

4 **Anti-inflammatory Effect of *Entandrophragma angolense***
5 **Bark Extracts on Acute Edema of the Rat's Paw Induced**
6 **by Carrageenan**

7
8 **ABSTRACT**

9 **Aims:** Our work was to evaluate the anti-inflammatory activity of the bark of
10 *Entandrophragma angolense* in the laboratory.

11 **Methods:** The experiments were carried out on the model of acute edema of the rat's paw
12 induced by carrageenan and the determination of C-reactive protein (CRP). The aqueous and
13 ethanolic extracts, at doses of 100 and 200 mg/kg body weight were administered orally to
14 the rats. The extracts were administered an hour before induction of acute inflammation with
15 1% carrageenan. The results obtained were compared to those of Diclofenac Sodium and
16 those of physiological control.

17 **Results:** After administration of the distilled water, the carrageenan induced edema which
18 increased gradually with a maximum of 38.41% after five hours of observation. The
19 administration of Diclofenac Sodium at dose of 10 mg/kg b.wt as well as aqueous and
20 ethanolic extracts of *E. angolense* significantly prevented the increase in the diameter of the
21 rat's paw. From the 1st to the 4th hour, the aqueous extract at dose of 200 mg/kg b.wt
22 respectively shows a percentage of inhibition similar to that of Diclofenac Sodium. Then, the
23 administration of the extracts and Diclofenac Sodium decreased the concentration of CRP
24 induced by the injection of carrageenan compared to the control.

25 **Conclusion:** *E. angolense* has a mechanism of anti-inflammatory action similar to that of
26 non-steroidal anti-inflammatory drugs which could be attributed to its phytochemical
27 constituents.

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29 **Keywords :** *Entandrophragma angolense* ; inflammation ; CRP ; anti-inflammatory
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31 **1. INTRODUCTION**

32 Inflammation is a complex reactional process of the vasculo-conjunctive system of the body
33 in response to an aggression that may be physical, biological or infectious origin, in order to
34 maintain its homeostasis and integrity [1]. It is usually a beneficial process, but it can be
35 harmful because of the aggressiveness of the pathogen, its persistence, the site of
36 inflammation due to abnormality of the regulations of inflammatory process or by
37 quantitative or qualitative abnormality of the cells Involved in inflammation [2].

Inflammation, when it is visible, manifests classically itself by four clinical signs: redness, pain, swelling and an increase in heat. These clinical manifestations are treated by the anti-inflammatories which are drugs permitting to suspend or slow down the inflammation process. A lot of non-steroidal anti-inflammatory drugs (NSAIDs) are prescribed because of their effectiveness. However, their therapeutic use in the long term is often associated with adverse effects such as gastro-intestinal ulcers and renal insufficiency [3]. Moreover, plants are sources, rich in drugs because they produce many bioactive molecules, most of which probably play the role of chemical defense against predators or infectious agents [4]. These plants could constitute an alternative to the anti-inflammatory therapeutics because of their better accessibility and their less toxicity in general. *Entandrophragma angolense*, subject of our work, is used in the treatment of malaria, peptic ulcers, rheumatic or arthritic pains... [5-6]. The stem bark, in decoction, is used for the treatment of fever or malaria in Cameroon and Ivory Coast. In Congo, it is used as analgesic and anti-inflammatory [7]. The aim of this study was to investigate the anti-inflammatory activity of the aqueous and ethanolic extracts of the bark of *E. angolense* on acute inflammatory edema of the rat paw and the serum concentration of CRP induced by carrageenan.

2. Materials and Methods

2.1 Plant Material

The stem bark of *E. angolense* was collected in the area of Abidjan (southern Ivory Coast) in March 2014. It was identified at the National Floristic Centre of University Felix Houphouët-Boigny where a herbarium specimen of the plant was deposited. The bark was cut out then dried in the shade, at the room temperature for two weeks. Then, it was pulverized using an electric crusher in order to obtain a powder.

2.2 Experimental Animals

Female and male Rats of Wistar strain of body weight ranging between 110-170g were used for this study. The animals were housed in plastical cages and acclimatized for two weeks in the animal house of the Higher Teacher Training School. They had been maintained under standard conditions (room temperature $25^{\circ}\text{C} \pm 3^{\circ}\text{C}$, humidity 35 to 60%, light and dark period 12/12 hours). All animals had regular supply of clean drinking water and food.

2.3 Preparation of Extracts by Decoction or Maceration

One hundred grams (100g) of plant powder was boiled for 20 minutes in 2 liters of distilled water. The cooled juice was filtered thrice off through a cotton plug and once with a Whatman No.3 filter paper. The filtrate obtained was concentrated under reduced pressure at temperature below 50°C through a Büchi rotary evaporator and gave the dry aqueous extract of *E. angolense*. As for the maceration, one hundred grams (100g) of plant bark powder and 1 liters of 70% ethanol were introduced into an Erlenmeyer and subjected to magnetic stirring for 24 hours at room temperature. The mixture was then filtered thrice on cotton and one on Whatman N.3 filter paper and was dried at temperature below 50°C through a Büchi rotary evaporator. The powder obtained is called ethanolic extract [8].

