

Full Length Research Paper

Assessment of *Cassia siamea* stem bark extracts toxicity and stability in time of aqueous extract analgesic activity

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***Cassia siamea* Lam (Fabaceae), widely used in tropical countries for its nutritional and pharmacological properties, has been toxicologically evaluated. Oral administration of aqueous (CSE4) and ethanol (CSE3) *Cassia siamea* stem bark extracts did not show mortality up to 3000 mg/kg in albinos Wistar rats. LD 50 of petroleum ether (CSE1) and dichloromethane (CSE2) extracts were estimated at 1300 and 1275 mg/kg respectively. None of the extracts has cytotoxicity activity on KB and Vero cell lines. There were no differences in body weight and macroscopic examinations. Haematocrit, creatinine, alkaline phosphatase (ALP), alanine amino transferase (ALT) and Asparatate Amino Transferase (AST) parameters did not show difference of levels. Contrariwise, a significant increase in animal weight and blood glucose were observed in animals treated group. This increase can be explained by the presence of polysaccharides and phytosterols (stigmasterol, beta-sitosterol) in this plant. The lyophilised aqueous extract keeps its analgesic property for over two years.**

Key words: *Cassia siamea*, stem bark extracts, cytotoxicity, subchronic toxicity, pharmacological stability, analgesic activity.

INTRODUCTION

Cassia siamea Lam (Fabaceae) is a medicinal plant widely used in Asia, Africa, Australia and South

America (Kusamran et al., 1998; Sanon et al., 2003; Koyama et al., 2001). Its stem bark is traditionally used

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against constipation, malaria, and associated diseases such as fever and jaundice (Ahn et al., 1978; Nsonde et al., 2009; Kaur et al., 2006). A decoction of bark is given to diabetic patients while a paste is used as dressing for ringworm and chilblains. The roots are used as antipyretic preparations and leaves are present in remedies for constipation, hypertension, insomnia, and asthma (Ahn et al., 1978). Its antioxidant properties present the advantage to protect against heart disease people who consume it regularly as food (Kaur et al., 2006). Antiplasmodial, analgesic and antiinflammatory activities have been validated by pharmacological studies (Gbeassor et al., 1989; Nsonde et al., 2005; Mbatchi et al., 2006), as well as antioxidant and antihypertensive activities (Kaur et al., 2006), laxative activity (Elujoba et al., 1989) and sedative activity (Thongsaard et al., 1996; Sukma et al., 2002), antibacterial activity (Lee et al., 2014) and hepatoprotective (Kannampalli et al., 2005; 2007). It was recently reported insecticide activity of *Cassia siamea* extracts and pure compounds (Kamara et al., 2011; Mamadou et al., 2014).

Given its ethnomedical and traditional uses, its pharmacological, nutritional and phytochemical properties, *C. siamea* has proved to be a plant of great interest and a therapeutic food. Since this study ultimate goal is the development of improved traditional plant medicine, it is essential to investigate thoroughly the toxicity of active extracts in order to direct any form of exploitation of this plant in medicine or pharmaceutical industry.

This study aims to assess the cytotoxicity, the acute and subacute toxicity of aqueous and alcoholic extracts of *C. siamea* stem bark, and the stability of the lyophilised aqueous extract.

MATERIALS AND METHODS

Plant material

Stem barks of *C. siamea* Lam were collected from Mindouli (Pool, Congo) in May, 2007 and authenticated by the botanists of Centre d'Etudes sur les Ressources Végétales (CERVE), Congo Brazzaville. A voucher specimen has been deposited at the Herbarium of the botanic laboratory under reference number 128/16/01/1960/coll:P.Sita.

Preparation of extracts

Dried and powdered stem barks (1000 g) were successively extracted for 48 h by maceration at room temperature with petroleum ether (CSE1), dichloromethane (CSE2) and ethanol (CSE3). For each organic extract, 5 L of solvent was used free time successively. All organic extracts were concentrated to dryness under reduced pressure in a rotary evaporator to give the following yields: 0.62; 0.92 and 0.8% (w/w) respectively. The marc resulting from the extraction with ethanol was extracted for 10 min in boiling water (1.5 L). The cold aqueous extract was centrifuged (7000 rpm during 30 min.) and concentrated under reduced pressure with a rotavapor before lyophilization to give CSE4 (yield: 1.1%).

