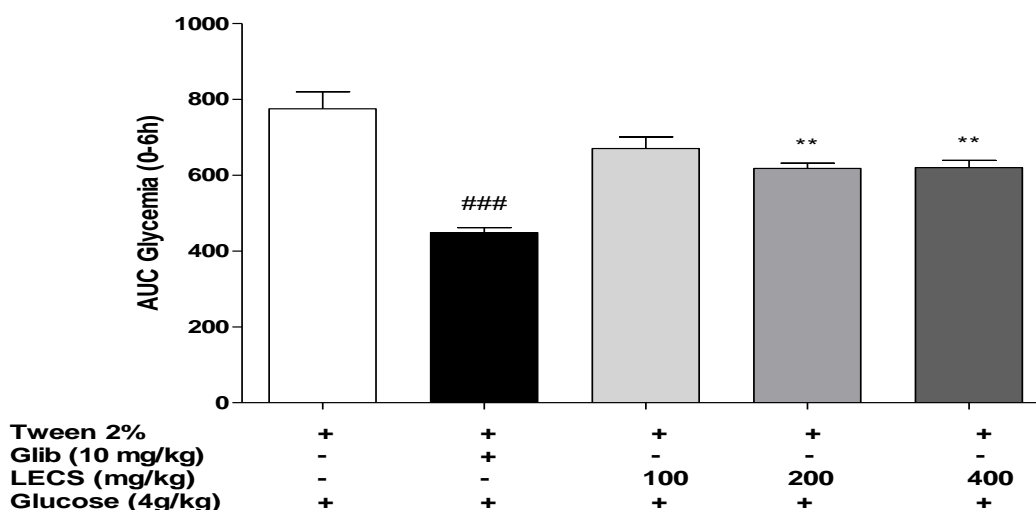**Figure 2a**. Effect of *Cassia siamea* on glucose-induced hyperglycemia.

Figure 2b. AUC glycemia of rats treated with *Cassia siamea compared from the* glucose-induced hyperglycemia.
LECS significantly reduced hyperglycemia at the dose of 200 mg/kg and 400 mg/kg (p<0.01).Control: rats received 2%Tween 80; Glib: rats received standard drug 10 mg/kg; LECS: rats received ethanol extract of *C. siamea*. Results are express as mean ± SEM. #significantly different from the Control rats (Tween); *statistically different from Control (One way ANOVA followed by Turkey's posttests, P<0.05.

Effect of LECS on Oral Glucose-induced Hyperglycemia

In the control group, acute oral glucose administration progressively increased blood glucose levels, reaching a peak at 30 min. As expected, the standard drug glibenclamide reduced glucose levels and the associated area under the curve (AUC) by 42.2% (p < 0.001) compared to the control. Interestingly, at doses of 200 and 400 mg/kg, both LECS significantly reduced the peak of blood glucose levels compared to the control group

(Figure 2a). Consequently, the AUC for glycemia was reduced by 20.7% for a dose of 200 mg/kg and by 20.2% for 400 mg/kg of LECS compared to the control group, respectively (p < 0.01) (Figure 2b). Hence, the LECS dose of 200 mg/kg was chosen for the following experiments.

