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Anti-Inflammatory Activity of Methanolic Extract of the Leaves of *Rhopalocarpus similis* (*Sphaerosepalaceae*) in Mice

Rama Michela Stephanie[1*], Roukia Djoudi[1], Razafindramanana Jeenath Francelline[1], Youssouf Loutfi[1], Ramanitrahasimbola David [2] Rajaonarison Jean Francois[1]

[1]*Department of Biotechnology and Environment Research Laboratory, University of Mahajanga, Mahajanga, Madagascar*

[2]*Department of Applied Pharmacognosy Laboratory, Malagasy Institute of Applied Research, Antananarivo, Madagascar*

ABSTRACT

Rhopalocarpus similis is a plant endemic to Madagascar, traditionally to treat edema and joint pain. Inflammatory paw edema was caused by injection of carrageenan (2%). Pains manifested by abdominal contortions and licking of the sore paw were induced by injection of acetic acid (1.5%) and formalin (2%), respectively. The plant extract at doses of 150 mg/kg and 300 mg/kg, significantly inhibits ($p<0.05$) edema at 48.27 ± 8.27% and at 52.74 ± 7.90%), respectively. Indomethacin (10 mg/kg) used as a reference anti-inflammatory drug exerts an inhibition of 53.44 ± 7.10%. Untreated edemas of the paws of control mice were inhibited by 19.53 ± 2.79%. The plant extract also decreases the pain manifested by a significant inhibition ($p<0.05$) of abdominal contortions to 82.98 ± 1.74% with the two doses versus 81.91 ± 2.30% with the indomethacin. At doses of 200 mg/kg and 400 mg/kg of plant extract, the frequencies of licking of the paw were also inhibited at 63.07 ± 3.32% and at 78.46 ± 2.17% ($p<0.05$), respectively, versus 83.07 ± 3.32% with paracetamol (300 mg/kg) used as reference analgesic. Furthermore, the plant extract does not cause any lethal toxicity at doses of 1.5 g/kg and 3.0 g/kg.

Keywords: *Rhopalocarpus similis*; Pain; Anti-inflammatory; Analgesic

INTRODUCTION

Inflammation is the body's innate defense reaction against aggression. Its purpose is to eliminate pathogens in order to restore damaged tissues. It becomes pathological when it is exaggerated, hence the need to use anti-inflammatory drugs. According to the World Health Organization (WHO), nearly 80% of the world's population use herbal medicines and traditional therapy to treat various diseases. In Madagascar, medicinal plants represent for the majority of Malagasy the most practical means to treat various diseases. Thus, in the case of exacerbated inflammation, decocts and macerates of plants are taken to alleviate joint pain and poultices are applied to edemas and fractures to accelerate their healing [1].

Rhopalocarpus similis belongs to the family of *Sphaerosepalaceae* which is endemic to Madagascar. It is traditionally used by the Malagasy population to treat swelling (edema) and joint pain. It is also described as having wound healing properties. This plant has not yet been the

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Corresponding author: Rama Michela Stephanie, Department of Biotechnology and Environment Research Laboratory, University of Mahajanga, Mahajanga, Madagascar; E-mail: michelastephanie2408@gmail.com

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subject of any scientific study for anti-inflammatory activity. This study was undertaken in order to enhance the *Rhopalocarpus similis* plant by scientifically demonstrating its anti-inflammatory potential, thus allowing to better understand its use in traditional medicine.

MATERIALS AND METHODS

Experimental Animals

Male and female OF-1 mice varying in weight from 30 g to 35 g and 6 weeks old were used for the anti-edema and acetic acid analgesic tests. They were from the animal facility of the Malagasy Institute for Applied Research (IMRA), Suzanne and Albert Rakoto Ratsimamanga Foundation, in Antananarivo (Madagascar).

Male and female SWISS mice varying in weight from 25 g to 30 g and 6 weeks old were used for the analgesic test with formalin and the study of acute toxicity. These animals were from the Biotechnology, Environment and Health Research Laboratory (LRBES) of the life engineering and modeling doctoral school (EDGVM) of the university of Mahajanga [2].

