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Anti-Inflammatory Activity of Hydro Alcoholic Extract of *Cladogelonium madagascariense* L. (Euphorbiaceae) in Mice

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ABSTRACT

The aim of this work is to investigate the activity of the hydroalcoholic extract of *Cladogelonium madagascariense* L. (EUPHORBIACEAE). The study was carried out on experimentally induced inflammation, in SWISS strain mice. Edema was provoked by intradermal injection of 1% carrageenan solution in paw, while the pain was induced by intraperitoneal injection of 0.6% acetic acid, and hyperthermia by subcutaneous injection of 12 % of yeast suspension. At doses from 62.5 to 250 mg/kg, the extract reduces the edema from 0.53 ± 0.02 ml in the control group to 0.43 ± 0.03 and 0.37 ± 0.01 ml respectively ($p<0.05$). The number of abdominal contortions reduces from 42.8 ± 1.1 during 20 minutes to 36.6 ± 1.5 and 20.2 ± 0.91 respectively ($p<0.05$). and the hyperthermia reduces from 39.30 ± 0.03°C to 39.03 ± 0.01 and 38.80 ± 0.01°C respectively ($p<0.05$). These results show that *Cladogelonium madagascariense* L. extract possesses an anti-inflammatory activity *in vivo*.

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I. INTRODUCTION

Inflammation is a physiological mechanism to protect the body against external or internal harmful stimuli, such as irritants or pathogens. Its semiology is characterized by edema and hyperthermia due to local vasodilation, and pain [1]. Its treatment is based on corticoids and non-steroids anti-inflammatories. Even though these are efficient, their use is limited by their secondary effect on stomach [2]. Beside the pharmaceutical products, medicinal plants are also used as alternative to treat inflammation, for example *Zygophyllum gaetulum* (ZYGOPHYLLACEAE) [3], *Erica arborea* (ERICACEAE) [4], *Aegle marmelos* (RUTACEAE) [5], *Acanthus montanus* (ACANTHACEAE) [6], *Moringa oleifera* (MORINGACEAE) [7], *Aloe vera* (ASPHODELACEAE) [8] and *Curcuma longa* (ZINGIBERACEAE) [9].

In Madagascar, the decoction of the leaves of *Ageratum conyzoides* L. (ASTERACEAE), is used to relieve dysmenorrhea and to clean infected wounds and skin ulcer. The hydroalcoholic extract of its leafy branches possesses analgesic and anti-inflammatory properties [10]. *Centella asiatica* (L.) Urb. (APIACEAE) has also been shown to have an anti-inflammatory, wound healing and anti-ulcer activities [11]. The leaves decoction of *Gambeya boivini* Pierre (SAPOTACEAE) is also used to treat fever, and its hydroalcoholic extract has an anti-inflammatory activity [12].

Cladogelonium madagascariense L. (vern. *tsontso*) (EUPHORBIACEAE) (Fig.1) is found in the northern region of Madagascar. It is monogenic, mono species and endemic to the region [13]. The leaves of this plant are used as cataplasm to relieve pain and inflammation, and its decoction to treat fever. This study is based on this empirical information collected in Sadjoavato, district of Antsiranana, and the absence of investigation on anti-inflammatory activity of this plant.

Fig. 1: Leafy branches of *Cladogelonium madagascariense* L. (tsontso) (EUPHORBIACEAE)

II. MATERIALS AND METHODS

Preparation of the extract

The leafy branches of *C. madagascariense* were collected in the District of Sadjoavato, Antsiranana II in August. They were dried under shade and ground. Two hundred grams of the powder was macerated in 3.5 liters of a mixture of ethanol and water (80:20) for 72 hours at room temperature. The macerate was filtered and evaporated to dryness at 60°C, using a vacuum rotative evaporator (Büchi ®). The dried hydroalcoholic extract was dissolved in distilled water and administered orally at the doses 62.5, 125 and 250 mg/kg in a volume of 10 ml/kg.

A. EXPERIMENTAL ANIMALS

Male and female albino mice, Swiss strain, 6 to 8 weeks old, and weighing 25 to 30 g were used in this study. They were kept under standard laboratory conditions, at room temperature, with 12/12 hours light/dark cycle at the animal house of the "Laboratoire de Pharmacologie Générale, de Pharmacocinétique et de Cosmétologie" (University of Antananarivo). The animals were allowed food and water *ad libidum*. They were fastened for 18 h prior to tests with free access to water. The mice were divided into 5 groups of 6: 1 control group, 1 reference group, and 3 groups treated with 3 doses of the extract. The Committee of Animal Ethics at the Sciences Faculty, University of Antananarivo, has approved all the experiments.