Effect of LECS on intestinal glucose absorption

As expected, oral administration of the standard drug

After 1h

Tween 2%	+	+	+
Metformin (15 mg/kg)	-	+	-
LECS (200 mg/kg)	-	-	+
Glucose (2 g/kg)	+	+	+
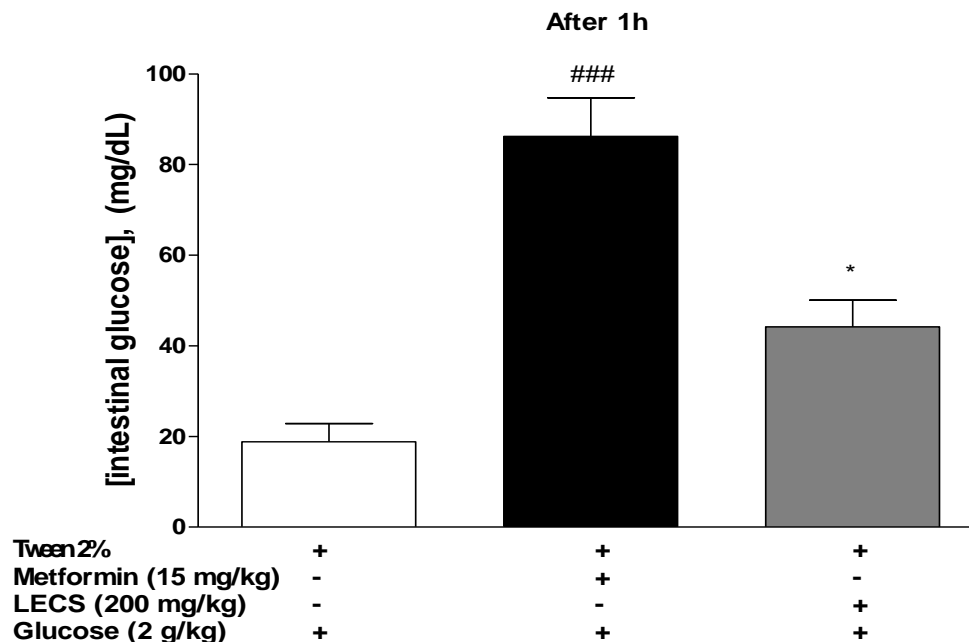
Figure 3. Effect of *Cassia siamea* on glucose absorption in non-diabetic rats.
LECS (200 mg/kg) exhibited significantly inhibition of intestinal glucose absorption (p<0.001). Control: rats received 2%Tween 80; Metformin: rats received standard drug 15 mg/kg; LECS: rats received ethanol extract of *C. siamea*. Results are express as mean ± SEM. Significantly different to Control rats (Tween); *Statistically different from the control (one way ANOVA followed by Turkey's tests, P<0.05.

Table 1. Effect of *Cassia siamea* on food intake in diabetic rats.

	Food intake (g)			
	Week 1	**Week 2**	**Week 3**	**Week 4**
ND	116.6±5	103.9±7.4	104.33.7	110.8±6.8
D	152±7.5$^{\#\#}$	164±3.4$^{\#\#\#}$	185±4.0$^{\#\#\#}$	191±9.5$^{\#\#\#}$
D+Glib 10 mg/kg	165.9±5.3	121.2±5.2***	125.8±4.4***	128.8±10.2***
D+LECS 200 mg/kg	164.6±11.2	162.8±5.2	158.2±8.5*	158.0±7.2**

ND: Normal rats, and D: diabetic rats. Both received 2%Tween 80; D+Glib: diabetic rats treated with glibenclamide 10 mg/kg; D+LECS: diabetic rats treated with ethanol extract of *C. siamea* 200 mg/kg. Results are express as mean ± SEM. Statistically different to Control (Two way ANOVA followed by Bofferroni posttests. p<0.05); $^{\#}$Significantly different to ND; *Significantly different to DC, $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001, *p<0.05, **p<0.01, ***p<0.001.

metformin (15 mg/kg b.w) significantly increased (p < 0.001) intestinal glucose concentration compared to the control group. Additionally, LECS (200 mg/kg b.w.) exhibited an increase in intestinal glucose levels compared to the control (p < 0.01). However, the effect induced by the extracts was significantly lower than that of metformin (Figure 3).

Effect of LECS on Diabetic Rats' Nutritional State

Tables 1, 2, and 3 present the levels of body weight, food intake, and fasting blood glucose of experimental rats

after 1, 2, 3, and 4 weeks of drug treatment, respectively. The body weight of the non-diabetic group was significantly higher (p < 0.001) than all diabetic groups throughout the entire experimentation period. Food intake was higher in the diabetic control group compared to all other groups. The supplementation of LECS (200 mg/kg, b.w.) to diabetic rats significantly reduced food intake compared to the diabetic control (p < 0.01), but no significant effect was observed on body weight (Table 1, 2). Initially, all diabetic groups showed significantly higher fasting blood glucose (FBG) levels compared to the normal control group (p < 0.001). After treatment with LECS (200 mg/kg/day), the FBG level of diabetic rats

Table 2. Effect of *Cassia siamea* on body weight in diabetic rats.