Animals

Male and female Wistar rats (150 and 250 g) obtained from the Health Science Faculty of Brazzaville were used. They were housed under standard conditions (25±5°C, 40 to 70% RH, 12 h light/dark cycle) and fed with a standard feed and water *ad libitum*. The rules of ethics published by the International Association for the Study of Pain (Zimmermann, 1983) have been considered.

Acute toxicity study

Wistar rats, male and female, were divided into groups of ten animals. The control groups received p.o. distilled water (10 ml/kg). Aqueous (CSE4), ethanol (CSE3), petroleum ether (CSE1) and dichloromethane (CSE2) extracts were given p.o. at the doses of 100, 400, 1000, 2000, 2600, 3000 mg/kg from one to seventh groups. The mortality rate within 72 h period was determined, and the LD₅₀ was estimated according to the method described by Miller and Tainter (1944).

Cytotoxicity

The toxicity of cell extracts was tested on KB cells (cancerous cells of the human epidermis) and Vero cells (kidney cells from African green monkey) using the protocol described by Mbatchi et al. (2006). These cells were grown in a culture medium consisting of RPMI 1640 (Gibco BRL, Paisley, Scotland), 25 mM HEPES, 30 mM NaHCO₃ (Gibco BRL) and 5% fetal calf serum (Boehringer, Germany). Culture was kept in an incubator at 37°C and 5% CO₂. For the assessment of cytotoxicity, cells were distributed on a culture plate of 96 costar wells and then the concentration of 2x10⁴ cells diluted in a volume of 100 ml were added in each well. The controls were cultured without extracts. Cell growth was estimated by the incorporation of tritiated hypoxanthine (3H) after 72 h of incubation. The results of the test groups were compared with controls. The reference used was taxotere (10µg/ml) provided by Aventis Pharma (Anthony, France). The tests were performed in triplicate.

Subchronic toxicity

This study concerns only the aqueous extract. This extract was chosen because of its popularity in traditional medicine, its ease of preparation, the pharmacological effects against malaria, oedema and pain that the study have highlighted (Nsonde et al., 2010).

Forty Wistar rats, males and females, were divided into eight groups of five rats each. These eight groups were then divided in two lots of four groups each. The lot of control group was treated with 10 ml/kg/ day of distilled water per os for 39 days maximum. The lot of the test group was treated with 200 mg/kg/ day of aqueous extract (CSE4) per os for 39 days maximum. Evaluation of toxicity was performed at days 7, 21, 28 and 40. At each assessment, animals were subjected to ether anaesthesia, a volume of blood was collected at the pre-orbital plexus. A portion of blood was collected in vacutainer tubes on the lithium heparin for determination of haematocrit. A drop of each blood sample was deposited on a strip for the determination of glycaemia. Remaining blood was centrifuged at 3000 rpm for 10 min. The serum aliquots were collected for enzymatic analysis (King, 1965b; Panigrahi et al., 2014). Biochemical parameters measured were AST (Aspartate Amino-Transferase), ALT (Alanine Amino-Transferase), Creatinine and ALP (Alkaline Phosphatase) (Luximon-Ramma et al., 2001).

Noble organs (liver, heart, kidneys, and brains) were removed, weighed and observed macroscopically. The weights of animals were determined at each evaluation. GOT or AST, GTP and ALT

Table 1. Cytotoxicity of *C. siamea* bark extracts on cell KB and Vero lines.

