

Evaluation of Anti-inflammatory Activity of *Crinum scillifolium* Extracts in Wistar Rats

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Abstract Aim of study: Inflammation was associated with many diseases in humans. *Crinum* species have a considerable medicinal reputation as potent folkloric remedies. The main objective of the study was to evaluate the anti-inflammatory activity of aqueous and hydroethanolic extracts of *Crinum scillifolium* bulbs in *in vivo* models. Materials and methods: The anti-inflammatory effect of *Crinum scillifolium* extracts was also evaluated in carrageenan-induced paw edema models and C-reactive protein (CRP) levels was measured. Two doses 100 and 200 mg/kg body weight for each extract, were tested. The results obtained were compared with those of the standard drug (Diclofenac at 25 mg/kg body weight) and those of the control (normal saline). Results: The results showed a highly significant decrease in the edema size ($p < 0.01$) and significant decrease in CRP values ($p < 0.01$) compared to control group when the animals were treated with diclofenac at 25 mg/kg, and 200 mg/kg of aqueous and hydroethanolic extracts. Conclusion: The study suggests that the extracts possess enough potential to reduce inflammation on rat model and directs the importance of further research and development of novel anti-inflammatory agents.

Keywords *Crinum scillifolium*, Inflammation, CRP, Carrageenan

1. Introduction

Inflammation is a transitory nonspecific biological response of the microcirculation to tissue damage or pathogen infection. This process occurs as a defensive mechanism, which induces profound physiological adaptations in an attempt to limit tissue damage and

remove the pathogenic insult [1]. Usually, during acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. This mitigation process contributes to restoration of tissue homeostasis and resolution of the acute inflammation. However, uncontrolled acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases such as neurodegenerative and cardiovascular diseases [2, 3].

The only available medicine in modern practice are cyclooxygenase (COX) inhibitors i.e. NSAIDs. The use of these classical medicine for long term treatment, produces severe adverse effects such as renal damage, gastrointestinal disturbances, respiratory depression, and possible dependence [4]. Therefore, new anti-inflammatory drugs lacking those effects are being searched all over the world as alternatives. Medicinal plant have great value so that, the study of plants that have been traditionally used as anti-inflammatory killers should still be seen as a fruitful and logical research strategy in the search for new anti-inflammatory [5].

Crinum species have a considerable medicinal reputation as potent folkloric remedies. Their use extended from the ancient times to nowadays especially in Africa, tropical Asia and South America [6, 7]. Several *Crinum*s are commonly used in treatment of various painful and inflammatory disorders such as rheumatism, earache, lumbago, edema, headache, swelling, backache, wounds and haemorrhoids [7, 8, 9, 10], and pharmacological investigation of the effects of total extracts obtained from different parts of *Crinum*s using many algometric and inflammatory models showed their potential for treatment of various pains and inflammatory processes [11]. From the above context, the study was designed to evaluate the anti-inflammatory effect of *Crinum scillifolium* in rat model.

2. Materials and Methods

2.1. Extracts Preparation

2.1.1. Aqueous Extract

Fifty gram (50 g) of the bulbs powder was macerated in 500 ml of distilled water for 48 hours with stirring at 25° C. The liquid extract obtained after filtration through hydrophilic cotton followed by Whatman filter paper was evaporated to dryness in an oven at a temperature of 40 ° C. The extract was stored in refrigerator (4°C) until ready use. Various concentration were reconstituted in distilled water for biological tests.

2.1.2. Hydroethanolic Extract

Guédé-Guina (1990) method was used with slight modification: fifty gram (50g) of bulbs powder was macerated in 500 ml of hydroethanolic solution 90 % (ethanol/water 90:10) for 48 hours with stirring at 25° C [12]. The liquid extract obtained after filtration through Whatman filter paper was evaporated to dryness in an oven at a temperature of 40 ° C. The extract was stored in refrigerator (4°C) until ready use. Various concentration were reconstituted in distilled water for biological tests.

2.2. Animals

Wistar albinos rats (150 – 180 g) of either sex were used in this study. The animals were housed in appropriate cages at 24°C on a 12 h light/dark cycle with free access to food and water in the Laboratory Animal (UFR Pharmaceutical and Biological Sciences; University Félix Houphouët-Boigny). In all the experimental studies, each group consisted of six animals. Each animal was used only once. The investigation conforms to the recommendation of the Organization for Economic Co-operation and Development (OECD) in 2008 [13]. Before the experiment, the mice were divided into homogeneous lots by weight.