	Body weight					
	Injection of alloxan	Period of treatment after Injection of alloxan				
	T - 3 days	T0	Week 1	Week 2	Week 3	Week 4
ND	222.5±14.9	223.8 ±15.0	231.6±15.5	235.7±15.3	240.9±16.1	242.8±16.2
D	212.3±12.2	180.1±8.8###	191.24±9.4###	194.42±9.3###	197.94±9.7###	199.7±9.6###
D+Glib 10 mg/kg	209.3±9.9	175.0±7.6	180.6±7.8	183.6±7.8	183.1±7.6	187.2±9.4
D+LECS 200 mg/kg	216.4±14.4	175.7±12.5	182.9±12.51	187.08±12.4	188.25±12.45	191.0±12.1

ND: Normal rats, and D: diabetic rats. Both received 2%Tween 80; D+Glib: diabetic rats treated with glibenclamide 10 mg/kg; D+LECS: diabetic rats treated with ethanol extract of *C. siamea* 200 mg/kg. Results are express as mean ± SEM. Statistically different to Control (Two way ANOVA followed by Bofferroni posttests. p<0.05); #significantly different to ND, #p<0.05, ##p<0.01, ###p<0.001.

Table 3. Effect of *Cassia siamea* on blood glucose level in diabetic rats.

	Blood glucose (mg/dL)				
	T0	Week 1	Week 2	Week 3	Week 4
ND	94.2±3.7	91.0±2.3	89.3±2.5	103.3±3.5	97.7±2.3
D	364.7±23.4###	376.7±17.0###	383.0±17.6###	386.2±17.3###	394.2±15.5###
D+Glib 10 mg/kg	330.8±24.2	286.5±15.5**	213.3±15.4***	173.3±14.7***	115.5±12.8***
D+LECS 200 mg/kg	365.2±29.0	305.3±21.2*	258.2±19.5***	188.3±22.9***	132.8±22.1***

ND: Normal rats, and D: diabetic rats. Both received 2%Tween 80; D+Glib: diabetic rats treated with glibenclamide 10 mg/kg; D+LECS: diabetic rats treated with ethanol extract of *C. siamea* 200 mg/kg. Results are express as mean ± SEM. Statistically different to Control (Two way ANOVA followed by Bofferroni posttests. p<0.05); #Significantly different to ND; *Significantly different to DC, #p<0.05, ##p<0.01, ###p<0.001, *p<0.05, **p<0.01, ***p<0.001.

Table 4. Effect of *Cassia siamea* on Serum glucose level and lipid profile in diabetic rats.

	Serum glucose (mg/dL)	Lipid profile (mg/dL)			
		TC	TG	HDL-C	LDL -C
ND	50.2±5.1	74.2±3.2	80.3±6.3	26.5±1.4	31.6±3.2
D	253.8±13.2###	97.7±7.5	110.3±8.9##	17.7±0.9###	57.9±2.6###
D+Glib 10mg/kg	127.3±7.2***	84.2±7.41	79.7±4.8**	25.7±0.9**	38.0±2.7***
D+LECS 200 mg/kg	136.7±12.4***	79.2±4.6	54.3±6.2**	24.0±1.2**	39.5±4.4**

ND: Normal rats, and D: diabetic rats. Both received 2%Tween 80; D+Glib: diabetic rats treated with glibenclamide 10 mg/kg; D+LECS: diabetic rats treated with ethanol extract of *C. siamea* 200 mg/kg. Results are express as mean ± SEM. Statistically different to Control (Two way ANOVA followed by Bofferroni posttests. p<0.05); #significantly different to ND; *significantly different to DC, #p<0.05, ##p<0.01, ###p<0.001, *p<0.05, **p<0.01, ***p<0.001.

decreased by 64.8% (365.2 ± 29.0 to 132.8 ± 22.1 mg/dL), compared to non-treated diabetic animals with a 10.1% FBG variation (364.7 ± 23.4 to 394.2 ± 15.5 mg/dL) (Table 3).

Analysis of biochemical parameters

Serum glucose level

The serum glucose level was significantly increased in diabetic control rats compared with normal control rats (p

< 0.001). Oral administration of LECS (200 mg/kg b.w.) to diabetic rats significantly reduced the changes in the level of glucose to near normal levels during the study period (p < 0.001) (Table 4).