Extracts	Cytotoxicity (in% inhibition) at 10 µg / ml	
	KB	Vero
CSE1	10	15
CSE2	12	15
CSE3	3	2
CSE4	0	0
Taxotere (2.5 x10 ⁻¹⁰ M)	75	29

were assayed by the method described by Wooten (1964). This method is based on the ability of enzymes to form pyruvate, which reacts with 2,4-dinitrophenylhydrazine in hydrochloric acid to give the hydrazone. The hydrazone formed turns into an orange complex in alkaline medium, where the colour was measured spectrophotometrically at 540 nm. The alkaline phosphatase (ALP) was measured by the method described by King (1965a). This method is based on the determination of phenol liberated by enzymatic hydrolysis in phenyl disodium phosphate at pH = 10 (A vérifier, la phrase initiale n'était pas très claire). This rate of phenol was estimated by spectrophotometry at 640 nm. Creatinine forms a complex photometer with alkaline picrate. A kinetic assay at 490 nm helped to overcome the interference.

Study of the stability of analgesic activity in time

The study aimed at estimating an occasional way, over 2 years, the analgesic activity of the aqueous extract (CSE4). CSE4 appears under the shape of a lyophilised powder, kept away from heat, maintained under standard conditions of temperature and pressure of the laboratory. It was placed in a glass bottle with the possibility to close tightly after use, the whole being wrapped in aluminium foil. *C. siamea* Lam stem bark analgesic activity on the animal paw pressure was studied according to Randall and Sellitto method (1957), using an analgesimeter (Ugo basile 1740, Italy). Wistar rats were assigned to three groups of five animals each. The first group received distilled water (10 ml/kg) p.o. CSE4 was given at the dose of 200 p.o. The third group, which served as control, received morphine (2 mg/kg). Analgesic activity was measured 1 h after administration of test and standards drugs (Winter et al., 1962; Abena et al., 2003). In rat subjected to pressure of the left leg, the study measured the intensity of the threshold pain that triggers the withdrawal of the animal paw and determined the reaction time (Woolfe and MacDonald, 1994).

Statistical analysis

Values were expressed as mean ± S.E.M. Statistical significance for analgesic activity was calculated using a one-way analysis of variance (ANOVA). Significant differences between means were determined by Duncan's multiple-range tests. Values of $p < 0.05$ were considered significant.

RESULTS

Study of acute toxicity

No mortality was observed in groups of rats treated with aqueous (CSE4) and ethanol (CSE3) extracts. LD₅₀ values for petroleum ether (CSE1) and dichloromethane

(CSE2) extracts were estimated at 1300 and 1275 mg/kg. The minimum lethal dose and maximum tolerated dose were 1000 and 1600 mg/kg for CSE1 and CSE2. Signs of toxicity include dyspnoea and disorders of motor function.

Cytotoxic study

The values for the cytotoxicity study are given in Table 1. CSE3 and CSE4 are completely devoid of toxicity against cell lines KB and Vero and the two other extracts present a very low cytotoxicity (maximum: 15% of inhibition of cell growth).

Subchronic toxicity

There is no difference in body weight, haematocrit and macroscopic observation of organs between the control group and test group (Figure 1). There is a very significant and progressive increase of weight of the animals treated daily for 40 days as compared to controls (Figure 2). Except for the glucose level, which increases significantly from day 21, there is no significant difference on biochemical parameters levels (Table 2).

Stability of analgesic effect

Kept in ordinary conditions, away from heat and light, aqueous extract, prepared by lyophilisation, in the form of powder retained its analgesic property for over two years, as one can see in Figure 3a and b.

DISCUSSION AND CONCLUSION

Toxic or beneficial effects of a drug have an intensity which depends on the dose and its plasma concentration or tissue. The effects of toxicities affecting organs are characterized by hypertrophy of these organs or weight loss when they are very important. There is no toxicity of aqueous extract (CSE4) affecting the weight of noble organs: heart, kidney, liver, pancreas and brain. Some biochemical parameters (AST, ALT, ALP) are the most sensitive markers employed in the diagnosis of hepatic

Table 2. Effect of lyophilised aqueous extract of *C. siamea* stem bark on blood serum biochemical in rats, values are expressed as mean ± standard deviation of five animals, *** p < 0,001 compared with control.

