

Full Length Research Paper

Anti-inflammatory and analgesic effects of leaves of *Chromolaena odorata* L. (King and Robinson)

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This study was to evaluate anti-inflammatory and analgesic effects of the aqueous extract of leaves of *Chromolaena odorata* (Asteraceae) collected in Brazzaville-Congo. Acute inflammation was induced by using the carrageenan and formaldehyde models, and chronic inflammation by the cotton pellet induced granuloma model. Analgesic effect was evaluated by using the acetic acid-induced writhing, the pressure induced by the analgesymeter as well as the pain induced by formaldehyde. The results obtained show that aqueous extract (400 and 800 mg/kg) inhibits the edema induced by the carrageenan and formaldehyde. Moreover, this extract at the doses used (400 and 800 mg/kg) significantly inhibits granuloma fabric induced by cotton pellet. In addition, aqueous extract (400 and 800 mg/kg) inhibits significantly the pain induced by the three methods used. In conclusion, aqueous extract of *C. odorata* has anti-inflammatory and analgesic effects. These observations justify the traditional use of this plant in the treatment of inflammatory pathologies and the pain.

Key words: *Chromolaena odorata*, anti-inflammatory, analgesic.

INTRODUCTION

In Africa, therapeutic properties of the medicinal plants had been known for several years. However, few medicinal plants were studied scientifically at the

moment. In Africa, about 80% of the populations use them in the treatment of several pathologies (Etchike et al., 2011; Epa et al., 2015). In spite of the development

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of synthetic drugs, the vegetable drugs in his various forms occupy a special place. Moreover, WHO recognize the traditional medicine like one of the essential elements of primary care of health: «*save the plants which save the life*» such is a WHO slogan which summarizes his strategy in this domain (WHO, 2014). So, to find a simple methodology of evaluation of effectiveness and security of medicinal plants is significant. Vegetable plant in these different origins contributes to the discovery of new drug used in the treatment of several inflammatory and analgesic pathologies (Abena et al., 1996). Inflammation is one reactional phenomenon operated by the body every time the integrity of its morphological and biological constants is threatened. Inflammation is not synonymic of an infection, but the infection can be the cause of an inflammation (Bokia, 2016). It can present diverse symptoms such as the edema, the pain and the heat or fever. Inflammation is always accompanied by pain, so the evaluation of the pain treatment constitutes a real major stake in public health. So, searching new anti-inflammatory and analgesic drugs is necessary. *Chromolaena odorata* is a medicinal plant used in Congolese traditional medicine. The juice of the leaves is used to cure the wound (Bissangou and Ouamba, 1997). The previous studies undertaken in other countries give it some pharmacological properties such as: analgesic, anti-inflammatory and antipyretic (Owoyele et al., 2008), antibacterial (Etchike et al., 2011; Agban et al., 2013), anti-oxidative (Kavitha et al., 2013) and antifongic (Kra et al., 2009). This is the reason why anti-inflammatory and analgesic effects of leaves of *C. odorata* (Asteraceae) collected in Brazzaville-Congo in this study were investigated..

MATERIALS AND METHODS

Plant

The leaves of *C. odorata* were collected in Brazzaville (February, 2014). Botanical identification of the plant material was done by Dr. A. Mousamboté, botanist systematist at the Institute of Rural Development and confirmed at the herbarium where the samples of *C. odorata* were compared with the reference samples (number 1183/07/1965). After identification, the plant material was dried and pulverized. 250 g of powder of leaves were mixed with 2500 ml of distilled water in a heating balloon. The mixture was boiled for 15 min. After cooling and filtration, the filtrate obtained was concentrated on a double boiler (60°C). The aqueous extract obtained was kept for the experiments.

Animals

Albino rats (150 to 200 g) and albino mice (20 to 30 g) of either sex obtained from the Faculty of Science and Technology 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 h light and 12 h dark) and at the temperature of $27 \pm 1^\circ\text{C}$. The rules of ethics published by the International Association for the Study of Pain (Zimmermann, 1983) have been considered.

