

Anti-Inflammatory and Central Analgesic Effect of the Aqueous Extract of the Leaves of *Vitex Madiensis* Oliv (Lamiaceae-Viticoideae)

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DOI: [10.36348/sijtcm.2020.v03i01.002](https://doi.org/10.36348/sijtcm.2020.v03i01.002)

Received: 03.01.2020 | Accepted: 10.01.2020 | Published: 27.01.2020

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Abstract

Vitex madiensis Oliv is a plant of the family of the Lamaceae-Viticoideae used in traditional medicine in Congo against: dysenteriform diarrheas, barrenness of the women, madness, otitis, tooth decay, oral affections, malaria, inflammation of ganglia and stiffness. The aqueous extract of leaves of *Vitex madiensis* oliv. was the subject of an investigating by the carraghenine and the histamine induced inflammation, and on the central pain of thermal origin at the doses of 50, 100, 200 and 400 mg/kg per os. A significant anti-inflammatory effect (**p < 0,001) at the dose of 400 mg/kg from the thirteenth hour and at the dose of 200 mg/kg from 3 hours was observed. A significant (**p < 0,001) analgesic effect was observed at the dose of 400 mg/kg from 30 minutes. These effects are due to the presence of the numerous metabolites contained in this extract.

Keywords: *Vitex madiensis*, aqueous extract, anti-inflammatory, analgesic.

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INTRODUCTION

In Congo, the traditional medicine is a non-negligible remedy for the population. Also, to improve its performance, the Congolese government has launched a promotion strategy aimed at integrating it into the health system through the development of medicinal plants [1]. This strategy is motivated on the one hand by the richness of the culture and the traditions on the use of the medicinal plants and on the other hand by the richness and the diversity of the flora.

Indeed, the flora of the Republic of Congo includes many medicinal plants with therapeutic properties, which can remedy multiple pathological disorders including those related to inflammation [2, 13, 4, 5-8]. It is in this context that we wanted to contribute to the development of plants with anti-inflammatory and analgesic effects used in traditional Congolese medicine to fight against pain and inflammation. The genus *vitex* is reported to be rich in medicinal virtues; it is used as food, for the treatment of post-delivery bleeding, treatment of premenstrual and gynecologic affections, sexual enhancer, to control the foul odor of external genitalia, gastrointestinal disorders, as anthelmintic, astringent, stomachic, bacterial infections,

pain, inflammation, diarrhea, fever, as a tonic, cramps, malaria, convulsion, insect repellent, against stings of venomous animals and in wound healing [9-12].

Aqueous extract of *V. glabrata* is reported to have Human Immunodeficiency Virus-1 (HIV-1) reverse transcriptase inhibitory activity. *Vitex agnus-castus* L has been used to treat pain, swelling, inflammation, headaches, rheumatism, and sexual dysfunction [13]. *Vitex mollis*, *Vitex pyramidata*, *Vitex pubescens*, *Vitex agnuscastus* and *Vitex gaumeri* are reported to possess antidiarrheal, analgesic, anti-inflammatory, anti-tumor activities [12].

Vitex madiensis is used in the Congo to treat dysenteriforme diarrhea, female sterility, insanity, ear infections, fever, pain, dental caries, oral diseases, malaria, inflammation of the ganglions and body aches [14, 15]. Thus, in order to provide a scientific basis for the use of *Vitex madiensis* Oliv. Against pain and inflammation in traditional medicine, this study aims to evaluate anti-inflammatory and analgesic effects of the aqueous extract of leaves of *Vitex madiensis* Oliv on the pain of central type.

MATERIAL AND METHODS

Plant material

The assessed plant material consists mainly of *Vitex madiensis* Oliv leaves collected at Makana village (pool department), in April 2017. Plant species was identified in the Congo National Herbarium by botanists from the Botany Laboratory of the Institut National de Recherche en Sciences Exactes et Naturelles (IRSEN) of Brazzaville-Congo. The sample was dried at room temperature in the Laboratoire de Physiologie et Physiopathologie Animales of Faculté des Sciences et Techniques of Université Marien NGOUABI of Brazzaville-Congo. The dry leaves were pulverized and the resulting powder was used to prepare the extract.

Animal material

The treated animals are male and female, Wistar rats weighing between 175 and 200 g, and mice weighing between 18 and 20 g, raised at the Faculté des Sciences et Techniques (FST) animalaria under standard conditions (25 ± 5 ° C, 40-70 RH), with a diurnal cycle of 12 hours of light and 12 hours of darkness. These rats had free access to water and a standard food. The ethical rules of animal experiments published by the International Association for the Study of Pain was respected [16, 17].