Treatment	Group	Day 7	Day 21	Day 28	Day 40
AST (UI/dl)	Control	4.0±0.79	5.2±0.42	6.4±0.57	8.4±0.57
	Test	4.6±0.67	4.2±1.03	5.6±1.15	9.6±0.57
ALT (UI/dl)	Control	4.8±0.74	4.6±0.76	7.2±0.74	11.2±0.89
	Test	6.2±0.42	5.4±0.76	7.0±1.06	12.6±0.84
ALP (UI/dl)	Control	110.2±1.23	108.1±0.89	116.1±2.6	114.9±2.1
	Test	108.1±0.98	113.0±0.97	115.5±1.75	116.6±1.5
Creatinine (UI/dl)	Control	3.26±0.36	3.54±0.33	3.90±0.57	4.46±0.64
	Test	3.16±0.4	3.74±0.48	3.62±0.57	3.9±0.38
Glucose (mg/dl)	Control	85.2±2.07	84.6±4.6	88.4±4.04	180.4±4.99
	Test	87.2±3.31	100.6±4.35*	158.4±7.9***	250.4±28.17***
Haematocrit (%)	Control	21.2±0.65	31.2±0.65	31.8±0.42	31.8±0.65
	Test	31.2±1.08	31.2±0.42	32.3±0.65	33.6±0.48

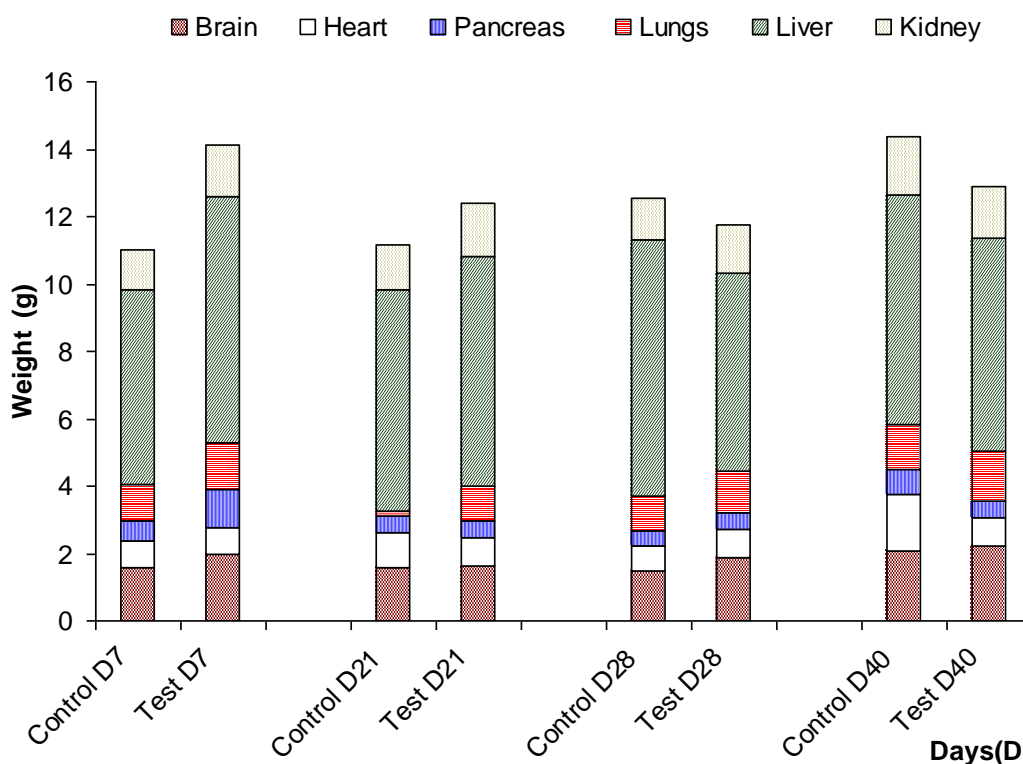


Figure 1. Effect of the lyophilised aqueous extract of *C. siamea* stem bark on organ weights of rats subjected to subchronic treatment for 40 days, values are expressed as mean ± SD of five animals.

lesions due to their localization in the cytoplasm of liver cells. They are released into the blood stream after cellular damage in the liver (Pradeep et al., 2007; Sallie et al., 1991).

