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Possible mechanisms of anti-inflammatory and analgesic effects of the aqueous extracts of *Ceiba pentandra* Gaertn (Bombacaceae) and *Chromolaena odorata* L (King and Robinson) (Asteraceae) in rat

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Abstract

The aim of this study was to investigate the probable mechanisms of anti-inflammatory and analgesic effects of the aqueous extracts of *C. pentandra* and *C. odorata*. The possible mechanisms of inflammatory effect were investigated in rat by the local administration into the plantar aponeurosis of the histamine, serotonin and the arachidonic acid. The results obtained show that the aqueous extracts of *C. pentandra* (400 and 800 mg/kg) and of *C. odorata* (400 and 800 mg/kg) inhibit the histamine, serotonin and the arachidonic acid inflammation. This suggests that these two aqueous extracts could interfere with their inflammatory mechanisms. The probable mechanisms of analgesic effect were investigated in rat by the local administration into the plantar aponeurosis of formaldehyde solution in the naloxone presence. The results obtained showed an inhibiting pain of the two extracts at the doses used on the two phases (neurogenic response and inflammatory response) of pain in spite of the preliminary administration of naloxone. This result suggests that these two extracts could act by not opioid mechanism.

Keywords: Mechanism of effect, anti-inflammatory drug, analgesic drug, *Ceiba pentandra* *Chromolaena odorata*

1. Introduction

The inflammation is defined as a whole of reactional phenomena which take place within connective tissue after a lesion [1]. It constitutes a system of defense of the organism against the exogenous aggressions (traumatism or infection), endogenous (immunological disturbance); from where it's beneficial state for the organism. The inflammation is often associated with the pain and the swelling which take place after a traumatism witch it comes from an injury or a wound. In fact, the inflammation is also implied in a great number of other pathological, in particular including the diseases like hypertension, cancer or cardiovascular diseases. The world epidemiologic data of diseases associated with the inflammation show their impact on the population. The percentages of these diseases are very significant with very high pathological frequencies [2]. The pain can be regarded as a protective function, a warning signal, after a lesion or of a traumatism of a peripheral tissue of the organism generating an acute inflammatory defense [3]. The studies on the pain show that it represents the main reason for clinical consultation. Bassols *et al.*, (1999) think that, pain is a leading public health problem, as well as a source of personal and family suffering, and it constitutes a problem which goes beyond the individual and becomes a social illness [4]. It is estimated that on three cases of consultations, two concern the pain. In front of the multiples problems of health involved in the inflammatory diseases which are treated only with the pharmaceutical products, at the present time, it is to advise by WHO to use various therapeutic means in order to treat a given disease by also making recourse to the treatment by the medicinal plants [2]. *C. pentandra* and *C. odorata* are two plants of the Congolese flora used in the treatment of several pathologies of which the inflammation and the pain. *C. pentandra* is variously employed in traditional medicine in Congo [5]. His stem barks are used for the treatment of the stomach aches, asthma, rickets, gastric ulcer and the diarrhoea [6, 5]. The previous studies carried out with this plant showed many pharmacological activities such as hypoglycemic [7], antiulcer [8], antidiabetic [9], antibacterial [10, 11], anti-inflammatory and analgesic [12]. However, *C. odorata* is used like disinfecting for the wounds in our traditional medicine [13]. Previous studies on this plant shows that it has several pharmacological properties such as analgesic and anti-inflammatory [14], antipyretic [15], antibacterial [16, 17], antioxidative [18] and antifongic [19]. None of these studies does not specify by which mechanism the extracts used would exert their

pharmacological effects. However, the mechanisms of the anti-inflammatory effects and the traditional analgesics are known. For example, the traditional anti-inflammatory drugs inhibit the phospholipase A2, it is the case of the steroidal anti-inflammatory drugs (SAIDs) or inhibits cyclo-oxygenase it is the case of non steroidal anti-inflammatory drugs (NSAIDs). In addition to their anti-inflammatory and antipyretic effect, the NSAIDs have a real non narcotic analgesic effectiveness. The mechanism of action still passes by the inhibition of the prostaglandins synthesis because they have the property to heighten the peripheral noxious receptors to the action of nociceptive substances such as the bradykinine, histamine, serotonin and the substance P [3]. On the other hand, a traditional analgesic inhibits the pain either at the peripheral level by an action on the vasoactives amines or at the central level by preventing the increase of the impulse generated at the peripheral ends of the fibers C and A δ by an action on the ascending ways of the pain [20]. Taking into consideration all that precedes, we aimed investigated the possible mechanisms of anti-inflammatory and analgesic effects of the aqueous extracts of *C. pentandra* and *C. odorata*.