B. BIOLOGICAL TESTS

1. Study of the extract effect on experimental edema

Experimental edema was induced by injecting the right hind paw of the animals with 1% saline (NaCl 9 ‰) solution of carrageenan intradermally [14]. The activity of the extract was studied by measuring the inflamed paw's volume of the treated animals versus the control group.

The paw's volume was measured with plethysmometer (Ugo Basile 7140), and the initial volume 'V_0' was noted. The control group animals received 10 ml/kg of distilled water orally; and the extract was given to the 3 groups at the doses 62.5, 125 and 250 mg/kg respectively, while the animals of the standard group received 100 mg/kg of phenylbutazone [15]. The extract and phenylbutazone were administered orally in 10 ml/kg of distilled water.

Thirty minutes after, inflammation was induced on the right hind paw of the animals by injecting 50 µl of 1% saline solution of carrageenan. The paw volume was measured 1 and 6 hours after the carrageenan injection [16].

2. Study of the extract effect on experimental pain

Pain is one of inflammation signs [17], acetic acid-induced writhing on mice was used to study the analgesic activity of the extract [18].

The study was carried out on 5 groups of mice: 1 control group which received orally 10 ml/kg of distilled water, 1 standard group treated with 100 mg/kg of salicylic acid, and 3 groups treated with 62.5, 125 and 250 mg/kg of the extract. Both extract and acetylsalicylic acid were administered by oral route in 10 ml/kg of distilled water.

Thirty minutes after, the animals were injected intraperitoneally with 10 ml/kg of 0.6% acetic acid. They were individually put in glass recipient of 14 cm diameter and 30 cm height for 20 min. The muscular reaction during this period was observed, and the number of contortions was counted [19].

The reduction of the nociception (P) was expressed in % and calculated using this formula:

$$P = \frac{N_T - N_t}{N_T} x100$$

N_T= Number of contortions in the control group

N_t= Number of contortions in the treated groups

3. Study of the extract effect on experimental hyperthermia

The extract effect on hyperthermia was studied on experimentally induced hyperthermia by subcutaneous injection of 12 % of yeast suspension, prepared in NaCl 9‰. The animals were injected with 10 ml/kg of this suspension subcutaneously in the subscapular zone [20].

The rectal temperature of the animals was measured before injection of the yeast suspension. Eighteen hours after, the animals presenting an increase of 0.5°C were selected [21]. They were divided into 5 groups: 1 control group received 10 ml/kg of distilled water, 3 treated groups with the extract at doses 62.5, 125 and 250 mg/kg, and the standard group received 100 mg/kg of acetylsalicylic acid. The extract and the acetylsalicylic acid were administered orally in 10 ml/kg of distilled water. The rectal temperature was measured, each 60 min, during 3 h [20, 21].

The effect of the extract was expressed in % of reduction of the temperature:

$$\% \text{ of reduction of temperature} = \frac{(B-C)}{(B-A)} \text{ x } 100$$

A: initial temperature

B: temperature 18 h after yeast suspension injection

C: temperature at (1, 2 and 3 h) after products administration

B. STATISTICAL ANALYSIS

The experimental data were expressed as the mean ± s.e.m. Data were assessed by statistical analysis using unpaired Student's 't ' test, p values < 0.05 were considered as significant.

III. RESULTS

After evaporation of the solvent used to extract 200 g of the plant powder, 12.2 g of dry extract were obtained. Since edema, pain, and hyperthermia characterize inflammation, they were experimentally induced in mice to investigate the anti-inflammatory activity of the extract of *C. madagascariense*.

1. Effect of the extract on experimental edema

The anti-inflammatory activity of *C. madagascariense* extract was investigated on carrageenan-induced edema. The initial volume of the mice hind paw is 0.26 ± 0.005 ml. One hour after intradermal injection of carrageenan, the paw volume of animals of the control group increases to 0.59±0.01 ml. The paws volume of animals in the treated groups also increases, but there is no significant difference between the paw volume of control group and groups treated with the extract ($p>0.05$). The volume of the standard group animal's paws increases to 0.35±0.01 ($p<0.05$). Six hours after injection, the paw volume of the treated animals is inferior to the control animals. The control group paw's volume is 0.53 ± 0.02 ml, while the volume of the paw of the animals treated with the extract at doses from 62.5 to250 mg/kg was respectively 0.43 ± 0.03 and 0.37 ± 0.01 ml ($p<0.05$). At the doses used, the extract is less effective than the phenylbutazone, where the paw's volume is 0.27 ± 0.01 ml ($p<0.05$) (Table 1).