Evaluation of anti-inflammatory effect of aqueous extract of *C. odorata*

Carrageenan induced inflammation

Method described by Elion Itou et al. (2014) was used. The animals were divided into groups of 5 rats each. Different doses of aqueous extract 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, 1 h prior to the local injection of carrageenan (0.05 ml, 1%) into the plantar aponeurosis. Edema was measured by using pletysmometer (Ugo Basile, Italy) at 1/2, 1, 2, 3, 4, 5, 6 and 24th h. The anti-inflammatory effect is evaluated by the inhibition of edema (Elion Itou et al., 2014).

Formaldehyde induced paw inflammation

The rat paw edema was induced with formaldehyde 2.5% (0.2 ml/rat). The animals were divided into groups of 5 rats each. Different doses of aqueous extract 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 h prior to the local injection of formaldehyde into the plantar aponeurosis. Edema was measured by using pletysmometer (Ugo Basile 7140, Italy) at 1, 2, 3 and 4th hour after formaldehyde administration (Mbiantcha et al., 2010). The anti-inflammatory effect is evaluated by calculating the inhibition of edema.

Cotton pellet induced chronic inflammation

The effect of aqueous extract of *C. odorata* on granuloma formation was studied according to the method described by Elion Itou et al. (2014). 100 mg of cotton pellet were sterilized at 60°C during 24 h and placed interscapular region of rat after ether anesthesia and incision. Distilled water (control group), diclofenac (standard drug) and aqueous extract of *C. odorata* (400 and 800 mg/kg) were administered orally during seven (7) days. On the eighth day, the cotton pellet was removed, cleared of from all adhering tissue and dried at 60°C for 24 h and weighed. The anti-inflammatory effect was given by the inhibition (I) of the granuloma formation (Elion Itou et al., 2014):

$$I = (B - A) / A \times 100$$

where *A* = weight of cotton pellet before implantation (100 mg); *B* = weight of dried cotton pellet after implantation.

Evaluation of analgesic effect of aqueous extract of *C. odorata*

Acetic acid-induced abdominal writhing in mice

The pain was induced in the mice by using 0.6% acetic acid solution (Sawadogo et al., 2011; Elion Itou et al., 2014). The animals were divided into groups of 6 mice each. Different doses of aqueous extract of *C. odorata* (400 and 800 mg/kg), paracetamol (standard drug, 50 mg/kg) and distilled water (control group, 0.5 ml/100 g) were administered orally to groups, 1 h prior to the local injection of acetic acid (10 ml/kg, IP). 5 min after acetic acid injection, the number of abdominal writhing made by each mouse was recorded during 20 min. The analgesic effect was given by the inhibition (I) of the abdominal writhing (Elion Itou et al., 2014).