Lipid profile

The lipid profile showed a significant increase in triglycerides (TG) (p < 0.05), total cholesterol (TC) (p < 0.05), and low-density lipoprotein (LDL) (p < 0.001), along with a decrease in high-density lipoprotein (HDL) (p

Table 5. Effect of *Cassia siamea* on hepatic and kidney enzymes activities markers in diabetic rats.

	Kidney biomarkers (mg/dL)			Liver biomarkers (U/L)		
	Urea	Creatinine	Uric acid	AST	ALT	AlkP
ND	41.7±6.4	0.3±0.03	2.7±0.2	362.5±67.4	91.8±5.1	400.9±34.3
D	102.3±6.4[###]	0.5±0.05[#]	2.8±0.24	691.4±124.0[###]	139.1±9.4[###]	1031.9±269.2[###]
D+Glib 10 mg/kg	79.5±5.8	0.4±0.03	3.0±0.5	586.8±129.1	107.5±16.5	724.7±80.5
D+LECS 200 mg/kg	94.2±4.2	0.4±0.02	3.5±0.5	494.6±106.8	118.5±5.9	923.3±167.2

ND: Normal rats, and D: diabetic rats. Both received 2%Tween 80; D+Glib: diabetic rats treated with glibenclamide 10 mg/kg; D+LECS: diabetic rats treated with ethanol extract of *C. siamea* 200 mg/kg. Results are express as mean ± SEM. Statistically different to Control (Two way ANOVA followed by Bofferroni posttests. $p<0.05$); [#]Significantly different to ND, [#]$p<0.05$, [##]$p<0.01$, [###]$p<0.001$.

< 0.01) in diabetic control compared to the normal control. LECS (200 mg/kg b.w.) produced a beneficial decrease in TG and LDL in diabetic rats ($p < 0.01$) and increased HDL ($p < 0.05$) (Table 4).

Hepatic and kidney enzymes activities markers

Levels of urea and creatinine, biomarkers of kidney damage, were elevated in alloxan-induced diabetic rats compared with normal rats. Additionally, serum concentrations of AST, ALT, and AlkP, biomarkers of liver damage were elevated in alloxan-induced diabetic rats when compared with non-diabetic controls. The treatment of diabetic rats with LECS exhibited no significant reduction in the serum concentration of biomarkers compared to control diabetic rats (Table 5).

Effect of LECS on pancreas histopathology

Figure 4 shows the histological profile of the pancreas in normal, alloxan-induced diabetic untreated and alloxan-induced diabetic treated Wistar rats. The normal control rat exhibited a normal histological architecture with many rounded normal proportions of the islet of Langerhans found around the pancreatic acini. Prominent nuclei with well-arranged lobules and surrounding islet cells were observed in normal control rats (Photograph A). In alloxan-diabetic rats, the sections of pancreatic tissues showed extensive β-cell degranulation, reduction of islet cells, islet diameters, and their size (Photograph B). In diabetic rats treated with Glibenclamide 10 mg/kg (Photograph C) and LECS 200 mg/kg (Photograph D), β-cell granulation, cellularity, and islet size were improved.

DISCUSSION

Alloxan-induced diabetes in rats mimics experimental diabetes observed in humans (King, 2012). Alloxan is known to be rapidly taken up by beta cells, causing fragmentation of β-cell DNA and specific destruction of β-cells through the formation of free radicals (Szkudelski, 2001). Consequently, this leads to reduced insulin secretion and clinical conditions such as hyperglycemia, polyphagia, polydipsia, polyuria, and weight loss (Nerup et al., 1994). Interestingly, the ethanol extract of *C. siamea* leaves induced an improvement in the histological architecture of pancreas β-cell islets in alloxan-induced diabetic rats. This protective activity on pancreas β-cell function is likely due to its antioxidant properties. LECS reduced the production of reactive oxygen species (ROS) in HUVEC cells in culture with H_2O_2 stimulation. Indeed, the exogenous addition of H_2O_2 at 0.1% induces intracellular ROS production, affecting cell metabolism and leading to cell destruction (Jiang et al., 2015). This β-cells protective activity of *C. siamea* could justify its antihyperglycemic effects, as pancreatic β-cells are responsible for the production of endogenous insulin, the hypoglycemic hormone (McCall, 2012).

