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Original Research Article

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

ABSTRACT

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

Methods: The experiments were carried out on the model of acute edema of the rat's paw induced by carrageenan and the determination of CRP. The aqueous and ethanolic extracts, at doses of 100 and 200 mg/kg were administered orally to the rats. The extracts were administered an hour before the induction of acute inflammation with 1% carrageenan. The results obtained were compared to those of Diclofenac Sodium and those of physiological control.

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

Conclusion: *E. angolense* has a mechanism of anti-inflammatory action similar to that of non-steroidal anti-inflammatory drugs and could be allotted to these rich phytochemical compounds.

Keywords : Entandrophragma angolense ; inflammation ; CRP ; anti-inflammatory

1. INTRODUCTION

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

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process. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely 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 a rich source of 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 in 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 and certain diseases [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 objective of this study was to determine 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 during 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°C ± 3°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 were boiled during 20 minutes in 2 liters of distilled water. The cooled decoction was filtered three times on cotton and once on filter paper Whatman N.3. The filtrate obtained was then dried in a hot air oven at 50°C to give 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 three times on cotton and on Whatman N.3 filter paper and was dried in a hot air oven at 50°C. The powder obtained is called ethanolic extract [8].

2.4 Inhibition Test of Rat Paw Edema to Carrageenan

The rats were fasted for 16 hours before the experiment with free access to water. At the time of experiment, they were weighed and divided into six groups of six rats. For each rat, the diameter at time T0 of the right hind paw (D0) was determined using the caliper rule. Then, the distilled water (10 ml/kg) was orally administrated to rats of group 1, those of group 2,

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Diclofenac Soduim solution at the dose of 10 mg/kg, groups 3 and 4 respectively 100 and 200 mg/kg of the aqueous extract of *E. angolense* and groups 5 and 6 respectively 100 and 200 mg/kg of the ethanolic extract of *E. angolense*. One hour after drug treatment, 0.1 mL of 1% (w/v) carrageenan solution was injected subcutaneously into the subplantar aponeurosis of the right hind paw of each rat. The evolution of right hind paw edema was measured at 1 h, 2 h, 3 h, 4 h, 5 h, 6 h and 24 h [9]. The anti-inflammatory activity expressed as inhibition percentage (% Inh) of the edema was calculated for each group of rats treated compared with control group.

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

Pc = percentage of increase of the paw of control group;
Pt = percentage of increase of the paw of treated group.

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

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

Do = Paw diameter in mm, before carrageenan administration;
Dt = Paw diameter in mm, at time t, after carrageenan administration.

2.5 Determination of CRP

Before the determination of CRP, the rats were put in 16 hours water diet. They were weighed and divided into five groups of five rats. The various drugs were administrated orally in the following way:
-groups 1(normal) and 2 (control), distilled water (10 ml/kg);
-group 3, Diclofenac Sodium solution (10 mg/kg);
-group 4, aqueous extract of *E angolense* (200 mg/kg);
-group 5, ethanolic extract of *E angolense* (200 mg/kg).
One hour after administration, the rats of groups 2, 3, 4 and 5 received 0.1 mL of 1% (w/v) carrageenan solution subcutaneously into the subplantar aponeurosis of the right hind paw. Five hours after injection, blood was collected in the rats by incision of the tail. The determination of CRP was made from serum of the animals by the technique of immunoturbidimetry on Hitachi (Cobas C311. The principle is based on the photometric measurement of the disorder brought by the antigen-antibody reaction in the final point method at 340 nm [10].

2.6 Statistical Analysis

The results were expressed in mean followed by the standards errors on mean (mean ± SEM). The graphic representation of data was carried out from the software Graph Pad PRISM 5.0 (Microsoft U.S.A). The statistical analysis of the results was carried out by using the analysis of variances (ANOVA ONE WAY). The differences between the averages were determined according to Dunnett's comparison test. $P < 0.05$ is considered as significant.

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3. RESULTS

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

Edema was created at the right hind paw of the 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 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 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 significantly ($P < 0.05$) prevents acute edema of the rat paw during 24 hours. The effect of the ethanolic extract of *E. angolense* is less pronounced than 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 respectively shows inhibition percentages similar to those of Diclofenac Sodium® at dose of 10 mg/kg (Table 2).

