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Laxative, Antiinflammatory and Analgesic Effects of *Cassia Siamea* Lam (Fabaceae) Leaves Aqueous Extract

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Abstract
Purpose: *The objective of this study is to evaluate laxative, anti-inflammatory and analgesic activities of the aqueous extract of C. siamea leaves to contribute to the development of an improved traditional phytomedicine based on this plant.*
Material and Methods*: At doses of 400 and 800 mg / kg administered orally, the aqueous extract of Cassia siamea (Fabaceae) leaves was evaluated in rats.*
*Results: in normal and constipated rats the aqueous extract of the leaves of Cassia siamea significantly decreases, the latency of the first fecal excretion (*** *p <0.001) and significantly increased the fecal excretion rate (** * p <0.001), and the total amount of excreted feces (*** p <0.001 and * p <0.05). This extract, also, showed interesting activity against acute inflammation of the paw edema induced by carrageenan, very significant after 1:00 (*** p <0.001), and against chronic inflammation of the granuloma cotton pellet (***p <0.05). The analgesic activity against cramps induced by acetic acid was less significant (* p <0.05). Alkaloids, polyphenols, terpenoids, steroids, anthraquinones, cardiotonic glycosides and anthocyanins have been identified in this plant.*
Conclusion: *Already used as food, the leaves of Cassia siamea are likely to serve, can be use in the development of an efficient and better tolerated laxative and anti-inflammatory medicine.*
Keywords: *Cassia siamea, leaves, aqueous extract, traditional medicine, laxative, anti-inflammatory, phytochemistry.*

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I. Introduction

Given the number of publications related, the many medicinal uses, the many pharmacological activities and the many bioactive molecules as well as safety, *Cassia siamea* (Fabaceae) can be considered as a natural pharmacy (Ali et al., 2011 ; Mohammed et al., 2012 ; Mamadou et al., 2014 ; Nsonde Ntandou et al., 2016). Among organs used in this plant leaves are very promising because they are regenerated and their use does not threaten the survival of the plant (Webro et al., 2011). They are already used as food in Asia (Kaur et al., 2006). The literature review does not report toxicity associated with its use. In traditional medicine, the decoction or its aqueous maceration is used, among other things, against pain, oedema, and constipation. It also reported that they would have an inhibitory effect on the pain induced by acetic acid and contain molecules that would act on the intestinal motility (Sukma et al., 2002; Ayutthaya et al., 2005; Matsumoto et al., 2010). Furthermore, in our previous studies, analgesic and interesting antiinflammatory activities have already been demonstrated in this plant, but much more in the sterm bark (Nsonde Ntandou et al., 2015).The bibliography also reported that this plant has already been the subject of extensive chemical studies (Ogbole et al., 2014 ; Mamadou et al., 2014 ; Qiu-Fen et al., 2015). Because of the environment which to influence the chemical composition of plants and indirectly the pharmacological effects and the need to confirm the traditional uses in Congo or our preliminary studies, this study aimed to assess the laxative, anti-inflammatory and analgesic *C.siamea* leaves aqueous extract activities to contribute to the development of an improved traditional phytomedicine based on this plant.

II. Material and Methods

1**. Animal**

Male and female Wistar rats, weighing between 150 and 238 g were used. These rats were elevated at the animaleria of the Faculté des Sciences et Techniques at the Marien NGOUABI University, under standard conditions (25 ± 5 ° C, 40-70% relative humidity and 12 h light / 12h dark cycle). They had free access to standard food and water. The ethical rules of animal experiments published by the International Association for the Study of Pain was respected (Zimmermann, 1983). One week before the experiment the animals were placed in individual metabolic cages each day, for 8 hours for acclimatization to experimental conditions.

2. Plant material

The leaves of *Cassia siamea* lam (Fabaceae) were collected in May 2010 in Brazzaville. A sample of this plant was identified by Dr Kami, a botanist at the National Herbarium of the Centre d'Etudes sur les Ressources Végétales (CERVE), after comparing the specimen with No129/1981P.Sita.

3. Preparation of extract

Cassia siamea leaves were dried, under the ambient atmospheric conditions of the laboratory of Biochemistry and Pharmacology of the Faculty of Health Sciences, protected from heat and sun. 50 g of powder were put into 500 ml of distilled water boiling at 100 ° c during 30 min. After cooling, the resulting solution is filtered, concentrated and lyophilized to give a yield of 10%.

4. Treatment of animals

Treatments were administered to different groups (n = 5) orally. The distilled water used as a control of the various tests was administered at 10 ml / kg. Paracetamol, voltaren, castor oil and tramadol, used as reference, were respectively administered at 100 mg / kg, 10 ml / kg, 5 mg / kg and 15 mg / kg. The extracts were administered at the doses of 400 and 800 mg / kg.