2. Materials and Methods

2.1 Plant material

The stem barks of *C. pentandra* and leaves of *C. odorata* were used. Botanical identification of the plant material was done by Mousamboté, botanist systematist of (Higher Normal School of Agronomy and Forestry HNSAF) and confirmed at the botanical laboratory of Research Institute in Exact and Natural Sciences (RIENS) in Brazzaville where the samples of *C. pentandra* and *C. odorata* were compared with the reference samples of the herbarium to the number 2529, 20/06/1968 for *C. pentandra* and number 1183,07/ 1965 for *C. odorata*. After that, plant material were dried and pulverized with a mortar. The aqueous extract of stem barks of *C. pentandra* and that of the leaves of *C. odorata* were prepared by decoction. 100 g of powder of each plant are mixed with 1000 mL of distilled water. The mixture was boiled for 15 min. After cooling and filtration, the filtrate obtained was concentrated on a double boiler (60 °C). The concentrate obtained was preserved to evaluate the mechanisms of the anti-inflammatory and analgesic effects.

2.2 Animals

Albino rats (150-200 g) of either sex obtained from the Faculty of Science and Technical of Marien NGOUABI-University were used. They were fed with a standard feed and water *ad libitum*. They were acclimatized during one week before experimentation and were housed under standard conditions (12 hours light and 12 hours dark) and at the temperature of 27 \pm 1 °C. The rules of ethics published by the International Association for the Study of Pain have been considered [21].

2.3 Possible mechanisms of anti-inflammatory effect of aqueous extracts of *C. pentandra* and *C. odorata*

The plantar aponeurosis administration of histamine, serotonin as well as the acid arachidonic causes an edema whose volume increases according to time. The anti-inflammatory drugs reduce this edema evolution compared to control group.

2.4 Histamine and serotonin-induced paw edema in rat

Method described by Nguemfo *et al.* (2007) [22] was used. The animals were divided into groups of 5 rat each. Different doses of aqueous extracts of *Ceiba pentandra* (400 et 800

mg/kg) and of *C. odorata* (400 and 800 mg/kg), diclofenac (standard drug, 5 mg/kg) and distilled water (control group, 0.5 mL/100 g) were administered orally to groups, one hour prior to the local injection of histamine (1 mg/kg) and of serotonin (1mg/kg) into the plantar aponeurosis. The paw volume was recorded during 4th hours by using pletysmometer (Ugo Basile, Italy) 1 hour after histamine (σ) injection and 30 min after serotonin (σ) injection. The anti-inflammatory effect is evaluated by the inhibition of edema [12].

2.5 Arachidonic acid-induced paw edema in rat

Method described by Nguemfo *et al.* (2007) [22] was used. The animals were divided into groups of 5 rat each. Different doses of aqueous extracts of *Ceiba pentandra* (400 et 800 mg/kg) and of *C. odorata* (400 and 800 mg/kg), diclofenac (standard drug, 5 mg/kg) and distilled water (control group, 0.5 mL/100 g) were administered orally to groups, 30 mn prior to the local injection of 1 mg/kg of arachidonic acid (Buffer pH4 citric acid /NaOH) into the plantar aponeurosis. The paw volume was recorded during 4th hours by using pletysmometer (Ugo Basile, Italy). The anti-inflammatory effect is evaluated by the inhibition of edema [12].

