

# Anti-Inflammatory and Analgesic Effects of Aqueous Extract of Stem Bark of *Ceiba pentandra* Gaertn

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Received 27 September 2014; revised 26 October 2014; accepted 15 November 2014

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## Abstract

Anti-inflammatory and analgesic effects of the aqueous extract of the stem bark of *Ceiba pentandra* Gaertn (Bombacaceae) were recorded in rat and mice. Inflammation was induced by carrageenan and cotton pellet. The pain was studied using analgesymeter, Koster and hot plate Methods. Aqueous extract (400 and 800 mg/kg) of *Ceiba pentandra* presents a significant anti-inflammatory and analgesic activity. Flavonoids present in the extract seem to be responsible for the activity.

## Keywords

Analgesic, Anti-Inflammatory, *Ceiba pentandra*

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## 1. Introduction

*Ceiba pentandra* (Bombacaceae) is a large tree reaching a height of 50 m and 2 m of diameter [1]. The plant is widely used in traditional medicine in the world [2]. In the African pharmacopeia, the plant is known for its antiseptic, anti-inflammatory, antispasmodic and diuretic properties [3]. In Republic of Congo, *Ceiba pentandra* Gaertn (Bombacaceae) is used in traditional medicine to treat stomach aches, asthma, rickets and diarrhoea [4]-[8]

however no scientific data on its anti-inflammatory and analgesic effects is available in Congo-Brazzaville. That's why in this study, we aimed investigated anti-inflammatory and analgesic activities of *Ceiba pentandra* stem bark collected in Brazzaville, capital of the Republic of Congo.

## 2. Materials and Methods

### 2.1. Materials

#### 2.1.1. Vegetal Materiel

The stem bark of *Ceiba pentandra* was collected in Brazzaville (Lycée Pierre Savorgnan de Brazza) in June 2008. Botanical identification of the plant material was done by Dr. A. Mousamboté, botanist systematist at the Institute of Rural Development. A voucher specimen was deposited at the Herbarium of Center of Studies on the Vegetable Resources (C.E.R.V.E.) of Brazzaville and registered under the number 2529 of 20/06/1968.

#### 2.1.2. Animals

Albinos rats weighing between 200 - 250 g of Faculty of Health Sciences of Marien Ngouabi University, and male and female albinos mice weighing 20 - 35 g provided by the National Center for Support and Fight against Diseases (CNAM) of Bamako in Mali were used. Animals were acclimated during one week before the experiments. They were fed and maintained under standard lighting conditions (12 hours light and 12 hours dark) at a temperature of  $24^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . They were fasted for 24 hours before experiments, while water was given *ad libitum*.

### 2.2. Methods

#### 2.2.1. Aqueous Preparation

600 g of pulverized powder of *Ceiba pentandra* were mixed with 6 liters (L) of distilled water, and the mixture was boiled for 15 min. After cooling and filtration, the filtrate was concentrated under vacuum in a rotary evaporator Büchi R-210 at a temperature of  $55^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . The thick solution was then freeze-dried using a system Heto Drywinner Edwards Pump. The dried extract obtained was kept for experiments.

#### 2.2.2. Anti-Inflammatory Activities

Anti-inflammatory effect of aqueous extract of *Ceiba pentandra* was evaluated using two standard methods: the carrageenan induced paw oedema [9] and cotton pellet induced granuloma (chronic inflammation) models [10]. For each method, four groups of 5 rats were used. Animals were treated as follow: group control was treated with distilled water at the dose of 0.5 ml/100g; group 2 was treated with Diclofenac (Voltaren\*), used as the reference drug, at a dose of 5 mg/kg; group 3 and group 4 were treated with aqueous extract of *Ceiba pentandra* at the dose of 400 and 800 mg/kg.