5. Laxative effect

5**.1. In normal rats**

The study was performed according to the method described by Takahura et al. (2002). Rats which fasted twelve (12) hours prior to testing are placed in individual cages lined with filter paper. These rats were divided into four groups of five (5) animals each. The reference used here is castor oil. Observations have been done first for 8 h and then at 16th hour from the time of administration of the products. The following parameters were evaluated: latency time of the first faeces excretion, frequency of fecal excretion, and mass of dried fecal material excreted. The physical properties of faeces, particularly hardness, flow, color and odour were also assessed.

5.2. After constipation induced by loperamide

Constipated and normal rats were treated in the same conditions with the difference that 1 hour after product administration; all the animals were treated orally with 5 mg / kg of loperamide.

6. Analgesic effect

6.1. Abdominal cramps test

This test was performed according to the method described by Koster et al. (1959). Here, Paracetamol was used as a reference molecule. 45 minutes after product administration, intraperitoneal injection of 0.6% acetic acid (0.1 ml / 10g weight) was done. This injection causes in mice, cramps: stretching of the hind legs and of dorsoventral musculature, widening flanks (Itou Elion et al, 2014). The number of cramps is counted during 10 minutes after the acetic acid injection. The analgesic substances are supposed to cause a significant decrease of cramps.

6.2. Pressure paw test

The reference product used is tramadol. This test is based on applying a mechanical stimulus that can induce pain. Increasing pressure is applied to the right hind paw until the pain threshold. This threshold leads to a voluntary withdrawal of the leg accompanied by a stereotyped flinch reaction. The maximum weight that can be applied is 750g (Randall and Selitto, 1957). . One hour (1h) after administration of the product, activity was evaluated.

7. Study of the Anti-inflammatory activity

7.1. Acute inflammation

Inflammation is induced by 20µl (2%) carrageenin injection into the hind paw of the animal (Elion Itou et al., 2014), diluted in 0.9% NaCl buffered to pH 7, 1 hour after the administration. The development of

oedema is followed by measuring the volume of the paw with a plethysmometer (Ugobasile 7140, Italy). Measurements are carried out in blind. The paw volume was measured to the level of the tibiotarsale articulation. The measurements were performed at 0 min (before injection of carrageenan) and 30mn, 60mn, 90mn, after the injection of carrageenan. Anti-inflammatory substances reduce the volume of the paw. Diclofenac was used as the reference drug.

7.2. Chronic inflammation

The study was conducted in rats. After ether anesthesia of the animals, 100 mg of sterilized cotton pellets (60 ° C) were placed in the incised interscapular region. The incision was then closed with a suture. The animals were treated with test products for seven (7) days. On the eighth day the cotton pellet is removed, freed from adhering tissue and dried in at 60 °C for 24 hours before being weighed. The antiinflammatory effect is given by the percentage inhibition of granuloma (PI%):

PI%= (PG/A) x 100 PG=B-A PG: weight of granuloma A = weight of the cotton pellet before implantation (100 mg)

B = weight of cotton after pellet weighed.

8. Chemical Screening

Conventional phytochemical tests were used to determine the different chemical groups present in this extract (Ogbole et al., 2014).

4. Statistical Analysis

The results were expressed as mean ± standard error and subjected to analysis of variance followed by a factor a test << t >> Student-Fischer. The significance level was set for * p <0.05; ** p <0.01 and *** p <0.001

III. Results

1. Laxative effect

In normal rats, the aqueous extract of the leaves of *Cassia siamea* showed a very significant laxative effect marked by significant differences (*** p <0.001), the latency time of the first faeces excretions (Figure 1), of the frequency of faeces excretion (Figure 2); and the total mass of excreted faeces (Figure 3), at doses of 400 and 800 mg / kg. In loperamide constipated rats this extract showed very significant differences (p <0.001) as well as on the latency of the first faecal than on the frequency faecal excretion, at both doses (Figures 4 and 5). The total quantity of faeces excreted is not very significant (* p <0.05).

2. Antiinflammatory effect

The aqueous extract of *Cassia siamea* leaves *showed* a significant anti-inflammatory effect after 1h, on acute inflammation (Figure 7), and after 7 days on chronic inflammation (Figure 8), at doses of 400 and 800 mg / kg .

3. Physical characteristic of faeces

The faeces were obtained in the form of paste and / or soft tissue (in normal and constipated rats) with the aqueous extract of the leaves of *Cassia siamea* and castor oil. With the extract the odor was characteristic of normal faecal mass. The faecal mass obtained from animals treated with castor oil was nauseating.

4. Chemical Constituents

The leaves of *Cassia siamea* are very rich in polyphenols, anthraquinone and anthocyanins; rich in alkaloids, cardiotonicglycosides, saponins, steroids and terpenoids; but do not contain tannins.