The high level of transaminase is seen as an indicator of liver damage. The elevation of serum ALT is

considered as the most sensitive indicator (Zimmerman et al., 1993.; Ha et al., 2001; Huseby et al., 1993). The level of AST, ALT, and ALP in the serum shows that the aqueous extract does not involve the release of cytoplasmic enzymes in the blood. CSE4 may not show any liver toxicity observed in these conditions. Moreover,

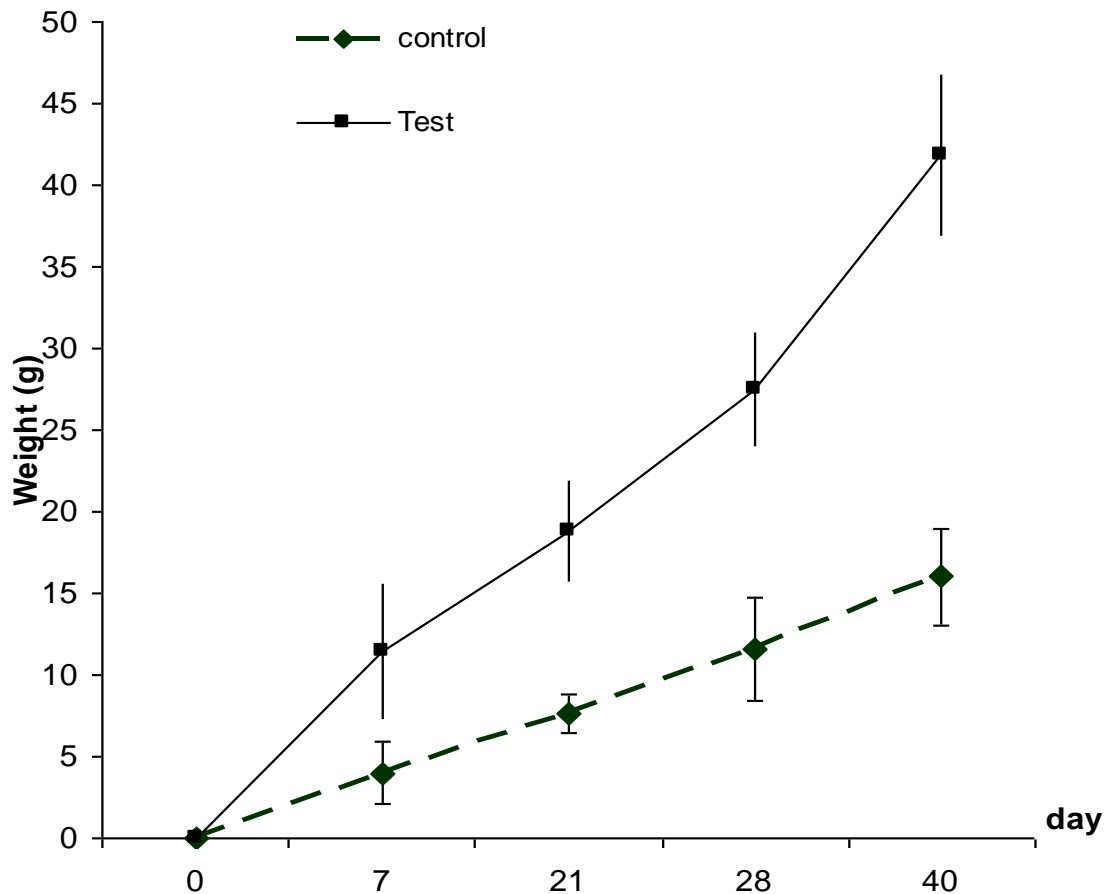


Figure 2. Weight gain of animals chronically treated with *Cassia siamea* stem bark aqueous extract (CSE4) for 40 days. The results are expressed as geometric mean \pm standard deviation, $n = 5$.