2.3. Test for Anti-inflammatory Activity

2.3.1. Carrageenan Induced Paw Edema in the Rat

Adult male and female Wistar rats were used for the study. An edema was induced on rat's right hind paw by subplantar injection of 0.2 ml of 1% carrageenan in 0.9% saline. The experimental groups consisted of 36 rats divided into six groups

- Group 1 served as the negative control and 10 ml/kg bw NaCl 0.9 % was orally administered;
- Groups 2 and 3 were administered the aqueous extract respectively at the dose of 100 and 200 mg/kg bw was orally administered;
- Groups 4 and 5 were administered the hydroethanolic extract respectively at the dose of 100 and 200 mg/kg bw was orally administered;

- Group 6 served as the positive control and Diclofenac at a dose of 25 mg/kg bw was intraperitoneally administered;

One hour after the pretreatment, 0.2 mL of Carrageenan (1%) was injected into the subplantar tissue of the right hind-paw of each rat. The diameter of each paw was measured using digital paw edema meter. The measures were carried out at 0 h (D_0 : before carrageenan injection) and 1, 2, 3, 4, 5, 6 and 24 h after induction of inflammation (D_T). The difference between D_T (1, 2, 3, 4, 5, 6 and 24 h) and D_0 was taken as the edema diameter value. The percentages of inhibition were calculated according to the following formula [14, 15]:

$$\% \text{ Inhibition} = \frac{[(D_T - D_0)_{\text{control}} - (D_T - D_0)_{\text{treated group}}]}{(D_T - D_0)_{\text{control}}} * 100$$

2.3.2 Quantitative Measurement of Rat C Reactive-Protein (CRP) in Serum

Thirty six new rats either sex were divided into six groups (n=6)

- Group 1 served as the negative control and 10 ml/kg bw NaCl 0.9 % was orally administered;
- Group 2 served as the normal control and 10 ml/kg bw NaCl 0.9 % was orally administered;
- Groups 3 was administered the aqueous extract at 100mg/kg bw was orally administered;
- Groups 4 was administered the hydroethanolic extract at 100 mg/kg bw was orally administered;
- Group 6 served as the positive control and Diclofenac at a dose of 25 mg/kg bw was intraperitoneally administered;

One hour after the pretreatment, 0.2 mL of Carrageenan (1%) was injected into the subplantar tissue of the right hind-paw of each rat without group 2. After 5 h carrageenan administration, all the animals were sacrificed and blood were collected and serum was separated. The dosage of the CRP was performed according to the method of Immunoturbidimetry improved particles in the Erba XL 180 (Mannheim) [16]. It is based on the principle of agglutination of latex particles coated with specific antibodies.

3. Results

3.1. Carrageenan-induced Paw Edema

The results obtained show that all rat injected with carrageenan developed a paw edema. The volume of paw edema varies with time. After one hour of carrageenan injection, the statistical study showed no significant difference between the control group paw diameter and those of the groups treated with aqueous and hydroethanolic extract of *Crinum scillifolium* (100 and 200 mg/kg body weight) and Diclofenac (25 mg/kg body

weight). For animal received saline solution, the edema increased to reach a peak characteristic of an inflammatory reaction at the 4rd and 5rd hour and then decreased at the 6th hour, without reaching the initial volume (normal state). The anti-inflammatory activity data indicated that all the test concentrations (100 and 200 mg/kg) were able to significantly reduce the carrageenan-induced edema at different time after the injection of the phlogistic agent, in comparison to control ($p < 0.001$) in a dose-dependent manner with increased activity after 2 hours. Hydroethanolic extract at 200 mg/kg reduced edema significantly ($p < 0.01$) compared with the edema of the control group at 2, 3, 4, 5, 6 and 24 hour (Table I). The hydroethanolic extract at 100 mg/kg showed significant inhibition of carrageenan-induced rat paw edema from 4 hours following inflammation induction, compared to the control group. Oral administration of aqueous extract at 100 and 200 mg/kg reduced edema significantly ($p < 0.01$) compared with the edema of the control group at 4, 5, 6 and 24 hour. Interestingly, the reduction of the edema by aqueous extract of *Crinum scillifolium* at the dose of 200 mg/kg was similar to the standard used (diclofenac) throughout the entire period of observation (Table I). Anti-inflammatory activity of *crinum scillifolium* extracts was expressed as a percentage of inhibition of inflammatory edema (Table II). During the first six hours of the experiment, the results showed that the Diclofenac at