IV. Discussion

The main purpose of this study was to investigate the potential laxative, anti -inflammatory and analgesic activities of the aqueous extract of *Cassia siamea* leaves.The consideration of the latency time of the first excretion and the frequency of excretion of faeces , next to the total mass of faeces excreted , have already allowed the identification of a laxative effect (Nsonde Ntandou et al. , 2016) . An increase of water in feces and the frequency of defecation are among the most significant characteristics of the laxative (Croci et al. , 1996). To create a disease model, we used an antidiarrheal or constipating drug; The Loperamide which acts on the intestinal nerve system to induce constipation (Kakino et al., 2010) . Furthermore, soft, loose stools assume the existence of mechanisms of action that pass stimulation of intestinal motility and osmotic or electrolyte disturbances. The aqueous extract of the leaves of *Cassia siamea* at doses of 400 and 800 mg / kg given orally showed a significant laxative activity both in normal rats and in constipated rats. Stools were soft in constipated rats and diarrhea in normal rats.The aqueous extract of the leaves of *Cassia siamea* have very powerful laxative

as the castor oil. This effect is due to the presence of polyphenols and anthraquinones mainly barakol (Thongsaard et al., 2001 ; Ayutthaya et al., 2005). Two mechanisms of action can be possible; the increasing of intestinal motility as for barakol on the one hand (Sukma et al., 2002), and on the other hand, a mechanism via osmotic disturbances because of a portion of pasty in the faeces as with the Castor oil (Washabau et al., 2003 ; Paguigan et al., 2014). These results agree with those of our predecessors who demonstrated the same activity in other species of the same genus and sometimes (Guarise et al., 2012) .Senna or castor oil are reported to induce diarrhea caused by inhibition of water absorption in the large intestine which is a water absorptive organ (Kakino et al.,2010). This suggests the extract may induce its laxative effects, at least partly, via increased intestinal osmolarity and may stimulate intestinal electrolyte secretion. This is the case of Senna cascara , another species of the same family (fabaceae) famous for its laxative effect which acts via Increased electrolyte secretion from intestinal crypts (26)The methods of the cotton pellet granuloma to induce chronic inflammation and the carrageenan-induced paw oedema for acute inflammation are effective and well known (Adiukwu et al., 2013). A fast and very powerful anti-inflammatory effect on carragenane induced inflammation methode, greater than that of diclofenac under the experimental conditions this confirms the results found by nsonde Ntandou et al., (2010). This effect would be of the triterpene, terpenoids and polyphenols present in this plant mainly the lupeol, the emodin, the betasistosterol the luteolin and the kampferol (Mamadou et al., 2014).
The acetic acid test allows study analgesic activity of substances that act in the peripheral route (Koster et al., 1959). The abdominal constriction is associated with the sensitization of nociceptors to prostaglandins (Bose et al., 2007). The number of abdominal constriction reflects the intensity of the pain. The test of mechanical paw pressure is also very safe for the evaluation of the analgesic effect (Nsonde Ntandou et al., 2010). The aqueous extract of the leaves of *Cassia siamea* showed a very significant analgesic activity to be due to the presence of these numbers secondary metabolites. These results confirm the anti-inflammatory and analgesic effects already highlighted in the aqueous extracts of the bark of the same plant. Leaves and bark of *Cassia siamea* have some identical secondary metabolites.These results which confirm the use of this plant in traditional medicine encourage the development of a phytomedicine enhanced public interest.

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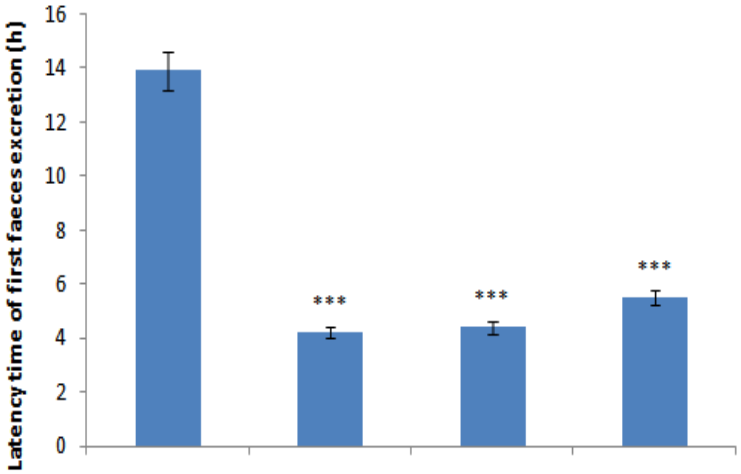

Figure 1: Effect of *Cassia siamea* leaves extract on latency time of feaces output in normal rats. Values given are mean ± S.E.M of five observations, ***p < 0.001 compared with the control group.

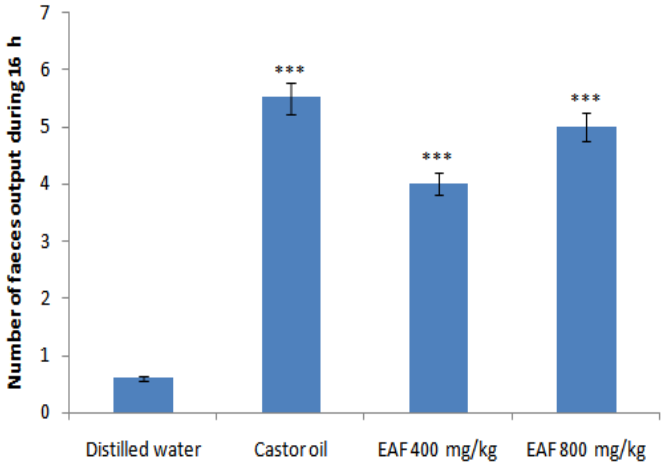

Figure 2: Effect of *Cassia siamea* leaves extract on frequency of feaces output in none constipated rats. Values shown are mean ± S.E.M (n = 5) and the values are compared with control group and considered significant at ***p < 0.001.