Analgesymeter test

The nociceptive response to pressure was measured as described

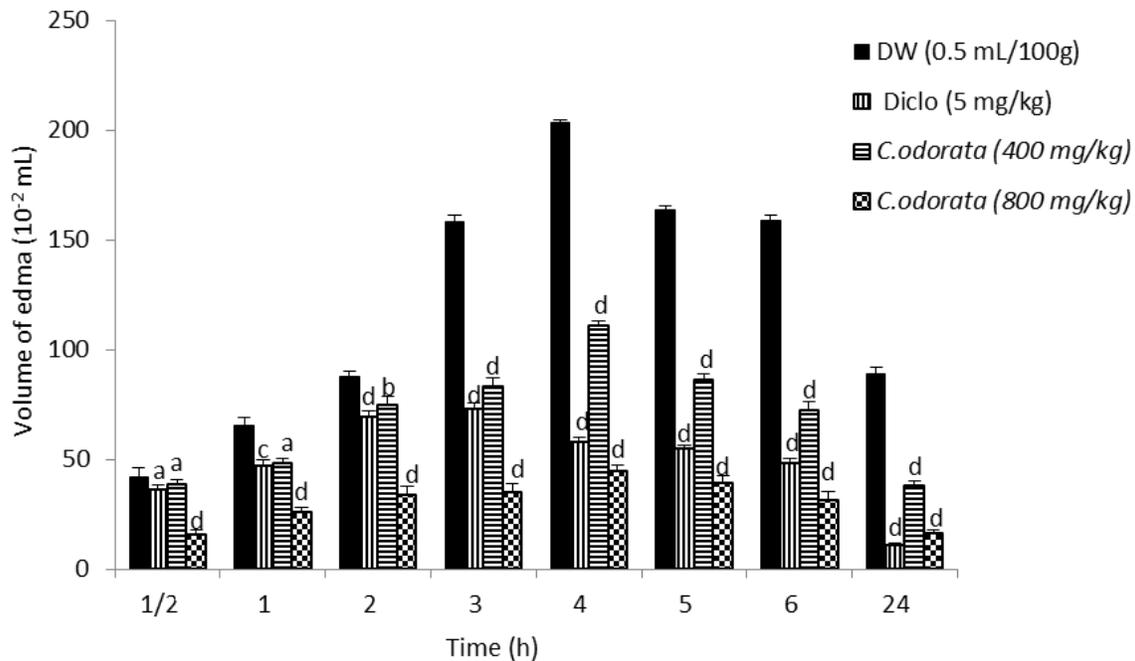


Figure 1. Effect of the aqueous extract of *Chromolaena odorata* on edema induced by carrageenan in rat. Each value represents the mean \pm ESM; a= $p>0.05$; b= $*p<0.05$; c= $**p<0.01$; d= $***p<0.001$ (Student t-test), versus control group. DW: Distilled water, Diclo: diclofenac, *C. odorata*: *Chromolaena odorata*.

by Mbiantcha et al. (2010) using an analgesimeter (Ugo Basile, Italy). This instrument generates a linearly increasing mechanical force or pressure on the dorsal surface on the rat's hind paw. The nociceptive threshold was the point where the animal withdraws its paw. The animals were divided into groups of 6 rats each. Different doses of aqueous extract of *C. odorata* (400 and 800 mg/kg), paracetamol (standard drug, 50 mg/kg) and distilled water (control group, 0.5 ml/100 g) were administered orally to groups 1 h before the measure of nociceptive threshold. After determination of the nociceptive threshold, the reaction time was calculated (Elion Itou et al., 2014).

Formaldehyde-induced paw licking

The formaldehyde-induced paw licking was studied in rat using the method described by Freitas et al. (2013) and Sudo et al. (2015). The animals were divided into groups of 5 rats each. Different doses of aqueous extract 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 h prior to the local injection of formaldehyde subcutaneous tissue of the plantar surface of the right paw. 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 to 10 min for neurogenic pain response and 10 to 30 min for inflammatory pain response. The analgesic effect was given by the inhibition of the pain.

Statistical analysis

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

RESULTS

Anti-inflammatory effect of aqueous extract of *C. odorata*

Effect on carrageenan edema

Subplantar injection of the carrageenan produced an inflammatory edema which increased gradually with a maximum between at the 4th hour after injection (Figure 1). Aqueous extract of *C. odorata* (400 and 800 mg/kg) induced significant ($p<0.05$, $p<0.01$ and $p<0.001$) anti-inflammatory effect that increased gradually and reached a maximum (56.98 and 81.75%) at the 24th hour, respectively (Figure 2). Diclofenac (5 mg/kg) shows anti-inflammatory effect ($p<0.01$ and $p<0.001$) with a maximum (80.07%) at the 6th hour (Figures 1 and 2).

Effect on formaldehyde edema

The results are shown in Table 1. Aqueous extract of *C. odorata* (400 and 800 mg/kg) has no reduced the edema evolution at the 1 and 4th hour ($p>0.05$). However, aqueous extract (400 and 800 mg/kg) induced significant ($p<0.05$, $p<0.01$ and $p<0.001$) anti-inflammatory effect that increased between 2 and 3th hour after the injection of carrageenan. Tramadol (10 mg/kg) shows anti-inflammatory effect ($p<0.001$) and was maintained all along the experiment with a maximum of 70.03% at the