2.6 Possible mechanisms of analgesic effect of aqueous extracts of *C. pentandra* and *C. odorata*

The administration into the plantar aponeurosis of formaldehyde 2,5% induced a neurogenic and an inflammatory pain in rat. An analgesic drug will decrease this pain according to time. Naloxone (2 mg/kg, s.c.) was administered 15 min prior to the extracts or tramadol injections [23]. The animals were divided into groups of 5 rats each. Different doses of aqueous extracts of *Ceiba pentandra* (400 et 800 mg/kg) and of *C. odorata* (400 and 800 mg/kg), tramadol (standard drug, 10 mg/kg) and distilled water (control group, 0.5 mL/100 g) were administered orally to groups, 1 hour prior to the local injection of formaldehyde (0.2 ml/rat) into the plantar aponeurosis [24, 25]. An additional group was treated with the tramadol without naloxone, and the formaldehyde was injected has previously. Immediately, animals were placed in various cages to observe the noxious effects. The frequency that the animal licks or bites its paw was monitored over 0-10 min for neurogenic pain response and 10-30 min for inflammatory pain response [14]. The analgesic effect was given by the inhibition of the pain.

2.7 Statistical Analyze

All values were expressed as mean \pm ESM. Analysis of variance followed by Student-Fischer t test "t" was performed. The significance level was set at $p < 0.05$

3. Results

3.1 Effect on histamine and serotonin-induced paw edema

The plantar aponeurosis administration of histamine and serotonin produced the edema which increased gradually with a maximum at 4th hours and 1 hour respectively (figures 1-3). The aqueous extracts of *C. pentandra* and *C. odorata* as well as the diclofenac induced significant reduction ($p < 0.01$ and $p < 0.001$) the evolution of edema compared to control group. Aqueous extract of *C. pentandra* (800 mg/kg) better inhibits histamine edema compared to the aqueous extract of *C. odorata* (800 mg/kg) and diclofenac with a maximum at 2th hours of 50.83; 19.50 and 21.67% respectively (figures 2). However, in serotonin edema, diclofenac better inhibits edema compared to the aqueous extracts of *C. pentandra* (800 mg/kg) and *C. odorata* (800 mg/kg) with a maximum at 2th hours of 38.55; 35.61 and 31.78% respectively (figure 4).

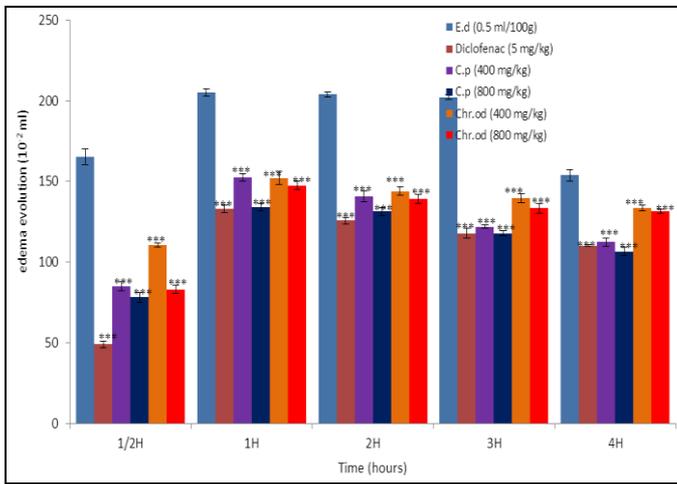


Fig 1: Effect of aqueous extract of C.p and Ch.od in the volume of edema induced by histamine. Each value represents the mean ± ESM; ** $p < 0.01$; *** $p < 0.001$ (Student t-test), versus control group (E.d) E.d= distilled water; C.p= *C. pentandra*; Ch.od= *C. odorata*

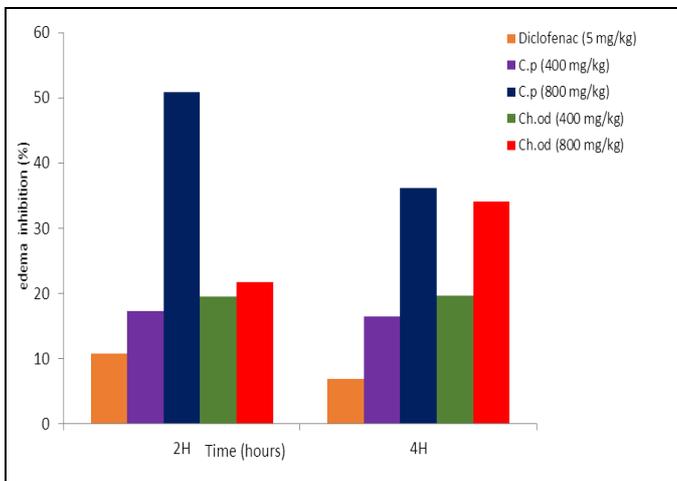


Fig 2: Antiinflammatory effect of aqueous extract of C.p and Ch.od in the edema induced by histamine in rat. C.p= *C. pentandra*; Ch.od= *C. odorata*