The present study demonstrated that LECS at a dose of 200 mg/kg exhibited antihyperglycemic effects and improved lipid metabolism in alloxan-induced diabetic rats. Food intake of diabetic rats was significantly decreased by LECS administration, but no change in body weight was observed. Additionally, LECS markedly reduced oral glucose-induced hyperglycemia without affecting fasting blood glucose levels in non-diabetic rats. These results are consistent with those reported in streptozotocin-induced diabetic rats (Kumar et al., 2010), alloxan-induced diabetes rats (Mohammed, Atiku, 2012), and leptin-deficient obese mice (Koffi et al., 2019). The antihyperglycemic effects of *C. siamea* are thought to be due to its inhibitory action on intestinal glucose absorption. Preliminary studies showed that the methanolic extract of *C. siamea* reduced intestinal absorption in the rat model (Koffi et al., 2022). This study confirmed that it increased luminal intestinal glucose levels after oral glucose administration in non-diabetic rats. Therefore, *C. siamea* could decrease postprandial glucose levels, most likely by reducing intestinal glucose absorption. C. siamea contains flavonoids such as quercetin (Shafiullah and Kamil, 1995), which are known to inhibit intestinal glucose absorption by inhibiting
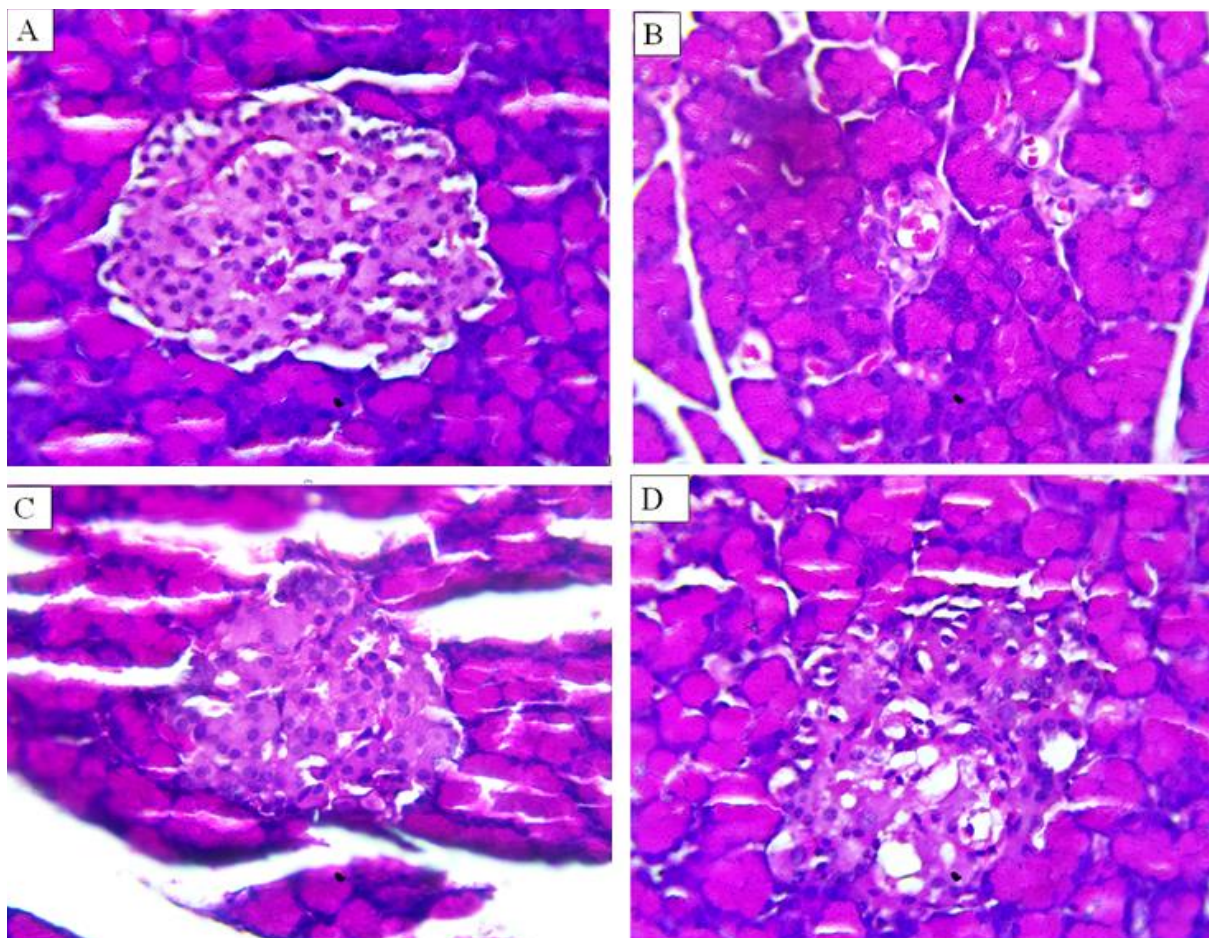
Figure 4. Effect of *Cassia siamea* on pancreas histopathology in diabetic rats.
*C.siamea (*LECS) improved histological architecture of pancreas β-cell islets in alloxan-induced diabetic rats. (Heamatoxylin, and Eosin X 400). A: Photography of pancreas of normal control rats; B: Photography of pancreas of diabetic controls rats; C: Photography of pancreas of diabetic rats treated with glibenclamide; D: Photography of pancreas of diabetic rats treated with *C. siamea*.