METHODS

Preparation of extracts

The aqueous extract was prepared by the decoction method. 50 g of *Vitex madiensis* leaves powder were placed in 500 mL of distilled water and boiling at 100 ° C during 30 minutes, then filtered with cotton, concentrated at reduced pressure at 50-60 ° C and kept at 4 ° C. The dry extract obtained was used to prepare the various test solutions.

Anti-inflammatory activity

In order to evaluate the anti-inflammatory effect of the aqueous extract of *Vitex madiensis* leaves, a model of acute inflammation was produced in vivo in male rats weighing between 100 and 250 g, using the carrageenan and histamine. Animals are deprived of food (but no water) for 18 hours before the test.

Acute inflammation induced by the carrageenan

The injection of 0.05ml of 1% carrageenan into the plantar region of the right hind paw of the rat induced edema 30 minutes later, acute inflammation is observed by increased the diameter of paw [18]. This edema can be reduced by treatment with anti-inflammatory substance.

Thirty (30) rats were selected and divided into six (6) groups of five (5) rats:

- group1 (control): treated with distilled water 10 mL / kg/rat per os;
- group 2 (reference): treated with diclofenac (50 mg) at a dose of 5 mg / kg/rat per os;

- group 3, 4, 5, 6 (test groups): treated with 50, 100, 200 and 400 mg / kg/rat per os of the aqueous extract of *Vitex madiensis* Oliv. An initial measurement (d0) of the paw is taken in each animal before induction of edema.

One (1) hour after the oral administration of the different substances, 0.05 ml of a fresh carrageenin suspension (1% carrageenin in 0.9% NaCl) was injected to all animals subcutaneously at the fascia of the right hind paw (PPD).

After the injection of the oedematogenic agent, the evolution of the edema is controlled by measuring the diameter (dt) of the PPD at 1 / 2h, 1h, 2h, 3h, 4h and 24h using a caliper. The percentages of inhibition of the edema (PINH) are calculated according to the following formula:

$$PINH = M (dt - d0)$$

dt: diameter of the paw after induction of inflammation at time t;

d0: diameter of the paw before induction of inflammation;

M: average.

Acute inflammation induced by the histamine [24]

Indeed, histamine is a polysaccharide that injected into an animal, causes 30 minutes after acute inflammation during which the volume of the paw increases with time. An anti-inflammatory substance inhibits this increase compared to the control that received distilled water. The induction of inflammatory edema on the rat's paw with histamine was carried out as follows:

Twenty (20) animals were selected, divided into four (4) groups of 5 rats and treated as follows:

- groupe 1 (control): treated with distilled water 10 mL / kg/rat per.os;
- groupe 2 (reference): treated with diclofenac at a dose of 5 mg / kg/rat per os;
- groupe 3 (test group): treated with the aqueous extract of *Vitex madiensis* Oliv at 400 mg / kg/rat per os;
- groupe 3 (test group): treated with the aqueous extract of *Vitex madiensis* Oliv at 200 mg / kg/rat per os;

An initial measurement (d0) of the paw is taken in each animal before the induction of edema.

One (1) hour after the oral administration of the various substances, 0.05 ml of a fresh suspension of histamine was injected into all the animals by subcutaneous route at the level of the fascia of the right hind paw (PPD).

After the injection of the edematogenic agent, the development of the edema is monitored by measuring the diameter (dt) of the (PPD) at 1 / 2h, 1h, 2h, 3h, 4h and 24h using a caliper.

Analgesic activity: tail immersion test in hot water

We modified the method firstly described by D'Amour & Smith [20]. This test involves to induce pain by immersing the tail of the animal in a bath of hot water at a temperature of 57 ± 2 ° C. The animals are previously treated orally by different test products, 30 minutes after the administration of the products, they were kept in a vertical position to immerse its tail with a depth of 5 cm in hot water maintained at 57 ± 2 ° C. The reaction time (in seconds) that the animal takes to remove its tail from water was noted 30, 60, 90 and 120 minutes after administration of product. The maximum time that the animal must pass in hot water during this test is 30 seconds. Exceed this time the animal is not taken into account. The pain sensation is characterized by the rapid withdrawal of the tail of the animal. An analgesic would act by increasing the reaction time of the animal. Five (5) lots of five (5) mice each were made. The mice were treated as follows:

- the 1st group (control) received 10 mL / kg of distilled water per mouse;
- the 2nd group received 100 mg / kg of tramadol, used as reference molecule per mouse;

- the 3rd group received 200 mg / kg of the aqueous extract of the roots of *Nauclea latifolia* leaves per mouse;
- the 4th group received 400 mg / kg of the aqueous root extract of *Nauclea latifolia* leaves per mouse;
- the 5th group received 800 mg / kg of aqueous extract of *Nauclea latifolia* leaves per mouse;

STATISTICAL ANALYSIS

The results we obtained, are expressed as an average \pm ES for a number of 5 animals per group using the Microsoft Excel Windows 13 software. The results obtained in the test groups were compared to the negative control group using the t test. Student and significance was established with probabilities * $p < 0.5$; ** $p < 0.01$ and *** $p < 0.001$.

