

## INFLUENCE OF AQUEOUS EXTRACT OF *ANNONA SENEGALENSIS* LEAVES ON SOME PARAMETERS OF INFLAMMATION ON RAT SPECIALLY THOSE OF 'WISTAR' ORIGIN

Manouan Miézou Annick Marina\*, Yeo Dodéché, Thès Pehie Marie Epse Soumahoro,  
Djaman Allico Joseph

Laboratoire De Pharmacodynamie Biochimique, UFR Biosciences, Université de Cocody,  
22, BP 582 Abidjan 22, Côte-d'Ivoire.

Article Received on  
13 April 2018,  
Revised on 04 May 2018,  
Accepted on 25 May 2018  
DOI: 10.20959/wjpr201811-12014

### \*Corresponding Author

Manouan Miézou Annick  
Marina

Laboratoire De  
Pharmacodynamie  
Biochimique, UFR  
Biosciences, Université de  
Cocody, 22, BP 582 Abidjan  
22, Côte-d'Ivoire.

### ABSTRACT

The present study was carried out on medicinal plants had for target to establish the influence of the aqueous extract of *Annona senegalensis* leaves in various doses on the edema prevention and the quantity of protein C Réactive (CRP) during an inflammatory process. For this experiment, 20 rats of middle weight of 160 g were divided into 5 random group. One control group received no treatment, another reference batch received 1 ml/100g body weight of diclofenac and the other three lots received 250, 500 and 750 ml / mg / kg body weight respectively of Aqueous total extract (ETA) of *Annona senegalensis* leaves. The edema is caused 30 minutes later by the injection of carrageenan 1% in sub-plantar region right of paw of each rat. The diameter of the rats paw is measured before and after the injection of carrageenan up to 4 hours of experimentation. However, the ETA in

the dose of 750 ml/mg/kg PC had the best prevention of the edema with 55.02 percent of inhibition. The dosage of CRP in the serum of the rats of the various group revealed a lower quantity of CRP in the serum of rats having received the ETA in the dose of 750 ml / mg / kg PC. There was no significant difference between the effect of the diclofenac and the ETA in the dose of 750 ml / mg / kg PC on the inhibition of the edema and the decrease of the quantity of CRP in the serum. The anti-inflammatory activity of *Annona senegalensis* leaves demonstrated in this study is greater at the dose of 750 ml / mg / kg PC. This activity put the scientific foundation for the use of the leaves of this plant for the traditional treatment of

various inflammatory diseases. Nevertheless, studies must study the action mode of this plant and the purification of the active ingredients responsible for the effects of this plant.

**MOTS CLES:** *Annona senegalensis*, inflammation, carrageenan, edema, CRP.

## INTRODUCTION

The inflammation is a defense reaction of the body with diverse attacks. It is the usually beneficial process which can be fatal because of the aggressiveness of the pathogenic agent, its persistence, the place of inflammation, or by quantitative or qualitative anomaly of cells occurring in the inflammation (Agarwal *et al.*, 2001).

The current treatment for inflammation uses steroidal anti-inflammatory drugs and nonsteroidal anti-inflammatory drugs. Although these molecules are effective, they most often have undesirable effects that can hinder their long-term use. But, they remain the best-selling drugs (Gaziano *et al.*, 2006). The use of plants in therapy is very old and still plays a very important role in the therapeutic traditions and population life (Marc *et al.*, 2001). In Africa, *Annona senegalensis* is used in traditional African medicine to treat several pathologies including diarrhea, internal and external hemorrhages, dysentery and night blindness (Ouattara, 2004). Other authors have also highlighted antitumor and antibacterial activities (Kayodé *et Durodola*, 1976), anti-parasitic (Fall *et al.*, 2003), antivenomous (Adzu *et al.*, 2005) and anti-inflammatory agent (Yéo *et al.*, 2011) of *Annona senegalensis*.

The objective of this study was to verify the influence of the aqueous extract of the leaves of *Annona senegalensis* on the model of the inflammatory edema led by the carrageenan and to do the dosage of CRP in the serum of the rat of wistar origin.