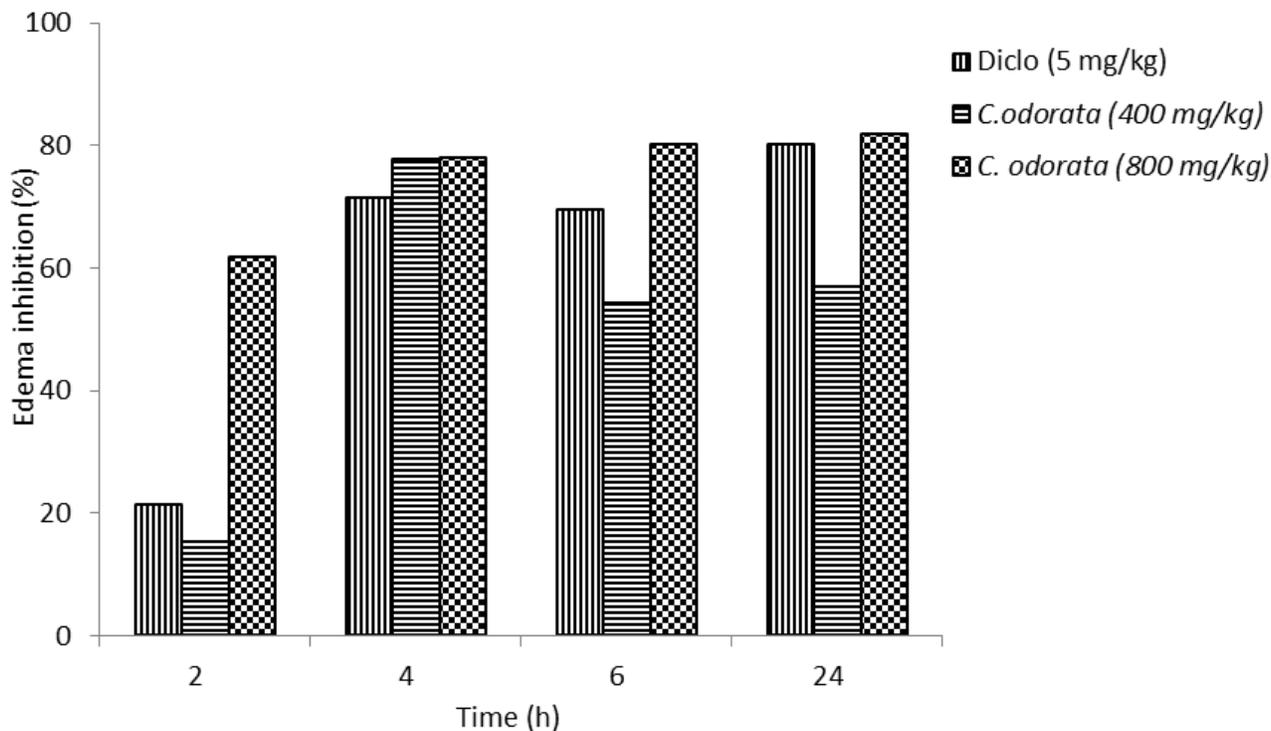


Figure 2. Anti-inflammatory effect of the aqueous extract of *C. odorata* on edema induced by carrageenan in rat. Diclo: diclofenac, *C. odorata*: *Chromolaena odorata*.

Table 1. Effect of the aqueous extract of *Chromolaena odorata* on edema evolution induced by formaldehyde 2.5 %.

Parameter	Doses	Edema volume (10^{-2} ml)				Inhibition (%)	
		1 h	2 h	3 h	4 h	2 h	4 h
Control group	0.5 ml/100 g	174.2± 1.74	218.2± 4.56	263.6± 2.83	280±2.21	-	-
Tramadol	10 mg/kg	73± 6.26***	83.6± 3.90***	79 ±5.20***	69.4± 2.58***	61.68	75.21
<i>Chromolaena odorata</i>	400 mg/kg	173± 1.81 ^{ns}	206.2±1.01 *	249.8± 1.56**	278.8± 2.78 ^{ns}	5.49	0.42
	800 mg/kg	160± 6.68 ^{ns}	204.8± 1.93*	245.6±1.80***	272.8± 3.12 ^{ns}	6.14	2.57

Each value represents the mean ± ESM; ***p<0.001 (Student t-test), versus control group.

4th hour after the injection of carrageenan.

Effect on cotton pellet granuloma

The effect of the aqueous extract of *C. odorata* (400 and 800 mg/kg) as well as diclofenac on the granuloma is shown in Table 2. They show that the aqueous extract of *C. odorata* (400 and 800 mg/kg) as well as diclofenac induced significant ($p<0.001$) the formation of cotton pellet granuloma compared to the control group. Diclofenac and aqueous extract (800 mg/kg) better inhibited the formation of cotton pellet granuloma than

aqueous extract (400 mg/kg) with a maximum of 49.59 and 49.84%, respectively against 19.38% for aqueous extract (400 mg/kg).