glucose transporter 2 (GLUT2) (Vinayagam and Xu, 2015) and α-glucosidase enzyme (Dej-adisai and Pitakbut, 2015).

Insulin deficiency in diabetes mellitus is known to stimulate lipolysis in adipose tissue, leading to an increase in serum triacylglyceride levels. There is a reciprocal influence between dyslipidemia and beta-cell function, as well as beta-cell dysfunction and lipid metabolism (Bardini et al., 2012). LECS was found to increase HDL levels and decrease serum TG and LDL in diabetic rats. These actions on lipid metabolism suggest that *C. siamea* may be beneficial against hyperlipidemia and the resulting cardiovascular diseases. The effect of C. siamea could be attributed to its inhibitory effects on pancreatic lipase (Kumar et al., 2013) and α-glucosidase (Dej-adisai and Pitakbut, 2015). Chronic consumption of α-glucosidase inhibitors is known to improve lipid profiles in animal models of diabetes (Standl and Schnell, 2012).

The results are supported by the work of Nuankaew et al. (2021), who demonstrated the potent effects of ethyl acetate and ethanol extracts of *C. siamea* against insulin resistance in zebrafish larvae. The compounds isolated from these extracts included resveratrol, piceatannol, dihydropiceatannol, chrysophanol, and emodin. Additionally, *C. siamea* contains flavonoids such as luteolin and D-pinitol. Resveratrol, piceatannol, and dihydropiceatannol isolated from *C. siamea* showed inhibitory effects against α-glucosidase. Chrysophanol and emodin inhibited PTP1B activity, while resveratrol exhibited DPP-IV inhibition effects via molecular docking (Nuankaew et al., 2021). These polyphenols may be involved in the antidiabetic properties of *C. siamea* (Kim et al., 2012; Zang et al., 2016). Further studies are needed to elucidate the mechanisms of action of these bioactive compounds in mediating the antidiabetic effects.

The liver plays a crucial role in glucose metabolism regulation, and serum glucose levels are influenced by

peripheral glucose use and liver storage and/or production (Cotrozzi et al., 1997). This central role of the liver makes it susceptible to diseases in individuals with metabolic disorders, particularly diabetes (Levinthal and Tavill, 1999). In line with expectations, the present study demonstrated that the diabetic control group exhibited relatively high serum levels of AST, ALT, and AlkP, indicating the hepatotoxic impact of alloxan (Lucchesi et al., 2015). Alloxan also caused a significant increase in serum urea and creatinine in diabetic animals compared to non-diabetic control. However, treatment with *C. siamea*, like glibenclamide, did not show improvement in these liver and renal function parameters in diabetic rats compared to diabetic control rats. Similar observations have been reported in a sub-chronic study using the aqueous extract of the stem bark of *C. siamea* in rats (Mohammed et al., 2012). In contrast, oral administration of *C. siamea* for 28 days showed a beneficial effect on hepatic transaminases (AST, ALT) in leptin-deficient obese mice (Koffi et al., 2019).

