Analgesic and anti-inflammatory activities of triterpenoid molecules isolated from the leaves of *Combretum glutinosum* Perr. Ex DC (COMBRETACEAE)

Running title: Anti-inflammatory activity and *Combretum glutinosum*

Abstract

Combretum glutinosum Perr. Ex DC (COMBRETACEAE) is a traditional medicinal plant, widely distributed in Senegal and Africa. The aim of that study was to investigate the analgesic and anti-inflammatory activities of two triterpenes, betulonic acid (DN7) and cabraleone (DN12), isolated from the leaves of *Combretum glutinosum* (COMBRETACEAE). Experiments were performed in acetic acid induced contorsions in mice and carrageenan rat paw edema. DN7 (3 mg/kg, per os) and DN12 (3 mg/kg, per os) significantly prevent contorsions in mice. The mean of contorsions is respectively 30±10 and 32±7 versus 72.6±6.64 in control group ($p<0.05$, n=5). DN7 (3 mg/kg, per os) significantly prevents the increased rat paw edema (31.84±6.76 vs 92.72±6.05 %) ($p<0.05$, n=5). DN12 (10 mg/kg, per os) induced rat paw edema prevention is similar to that of acetylsalicylic acid (10 mg/kg, per os) administered in the same conditions (29.28±5.88 vs 30.96±7.25 %) (ns, n=5). The analgesic and anti-inflammatory activities of triterpenes isolated from *C. glutinosum* leaves are similar to those of non steroidial anti-inflammatory drugs such acetylsalicylic acid, justifying the use of this plant in traditional medicine to treat pain and inflammation.

Key Words: *Combretum glutinosum*, betulonic acid, cabraleone, pain, inflammation

INTRODUCTION

Inflammation is a crucial biological process to maintain homeostasis. It is essential to successfully fight pathogens and repair tissue damage [1]. Pain is one of the most important symptoms of inflammatory disease, which is a pathophysiological process that activates defense mechanisms to protect organism again causal agent [2]. However, inflammatory process is linked to deleterious effects, which are an essential components of various diseases such as rheumatoid arthritis, type 2 diabetes, cancer, obesity, asthma, cardiovascular and neurodegenerative pathologies [3-5]. The drugs commonly used to prevent inflammatory response are non-steroidal anti-inflammatory and glucocorticoids. They are efficient, but responsible of serious adverse effects [6].

Medicinal plants are alternative for the discovery of new molecules with minimal adverse effects. In fact, bioactive natural products can be regard as very promising ones to develop new therapeutic agents, as well analgesic and anti-inflammatory drugs [7, 8].

Combretum glutinosum Perr. Ex DC is a plant of senegalese traditional pharmacopoeia, its leaves are used in the treatment of various diseases [9]. In west Africa, several traditional uses of *C. glutinosum* leaves have been described to treat hepatitis, cardiovascular, infectious, gastrointestinal and bronchial diseases [10-17].

Several studies had shown pharmacological properties of *C. glutinosum* leaf extracts [18-22].

Triterpenoid molecules, betulonic acid (DN7) and cabraleone (DN12) were isolated form *C. glutinosum* leaves. (**Figure 1**) [9].

It was shown that triterpenoids are widely distributed in the plant kingdom [24]. Several properties of triterpenes such as anti-inflammatory, wound healing, anti-bacterial, antiviral, hepatoprotective, antidiabetic and anti-tumoral effects, have been also described [25, 26, 27, 28, 29].

The aim of that study was to investigate anti-inflammatory and analgesic activities of betulonic acid (DN7) and cabraleone (DN12) on acetic acid contortions in mice and carrageenan-induced paw rat edema.

MATERIALS AND METHODS

a. Drugs and chemicals

Carrageenan, acetyl salicylic acid, betamethasone, acetic acid were obtained from Sigma. Betulonic acid (DN7) and cabraleone (DN12) were provided by the Laboratory of chemistry and Physics of materials of Assane Seck University of Ziguinchor (SENEGAL).

b. Animals

Adult Wistar KYOTO strain rats of 180 g and mice of 20 g body weight were used. The animals had free access to food and water. The experimental protocols were conducted in accordance with the guidelines on the care and use of laboratory animals (Senegal National Ethical Committee for Health Research).

c. Experimental procedures

i. <u>Carrageenan induced rat paw edema</u>

The anti-inflammatory study was carried out following the method described by Winter [30]. The rats were divided into 8 groups of 5. They were then fasted 12 hours before the tests.