25 mg/kg exhibited the highest anti-inflammatory activity, with percentage of inhibition ranging between 46.49 and 71.62 % followed by aqueous and hydroethanolic extracts at 200 mg/kg, with percentage inhibition ranging from 37.7 to 60.13 % and from 46.46 to 55.31 % respectively. While the aqueous and hydroethanolic extracts at 100 mg/kg allowed the lowest inhibitory power with percentages of inhibition ranging between 21 to 46. % and 11.11 to 32.18 % respectively. However, after 24 hours of the experimentation, aqueous extract at 200 mg/kg exhibited the highest anti-inflammatory activity than Diclofenac, with percentage of inhibition to 82.22% and 73.33 % respectively

3.2. Effect of *Crinum scillifolium* Extract on Serum Levels CRP at 5th Hour during Carrageenan induced Paw Edema in Rats

By results of biochemical researches it is established that the increase of the C reactive protein level is noted which is more expressed in control group (NaCl + carrageenan). Oral administration the plant extracts decreased a significant ($P < 0.001$) the concentration of CRP induced by the injection of carrageenan compared to the control. Intensity of this effect was comparable to that Diclofenac at 25 mg / kg bw (Figure 1)

Table I. Effect of Aqueous and hydroethanolic extracts of *Crinum scillifolium* bulbs and diclofenac on carrageenan rat paw edema.

Traitements (mg/kg)	Variations of paw edema (mm)						
	T 1h	T 2h	T 3h	T 4h	T5h	T 6h	T 24h
NaCl 10 ml/kg	0.99 ± 0.26	1.07 ± 0.17	1.41 ± 0.27	1.765 ± 0.56	1.56 ± 0.28	1.48 ± 0.43	1.35 ± 0.45
EA 100	0.78 ± 0.50	0.78 ± 0.35	0.92 ± 0.41	0.95 ± 0.41**	1.08 ± 0.42*	0.84 ± 0.31**	0.75 ± 0.33*
EA 200	0.62 ± 0.4	0.79 ± 0.48	0.9 ± 0.46	0.75 ± 0.25***	0.67 ± 0.22***	0.59 ± 0.16***	0.24 ± 0.09***
EHE 100	0.88 ± 0.4	1.18 ± 0.2	1.16 ± 0.22	1.197 ± 0.19*	1.26 ± 0.20	1.10 ± 0.17	0.87 ± 0.18
EHE 200	0.53 ± 0.4	0.54 ± 0.36*	0.63 ± 0.45**	0.80 ± 0.41***	0.80 ± 0.41***	0.90 ± 0.36*	0.55 ± 0.35***
Diclo 25	0.5 ± 0.17	0.62 ± 0.15	0.6 ± 0.19**	0.7 ± 0.20***	0.68 ± 0.09***	0.42 ± 0.06***	0.36 ± 0.08***

The results are expressed as means ± standard deviation at risk $\alpha = 5\%$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to control (NaCl) (ANOVA test followed by Dunnett test)

Table II. Inhibition (%) of inflammatory paw edema by *Crinum scillifolium* extracts and Diclofenac.

Traitements	% Inhibition of paw edema						
	T 1h	T 2h	T 3h	T 4h	T5h	T 6h	T 24h
EA 100 mg/kg	21	22.42	34.75	46.17	30.76	43.24	44.44
EA 200 mg/kg	37.37	26.16	36.17	57.50	57.05	60.13	82.22
EHE 100 mg/kg	11.11	11.11	17.73	32.18	19.23	25.67	35.55
EHE 200 mg/kg	46.46	49.53	55.31	54.67	48.71	39.18	59.25
Diclo 25 mg/kg	49.49	42.05	57.44	60.33	82.22	71.62	73.33