## MATERIAL AND METHODS

**Plant material:** The plant material consists of leaves of *Annona senegalensis* collected in the region of Tiébissou in June, 2017. Forwarded in Abidjan, they were dried in the ambient temperature shielded from the sunlight during four weeks and reduced powder fine by crushing.

**Animal material:** The experiences were realized to rats of origin Wistar the weight of which is between 150 and 200 g. These rats were raised to the pet shop of the pharmaceutical laboratory with a free access to the water and to the food. Rats were distributed at random in 5 homogeneous group of 4 rats.

❖ **Preparation of the aqueous total extract (ETA):** The preparation of this extract was made according to the method described by Guédé-Guina which consists in soaking 100 g of plant powder in 2L of distilled water, soaked is homogenized him during 24 hours by means of a magnetic agitator. The homogenate is filtered successively 3 times, first on white fabric, then on hydrophilic cotton and finally on Whatman 3 mm filter paper. The filtrate obtained is evaporated using an oven of the Med Center Venticell type, at 50 ° C. For 03 days to give a black powder which constitutes the aqueous total extract (ETA).

❖ **Anti-inflammatory activity**

**Inflammatory edema:** The anti-inflammatory activity was evaluated by the carrageenan-induced rat paw edema method. Injection of carrageenan under in plantar region of the right back paw of the rat causes an inflammatory reaction which can be reduced by the anti-inflammatory substances. For each rat, the diameter (V<sub>0</sub>) of the right back paw was measured using an slidind caliper electronic prior to treatment administration. The experiment is as follows.

➤ **Lot 1 control:** The rats of this batch receive the vehicle solution (physiological saline) at the dose of 2mL / 100 g of body weight by gavage, 30 min before the injection of carrageenan 1%.

➤ **Lot 2 reference:** The rats of this lot were treated by gavage with Diclofenac an anti-inflammatory used therapeutically, 30 minutes before the injection of carrageenan 1%. The administration of the anti-inflammatory is at a rate of 1 ml / 100 g of body weight.

➤ **The Lot 3, 4 and 5:** The extract to be tested is administered to rats by gavage in respectively increasing doses (250, 500 and 750 mg / mL / kg of body mass) 30 mn before the injection of the carrageenan 1%. The injection of carrageenan is 0.1 ml under in plantar region of the right back paw of the rat. The animals are then put back in their cage. The diameter of edema was measured in 1, 2, 3 and 4 hours after injection of the carrageenan 1%. The importance of the edema was estimated by the determination of the average percentage of increase % (AUG) of the diameter of the leg of rat according to the following formula:

$$\% \text{ AUG} = \frac{V_t - V_o}{V_o} \times 100$$

**V<sub>t</sub>:** Diameter of the paw at a time t

**V<sub>o</sub>:** Initial diameter of the paw

The anti-inflammatory activity of the products tested was estimated by calculating the average percentages of inhibition of the edema according to the formula:

$$\% \text{ d'inhibition} = \frac{\% \text{ AUG témoin} - \% \text{AUG traité}}{\% \text{AUG témoin}} \times 100$$

### Reactive Protein C (CRP) Assay

Rat blood was taken 6 hours after injection of carrageenan into dry tubes and centrifuged at 3000 rpm for 10 min. The serum was recovered and stored in eppendorff tube at 4 ° C. The CRP assay was done by the ELISA technique and the Abcam's kit was used. Abcam's kit consists of several articles for the quantitative measure of CRP in the urine, the plasma and serum. First, remove the excess microplate strips from the plate frame and immediately return them to the aluminum pouch with desiccant inside and close the pouch tightly to minimize exposure to water vapor. Then, add 50µL of the sample per well and cover them with a sealing tape for 2 hours. In addition, wash the plate six times with 300µL of 1X Wash buffer and then dab 4-5 times on an absorbent paper towel. In each well add 50µL of biotinylated 1X and incubate for one hour at 37 ° C. Wash the microplates again 6 times with 300µL 1X wash buffer before adding 50µL 1X Sp conjugate to each well and incubate them again for 30 min; turn on the microplate reader and combine the program in advance. Besides it, the wells must be washed 6 times with 300µL 1X wash buffer. Add 50µL of chromogenic substrate per well and incubate for 20 min, the color will turn blue. The last stage consists in tapping the plate to mix well while breaking bubbles in the well with the headland of a pipette; finally add 50µL of solution stop in every micropatch so that the color passes of the blue (bruise) in the yellow, and to stop reading immediately the absorbance to the wavelength 450 nm.