For each rat, the initial diameter (D_0) of the left hind paw was measured using digital caliper:

- Group 1 (control): Normal saline (10 mL/kg, *per os*)
- Group 2 (reference): Betamethasone (1 mg/kg, *per os*)
- Group 3 (reference): Acetyl salicylic acid (ASA) (10 mg/kg, *per os*)
- Group 4 and 5 (treated): DN7 (1 and 3 mg/kg, *per os*)
- Group 6, 7 and 8 (treated): DN12 (1, 3 and 10 mg/kg, *per os*)

The rat paw edema was induced by injection of carrageenan solution 1 % (100 µL) under neath the planter region of left hind paw of the rats, 1 h after oral administration of different solutions.

The increased edema was measured using digital caliper at 180 and 300 minutes (T_{3h} and T_{5h}) after carrageenan injection.

The importance of edema was assessed by determining the mean percentage increase **(% INC**) of diameter of rat paw according to formula:

$$\%\mathbf{INC} = \frac{\mathbf{Dt} - \mathbf{Do}}{\mathbf{Do}} \times \mathbf{100}$$

Dt: Paw diameter at t time;

$\mathbf{D_O}$: Initial paw diameter

ii. <u>Acetic acid induced writhing in mice</u>

The writhing test in mice was used [31]. Contortions were induced by intraperitoneal injection of acetic acid 3 %. Animals were divided in groups of 5 mices each. They were then fasted 12 hours before tests.

Mice were treated with the following solutions:

- Group 1 (control): Normal saline (10 mL/kg, *per os*)
- Groups 2 (reference): Acetyl salicylic acid (ASA) (10 mg/kg, *per os*)
- Group 3 and 4 (treated): DN7 (3 and 10 mg/kg, *per os*)
- Group 5 and 6 (treated): DN12 (3 and 10 mg/kg, *per os*)

Intraperitoneal injection of 3 % acetic acid solution was performed 1 h after gavage. The sensitivity to pain was evaluated by the contortions number counted during 30 min after latency time.

d. Statistical analysis

The experimental results are expressed as mean ± standard error of mean (SEM). The significance was evaluated using one-way ANOVA followed by Bonferroni's post hoc test compared with the control group. Values of $p<0.05$ were significantly different. n is the number of experiences in each group.

2. RESULTS AND DISCUSSION

Intraperitoneal acetic acid (3 %) induced contorsions (72.6±6.64) ($p< 0.05$, n=5) in mice which were treated with vehicle (10 mL/kg, *per os*). Pretreatment with betamethasone (300 µg/kg, *per os*), prevented significantly the whrites induced with acetic acid, compared to control group (24±4 vs 76.6±6.64) ($p< 0.05$, n=5). The prevention of contorsions induced with acetylsalicylic acid (10 mg/kg, *per os*) is significant versus control group, but less potent to that of betamethasone administered at a tiny dose (300 µg/kg, per os). It suggests that peripheral pain model of acetic acid induced contorsions, also discriminates the profile of analgesic effect between glucocorticoids and the non steroidial anti-inflammatory drugs. **(Figure 2)**

Prior administration of DN7 (3 mg/kg, *per os*) and DN12 (3 mg/kg, *per os*), significantly prevents acetic acid induced controrsions in mice. The mean of contorsions is respectively 30±10 and 27±7 versus 72.6±6.64 in control group ($p< 0.05$, n=5). The analgesic effect of DN7 and DN12, at 3mg/kg per os, is similar to that of acetylsalicylic acid (10 mg/kg) administered in the same conditions. **(Figure 2)**

The cyclooxygenase 2 (COX2) produces prostanoids that mediate pain, fever and inflammation processes [32]. Ribeiro et al. [33], showed that acetic acid causes nociception

by a mechanism involving eicosanoids such as prostaglandins, which also mediate the mechanical hyperalgesia induced by inflammatory stimuli as carrageenan. Several studies had shown the analgesic activity of triterpenes. In fact, asiatic acid, a pentacyclic triterpene compound from *Centella asiatica*, significantly inhibits acetic acid induced whrites. This effect may be caused by inhibition of arachidonic acid metabolites synthesis [34]. It was also shown that many molecules possess both analgesic and anti-inflammatory properties; their mechanism of action involves COX2 inhibition [35]. In this study, the analgesic effect of betulonic acid (DN7) and cabraleone (DN12), similar to that of acetylsalicylic acid, may involve COX2 inhibition such as asiatic acid of *C. asiatica*, a triterpenoid molecule.