#### 2.2.3. Acute Inflammation Induced by Carrageenan

Acute inflammation was induced by carrageenan [9]. One (1) hour after oral administration of products (distilled water, Diclofenac and aqueous extract), animals received 0.05 ml of 1% carrageenan by sub-plantar administration at the right hind paw. The volumes  $V_0$  and  $V_t$  of paw oedema were measured using an Ugo Basile 7140 pletysmometer, Italy. The anti-inflammatory effect was evaluated by determining the percentage of inflammation inhibition (PI%), using the following formula:

$$V = V_t - V_0$$

$$\%PI = \frac{(V_t - V_0)_t - (V_t - V_0)_{tr}}{(V_t - V_0)_t} \times 100$$

With:  $V$  = volume of œdema;  $V_t$  = volume of the paw oedema at a time  $t$ ;  $V_0$  = volume of the paw before induction of inflammation;  $(V_t - V_0)_t$  = volume of œdema of the control group of rats;  $(V_0 - V_t)_{tr}$  = volume of œdema in the group of treated rats.

#### 2.2.4. Chronic Inflammation Induced by Cotton Pellets

The effect of aqueous extract of dried and powdered bark of *Ceiba pentandra* was evaluated on the cotton pellet induced granuloma model [10]. 100 mg of cotton pellets were sterilized at  $60^{\circ}\text{C}$  during 24 h and placed in the right

interscapular region of animal after ether anesthesia and incision. The incision was closed by suture. Animals were then treated with drugs for seven (7) days. On the eighth day the granulation tissue with cotton pellet was removed, cleared of adhering tissue and placed at 60°C for 24 h and weighed. The anti-inflammatory effect was given by the percentage inhibition (PI) of the granuloma:

$$PG = B - A$$

$$PI = \frac{PG}{A} \times 100$$

PG: weight of the granuloma, A = weight of cotton pellet before implantation (100 mg); B = weight of dried cotton pellet after implantation.

### 3. Analgesic Effect

In this study, groups of 5 rats (male and female) and groups of 6 mice (male and female) were used and treated as follows: the control group treated with distilled water at a dose of 0.5 ml/100g; two groups treated with the aqueous extract of *Ceiba pentandra* at the doses of 400 and 800 mg/kg, one group treated with the standard drug which was either paracetamol (para) 100 mg/kg for analgesymeter (Cat. No. 37215 Ugo Basile, Italy) induced pain; or Koster (1959) method either, morphine (2 mg/kg) for experiments using heating plates.

#### 3.1. Analgesymeter Induced Pain Experiment

The pain is induced by the pressure exerted on the hind paw of animals by the analgesymeter [11]-[14]. One (1) hour after oral administration of the tested products, nociceptive thresholds were determined using the analgesymeter. A constantly increasing pressure was applied on the right hand paw until the rats withdrew the paw. The sensitivity threshold to pain was determined by the following formula:  $V = F/T$ .

With: V = Speed of sliding of the needle (16 g/sec), F = Force causing the withdrawal of the paw by the rat, T = threshold of pain sensitivity.

#### 3.2. Acetic Acid-Induced Pain

The pain was caused in mice by intraperitoneal injection of 0.6% acetic acid solution [12]. One hour after oral administration of products, 10 ml/kg of acetic acid was injected intraperitoneally to mice. After injection of acetic acid solution, and a latency period of 5 minutes, the number of writhing made by each mouse was determined within the next 20 minutes [5].

#### 3.3. Hot Plate Test

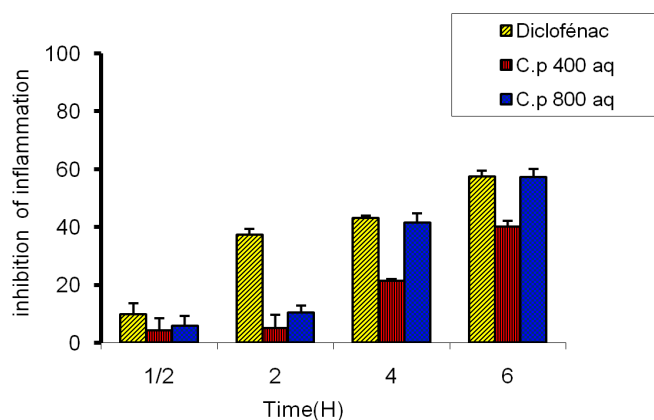
The pain was induced by the hot plate as described by Sabih *et al.* [3]. One (1) hour after oral administration of test products, animals were placed on the hot plate (56°C) and the reaction time (licking of the paws, jumping or screaming) was recorded. The maximum time was set at 30 seconds.