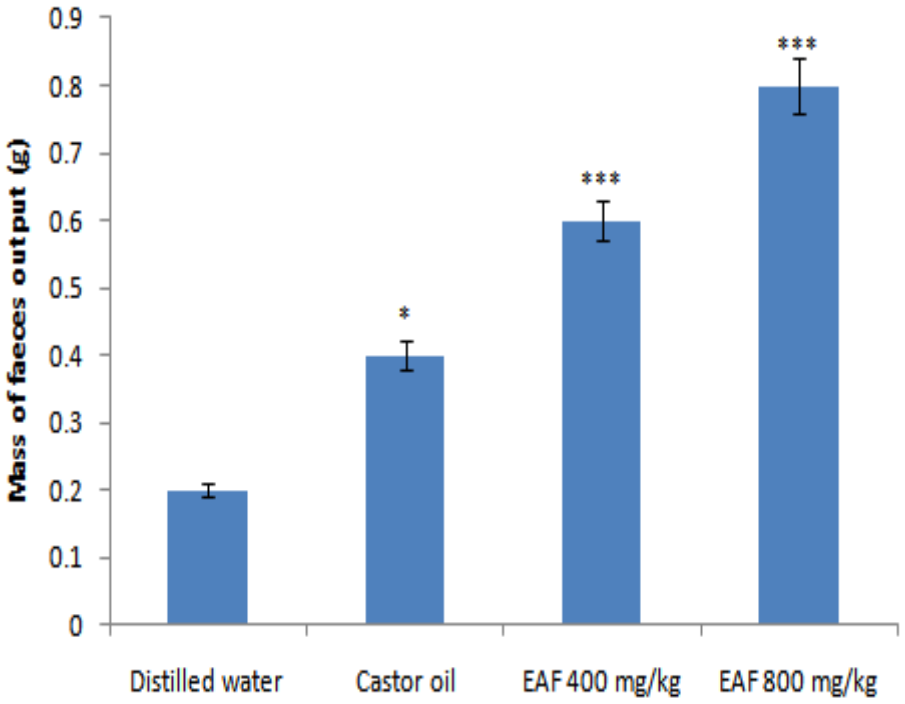

Figure 3: Effect of *Cassia siamea* leaves extract on total quantity of faeces output in normal rats. Values given are mean ± S.E.M of five observations, **p < 0.001 and *p < 0.05 compared with the control group.

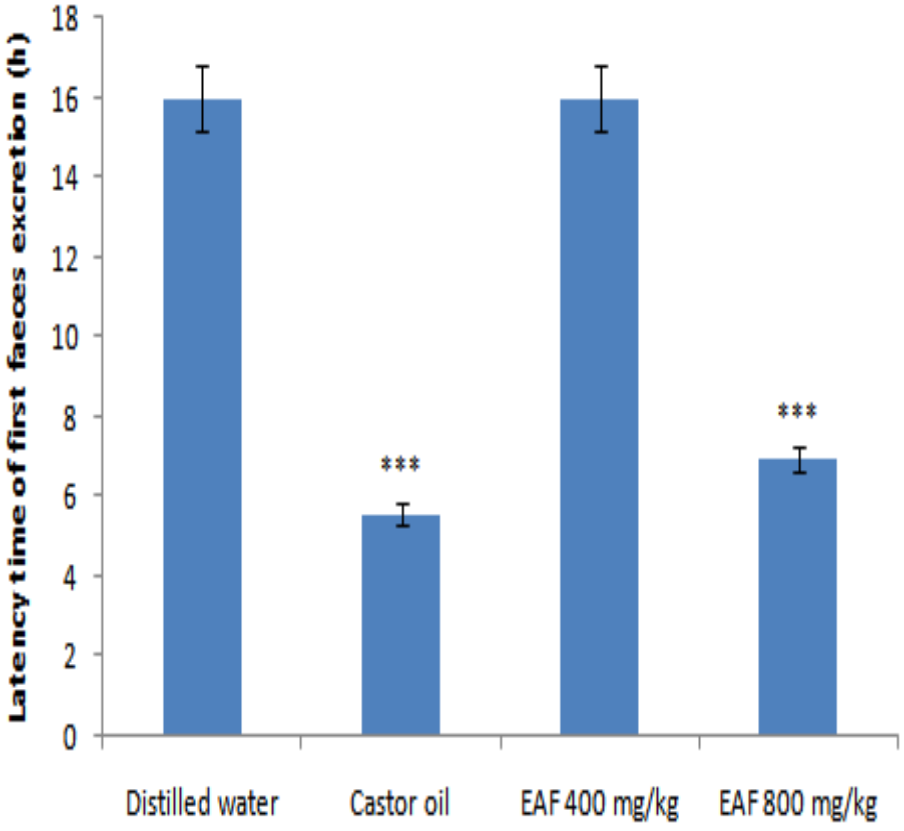

Figure 4: Effect of *Cassia siamea* leaves on latency time of faeces output on loperamide induced constipation rats. Values given are mean ± S.E.M of five observations,***p < 0.001 compared with the control group.