edema (92.72±6.05 %). Pretreatment with betamethasone (1 mg/kg, *per os*) significantly prevented the acute rat paw edema induced with carrageenan 1 %. The increase of paw edema is only 23.47±3.99 %, suggesting a power anti-inflammatory response of glucocorticoids in acute rat paw edema. In this model, the anti-inflammatory activity of glucocorticoids is more effective than that of non steroidial anti-inflammatory drugs such acetylsalicylic acid. In fact, pretreatment with acetylsalicylic acid (1 mg/kg, per os), significantly prevents rat paw edema (30.96±7.25 vs 92.72±6.05 %) ($p < 0.05$, n=5). DN7 and DN12 dose dependently prevent rat paw edema induced with carrageenan 1 %. At 10 mg/kg per os, the increase of paw edema is 29.28±5.88 vs 30.96±7.25 % in acetylsalicylic acid (10 mg/kg, per os) group, suggesting a similar profile of anti-inflammatory effect between acetylsalicylic acid and triterpenoid molecules from *C. glutinosum*. **(Table I)**

The development of paw edema following injection of carrageenan has been characterized as a biphasic event in which various mediators are involved to generate inflammatory response [36]. The acute phase of edema (0–2.5 h) contributes to the release of histamine, 5-hydroxytryptamine and bradykinin, which are not inhibited by non-steroidal anti-

inflammatory drugs [37]. However, a delayed phase of inflammation is correlated with overproduction of prostaglandins in tissues, mediated by COX2 [38].

The anti-inflammatory effect of DN7 and DN12 on carrageenan-induced rat paw edema is particularly significant in the second phase of inflammation, suggesting a possible prevention of eicosanoids production such prostaglandins, probably by COX 2 inhibition.

Several studies had shown the anti-inflammatory activity of triterpenoid molecules, involving the prevention of prostaglandins production. According to Begum et al. [39], coumaroyl lupendioic acid from *Careya arborea* stem barks, inhibits pro-inflammatory mediators on carrageenan induced inflammatory model. The underlining mechanism of action is associated with selective inhibition of COX2. Ursolic acid, pentacyclic triterpenoid, isolated to hexane extract of *Plantago major*, is also anti-inflammatory. This effect has been attributed to inhibition of prostaglandins synthesis associated with COX2 inhibition [40, 41]. Tetracyclic triterpenes isolated from gum resins of *Boswellia spp* possess anti-inflammatory activity involving COX2 inhibition [42]. Cabraleone (DN12) and betulonic acid (DN7), respectively a tetra- and pentacyclic triterpenoid molecules from *C. glutinosum*, may involve similar mechanism to prevent both pain and inflammation, justifying their use in African traditional medicine.