RESULTS

Anti-inflammatory effect

Inflammation induced by the carrageenan

The aqueous extract of *Vitex madiensis* showed a very significant anti-inflammatory effect at the dose of 400 mg / kg per os observable from 1 hour until the end of the experiment after 24 hours (Figure 1).

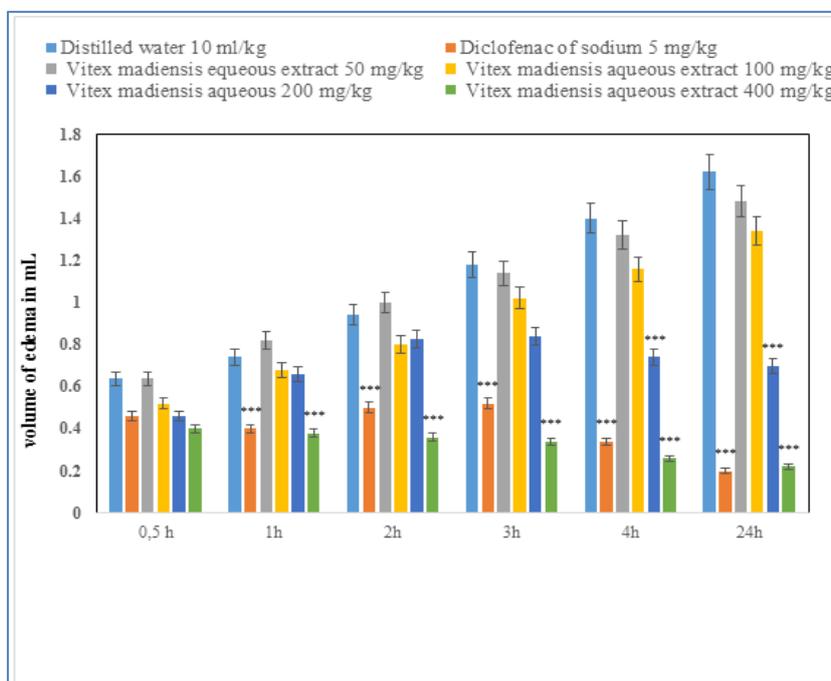


Fig-1: *Vitex madiensis* aqueous extract antiinflammatory activity on carrageenan-induced rat paw edema, results expressed by mean \pm SE, *P < 0.001, n = 5**

Inflammation induced by histamine

The aqueous extract of *Vitex madiensis* has an antinflammatory effect at 400 mg / kg per os very

significant (***) $p < 0.001$) on the histamine-induced inflammation observed after 2h (Figure 3).

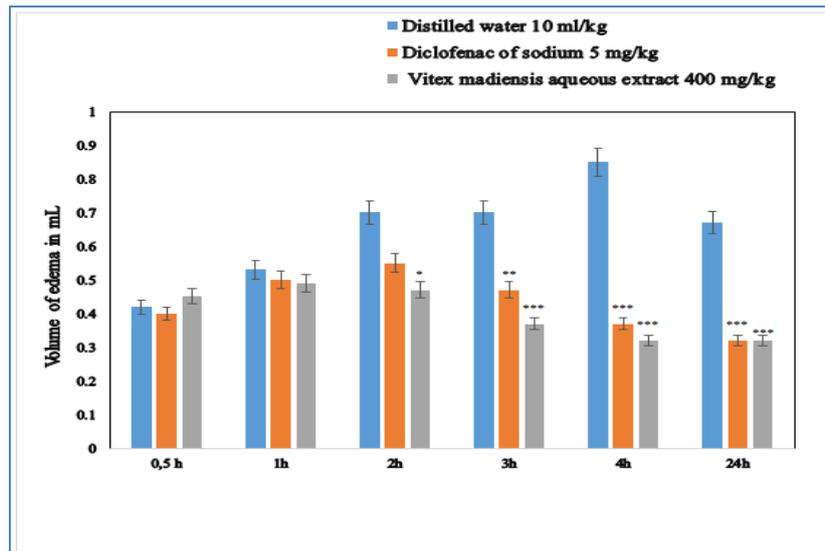


Fig-2: *Vitex madiensis* aqueous extract antiinflammatory activity on histamine-induced rat paw edema, results expressed by mean \pm SE, * $P < 0.05$, *** $P < 0.001$, $n = 5$

Analgesic effect

The aqueous extract of *Vitex madiensis* showed a very significant central type analgesic effect

(*** $p < 0.001$) at a dose of 400 mg / kg per os remarkable after 0.5h up to 2h (Figure 3).