2.4 Inhibition Test of Rat Paw Edema to Carrageenan

82 Rats were fasted for 16 hours before the experiment with free access to water. At the time of
 83 experiment, they were weighed and divided into six groups of six animals each. For each rat,
 84 the diameter (D0) at time T0 of the right hind paw was determined using the caliper rule.
 85 Then, the distilled water (10 ml/kg) was orally administrated to rats of group 1, those of
 86 group 2, Diclofenac Sodium solution at the dose of 10 mg/kg b.wt, groups 3 and 4
 87 respectively 100 and 200 mg/kg b.wt of the aqueous extract of *E. angolense* and groups 5 and
 88 6 received respectively ethanolic extract at dose of 100 and 200 mg/kg b.wt. One hour after
 89 drug treatment, 0.1 mL of 1% (w/v) carrageenan solution was injected subcutaneously into
 90 the subplantar aponeurosis of right hind paw of each rat. The evolution of right hind paw
 91 edema was measured at 1 h, 2 h, 3 h, 4 h, 5 h, 6 h and 24 h [9]. The anti-inflammatory
 92 activity expressed as inhibition percentage (% Inh) of the edema was calculated for each
 93 group of rats treated compared with control group.

$$95 \quad \%Inh = \frac{Pc - Pt}{Pc} \times 100$$

96 Pc = percentage of increase of the paw of control group;

97 Pt = percentage of increase of the paw of treated group.

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 99 The percentage of increase (% Inc) of the rat paw was given by the formula:

$$100 \quad \%Inc = \frac{Dt - Do}{Do} \times 100$$

102 Do = Paw diameter in mm, before carrageenan administration;

103 Dt = Paw diameter in mm, at time t, after carrageenan administration.

105 2.5 Determination of CRP

106 Before the determination of CRP, the rats were put in 16 hours water diet. They were
 107 weighed and divided into five groups of five rats. The various drugs were administrated
 108 orally in the following way:

109 -groups 1(normal) and 2 (control), distilled water (10 ml/kg);

110 -group 3, Diclofenac Sodium solution (10 mg/kg b.wt);

111 -group 4, aqueous extract of *E angolense* (200 mg/kg b.wt);

112 -group 5, ethanolic extract of *E angolense* (200 mg/kg b.wt).

113 One hour after administration, the rats of groups 2, 3, 4 and 5 received 0.1 mL of 1% (w/v)
 114 carrageenan solution subcutaneously into the subplantar aponeurosis of right hind paw. Five
 115 hours after injection, blood was collected in the rats by incision of the tail. The determination
 116 of CRP was made from serum of the animals by the technique of immunoturbidimetry on
 117 Hitachi (Cobas C311). The principle is based on the photometric measurement of the disorder
 118 brought by the antigen-antibody reaction in the final point method at 340 nm [10].

120 2.6 Statistical Analysis

121 The results were expressed as mean followed by the standards errors of mean (mean ± SEM).

122 The graphic representation of data was carried out from the software Graph Pad PRISM 5.0

(Microsoft U.S.A). Statistical analysis of results was performed using the analysis of variances (ANOVA ONE WAY). The differences between the means were determined according to Dunnett's comparison test. $P < 0.05$ is considered as significant.

3. RESULTS

3.1 Effect of Aqueous and Ethanolic Extracts of *E. angolense* on the Edema Induced by Carrageenan

Edema was created in the right hind paw of rat. The variation of edema was appreciated after administration of the aqueous and ethanolic extracts of *E. angolense* at dose of 100 and 200 mg/kg b.wt orally. The results obtained were compared with those of Diclofenac Sodium[®], a nonsteroidal anti-inflammatory and control (distilled water). After administration of distilled water, carrageenan induced edema which increased gradually with a maximum of 38.41% after five hours of observation (Table 1). Administration of Diclofenac Sodium at dose of 10 mg/kg b.wt significantly prevents the increase in the diameter of rat paw. It varies from 7.38% to 15.82% after administration of carrageenan (Fig. 1). The results are significantly different from those of control (distilled water) ($P < 0.05$). Administration of the aqueous extract of *E. angolense* at dose of 100 and 200 mg/kg b.wt significantly ($P < 0.05$) prevents acute edema of the rat paw during 24 hours. The effect of ethanolic extract of *E. angolense* is less pronounced compared to that of the aqueous extract in the prevention of acute edema of the rat paw. From the first hour to the fourth hour, the aqueous extract at dose of 200 mg/kg b.wt respectively shows inhibition percentages similar to those of Diclofenac Sodium[®] at dose of 10 mg/kg b.wt (Table 2).