Analgesic effect of aqueous extract of *C. odorata*

Effect on the pain induced by the acetic acid 0.6%

The results of the effect of the aqueous extract of *C. odorata* on abdominal cramps are shown in Table 3. Aqueous extract of *C. odorata* at doses used significantly reduced ($p<0.001$) the number of abdominal writhing

Table 2. Effect of aqueous extract of *Chromolaena odorata* on granuloma weight.

Parameter	Doses	Granuloma weight (mg)	Inhibition (%)
Control group	0.5 ml/100 g	95.48±1.74	-
Diclofenac	5 mg/kg	48.13±1.38***	49.59
<i>Chromolaena odorata</i>	400 mg/kg	76.97±1.24***	19.38
	800 mg/kg	47.89±2.14***	49.84

Each value represents the mean ± ESM; ^{ns}p>0.05; *p<0.05; **p<0.01; ***p<0.001 (Student t-test), versus control group.

Table 3. Effect of aqueous extract of *Chromolaena odorata* on abdominal writhes induced by 0.6 % acetic acid solution in mice

Parameter	Doses	Number of abdominal writhes	Inhibition (%)
Control group	0.5 ml/100 g	106.66±2.01	-
Paracetamol	50 mg/kg	100.16±1.01**	6.09
<i>Chromolaena odorata</i>	400 mg/kg	74.40±5.23***	30.24
	800 mg/kg	70.76±4.52***	33.65

p<0.01; *p<0.001 (Student t-test). Each value represents the mean ± ESM; versus control group .

compared to the control group. The effect observed with the aqueous extract at doses used is definitely better than paracetamol used as standard drug with a difference of inhibition of 24.15% at the dose of 400 mg/kg and 27.56% at the dose of 800 mg/kg of the aqueous extract of *C. odorata*.

Effect on the pain induced by the analgesimeter

The effect of the aqueous extract of *C. odorata* on the pain induced by the analgesimeter is as shown in Figure 3. It shows that the aqueous extract at doses used as well as paracetamol (used as standard drug) significantly increases (p<0.001) the reaction time of the animals against the pain compared to the control group. It is also noted that the effect obtained at the dose of 800 mg/kg of the aqueous extract is more significant than that obtained with the aqueous extract at 400 mg/kg and paracetamol.

Effect on the pain induced by formaldehyde in rat

The effect on the pain induced by formaldehyde 2.5% in neurogenic and inflammatory pain responses are presented in Table 4. On neurogenic and inflammatory pain responses, the results obtained show that the aqueous extract of *C. odorata* as well as tramadol

significantly reduce (p<0.001) the frequency of licking and biting the legs of the animals compared to the control group. In addition, the tramadol and aqueous extract (800 mg/kg) inhibits better the animals against neurogenic and inflammatory pain (Table 4).

DISCUSSION

Inflammation was evaluated by using the method of the acute inflammation induced by carrageenan and formaldehyde as well as the chronic inflammation induced by cotton pellet. Carrageenan is a mucopolysaccharide administered under plantar in rats which causes an acute inflammation which is traduced by edema (Elion Itou, 2010). Edema induced by the carrageenan is biphasic. First phase (1 h) is mediated by the inflammatory mediators (serotonin and histamine) and the second phase (2 h after carrageenan administration) is mediated by prostaglandins, products of cyclooxygenase (COX) (Perianayagam et al., 2006; Sudipta et al., 2011). Aqueous extract of *C. odorata* (400 and 800 mg/kg) inhibits the evolution of edema with a maximum of inhibition of 4 h after administration of the carrageenan compared to the control group. Moreover, aqueous extract at doses used is also opposed to acute inflammation induced by formaldehyde in rat. In addition, aqueous extract of *C. odorata* (400 and 800 mg/kg)