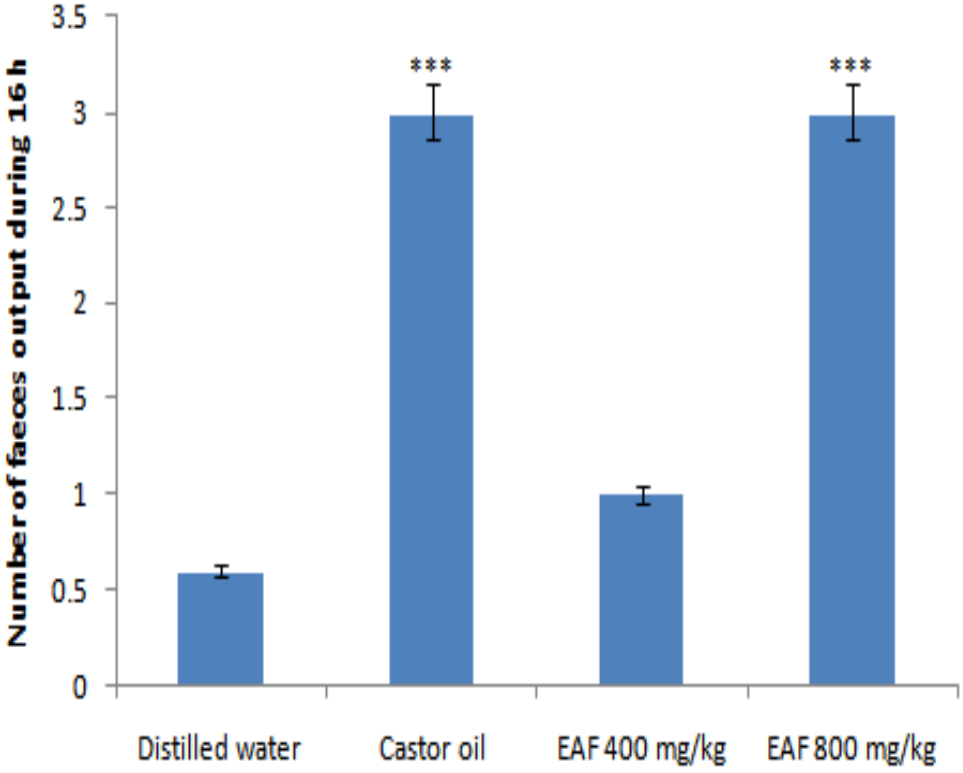

Figure 5: Effect of *Cassia siamea* leaves on frequency of faeces output on loperamide induced co nstipation rats. Values given are mean ± S.E.M of five observations, ***p < 0.001 compared with the control group.

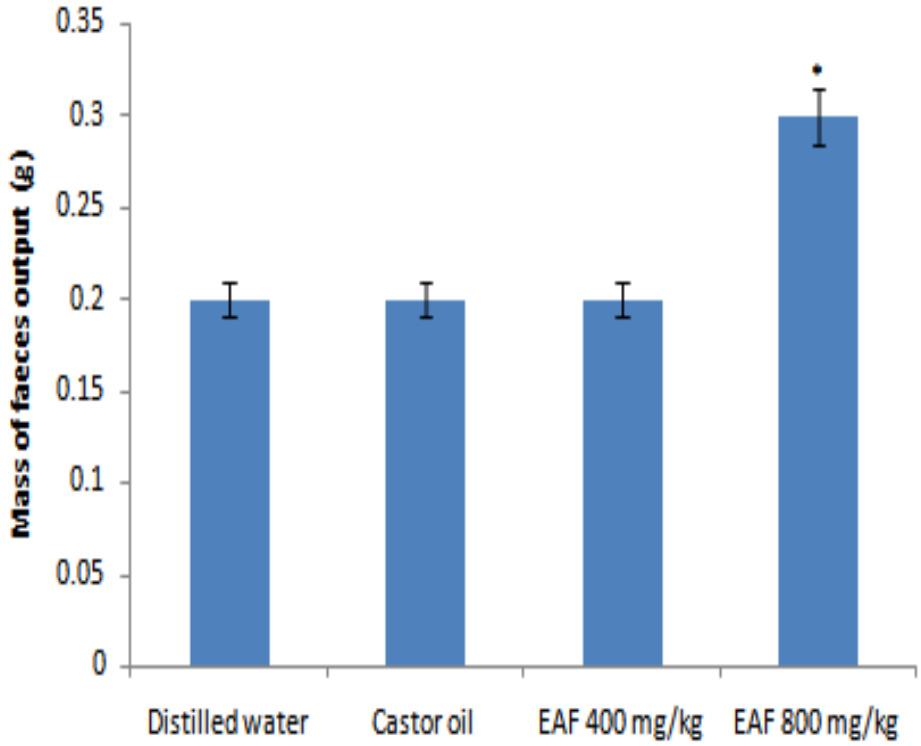

Figure 6: Effect of *Anchomanes difformis* leaves and rhizome on total quantity of faeces output on loperamide induced constipation rats. Values given are mean ± S.E.M of five observations, ***p < 0.001 and **p < 0.01 compared with the control group.