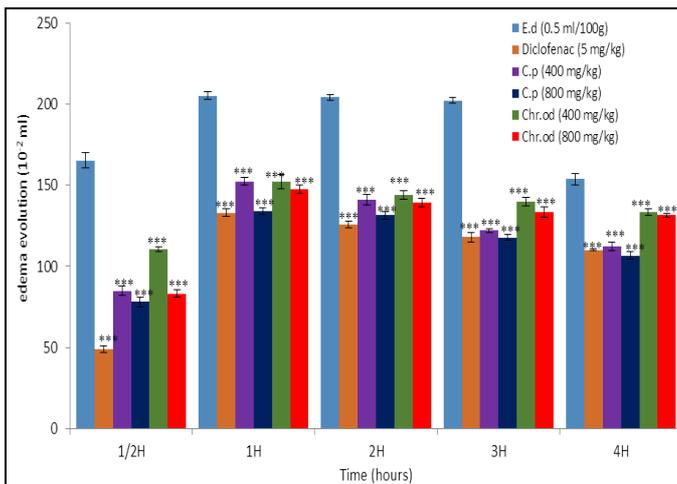


Fig 3: Effect of aqueous extract of C.p and Ch.od in the volume of edema induced by histamine. Each value represents the mean ± ESM; *** $p < 0.001$ (Student t-test), versus control group (E.d) E.d= distilled water; C.p= *C. pentandra*; Ch.od= *C. odorata*

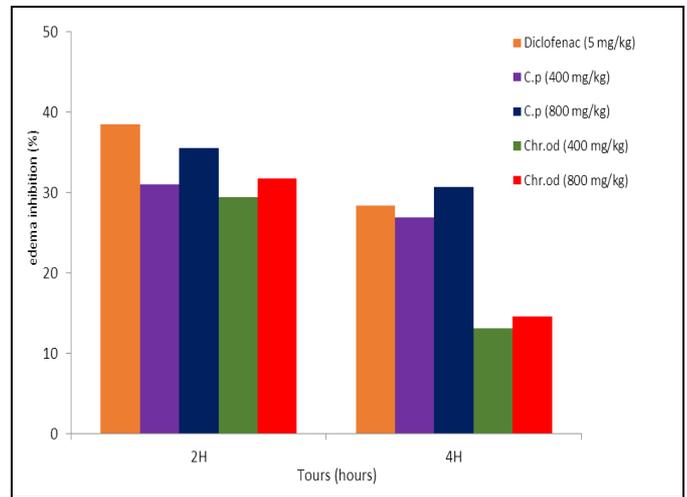


Fig 4: Antiinflammatory effect of aqueous extract of C.p and Ch.od in the edema induced by serotonin in rat. C.p= *C. pentandra*; Ch.od= *C. odorata*

3.2 Effect on arachidonic acid-induced paw edema

The plantar aponeurosis administration of arachidonic acid produced the edema which increased gradually with a maximum at 4th hours. Aqueous extracts of *C. pentandra* (400 and 800 mg/kg), *C. odorata* (400 and 800 mg/kg) and diclofenac (5 mg/kg) induced significant reduction ($p < 0.001$) the evolution of edema compared to control group (figure 5). This effect increased gradually and reached a maximum at 4th hours (66.18 and 67.23%) for aqueous extracts of *C. pentandra* (400 and 800 mg/kg), (51.337 and 69.33%) for Aqueous extracts of *C. odorata* (400 and 800 mg/kg) and 76.63% for diclofenac (figure 6). Nevertheless the diclofenac no significant induced (ns, $p > 0.05$) the edema volume 30 mn after arachidonic acid administration (figure 5).