### ❖ Statistical Analysis

The results (profits) were expressed on average ± standard error in the average. The differences between both averages were determined by analysis of variance ANOVA. A significant difference is represented by one p <0,05.

## RESULTS

**Inflammatory edema:** the table I shows the effects of the aqueous leaf extract of *Annona senegalensis*, and diclofenac on the evolution as a function of time, carrageenan -induced rat paw diameter. After injection of 1% carrageenan into the right hind paw of the rat, the average diameter of this tab increases from 2,97 ± 0,074 mm to 6,69 ± 0,288 after 1 hour, 7,96 ± 0,223 mm after 2 hours, 8,19 ± 0,23 mm after 3 hours and 8,30 ± 0,266 after 4 hours

for rats that received no treatment. Administration of the aqueous leaf extract of *Annona senegalensis* at a dose of 250 mg / ml / kg body weight prevents edema of the rat paw compared to the control group. The average diameter increases from  $3,15 \pm 0,15$  mm to  $5,98 \pm 0,30$  after 1 hour and from  $6,06 \pm 0,24$  mm after 4 hours to a maximum of  $6,58 \pm 0,08$  mm measured 2 hours after treatment. Rats receiving ETA at a dose of 500 mg / ml / kg body weight show less significant changes in diameter. In the presence of diclofenac (1 ml / 100g body weight) and the aqueous extract of *Annona senegalensis* (750 mg / ml / kg body weight), there are smaller increases in the diameter of the paw, the maximum of which is measured at 2 hours of the experiment. The increases are for diclofenac equal to 90.53% at 1 hour, 102.18% at 2 hours, 77.7% at 3 hours and 74.68% at 4 hours. For ETA at a dose of 750 mg / ml / kg body weight, 63.92% at 1 hour, 95.72% at 2 hours, 94.13% at 3 hours and 80.40% at 4 hours (**Table II**). Percent inhibition evaluation shows that the aqueous extract of *Annona senegalensis* leaves has a greater anti-inflammatory activity in the second phase of the inflammatory process (after 2 hours). However, a better inhibition of inflammatory edema of the rat paw was observed at 4 hours with a percentage inhibition for ETA at the dose of 750 mg / ml / kg body weight of 55.02 comparable to the reference group treated with diclofenac (1ml / 100g body weight) which shows an inhibition of 55.95% (**Figure 1**).

#### **Determination of C-reactive protein (CRP)**

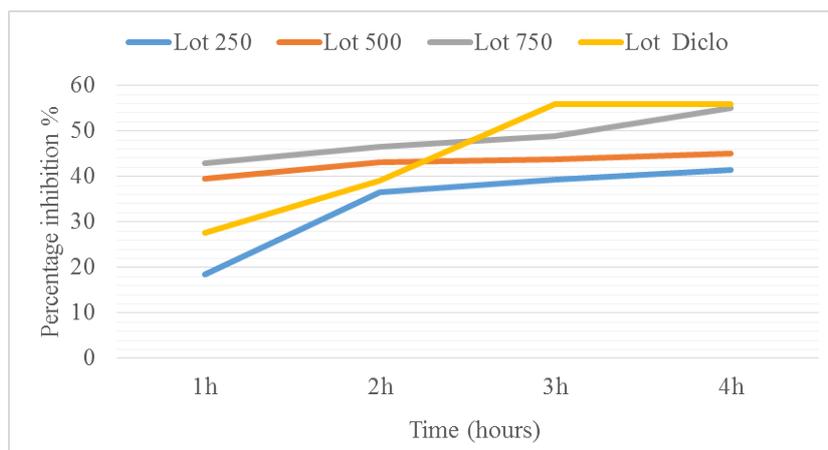
The reading of the different optical densities (OD) shows the evolution of the amount of CRP in the serum of the rats. Indeed, before the treatment, the amount of CRP in the serum is very high. For rats given diclofenac, the reference anti-inflammatory drug has a lower amount of CRP. As increasing doses of ETA are administered, there is a significant decrease in the amount of CRP in the serum to a minimum which is similar to the amount observed with diclofenac. ETA at a dose of 750 has an activity comparable to that observed with diclofenac in reducing the amount of CRP. These different ODs allowed us to plot **Figure 2** which represents the evolution kinetics of the amount of CRP in the serum of the rats.