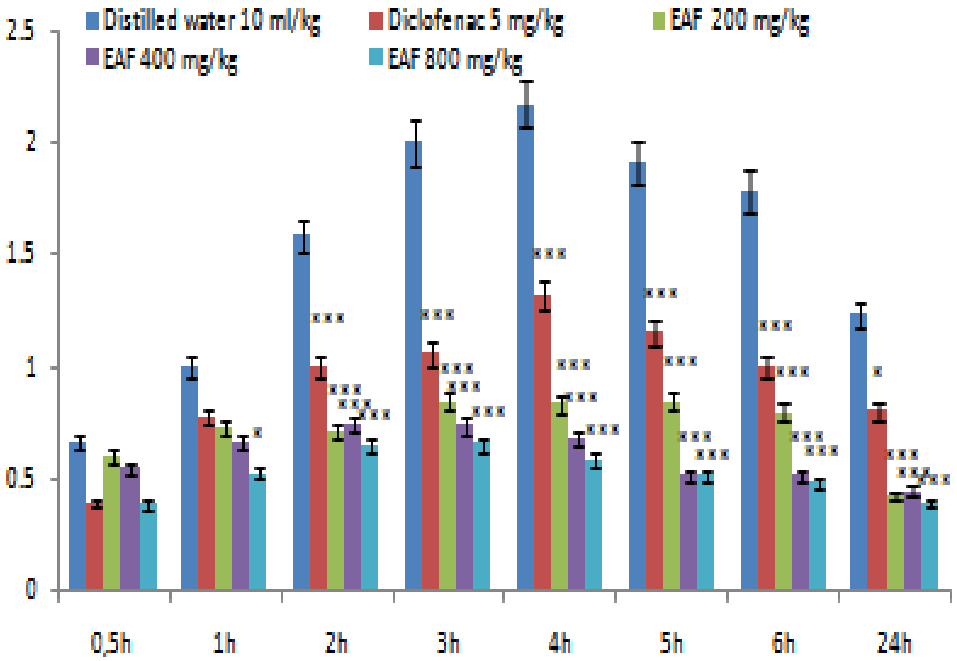

Figure 7: Effect of *Cassia siamea* leaves extract on the paw edema induced by carrageenan. Each value represents the mean± S.E.M of five animals per group***p < 0.001 and *p < 0.5 compared with the control group.

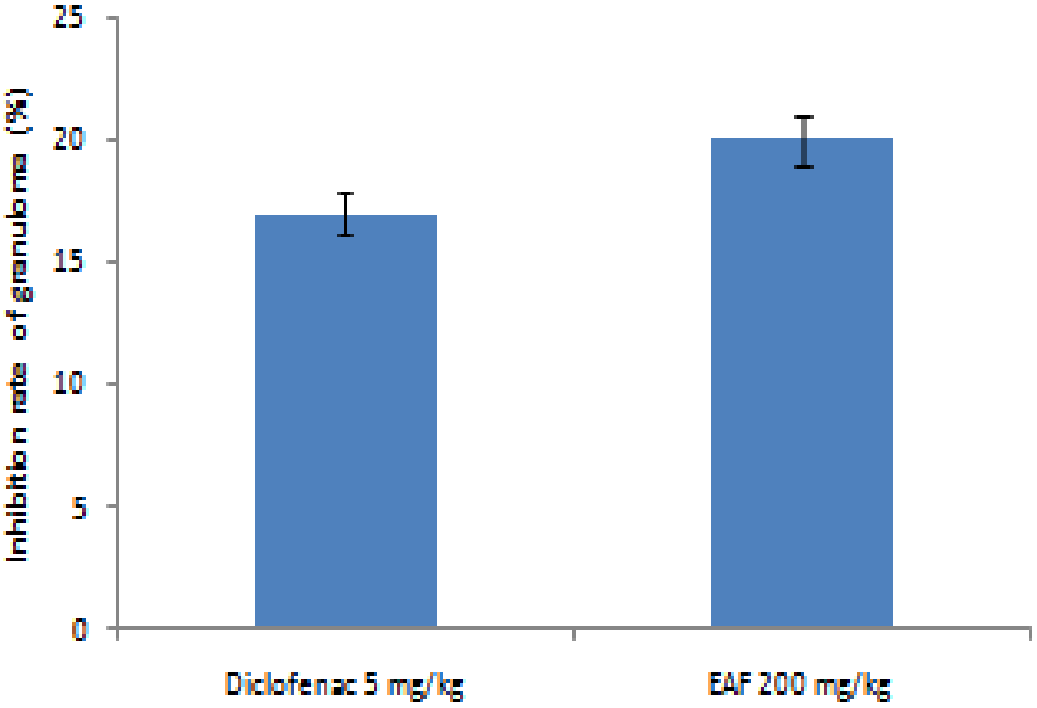

Figure 8: Effect of *Cassia siamea* leaves extract on the granuloma induced by cotton pellet. Each value represents the mean± S.E.M of five animals per group***p < 0.001 and *p < 0.5 compared with the control group.