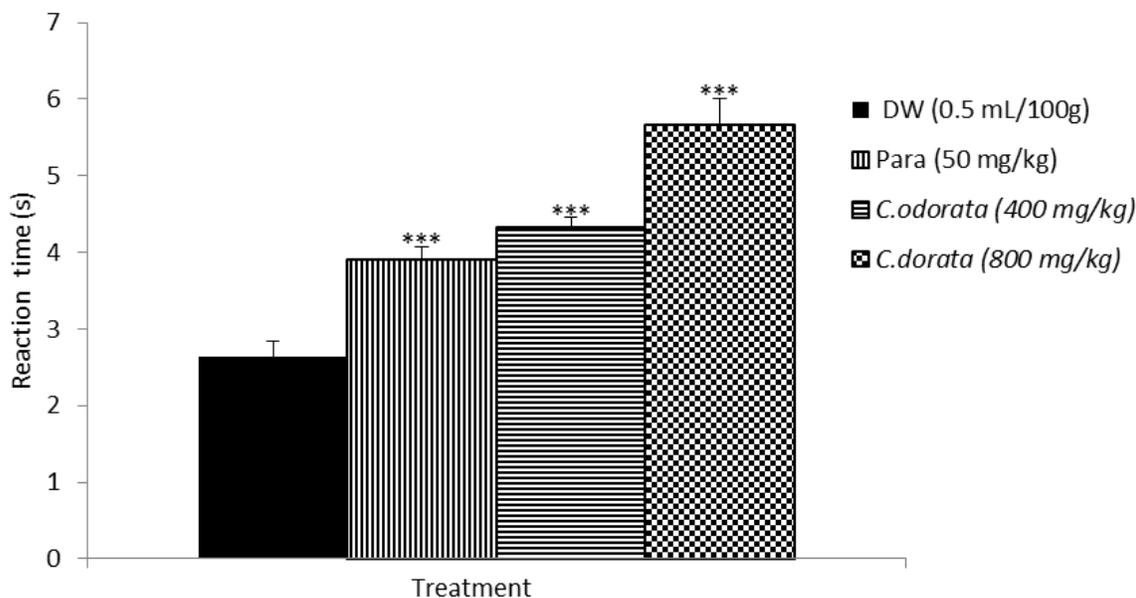


Figure 3. Analgesic effect of aqueous extract of *C. odorata* on pain induced by analgesymeter in rat. *** $p < 0.001$; (Student t-test). Each value represents the mean \pm ESM; versus control group. DW: Distilled water, para: paracetamol, *C. odorata*: *Chromolaena odorata*.

Table 4. Effect of aqueous extract of *Chromolaena odorata* on neurogenic and inflammatory pain response induced by formaldehyde in rat.

Parameter	Doses	Neurogenic pain response		Inflammatory pain response	
		Frequency of legs licking and of biting (0-10 min)	Inhibition (%)	Frequency of legs licking and of biting (10-30 min)	Inhibition (%)
Control group	0.5 ml/100 g	26 \pm 0.63	-	61 \pm 4.27	-
Tramadol	10 mg/kg	5.8 \pm 0.91***	77.69	7.2 \pm 0.27***	88.19
<i>Chromolaena odorata</i>	400 mg/kg	17 \pm 1.04***	34.61	35.6 \pm 0.73***	41.63
	800 mg/kg	8.8 \pm 0.58***	66.15	22.8 \pm 2.35***	62.62

*** $p < 0.001$; (Student t-test). Each value represents the mean \pm ESM; versus control group.

inhibits the formation of granuloma fabric compared to the reference group. These results suggest that the aqueous extract of *C. odorata* (400 and 800 mg/kg) has an anti-inflammatory effect. The inflammation can appear by various symptoms such as the edema (tumefaction or tumour), pain and heat or the fever. It is always accompanied by pain. This is why the evaluation of the analgesic effect is necessary. The analgesic effect of the aqueous extract was evaluated at the peripheral and central level. At the peripheral level, aqueous extract of *C. odorata* (400 and 800 mg/kg) showed an inhibiting effect on the pain induced by acetic acid and the analgesymeter, at the central level; it is opposed to the pain induced by formaldehyde like tramadol (standard drug). These results suggest that aqueous extract of *C.*

odorata (400 and 800 mg/kg) has an analgesic effect. The phytochemical study of the extract revealed the presence of saponins, alkaloids, cardiotonic-heterosids, steroids and terpenoids as well as tannins (Bokia, 2016). The presence of the flavonoïds could explain the anti-inflammatory effect (Bagli et al., 2004; O'Leary et al., 2004), flavonoïds and alkaloids would explain the analgesic effect (Borgi et al., 2007; Sudo et al., 2015).

Conclusion

This study shows that the aqueous extract *C. odorata* has anti-inflammatory and analgesic effects. This justifies the traditional use of this plant in the treatment of the

inflammation and the pain. This study deserves to be completed in order to clarify the mechanisms of action.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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