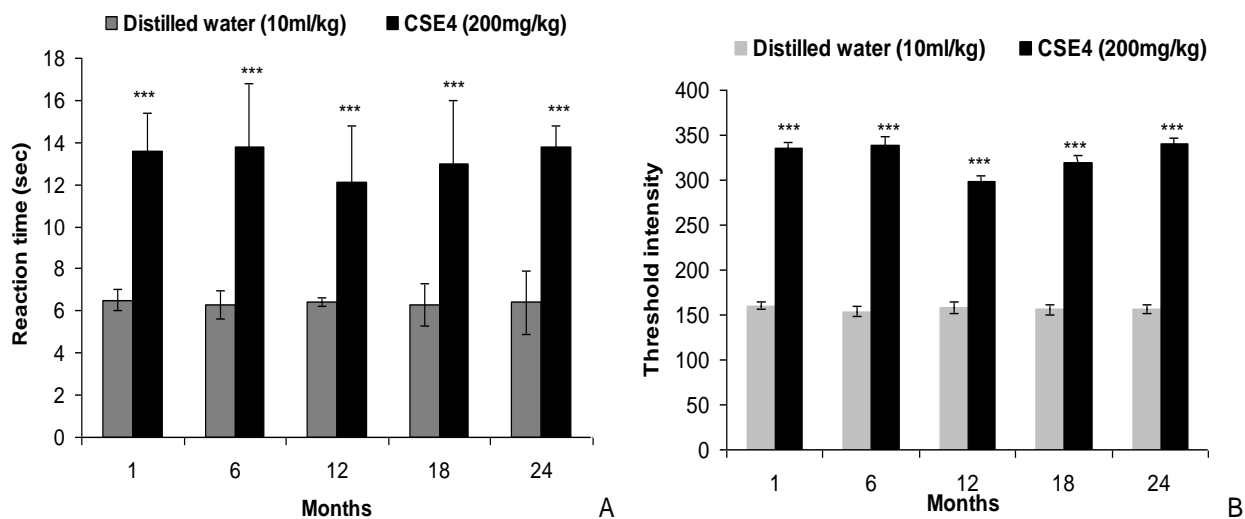


Figure 3 (a). Pharmacological stability in time of *Cassia siamea* aqueous extract analgesic activity expressed in time reaction. The results are expressed as geometric mean \pm standard deviation, $n = 5$. *** $p < 0.001$ compared with control. CSE4: bark aqueous extract of *C. siamea*, (b). Pharmacological stability in time of *Cassia siamea* aqueous extract analgesic activity expressed in threshold intensity. The results are expressed as geometric mean \pm standard deviation, $n = 5$. *** $p < 0.001$ compared with control. CSE4: bark aqueous extract of *C. Siamea*.

antioxidant and hepatoprotective properties in this plant have also been highlighted (Kusamran et al., 1998, Kaur et al., 2007).

Concerning creatinine, it is a very good indicator of glomerular function. The increase of creatinine level in the blood is followed to diagnose a possible renal dysfunction (Pradeep et al., 2005). The aqueous extract used in these conditions does not affect kidney. The increase of glucose level in the blood from the 21st day is probably due to a dysfunction of the glucose metabolism. Then, the weight gain could be explained by an increase in blood glucose as observed in the case of Type II diabetes. These results are completely opposite to those found in the study on the subchronic toxicity of barakol which is one of the active molecules isolated from this plant showing an increase in the level of ALT, and AST in the serum, weight loss and a reduction in amount of glucose (Ayutthaya et al., 2005, Pumpaisalchai et al., 2003).

Because of this pharmacological stability, the lyophilised aqueous extract of *C. siamea* bark could be considered as an enhanced phytomedicine, after a complete pharmacological study to understand the mechanism of weight gain induced by this extract. To avoid any kind of disturbance of glucose metabolism, the study advocate the use of this plant aqueous extract in treatment extended to seven days, or in repeated treatment at the reduced intervals for non diabetic patients and in any treatment for diabetic patients.

Conflict of Interests

The authors have not declared any conflict of interests.

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