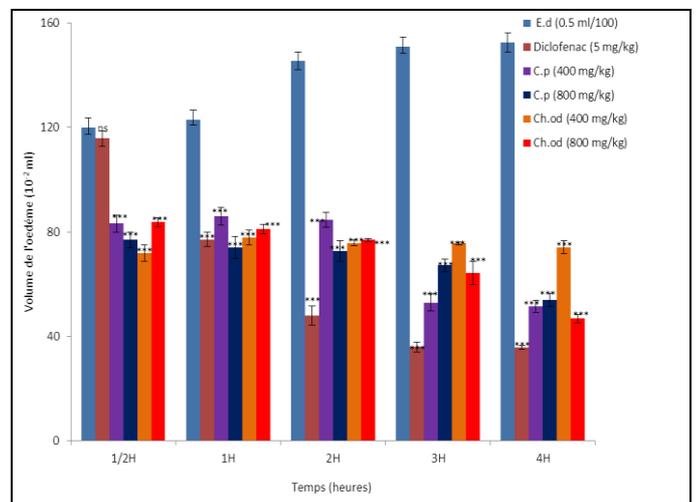


Fig 5: Effect of aqueous extract of C.p and Ch.od in the volume of edema induced by histamine. Each value represents the mean ± ESM; *** $p < 0.001$ (Student t-test), versus control group (E.d). (ns= $p > 0.05$) E.d= distilled water; C.p= *C. pentandra*; Ch.od= *C. odorata*

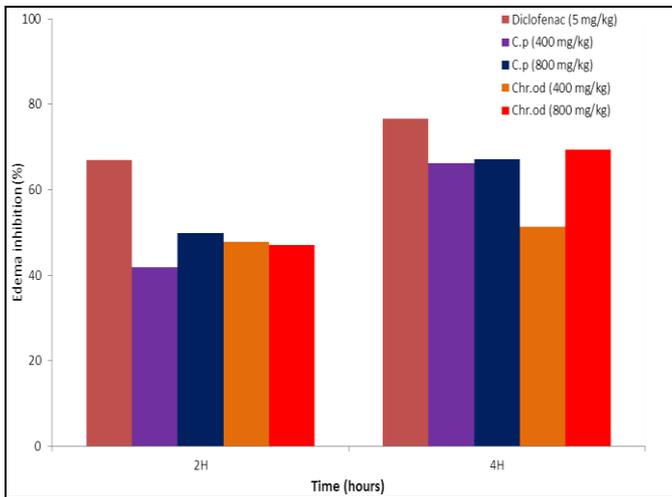


Fig 6: Anti-inflammatory effect of aqueous extract of C.p and Ch.od in the edema induced by arachidonic acid in rat. C.p= *C. pentandra*; Ch.od= *C. odorata*

3.3 Effect on formaldehyde-induced paw edema in naloxone presence

The plantar aponeurosis administration of formaldehyde produced neurogenic and inflammatory response (figures 7, 8, 9 and 10). On neurogenic and inflammatory pain responses the tramadol administrated without naloxone significantly reduce ($p < 0.001$) the frequency of licking and of biting the legs compared to the group control (figures 7, 9). However in naloxone presence, the tramadol does not reduce ($p > 0.05$) the frequency of licking and of biting the legs during the neurogenic and inflammatory pain responses (figures 7, 9). In addition, the two extracts at doses used in the naloxone presence significant reduce ($p < 0.05$; $p < 0.01$; $p < 0.001$) the frequency of licking and of biting the legs on neurogenic and inflammatory pain responses compared to the group control. Moreover, aqueous extract of *C. pentandra* (800 mg/kg) better inhibits neurogenic and inflammatory pain responses that aqueous extract of *C. odorata* (800 mg/kg). These effects are lower than those observed with the tramadol without naloxone (figures 8 and 10).