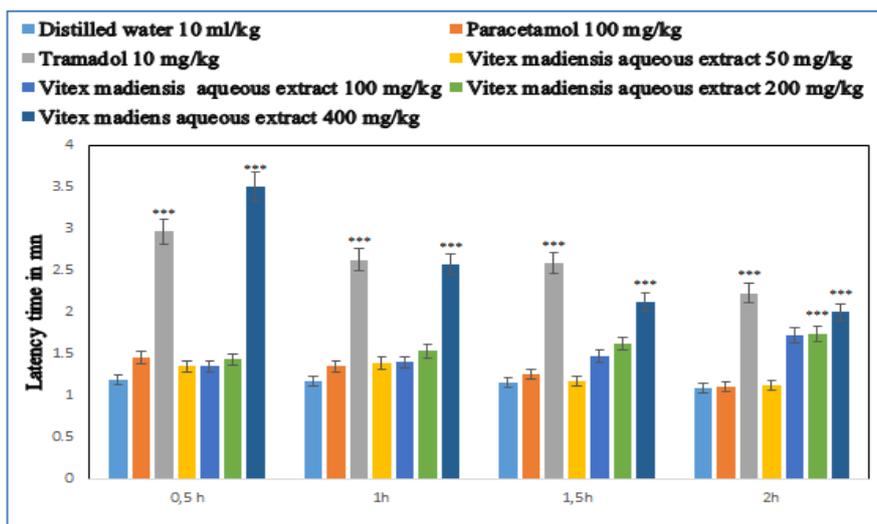


Fig-3: Analgesic activity of *Vitex madiensis* aqueous extract on tail flick method, results expressed by mean \pm SE, *** $P < 0.001$, $n = 5$

DISCUSSION

The purpose of this work was to evaluate the anti-inflammatory and analgesic effect of *Vitex madiensis* Oliv leaves aqueous extract on the pain of central type. An earlier study by Ngoka *et al.* [15] has demonstrated an analgesic effect on the pain of peripheral type. On this work we have demonstrated the anti-inflammatory activity and confirmed the analgesic activity on the pain of central type.

Carrageenan is a polysaccharide that induces local inflammation characterized by increased vascular permeability, edema and extravasation of neutrophils. Its action is done in two: the first phase (1/2 to 1 hour) involves the release of amino-vasoactive mediators (histamine, serotonin) and the second phase (1 hour

later) is mediated by prostaglandins. Carrageenin edema is an excellent, reliable model of acute inflammation [21-23]. The thermal test is well recognized for the evaluation of an analgesic activity having a central mechanism of action. The result obtained shows a very significant anti-inflammatory effect at a dose of 400 mg / kg. This extract inhibits the evolution of edema until the last phase of carrageenan-induced inflammation. This result is in agreement with those found by Iwueke *et al.* [24] which showed an anti-inflammatory effect of *Vitex doniania* which is of the same genus with *Vitex madiensis*. This effect could be explained by the inhibition of prostaglandin release and / or the reduction of their effect, as already demonstrated with *Vitex doniania* Sweet and *Vitex negundo* Linn [25, 24, 26, 27].

However, it should be noted that the anti-inflammatory effect induced by histamine is observed after about 1 hour 30 minutes of the edema induced by carrageenin (30 min). It may be thought that our extract does not block the action of histamine. The anti-inflammatory effect would therefore be mediated via inhibition of cyclooxygenase as it was already been demonstrated with other species of the genus vitex or with other botanical families [24, 4, 15, 27]. PGs are formed due to arachidonic acid metabolism catalyzed by the cyclooxygenase (COX) enzymes in cells [28, 29, 25, 30].

An analgesic effect of central type has been highlighted. Unlike the active doses (50, 100 and 200 mg / kg) used by N'goka *et al.* [15], here the activity is remarkable at 400 mg / kg. Three (3) hypotheses can be evoked to explain this difference: the first one is not the same physiopathological mechanism of pain induction, therefore is not the same mechanism of action of the "drug"; second it is not maybe the same active principles involved in the two types of actions; and the third is that the concentration of the active ingredient in the extract would be different either for reasons of samples that would have undergone an ecological or climatic influence or for reasons of manipulation during the preparation of the extract. Nevertheless, this result is in agreement with those of our predecessors who also demonstrated a central type analgesic effect using the hotplate test and the paw pressure test [24, 27]. These effects would be due to the presence of the secondary metabolites present in this extract [15].

CONCLUSION

The aqueous extract of *Vitex madiensis* has an anti-inflammatory effect and analgesic effect on central type of pain at 200 and 400 mg/kg per os in the rat.

ACKNOWLEDGMENTS

The authors are grateful botanists from the Botany Laboratory of the Institut National de Recherche en Sciences Exactes et Naturelles (IRSEN) of Brazzaville-Congo for botanical identification of plant. We are also grateful traditional healers for availing their facilities and time for some aspects of this study.

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