Plant material: The leaves of the plant were collected in the Sofia region (Madagascar). They were dried and crushed into a fine powder.

Extraction

307 g of powder of *Rhopalocarpus similis* leaves were macerated in 2.6 l of methanol for 72 h and at room temperature. The macerate was then filtered using Joseph paper, under vacuum and then evaporated using a rotary evaporator (BUCHI R-114), under reduced pressure, at 40°C. This process made it possible to obtain a crude methanolic extract (EB) with an extraction yield of 9.39%.

Preparation of Products

For anti-edematous and analgesic tests on pain caused by acetic acid, the vehicle used for EB and indomethacin (standard anti-inflammatory) was 5% Dimethyl Sulfoxide (DMSO) solution. For the acute toxicity study and the analgesic test on pain caused by formalin, the vehicle used for EB and paracetamol (standard analgesic) was distilled water. All the products tested were administered orally at a volume of 10 ml/kg [3].

Anti-Edema Test

The anti-edematous test was carried out using an experimental model of acute inflammation caused in mice according to the method described by Udegbunam et al. For this, the mice were fasted 16 h before experience. They were divided into 4 groups of 7 mice, including: A control group which received DMSO 5%, a reference group treated with indomethacin (10 mg/kg) and 2 groups treated with EB at the respective doses of 150 mg/kg and 300 mg/kg. One hour after administration of the products, carrageenan (2%) was injected into the animal's hind paw at a volume of 100 µl to induce inflammatory edema. The volumes of the paws of the mice were measured using a plethysmometer (UGO BASILE 71) before the injection of carrageenin on the non-inflamed paws (Vo), then immediately after the injection (Vto) to measure the edema formed, then at 1 h, 2 h, 3 h and 4 h for the study of the anti-inflammatory effect (Vt). The percentages of inflammation and inhibition as a function of time were then calculated respectively according to the formulas:

$$\text{Percentage of inflammation (PI \%)} = \frac{(Vt - Vo)}{(Vto - Vo)} X100$$

$$\text{Percentage inhibition of inflammation (\%)} = 100 - PI$$

Analgesic Test on Pain Caused by Acetic Acid

This test was carried out using an experimental model of pain caused in mice according to the method described by Koster et al. For this, the mice were fasted 16 h before the experiment. They were divided into 4 groups of 3 mice, including: A control group having received DMSO (5%), a reference group treated with indomethacin (10 mg/kg) and 2 groups treated with EB at the respective doses of 150 mg/kg and 300 mg/kg. One hour after the administration of the products, acetic acid (1.5%) was injected intraperitoneally at a volume of 5 ml/kg to induce the pain which was manifested by abdominal contortions in animals. The analgesic effect was determined by counting abdominal contortions for 30 min [4].

$$\text{Percentage of pain's inhibition (\%)} = \frac{Cto - Ct}{Cto} x100$$

Cto and Ct being the means of the number of contortions of the mice not treated and treated with EB on the one hand and the reference product on the other hand, respectively.

Analgesic Test on Pain Caused by Formalin

This test was carried out using an experimental model of pain caused in mice according to the method described by Hunskaar and Hole. For this, the mice were fasted 16 h and divided into 4 groups of 3 mice, including: A control group which received distilled water, a reference group treated with paracetamol (300 mg/kg) and 2 groups treated with EB at the respective doses of 200 mg/kg and 400 mg/kg. One hour after the administration of the products, formalin (2%) was injected into the plantar arch of the hind paw of the mice at a volume of 20 µl to cause the pain which is manifested by licking of the sore part. The analgesic effect was determined by counting the licks of the sore paw for 30 min [5].

$$\text{Pourcentage d inhibition de la douleur (\%)} = \left(1 - \frac{Lt}{Lo}\right) X100$$

Lo and Lt being the means of the number of licks of the legs of mice not treated and treated with EB on the one hand and the reference substance on the other hand, respectively.