Conclusion

In conclusion, the results indicate that the oral administration of the ethanol extract of *C. siamea* reduces intestinal glucose absorption, ameliorates the imbalance in glucose and lipid metabolism in diabetes by improving pancreatic function in diabetic animals. Additionally, the consumption of this medicinal plant, known for its antioxidant properties, did not exhibit toxicity on kidney and liver functions during diabetes treatment. Thus, the present study provides a pharmacological basis for the ethnomedicinal use of this plant for its antidiabetic properties, justifying the need for pragmatic trials aimed at developing improved phytomedicines against diabetes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

REFERENCES

Agouni A, Lagrue-Lak-Hal A, Mostefai HA, Tesse A, Mulder P, Rouet P (2009). Red wine polyphenols prevent metabolic and cardiovascular alterations associated with obesity in Zucker fatty rats (Fa/Fa). PLoS ONE 4(5):1-8.

American Diabetes Association (2008). Diagnosis and classification of diabetes mellitus. Diabetes Care 31(1):62-67.

Bardini G, Rotella CM, Giannini S (2012). Dyslipidemia and Diabetes: Reciprocal Impact of Impaired Lipid Metabolism and Beta-Cell Dysfunction on Micro- and Macrovascular Complications. The Review of Diabetic Studies 9:2-3.

Cotrozzi G, Casini Raggi V, Relli P, Buzzelli G (1997). Role of the liver in the regulation of glucose metabolism in diabetes and chronic liver disease. Annali Italiani di Medicina Interna 12(2):84-91.

Dej-adisai S, Pitakbut T (2015). Determination of α-glucosidase inhibitory activity from selected Fabaceae plants. Pakistan Journal of Pharmaceutical Sciences 28(5):1679-1683.

Jiang YH, Sun W, Li W, Hu HZ, Zhou L, Jiang HH (2015). Calycosin-7-O-β-D-glucoside promotes oxidative stress-induced cytoskeleton reorganization through integrin-linked kinase signaling pathway in vascular endothelial cells. BMC Complementary and Alternative Medicine 15(315):1-11.

Kamagaté M, Koffi C, Kouamé NM, Akoubet A, Yao NAR, Die-Kakou HM (2014). Ethnobotany, phytochemistry, pharmacology and toxicology profiles of *Cassia siamea Lam*. The Journal of Phytopharmacology 3(1):57-76.

Khavandi K, Amer H, Ibrahim B, Brownrigg J (2013). Strategies for preventing type 2 diabetes: an update for clinicians. Therapeutic Advances in Chronic Disease 4:242-261.

Kim HJ, Park KS, Lee SK, Min KW, Han KA, Kim YKl (2012). Effects of pinitol on glycemic control, insulin resistance and adipocytokine levels in patients with type 2 diabetes mellitus. The Annals of Nutrition and Metabolism 60(1):1-5.

King A (2012). The use of animal models in diabetes research. British Journal of Pharmacology 166:877-894.

Koffi C, Kamagaté M, Koffi E, Kouamé NM, Yao NAR, Balayssac E, Die-Kakou HM (2016). Aqueous extract of leaves of *Cassia siamea Lam* exhibited antihyperglycemic effect and improved kidney function in diabetic Wistar rats. International Journal of Pharmaceutical Research 6(11):336-342.

Koffi C, Kouame NM, N'zoue KS, Yobo BMS, Kee MYC, Toure M, Kamagate M (2022). Inhibitory effect of *Cassia siamea* methanol extract on intestinal glucose absorption in glucose-induced hyperglycemic Wistar rats. West African Journal of Pharmacology and Drug Research 36:62-66.

Koffi C, Soleti R, Nitiema M, Mallegol P, Hilairet G, Chaigneau J (2019). Ethanol Extract of Leaves of *Cassia siamea Lam* Protects against Diabetes-Induced Insulin Resistance, Hepatic, and Endothelial Dysfunctions in ob/ob Mice. Oxidative Medicine and Cellular Longevity 2019, ID 6560498: 1-11.