#### 3.4. Statistical Analysis

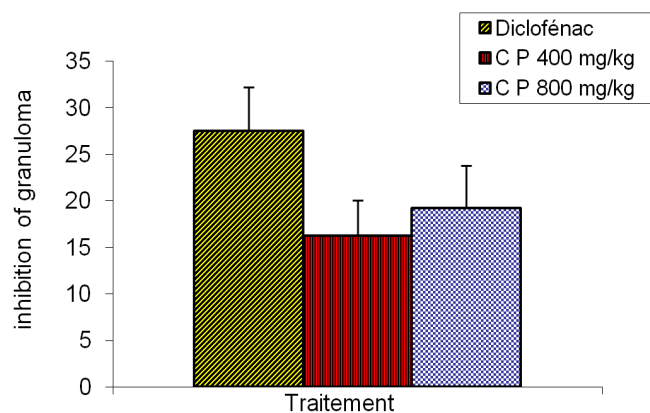
The results were expressed as mean  $\pm$  ESM. Analysis of variance followed by Student-Fischer *t* test “*t*” were performed. The significance level was set at  $p < 5\%$ .

## 4. Results and Discussion

Result of anti-inflammatory effects of *Ceiba pentandra* are shown in **Figure 1** and **Figure 2**. The aqueous extract (400 and 800 mg/kg) of *Ceiba pentandra* inhibits acute inflammation induced by carrageenan (**Figure 1**). This inhibition is much pronounced two hours after plantar injection of carrageenan with maximum values observed 5 hours after administration (**Figure 1**). However these effects are less important than those observed with diclofenac, a non steroidal anti-inflammatory used in our study as reference drug. The aqueous extract (400 and 800 mg/kg) inhibited significantly the formation of cotton pellet granuloma (**Figure 2**) with inhibition percentages of 16.23 and 19.22% at 400 and 800 mg/kg respectively. Indeed, carrageenan is a mucosaccharide whose administration in the intraplantar way to rats causes acute inflammation that induce edema, all under the influence of



**Figure 1.** Effect of aqueous extract of *Ceiba pentandra* on inflammation induced by 1% carrageenan in rats. Each value represents the mean  $\pm$  ESM, versus control group with N = 5 rats.



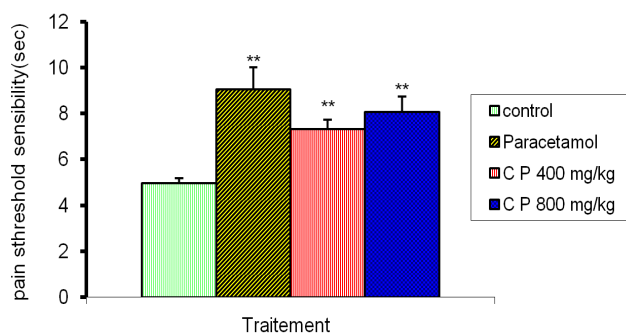
**Figure 2.** Effect of aqueous extract of *Ceiba pentandra* on the granulomatous tissue formation in rats. Each value represents the mean  $\pm$  ESM; versus control group with N = 5 rats.

vasoactive mediators [13]. The aqueous extract of *Ceiba pentandra* (400 and 800 mg/kg) inhibits the progression of edema to varying degrees. This suggests that it interferes with the effects which inhibit the release of mediators involved in these phases of inflammation.

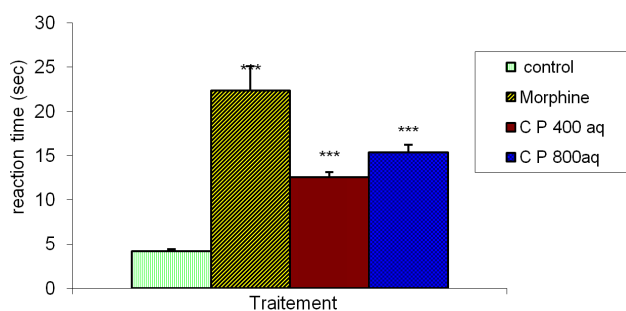
The cotton pellet granuloma is a model of chronic inflammation. The results obtained show that the aqueous extract inhibits the formation of granuloma. This lets suggest that aqueous extract inhibit the synthesis or effects of pro-inflammatory substances. Our results are in agreement with Lin *et al.*, (1992). Indeed, these authors, showed that this plant inhibited the edema induced by the carragenine under plantar in rat.