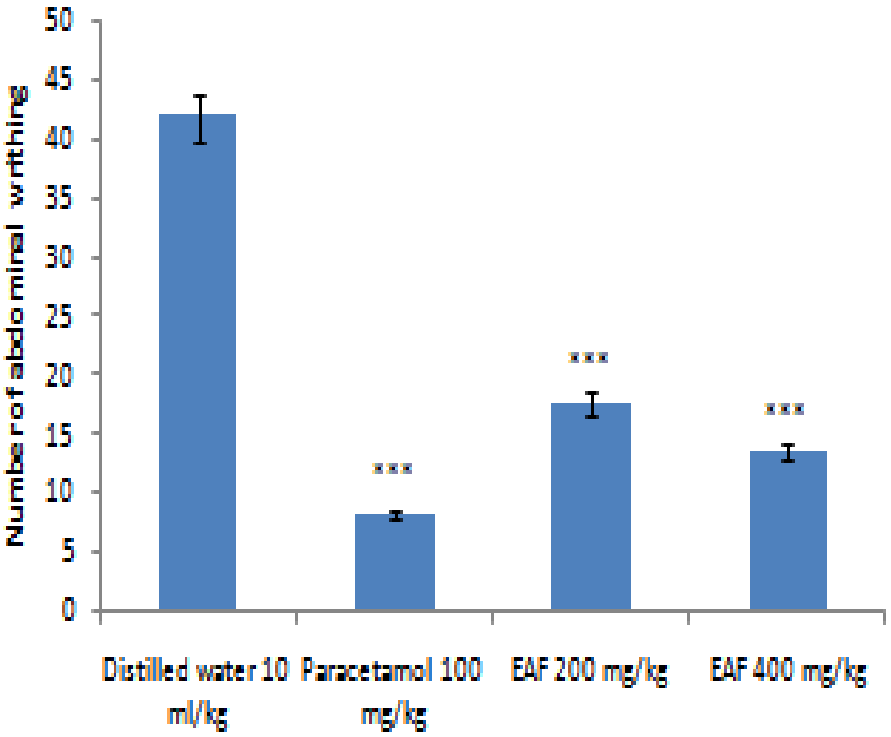

Figure 9: Effect of *Cassia siamea* leaves extract on the Acetic acid-induced abdominal constriction. Each value represents the mean± S.E.M of five animals per group***p < 0.001 and *p < 0.5 compared with the control group.

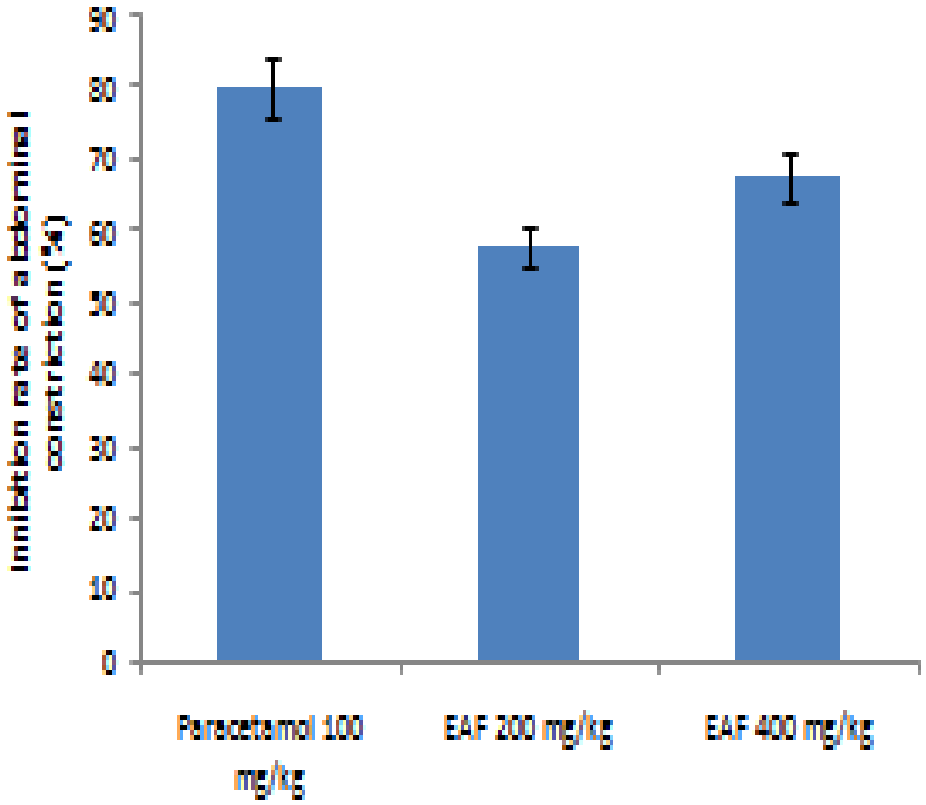

Figure 10: % percent inhibition of abdominal constriction induced by acetic acid of *Cassia siamea* leaves extract in mice. Each value represents the mean± S.E.M of five animals per group***p < 0.001 and *p < 0.5 compared with the control group.