**Table. I: Average Rat Paw Diameter as a Function of Time and Dose.**

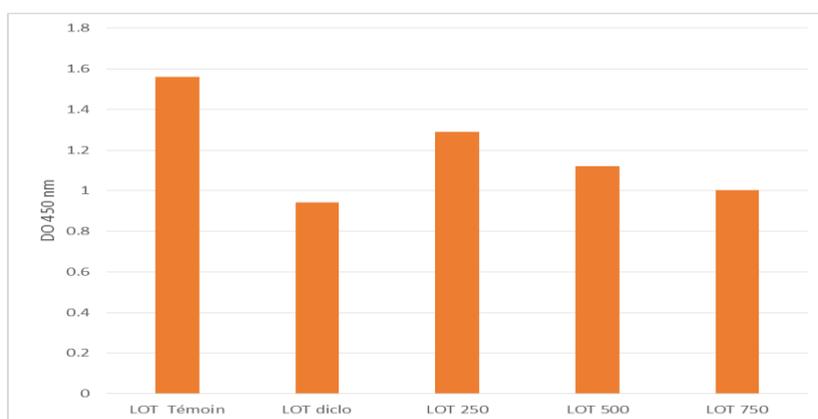
| Lots           | Doses                 | 0 h          | 1 h          | 2 h          | 3 h         | 4 h          |
|----------------|-----------------------|--------------|--------------|--------------|-------------|--------------|
| Lot 1 (Témoin) | 0,1 mL                | 2,97 ± 0,074 | 6,69 ± 0,288 | 7,96 ± 0,223 | 8,19 ± 0,23 | 8,30 ± 0,266 |
| Lot 3 (ETA)    | 250 mg/kg body weight | 3,15 ± 0,15  | 5,98 ± 0,30  | 6,58 ± 0,08  | 6,33 ± 0,35 | 6,06 ± 0,24  |
| Lot 4 (ETA)    | 500 mg/kg body weight | 2,96 ± 0,19  | 5,24 ± 0,04  | 6,35 ± 0,4   | 6,09 ± 0,31 | 5,87 ± 0,23  |
| Lot 5 (ETA)    | 750 mg/kg body weight | 3,06 ± 0,05  | 5,41 ± 0,26  | 6,17 ± 0,15  | 6,12 ± 0,20 | 5,34 ± 0,21  |
| Lot 2 (Diclo)  | 1ml/100g body weight  | 2,98 ± 0,053 | 5,68 ± 0,24  | 6,00 ± 0,19  | 5,30 ± 0,31 | 5,21 ± 0,24  |

**Table. II: Percentage increase in edema diameter as a function of time and dose.**

| Lots           | Doses                 | Percentage increase in edema diameter (%) |        |        |        |
|----------------|-----------------------|---|--------|--------|--------|
|                |                       | 1h  | 2h     | 3h     | 4h     |
| Lot 1 (Témoin) | 0,1 mL                | 125                                       | 168,01 | 175,75 | 178,79 |
| Lot 3 (ETA)    | 250 mg/kg body weight | 102,02                                    | 114,85 | 106,69 | 104,73 |
| Lot 4 (ETA)    | 500 mg/kg body weight | 71,1                                      | 101,59 | 98,86  | 98,31  |
| Lot 5 (ETA)    | 750 mg/kg body weight | 63,92                                     | 95,72  | 94,13  | 80,4   |
| Lot 2 (Diclo)  | 1ml/100g body weight  | 90,53                                     | 102,18 | 77,7   | 74,68  |