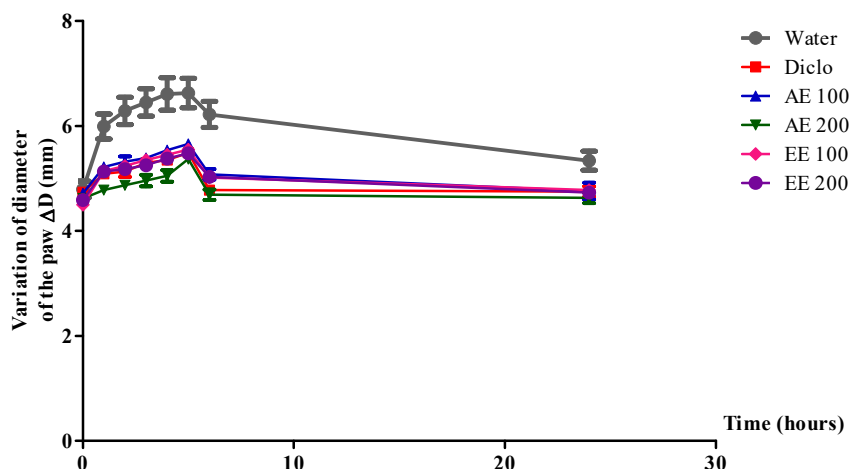


Figure 1: Variation of the rat paw diameter of the treated groups compared with

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control according to time

Expressed results in mean $\pm$ S.E.M (n = 6). $p < 0.05$ statistically significant compared to the control (DW), DW: Distilled Water (control); DICLO: Diclofenac; AE: Aqueous Extract at dose of 100 and 200 mg/kg b.wt; EE: Ethanolic Extract at dose of 100 and 200 mg/kg b.wt.

Table 1: Percentage of increase in edema of rats treated with diclofenac and extracts of *E. angolense* bark

Drugs	Dose	% of increase						
		T _{1h}	T _{2h}	T _{3h}	T _{4h}	T _{5h}	T _{6h}	T _{24h}
DW	10 ml/kg	25.05	31.31	34.65	37.99	38.41	29.85	11.48
DICLO	10	7.38	8.22	11.81	12.86	15.82	0.84	0.21
AE	100	10.59	12.71	14.19	17.37	19.91	7.62	0.84
AE	200	3.46	5.41	7.35	9.30	16.23	1.51	0.21
EE	100	14.22	16.44	18.88	21.11	23.33	11.55	6.22
EE	200	11.76	12.85	14.37	17.21	19.38	9.58	3.05

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DW: Distilled Water (control); DICLO: Diclofenac; AE: Aqueous Extract at dose of 100 and 200 mg/kg b.wt; EE: Ethanolic Extract at dose of 100 and 200 mg/kg b.wt.

Table 2: Inhibition percentage of edema of rats treated with diclofenac and extracts of *E. angolense* bark

Drugs	Dose	Inhibition percentage						
		mg/kg	T _{1h}	T _{2h}	T _{3h}	T _{4h}	T _{5h}	T _{24h}
Control		-	-	-	-	-	-	-
DICLO	10	70.53	73.74	65.91	66.14	58.81	97.18	98.17
AE	100	57.72	59.40	59.04	54.27	48.16	74.47	92.68
AE	200	86.18	82.72	78.78	75.51	57.74	94.94	98.17
EE	100	43.23	47.49	45.51	44.43	39.26	61.30	45.81
EE	200	53.05	58.95	58.52	54.69	49.54	67.90	73.43

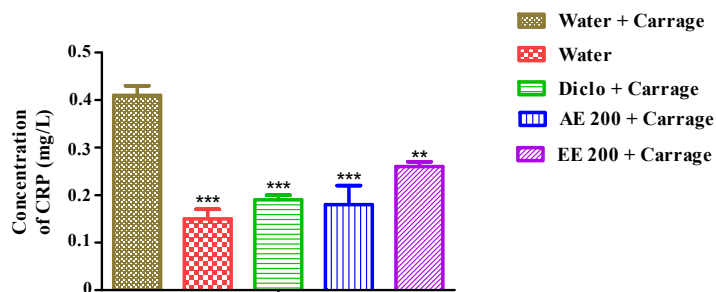
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Expressed results in mean $\pm$ S.E.M (n = 6). $P < 0.05$ statistically significant compared to the control (DW), DW: Distilled Water (control); DICLO: Diclofenac; AE: Aqueous Extract at dose of 100 and 200 mg/kg b.wt; EE: Ethanolic Extract at dose of 100 and 200 mg/kg b.wt.