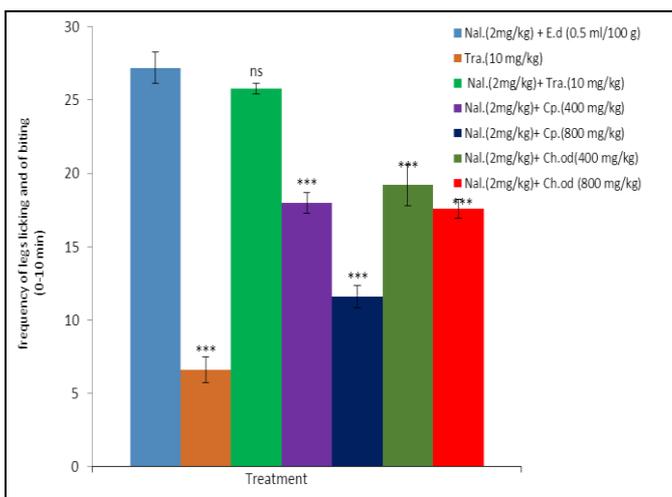


Fig 7: Effect of aqueous extract of C.p and Ch. On neurogenic pain response. Each value represents the mean \pm ESM; *** $p < 0.01$; (Student t-test), versus control group (E.d). ns= $p > 0.05$ E.d= distilled water; C.p= *C. pentandra*; Ch.od= *C. odorata*; Nal=naloxone

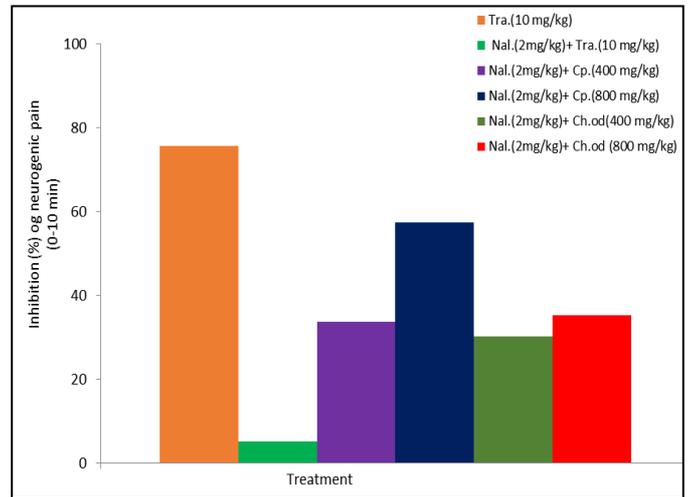


Fig 8: Analgesic effect of aqueous extract of C.p and Ch.od on neurogenic pain response in rat. C.p= *C. pentandra*; Ch.od= *C. odorata*; Nal= naloxone; Tra=tramadol

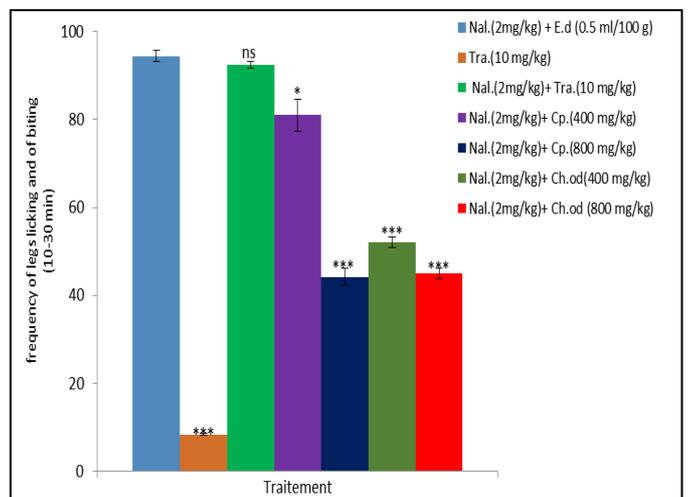


Fig 9: Effect of aqueous extract of C.p and Ch. On inflammatory pain response. Each value represents the mean \pm ESM; *** $p < 0.01$; (Student t-test), versus control group (E.d). ns= $p > 0.05$ E.d= distilled water; C.p= *C. pentandra*; Ch.od= *C. odorata*; Nal=naloxone; Tra=tramadol