**Figure. 1: Percentage middle inhibition in edema.**



**Figure. 2: Evolution kinetics of the amount of CRP in the serum.**

## DISCUSSION

The edema caused by carrageenan has two distinct phases: A first phase, which occurs between 0 and 2.5 hours after the injection of the carrageenan attributed to the action of mediators such as histamin, sérotonine and bradykinine to vascular permeability (Maity *et al.*, 1998) and the second phase the mediator of which is supposed to be the prostaglandins (Wang and Mineshita, 1996). This release of prostaglandins is associated with migration of leukocytes in the inflamed area. Prostaglandins are involved in acute or chronic inflammatory processes (Gilligan *et al.*, 1994). The carrageenan-induced edema test is of particular interest at this stage of our work in that it is a test in which the participation of COX derivatives produced during metabolism of acid arachnidonique, and the production of reactive oxygen species are well established (Smith *et al.*, 1974). Anti-inflammatory drugs generally intervene by opposing the effect of these chemical mediators histamine, sérotonine, kinines and prostaglandins. Our results would suggest that ETA leaves of *Annona senegalensis* inhibit mediators of inflammation, especially the production of prostaglandins such as diclofenac used in our test. The richness of the aqueous extract of *Annona senegalensis* in polyphenol constituents capable of trapping free radicals, would prevent the formation of prostaglandins that cause inflammation. These results provide a scientific justification for the traditional use of *Annona senegalensis* leaves in the treatment of rheumatism, diarrhea and dental pain (Ouattara, 2004). Traditional aqueous preparations would therefore find a therapeutic indication as analgesic and anti-inflammatory as diclofenac. The polyphenolic substances of the leaf decoction of *Annona senegalensis* may partly explain these activities. Yéo *et al.* (2011) research isolated of terpénoids, coumarines, flavonoïds and tanins in the leaves of the plant. On the other hand, they are poor of alcaloïdes and of quinoniques substance. The literature attributes of flavonoïds (quercétine, kaempférol), of phénols acid (protocatéchiq ue acid, gallique acid), of anthocyanes (poenidine), of xanthones (mangiférine) to anti-inflammatory activity (Jeffrey and Herbert, 1995). With regard to the structure activity relationship, flavonoids are inhibitors of 5-lypooxygenase, therefore of the production of prostaglandins and leucotriènes, which are mediators of inflammation and allergic manifestations (Kim *et al.*, 2004; Yoon *et Baek*, 2005). Thus, the anti-inflammatory activity of the aqueous extract of *Annona senegalensis* may be due in part to the presence of these aforementioned molecules including flavonoids. The effects of *Annona senegalensis* leaves are greater than those obtained with the aqueous extract of *Mangifera indica* leaves. The study of the anti-inflammatory activity of the extract of *Mangifera indica* at the dose of 1000 mg / kg PC over time, allowed us to confirm its

action on the acute phase of inflammation, 3 hours after injection of carrageenan (30.52%) (Aouissa, 2002).

The optical densities lifted in the control group indicates that the amount of CRP increases during the inflammatory process. Our results corroborate with the work of **Ficher *et al.* (1986)** who showed that CRP is the fastest performing kinetic inflammation protein for early diagnosis of inflammatory syndrome. It is the best biological marker of infection especially during bacterial infections where it can reach the highest values. The rapid kinetics of evolution of this protein made it possible to test the therapeutic efficacy of the extracts. Thus, compared to the control group, diclofenac and ETA of 750 mg / kg body weight were found to be more effective on the inflammatory process compared with ETA of 250 and 500 mg / kg body weight.

## CONCLUSION

This study showed an anti-inflammatory effect of the aqueous extract of leaves of *Annona senegalensis* on the model of carrageenan inflammatory edema in rats and a significant decrease in CRP. This activity is greater at the dose of 750 mg / kg body weight and is thought to be related to the inhibition of cyclooxygenases and lipoxygenases in the late phase of carrageenan inflammatory edema and these different secondary metabolites. The evaluation of the anti-inflammatory activity of the extract establishes the scientific basis for the use of *Annona senegalensis* leaves to prevent and treat various inflammatory diseases. Studies should also focus on the purification of the active ingredients responsible for the effects of this plant and on the determination of the molecular mechanisms involved for the preparation of improved forms of effective remedies based on *Annona senegalensis*.