Kumar D, Karmase A, Jagtap S, Shekhar R, Bhutani KK (2013). Pancreatic lipase inhibitory activity of cassiamin A, a bianthraquinone from *Cassia siamea*. Natural Product Communications 8:195-198.

Kumar S, Kumar V, Prakash OM (2010). Antidiabetic and anti-lipemic effects of *Cassia siamea* leaves extract in streptozotocin induced diabetic rats. Journal of Tropical Medicine 3(11):871-873.

Lima CR, Vasconcelosa CFB, Costa-Silvab JH, Maranhãoc CA, Costad J, Batistae TM (2012). Anti-diabetic activity of extract from *Persea americana Mill.* leaf via the activation of protein kinase B (PKB/Akt) in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology 141(1):517-525.

Luangpirom A, Saenbuaphan N (2006). Hypoglycemic effect of *Senna siamea (Lam.)* leaf extract in rats. KKU Research Journal 11:77-83

Lucchesi AN, Cassettari LL, Spadella CT (2015). Alloxan-Induced Diabetes Causes Morphological and Ultrastructural Changes in Rat Liver that Resemble the Natural History of Chronic Fatty Liver Disease in Humans. Journal of Diabetes Research ID 494578:1-11.

McCall AL (2012). Insulin Therapy and Hypoglycemia. Endocrinology and Metabolism Clinics of North America 41(1):57-87.

McGrath JC, Drummond GB, McLachlan EM, Kilkenny C, Wainwright CL (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. British Journal of Pharmacology 160:1573-1576.

Mohammed A, Atiku MK (2012). Antihyperglycemic and antihyperglycemic effects of leaves and stem bark methanol extracts *Cassia siamea* in alloxan-induced diabetic rats. Current Research in Cardiovascular Pharmacology 1(1):10-17.

Moradabadi L, Montasser KS, Fehresti SM (2013). Hypoglycemic Effects of Three Medicinal Plants in Experimental Diabetes: Inhibition of Rat Intestinal α-glucosidase and Enhanced Pancreatic Insulin and Cardiac Glut-4 mRNAs Expression. Iranian Journal of Pharmaceutical Research 12(3):387-397.

Nerup J, Mandrup-Poulsen T, Helqvist S, Andersen HU, Pociot F, Reimers JI (1994). On the pathogenesis of IDDM. Diabetologia 37(2):82-89.

Nuankaew W, Heemman A, Wattanapiromsakul C, Shim JH, Kim NW, Yasmin T, Jeong SY (2021). Anti-insulin resistance effect of constituents from *Senna siamea* on zebrafish model, its molecular docking, and structure–activity relationships. Journal of Natural Medicines 75(3):520-531.

OECD/OCDE 423 (2001). OECD guideline for testing of chemicals. Acute oral toxicity - acute toxic class method. 14 p. Available at: http://www.oecd.org/chemicalsafety/risk-assessment/1948370.pdf Accessed 17.09.20.

Shafiullah PM, Kamil MA (1995). New isoflavone C-glycoside from *Cassia siamea*. Fitoterapia 66:439-441.

Standl E, Schnell O (2012). Alpha-glucosidase inhibitors: cardiovascular considerations and trial evaluation. Diabetes and Vascular Disease Research 9:163-169.

Szkudelski T (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiological Research 50:537-546.

Tanty H, Permai SD, Pudjihastuti H (2018). In vivo anti-diabetic activity test of ethanol extract of the leaves of *Cassia Siamea Lam*. Procedia Computer Science 135 632–642.

Vinayagam R, Xu B (2015). Antidiabetic properties of dietary flavonoids: a cellular mechanism review. Nutrition & Metabolism 12:1-20.

Willcox ML, Elugbaju C, Al-Anbaki M, Lown M, Graz B (2021). Effectiveness of Medicinal Plants for Glycaemic Control in Type 2 Diabetes: An Overview of Meta-Analyses of Clinical Trials. Frontiers in Pharmacology 12:1-13.

Zang Y, Igarashi K, Li Y (2016). Anti-diabetic effects of luteolin and luteolin-7-O glucoside on KK-A(y) mice. Bioscience, Biotechnology, and Biochemistry 80(8):1580-1886.