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A. **B.**

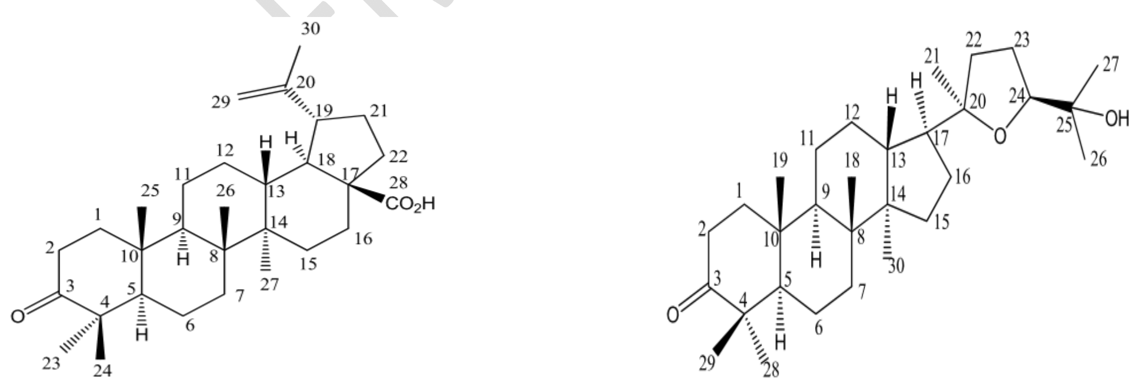
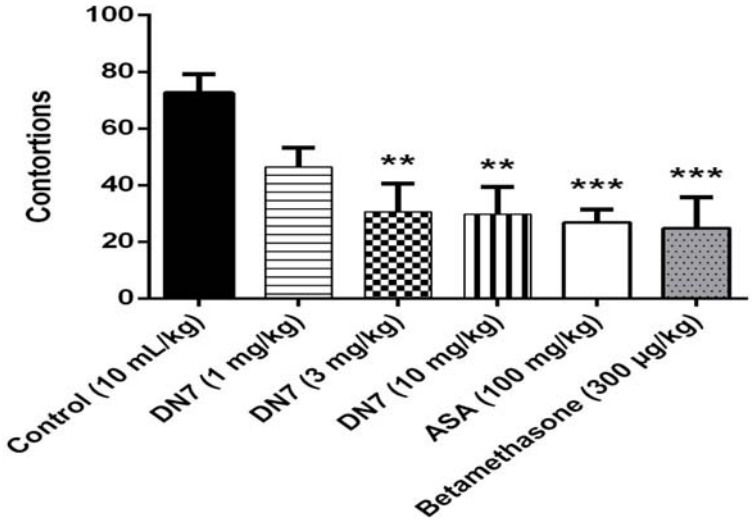


Figure 1: **Structure of betulonic acid (DN7) and cabraleone (DN12) isolated from leaf extracts of *Combretum glutinosum.* A: Betulonic acid, B: Cabraleone**

A.



B.

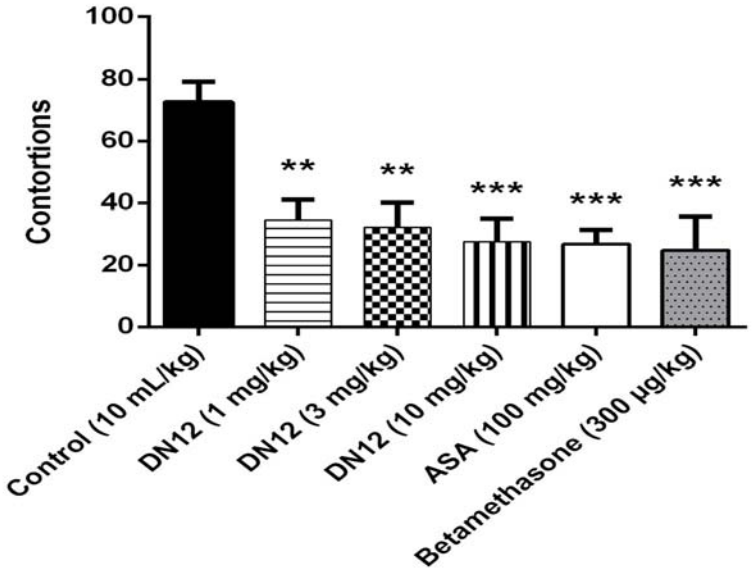


Figure 2: Analgesic effect of DN7 and DN12 on acetic acid induced contorsions in mice. **p < 0.01, ***p < 0.001 vs control group. DN7: Betulonic acid, DN12: Cabraleone.

Table I: Effect of DN7 and DN12 on carrageenan-induced paw edema test in rats. **p < 0.01, ***p < 0.001, ****p < 0.0001 vs control group. ASA: acetylsalicylic acid, DN7: betulonic acid, DN12: cabraleone

Treated groups	Dose (mg/kg)	Increased rat paw edema (%)	
		3H	5H
Control	**10 mL/kg**	67.77±6.79	92.72±6.05
ASA	**10**	33.77±7.08**	30.96±7.25****
DN7	**1**	31.56±7.35***	51.17±4.47***
	3	34.04±4.08**	31.84±6.76****
DN12	**1**	50.27±6.26**	56.49±8.29***

	3	37.67±4.91**	40.15±4.14****
	10	29.02±3.87***	29.28±5.88****