## REFERENCES

1. Adzu B., Abubakar M.S., Izebe K.S., Akumka D.D. & Gamaniel K.S., Effect of *Annona senegalensis* rootbark extracts on *Naja nigricotlis nigricotlis* venom in rats. *J. Ethnopharmacol.*, 2005; 96(3): 507-13.
2. Agrawal A., Shrive A., Greenhough T. & Volanakis J., Topology and Structure of the C1q-Binding Site on C-Reactive Protein. *J. Immunol*, 2001; 166(6): 3998-4004.
3. Aouissa I. W– R., étude des activités biologiques et de la toxicité aigüe de l'extrait aqueux des feuilles de *Mangifera indica*. Thèse de doctorat en pharmacie. Université de BAMAKO (Mali), 2002; 127.

4. Fall D., Badiane M., Ba D., Loiseau P, Bories C., Gleye, Laurens A. & Hocquemiller R., 2003.- Activité antiparasitaire d'Annonaceae du Sénégal utilisées en médecine traditionnelle. *Dakar Médical*, 48(2): 112-116.
5. Ficher M. & Page M.I., Clinical significance of extrême elevation of the érythrocyte sédimentation rate. *Arch Intern Med.*, 1986; 146: 1581-3.
6. Gaziano J. M. & Gibson C. M., Potential for drug-drug interactions in patients taking analgesics for mild-to-moderate pain and low-dose aspirin for cardioprotection. *Am J Cardiol*, 2006; 97: 23-9.
7. Gilligan J.P., Lovato S.J., Erion M.D. & Jeng A.Y., Modulation of carrageenan-induced hind paw edema by substance P. *Inflammation*, 1994; 18(3): 285-292.
8. Guédé-Guina F., Vangah-Manda M., Harounad D. & Bahi C., Potencies of MISCA a plant source concentrate against fungi, *J. Ethnopharmacol*, 1996; 14(2): 45-53.
9. Jeffrey B. H. & Herbert B., 1995.- *Phytochemical dictionary*; Edition Taylor et francis.
10. Kayode A. E. & Durodola J.I., Antitumor and antibiotic principles of *Annona senegalensis*. *Phytochemistry*, 1976; 15: 1311-1312.
11. Kim H.P., Kun H.S., Chang H.W. & Kang S.S., Anti-inflammatory plant flavonoids and cellular action mechanisms. *J. Pharmacol. Sci.*, 2004; 96(3): 229-245.
12. Maity T.K., Mandal S.C. & Mukherjee P.K., Studies on anti-inflammatory effect of *Cassia tora* leaf extract (fam leguminosae). *Phytother. Res.*, 1998; 12(3): 221- 223.
13. Marc T., Gerard W., Denis L., Classification des anti-inflammatoires in *Guide pharmacologie. Etudiants et professionnels paramédicaux*. 4eme Edition, 2001; 426.
14. Ouattara F., O., traitement traditionnel des infections sexuellement transmissibles au Mali : étude de la phytochimie et des activités biologiques de *Annona senegalensis* (ANNONACEAE) et de *Stachytarpheta angustifolia* (VERBENACEAE). Thèse en pharmacie. Université de Bamako (MALI), 2004 ; 237.
15. Smith M. J. H., Ford A. W., Elliot P. N. C. & Bolam J., Prostaglandin in the antiinflammatory activity of a human plasma fraction in carrageenan-induced paw oedeme in the rat. *J Pharm Pharmacol.*, 1974; 26: 692.
16. Wang L. M. & Mineshita S., Preventive effects of Unsei-in and Oren-gedoku-to, chinese traditional medicines, against rat paw oedem and abdominal constriction in mice. *J Pharm Pharmacol.*, 1996; 48: 327-331.
17. Yéo D., Dinica R., Yapi H. F., Furdui B., Praisler M., Djaman A. J. & N'Guessan J. D. Evaluation of the anti-inflammatory activity and phytochemical screening of *Annona senegalensis* leaves. *Thérapie*, 2011; 66(1): 73-80.

18. Yoon J.H. & Baek S.J., Molecular targets of dietary polyphenols with antiinflammatory properties. *Yonsei Med. J.*, 2005; 46(5): 585-596.