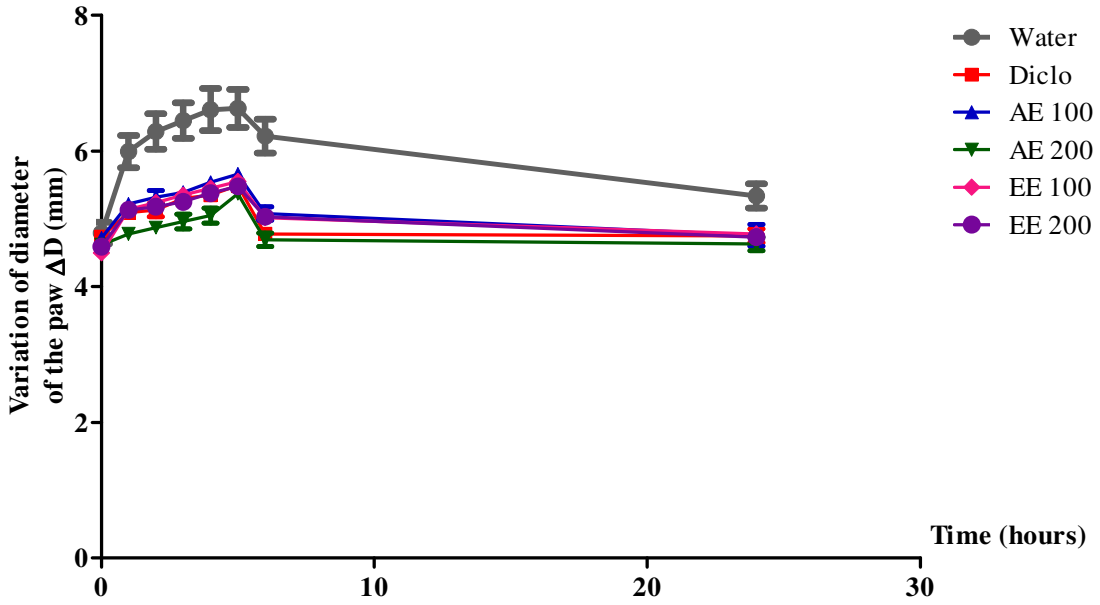

Figure 1: Variation of the rat paw diameter of the treated groups compared with control according to time

Expressed results in mean ± Standard deviation (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; EE: Ethanolic Extract at dose of 100 and 200 mg/kg.

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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 mg/kg	7.38	8.22	11.81	12.86	15.82	0.84	0.21
AE	100 mg/kg	10.59	12.71	14.19	17.37	19.91	7.62	0.84
AE	200 mg/kg	3.46	5.41	7.35	9.30	16.23	1.51	0.21
EE	100 mg/kg	14.22	16.44	18.88	21.11	23.33	11.55	6.22
EE	200 mg/kg	11.76	12.85	14.37	17.21	19.38	9.58	3.05

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

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_{6h}	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

Expressed results in mean ± Standard deviation (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; EE: Ethanolic Extract at dose of 100 and 200 mg/kg.

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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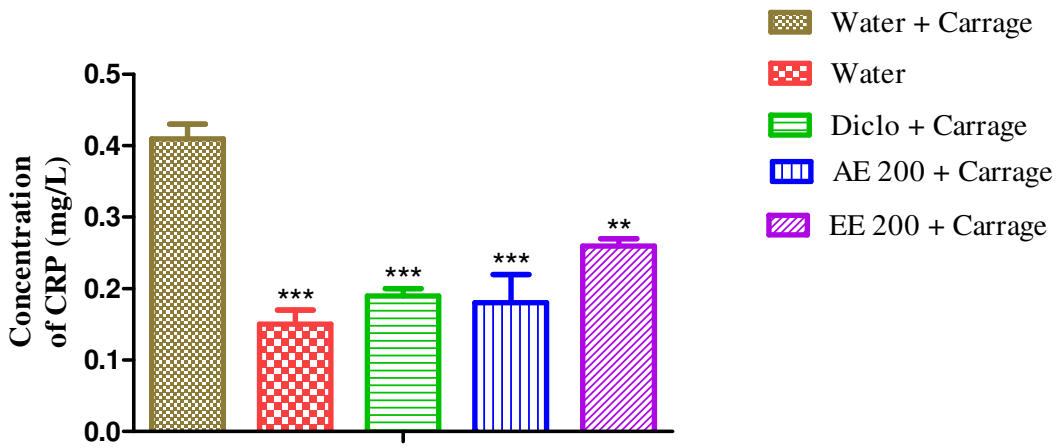

Figure 2: Effect of E.angolense extracts on serum CRP concentration 5 hours after injection of the carrageenan

*Values in Mean ± S.E.M (n = 5); ** P < 0.01 and *** p < 0.001 statistically significant compared to the Distilled water + carrageenan group.*

Distilled water + Carrageenan; Diclofenac + Carrageenan; Aqueous extract at 200 mg/kg + Carrageenan; Ethanolic extract at 200 mg/kg + Carrageenan

4. DISCUSSION

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

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would be due to 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 acute inflammation [20]. Its physiological roles are the activation of the 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 extract of the bark at dose of 200 mg/kg 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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