Table 1. ***Variation of mice hind paw volume 1 and 6 h after injection of 1% saline solution of carrageenan, in control group, treated with phenylbutazone (PNB) and the extract at different doses*** **($\overline{m}$ ± s.e.m.; n = 6, p < 0.05).**

	Hind paw volume (Vol.) and p values			
Animal groups	Vol at 1 h (ml)	p Vs control	Vol at 6 h (ml)	p Vs control
Control	0.59 ± 0.01	p>0.05	0.53 ± 0.02	p>0.05
Treated with extract 62.5mg/kg	0.58 ± 0.02	p>0.05	0.43 ± 0.03	p<0.05
Treated with extract 125mg/kg	0.55 ± 0.02	p>0.05	0.40 ± 0.01	p<0.05
Treated with extract 250mg/kg	0.53 ± 0.01	p>0.05	0.37 ± 0.01	p<0.05
Treated with 100 mg/kg PNB	0.35 ± 0.01	p<0.05	0.27 ± 0.01	p<0.05

2. Effect of the extract on experimental pain

Writhing induced by intraperitoneal injection of acetic acid was used to investigate the activity of the extract on experimental pain. Acetic acid causes algesia resulting in the writhing. The number of contortions in the control group is equal to 42.8 ± 1.1 during the 20 min of observation. *C. madagascariense* extract and acetylsalicylic acid, used as the standard drug, reduce the number of contortions. At doses 62.5 and 250 mg/kg, *C. madagascariense* extract reduces the number of contortions to 36.6 ± 1.5 and 20.2 ± 0.91 respectively, which are equal to 14.49 and 52.8 % nociception inhibition ($p<0.05$). The reference drug, acetylsalicylic acid, at 100 mg/kg is more potent with 12.8 ± 0.38 contortions, which corresponds to 70.1 % of nociception inhibition ($p<0.05$) (Fig.2).

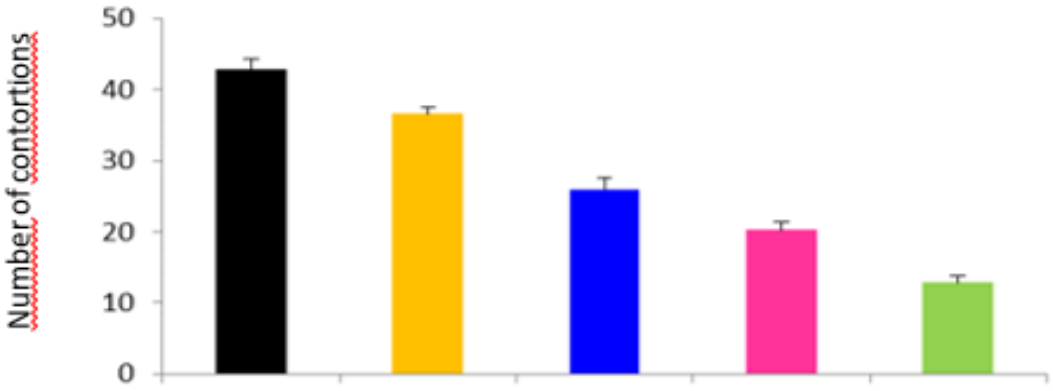

Fig. 2: Variation of the number of contortions in the control group ▬, treated with the extract at 625 mg/kg ▬, 125 mg/kg ▬ , 250 mg/kg ▬ and acetylsalicylic acid at 100 mg/kg ▬, in 20 min of observation, after intraperitoneal injection of acetic acid 0.6% ($\overline{m}$ ± s.e.m.; n = 6, p < 0.05).

3. Effect of the extract on hyperthermia

The animal's rectal temperature before the test is 37.48 ± 0.06°C. After 18 h of injecting yeast suspension, the temperature increases to 39.30± 0.03°C ($p<0.05$).

Three hours after oral administration of *C. madagascariense* extract at 62.5 and 250 mg/kg, the temperature reduces to 39.03 ± 0.01 and 38.80 ± 0.01°C respectively, which gives 15.76 and 34.78 % inhibition of the increase. However, acetylsalicylic acid is more effective, at

100 mg/kg, the rectal temperature of the animals is 38 ± 0.02 °C, which is 51.43 % inhibition of the temperature increase ($p<0.05$) (Fig.3).