The results of the analgesic effects of aqueous extracts are presented in **Figures 3-5**. The aqueous extract (400 and 800 mg/kg po) showed significant analgesic activity. This activity results in an increasing threshold of pain sensitivity (**Figure 3**), reaction time on the hot plate (**Figure 4**) and finally reduced number of abdominal writhing induced by 0.6% acetic acid (**Figure 5**). Indeed, tissue trauma induced by mechanical stimulation causes local swelling of the traumatized tissue responsible for the release of bradykinin, serotonin, potassium ions and hydrogen [6]. The aqueous extract (400 and 800 mg/kg) protects against the pain induced by the mechanical stimulation. This suggests that it interferes or inhibits the release of these mediators. These two methods are not really specific. So, we additionally performed the hot plate test using morphine as a reference central analgesic. The fact that the aqueous extract inhibits this type of pain, suggests that it may act as a central analgesic.

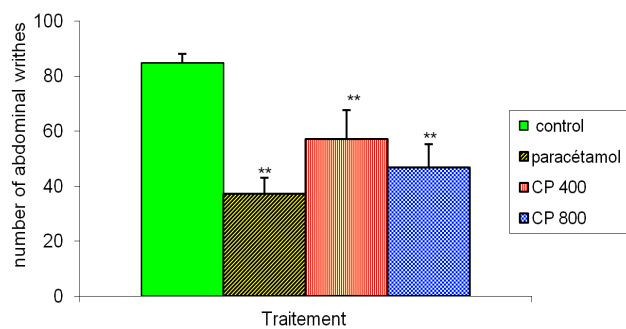
The phytochemical analysis of the aqueous extract of *Ceiba pentandra* previously performed [7] has revealed the presence of flavonoids, tannins, triterpene steroids, and quinones. The anti-inflammatory and analgesic properties of this extract observed in our study can be explained by the presence of flavonoids [9].



**Figure 3.** Effect of aqueous extra (aq) of *Ceiba pentandra* (CP) on the sensitivity threshold of the pain induced by analgesy-meter, with \*\* $p < 0.01$  (Student t-test). Each value represents the mean  $\pm$  ESM; versus control group with N = 5 rats.



**Figure 4.** Effect of aqueous extracts (aq) of *Ceiba pentandra* on the reaction time on the hot plate, with \*\*\* $p < 0.001$  (Student t-test). Each value represents the mean  $\pm$  ESM; versus control Grou; with N = 5 rats.



**Figure 5.** Analgesic effect of aqueous extract of *Ceiba pentandra* (Cp) against the pain induced by the 0.6% acetic acid solution in mice, with \*\* $p < 0.01$  (Student t-test) Each value represents the mean  $\pm$  ESM versus control group.

## 5. Conclusion

This study was initiated to evaluate the anti-inflammatory and analgesic effects of aqueous extract of stem barks of *Ceiba pentandra*. Indeed, the aqueous extract (400 and 800 mg/kg) on one hand, inhibits according to the time period, the development of edema induced by carrageenan and the formation of granulation tissue in rats, and on the other hand, the pain caused by analgesimeter and the hot plate in rats. It also protects mice against the abdominal writhes caused by 0.6% acetic acid. These observed effects could be explained by the presence of flavorous in this extract. These results may explain the traditional use of this plant in the treatment of inflammation and pain. However, its mechanisms of action remain to be clarified.

## Acknowledgements

Our sincere thanks to: the Traditional Medicine Department (MTD) of the National Institute of Public Health Research (INRSP) in Bamako, Mali, for achieving certain tests; the Agence Universitaire de la Francophonie (AUF) for mobility research grant.

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