3.2 Effect of Aqueous and Ethanolic Extracts of *E.angolense* on the Serum Concentration of CRP after Induction of Edema by Carrageenan

The CRP value of DW group + carrageenan group is significantly high ($p < 0.05$) compared to the treated groups. On the other hand, the administration of extracts and Diclofenac Sodium decreased the concentration of CRP induced by the injection of carrageenan compared to the control (Fig. 2).

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181 **Figure 2: Effect of *E.angolense* extracts on serum CRP concentration 5 hours after**
182 **injection of the carrageenan**
183 Values in Mean $\pm$ S.E.M ($n = 5$); ** $P < 0.01$ and *** $p < 0.001$ statistically significant compared
184 to the Distilled water + carrageenan group.
185 Distilled water + Carrageenan; Diclofenac + Carrageenan; Aqueous extract at 200 mg/kg b.wt +
186 Carrageenan; Ethanolic extract at 200 mg/kg b.wt + Carrageenan
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188 4. DISCUSSION

190 The anti-inflammatory activity was evaluated according to the carrageenan-induced rat paw
191 edema test [11] and the CRP determination method. Carrageenan (a phlogistic agent) induces
192 edema in the rat paw considered as a characteristic sign of inflammation [12]. This technique
193 has been used because of its simplicity of execution, its rapid induction of symptoms
194 characteristic of inflammation (development of edema in the hour which follows the
195 injection, with a maximum effect at the end of 5 hours) and also because of its
196 reproducibility. In the evaluation of anti-inflammatory activity, one of the non-steroidal anti-
197 inflammatory drugs is used for comparison of experimental and clinical parameters.
198 Diclofenac was chosen for comparison in this study because it is most commonly used
199 clinically [13]. Phytochemical analysis carried out on bark of *E. angolense* revealed the
200 presence of alkaloids, polyphenols, sterols, terpenes, tannins, flavonoids, leucoanthocyanins,
201 quinones, saponins and cardiotonic glycosides [14]. During this kinetic study, our extracts
202 gave an inhibition of the edema compared to the control. Already at first hour the aqueous
203 extract of *E.angolense* bark at dose 200 mg/kg b.wt gave the same effect as diclofenac
204 sodium at dose of 10 mg/kg b.wt with respectively 86.18% and 70.53 % inhibition of edema.
205 This gives the plant a mechanism of anti-inflammatory action similar to that of nonsteroidal
206 anti-inflammatory drugs and could be explained by the presence in the plant of tannins,
207 polyphenols and flavonoids. However, studies have showed that the anti-inflammatory effect
208 of flavonoids would be due to an inhibition of prostaglandin synthesis and polyphenols could
209 act on the enzymatic activities of arachidonic acid metabolism [15]. Tanins are chemical
210 substances known for their ability to bind to proteins with a tendency to impermeability of
211 the external layers and the protection of the underlying layers [16]. The extracts also possess
212 sterols and terpenes, compounds responsible for various activities in plants and have

beneficial effects in humans and animals. Thus, the effect of sterols could be due to an inhibition of pro-inflammatory cytokines and cyclooxygenase [17-18]. Terpenes are natural compounds which contribute to aroma of plants. Its anti-inflammatory properties would be due to the inhibition of inflammatory cell migration and a decrease in the release of pro-inflammatory cytokines [19]. C-reactive protein is a protein which reacts with pneumococcus polysaccharide C in the serum of patients with an acute inflammation [20]. Its physiological roles are the activation of classical complement pathway, mobilization and activation of leukocytes, stimulation of phagocytosis and cytokine secretion by monocytes [21]. Our results showed that administration of extracts and the reference molecule (Diclofenac Sodium) decreased the concentration of CRP induced by carrageenan injection compared to control (distilled water + carrageenan). The results confirm those obtained in the edema test. The various studies conducted on the protective effects of polyphenols in these pathological contexts have shown that these diminish the markers of inflammation.

5. CONCLUSION

The anti-inflammatory effect of the aqueous and ethanolic extracts of *E. angolense* was evaluated in this work. The results obtained show that the extracts have an anti-inflammatory activity. However, this activity is different according to the degree of solubility of secondary compounds in *E. angolense* extracts. During the carrageenin-induced rat paw edema test, the aqueous bark extract at dose of 200 mg/kg b.wt showed an anti-inflammatory activity more significant than the ethanolic extract. Also, the extracts decreased the CRP level induced by the injection of carrageenan.

Thus, these results constitute a scientific basis justifying the traditional use of *Entandrophragma angolense* in the management of pathologies with inflammatory component (malaria, ulcer, rheumatism ...).

ETHICAL APPROVAL

The experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences, University Felix Houphouët-Boigny. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals. All efforts were made to minimize pain of animals and reduce the number of animals used.

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