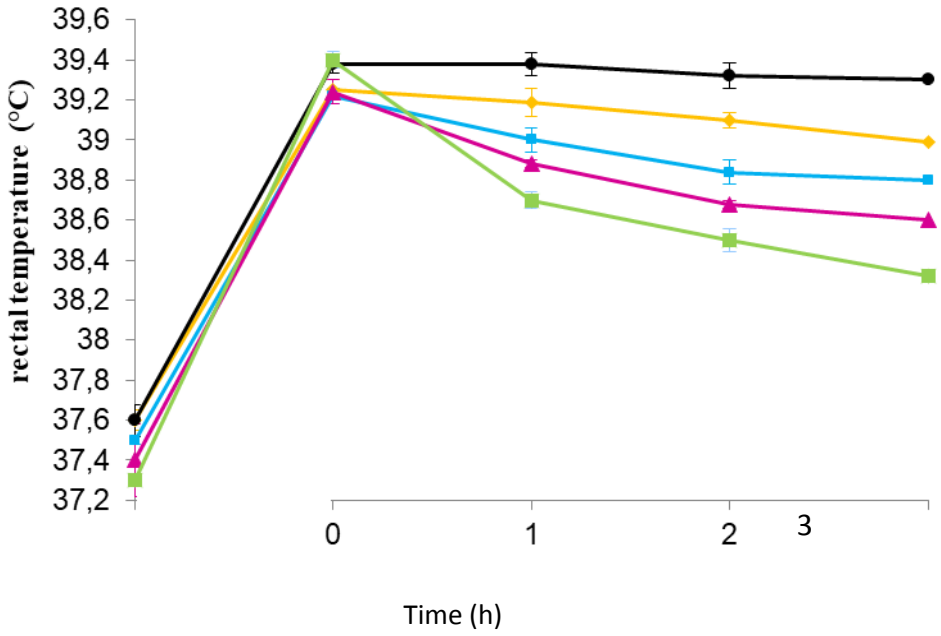

Fig 3: Variation of rectal temperature of the hyperthermic mice of the control group, treated with extract at 62.5, 125, 250 mg/ kg and acetylsalicylic acid at 200 mg/ kg, administered by oral route ($\overline{m}$ ± s.e.m., n = 6, p < 0.05).

IV. DISCUSSION

The present study establishes the anti-inflammatory activity of the hydroalcoholic extract of *C. madagascariense*. Since edema, pain, and hyperthermia characterize inflammation, they were experimentally induced in mice in this work. The anti-inflammatory activity of *C. madagascariense* extract was investigated on carrageenan-induced edema, acetic acid induced writhing and hyperthermia induced by yeast suspension, in mice. Administered orally, the extract reduces all these signs; however, its effect is less than acetylsalicylic acid and phenylbutazone used as standard drugs.

Carrageenan is recognized as an invading substance evoking immune reaction [22, 23]. The body reacts to it by releasing pro-inflammatory factors, such as IL1, TNFα, histamine, and prostaglandins from mastocytes and lymphocytes. Histamine and prostaglandins induce edema due to vasodilation [24, 25]. Body reaction to carrageenan injection has 2 phases; the first phase (0–2 h) is attributed to the release of histamine, serotonin, and bradykinin. The second phase (2–6 h) is correlated with NO, prostaglandins, TNF- α, IL-1β, and IL-6

production [26, 27]. According to the results obtained, the extract is efficient in the second but not in the first phase, which means, it probably inhibits the second phase mediators' action, their release or their biosynthesis.

Intraperitoneal injection of acetic acid used as an inducer of writhing syndrome causes algesia by prostaglandins release, which excites nociceptors, resulting in the writhing [28]. With the reduction of the number of contortions, one can advance a hypothesis, that *C. madagascariense* extract, either inhibit the release of prostaglandins or their activity.

In addition, subcutaneous injection of yeast suspension activates the immune system, which releases the pyrogen factors IL-1 and TNF-α in the bloodstream. Meanwhile, these factors stimulate COX2, responsible for PGE2 biosynthesis leading to the increase of the body's temperature [28].

These results seem to support the hypothesis that *C. madagascariense* extract influences the prostaglandins synthesis, because this mediator is responsible for edema, writhing and hyperthermia. This activity may be due to the inhibition of COX2 like acetylsalicylic acid, or the pro-inflammatory factors such as IL-1 and TNF-α responsible for COX2 activity [29, 30]. It may also contain a molecule which inhibits PGE2 receptor, like WO 20121/17768A1 and US-2014/0179750A1 which bind on EPE2, prostaglandin receptor [31].

V. CONCLUSION

The results obtained in this study show that *C. madagascariense* hydroalcoholic extract possesses anti-inflammatory activity at the investigated doses in mice. However, it is less effective than acetylsalicylic acid and phenylbutazone used as standard drugs. This activity may be mediated via prostaglandins biosynthesis inhibition. Further experiments on purified molecules from the extract will be conducted to elucidate this mechanism.

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