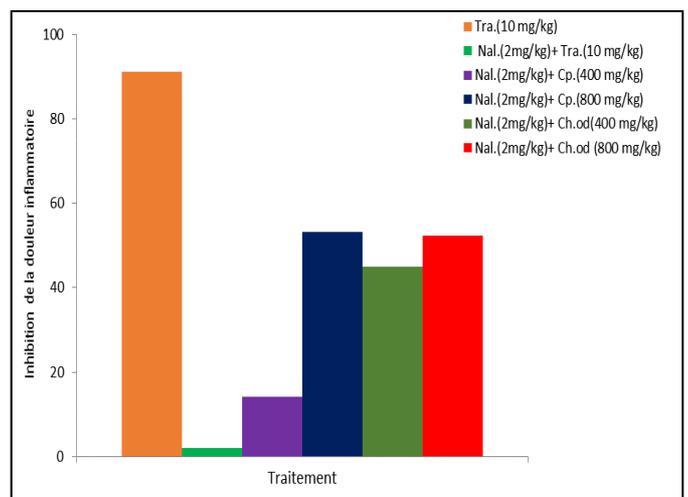


Fig 10: Analgesic effect of aqueous extract of C.p and Ch.od on Inflammatory pain response in rat. C.p= *C. pentandra*; Ch.od= *C. odorata*; Nal=naloxone; Tra=tramadol

4. Discussion

The edema was induced by histamine, serotonin and the arachidonic acid. The results obtained showed an inhibiting effect of the two aqueous extracts on the evolution of edema. Indeed, the edema is could be explaining by the vascular flight of plasmatic proteins following a modification of the organization of the wall venous under histamine and the serotonin influence which are two vasoactives amines. In addition, it is known that the prostaglandins which are derivatives of the arachidonic acid metabolism produce arteriole vasodilatation and contraction of the endothelial cells of the venous in the acute phase of the inflammation. These prostaglandins can be produced under the influence of the vasoactives amines ^[26]. The fact that the two aqueous extracts inhibit the inflammation induced by histamine, serotonin and the acid arachidonic suggest that they could interfere with their inflammatory mechanisms. The previously phytochimic studies of *C. pentandra* and of *C. odorata* revealed the presence of the flavonoïds, of saponosids, terpenoids and tannins for *C. pentandra* ^[8]; saponosids, alkaloids, heterosids cardiotonics, steroids/terpenoids *C. odorata* ^[27]. This anti-inflammatory effect could be explained by the presence of the flavonoïds in two extracts ^[28].

The pain induced by formaldehyde is biphasic. The first phase, the neurogenic response is explaining by the direct stimulation of the fibers C and A δ by the nociceptive agent, and the second phase, inflammatory response by the stimulation of these same fibers by histamine and serotonin after the substance P release (central neurotransmitter) ^[29]. The results obtained showed an inhibiting effect of the pain of the two extracts on neurogenic and inflammatory pain responses 15 minutes after administration of the naloxone. The tramadol used as standard drug is without effect in the presence of the naloxone. The naloxone is a competitive antagonist of opioids at the central level, which justifies his absence analgesic effect of tramadol which are a weak opioids. Indeed, the major part of the nervous cells reacts to the opioids by a hyperpolarization (increase of potassium permeability). The calcium impulse which occurs in the nervous cell during an excitation is decreased, reducing in fact the release of exiting neurotransmitters and the synaptic transmission ^[30]. However the transmission of the painful stimulus at the central level passes by the release in the synaptic space of peptides (substance P, Neurokinines A, CGRP, somatostatin, CCK, VIP) and the dorsal spinal cord level by the release of the exiting amino acids (glutamate, aspartate). The substance P and the glutamate seem to play a significant role in the transmission of the nervous message ^[20]. It is also known that the substance P takes part in the neurogenic inflammation by inducing a vasodilation and by stimulating the release of the histamine by the mastocytes which goes stimulating primary peripheral fibers ^[28]. The fact that these two aqueous extracts are opposed to this type of pain in the pretreated rats with the naloxone suggests that they could act differently than the tramadol. This effect could be explained by the presence of the flavonoïds and alkaloids already identified in these plants ^[31, 25].

5. Conclusion

C. pentandra and *C. odorata* are two plants largely used in traditional medicine in Congo. Their possible mechanisms of effects were evaluated on the inflammation induced by histamine, serotonin, and the arachidonic acid and counter the pain induced by formaldehyde. It comes out from this study that the anti-inflammatory drug effect of the aqueous extracts of the stem barks of *C. pentandra* and from the leaves of *C.*

odorata at the doses of 400 and 800 mg/kg would pass by an interference with the inflammatory mechanism of histamine, arachidonic acid serotonin. The analgesic effect of the aqueous extracts of the stem barks of *C. pentandra* and of the leaves of *C. odorata* to at the doses of 400 and 800 mg/kg seems to be not opioids mechanism.

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