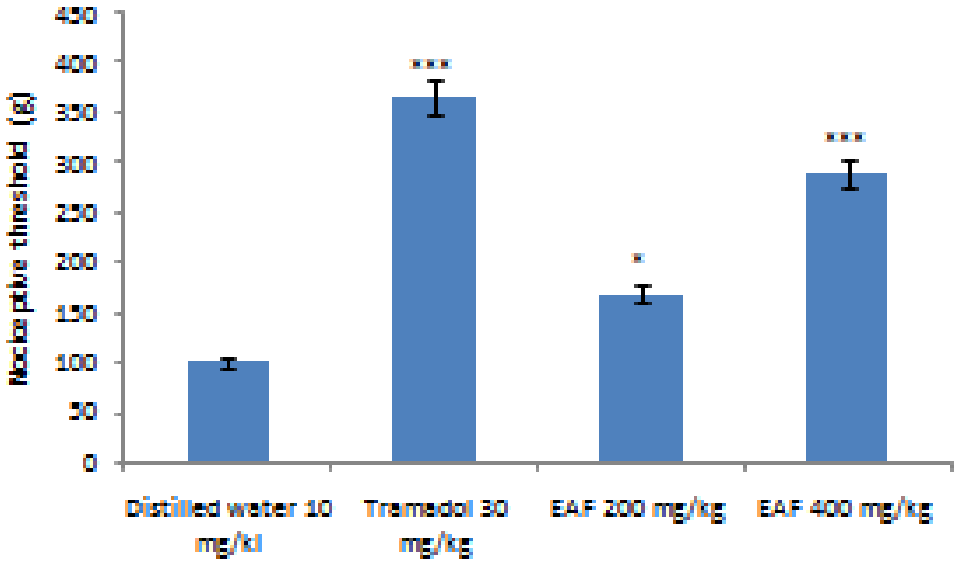

Figure 11a: Effect of *Cassia siamea* leaves extract on pain induced by mechanic paw pressure expressed on nociceptive threshold. Each value represents the mean± S.E.M of five animals per group***p < 0.001 and *p < 0.5 compared with the control group.

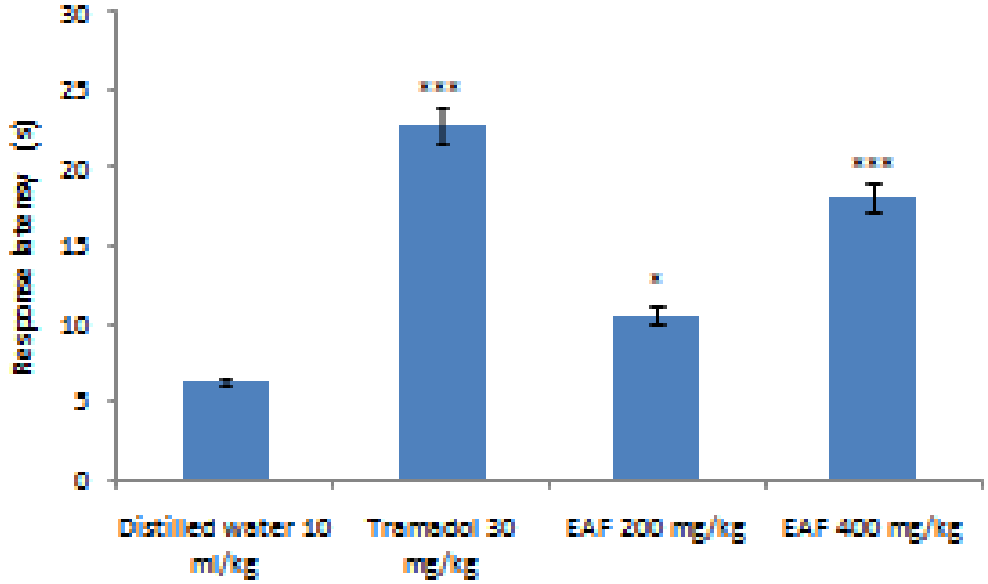

Figure 11b: Effect of *Cassia siamea* leaves extract on pain induced by mechanic paw pressure expressed by response latensy. Each value represents the mean± S.E.M of five animals per group***p < 0.001 and *p < 0.5 compared with the control group.

Nsonde Ntandou G.F." Analgesic, Antiinflammatory and Analgesic Effects of *Cassia Siamea* (Fabaceae) Leaves Aqueous Extract." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.1 (2018): 06-15.