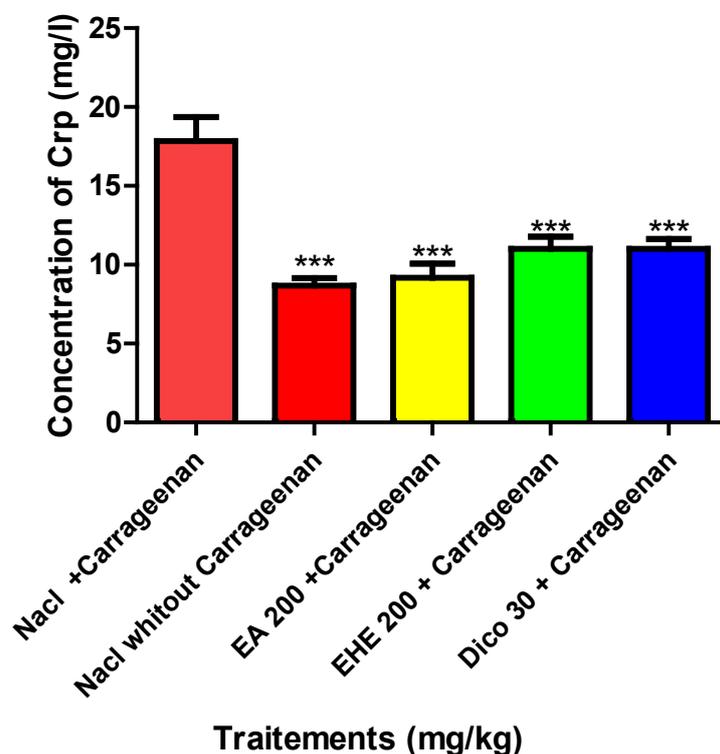


Figure 1. Effect of *Crinum scillifolium* extracts on serum CRP concentration 5 hours after injection of the carrageenan. The results are expressed as means \pm standard deviation at risk $\alpha = 5\%$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to control (NaCl) (ANOVA test followed by Dunnett test)

4. Discussion

The present investigation was aimed to study an anti-inflammatory activity of aqueous and hydroethanolic extracts of *Crinum scillifolium* in animal model. In order to evaluate this activity, the carrageenan paw model has been employed. This method has been widely employed as an animal model for screening anti-inflammatory drugs and most studies have used edema as the dependent measure [17, 18]. Two doses 100 and 200 mg/kg body weight for each extract, were tested. The results obtained were compared with those of the standard drug (Diclofenac at 25 mg/kg body weight) and those of the control (normal saline). It is well known that Carrageenan injection induces local inflammation caused by tissue injury, which results from the action of several chemical mediators, like prostaglandins, histamine, bradykinins, leukotriene and serotonin [19]. Indeed, the inflammatory response is a triphasic event. The initial phase (1–2 h) is attributed to the release of chemical mediators such as histamine and serotonin, which promotes vasodilation and plasma transudation. A second phase (2–3 h) involves kinins, principally bradykinin, as a mediator increasing vascular permeability and edema formation. The last phase (3–6 h) is due to the release of prostaglandins, arachidonate metabolites, neutrophil migration and release of oxygen free radicals [20, 21, 22]. The results showed that *Crinum scillifolium* extracts inhibited significant the formation of

the rat paw edema in the early and late phases (Hydroethanolic extract) and late phases (aqueous extract) and the standard anti-inflammatory drug Diclofenac significantly reduced the rat paw edema compared to the control group. The Diclofenac, used as a reference drug, is a nonsteroidal antiinflammatory drug (NSAID), which exert anti-inflammatory activity by the inhibition of cyclooxygenase (COX1 and COX2) and suppress production of chemical substances (prostaglandin, histamine and serotonin) which are involved to increase vasodilatation and vascular permeability [23, 24]. The decrease of the paw edema by the oral administration of *Crinum scillifolium* could be explained by the substances with antagonistic action to the histamine, the serotonin, the bradykinin and to the biosynthesis of prostaglandins. Anti-inflammatory activity of *Crinum scillifolium* extracts was evaluated too by estimation of levels CRP at 5 th hour during carrageenan induced paw edema in rats. Inflammatory reactions, infections, or tissue injury trigger acute phase proteins synthesis, such as CRP. It is synthesized mainly in the liver, but it may also be synthesized by local inflammatory cells in the area of tissue damage [25, 26]. The results found that its levels increased at 5h for group 1 (NaCl + carrageenan) and the extracts of *Crinum scillifolium* and diclofenac allowed to have a level of CRP getting closer to normal despite the injection of carrageenan. This indicates that *Crinum scillifolium* and diclofenac have potential inhibitory effects

on acute phase protein of inflammation. The genus *Crinum* have also been documented to present huge number alkaloids [27, 28]. While alkaloids are well known for their ability to inhibit the perception of pain and to be an anti-inflammatory [29]. The presence of these chemical compounds in the extract could be responsible for anti-inflammatory activity. Many studies have indicates that *Crinum* genus have been employed as anti-inflammatory agent. Pharmacological investigation of the effects of total extracts obtained from different parts of *Crinum* using many inflammatory models showed their potential for treatment of various inflammatory processes [30].

5. Conclusions

The present study confirms the anti-inflammatory effect of *Crinum scillifolium* and provides new evidences that a local carrageenan injection induces a systemic response, characterized by increased levels of acute phase proteins, such as CRP.

Ethical Approval

The experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences, University Félix 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 animal suffering and reduce the number of animals used.

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