Acute Toxicity Study

The acute toxicity of the extract was evaluated after single oral administration of EB at high doses. For this, the mice

were divided into 3 groups of 3 animals including: Control group which received distilled water and 2 groups treated with EB at the respective doses of 1.5 g/kg and 3 g/kg. The animals were then observed for 1 hour to detect deaths or any change in condition and/or behavior compared to the control group. Subsequently, brief observations at 2 h, 3 h, 4 h, 5 h, 6 h, 24 h, 48 h and 72 h after were made [6].

Statistic Study

All results are expressed as the mean ± standard error at the mean (m ± esm). Statistical analyzes were carried out using student's "t" test by comparing the means of the treated groups with those of the corresponding control group.

RESULTS

Anti-Edematous Effect

EB and indomethacin significantly ($p<0.05$ and $p<0.01$, respectively) inhibited inflammatory paw edema in mice from 2 and 3 h after injection of carrageenan, compared to that of the animals of the control group (Table 1).

Table 1: Anti-edema effect as a function of time of the crude methanolic extract of leaves of RMS96 (EB) on the inflammatory edema of the paw of mice.

	Inhibition of inflammatory edema (mean ± esm) (%)			
Processing	**1 hour**	**2 hours**	**3 hours**	**4 hours**
Control	7.45 ± 2.14	9.10 ± 2.07	15.52 ± 2.43	19.53 ± 2.79
EB (150 mg/kg)	14.02 ± 4.82	24.32 ± 4.84*	28.66 ± 5.79*	48.27 ± 8.27*
EB (300 mg/kg)	10.03 ± 3.71	16.62 ± 3.97	34.39 ± 6.22*	52.74 ± 7.90*
Indomethacin (10 mg/kg)	26.03 ± 6.01*	44.88 ± 4.37*	52.33 ± 6.33**	53.44 ± 7.10*

Note: *p<0.05 and **p<0.01 for n=7. Indomethacin has been used as reference

The results are shown as the mean ± esm of 7 independent determinations (n=7). The values of $p<0.05$ (*) and $p<0.01$ (**) are considered significant.

Analgesic Effect on Pain Caused by Acetic Acid

EB and indomethacin significantly inhibit pain ($p<0.05$) resulting in a decrease in the number of abdominal contortions in the mice of the treated groups compared to that of the animals of the control group (Table 2) [9].

Table 2: Analgesic effect of crude methanolic extract of RMS96 (EB) leaves on pain caused by acetic acid.

Processing	EB (150 mg/kg)	EB (300 mg/kg)	Indomethacin (10 mg/kg)
Pain inhibition (mean ± esm) (%)	82.97 ± 1.74*	82.97 ± 1.74*	81.91 ± 2.30*

Note: *p<0.05 for n=3. Indomethacin was used as the reference medicine

The results are represented as the mean ± esm of 3 independent determinations (n=3). The values of $p<0.05$ (*) are considered as significant.

Analgesic Effect on Pain Caused by Formalin

EB and paracetamol significantly inhibit pain ($p<0.01$) resulting in a decrease in the number of paw licks in the mice of the treated groups compared to that of the animals of the control group (Table 3) [10].

Table 3: Analgesic effect of crude methanolic extract of RMS96 (EB) leaves on pain caused by formalin.

Processing	EB (200 mg/kg)	EB (400 mg/kg)	Paracetamol (300 mg/kg)
Pain inhibition (mean ± esm) (%)	63.07 ± 3.32**	78.46 ± 2.17**	83.07 ± 3.32**

Note: **p<0.01 for n=3. Paracetamol was used as the reference medicine

The results are represented as the mean ± esm of 3 independent determinations (n=3). The values of $p<0.01$ (**) are considered significant.

Acute Toxicity

No mortality was noted during the 72 h observation after administration of EB at the high doses of 1.5 g/kg and 3 g/kg in mice [11].

DISCUSSION

Inflammation, whether local or systemic, is associated with an increase in the production of the main pro-inflammatory cytokines such as TNFα, IL-1, IL-6.6 Carrageenan induces a strong metabolism of membrane phospholipids and the synthesis of Cyclo-Oxygenase 2 (COX-2). Inflammation due to carrageenan is described as being biphasic. The mediators released in the first phase (the first hour after injection of carrageenan) are histamine, serotonin, bradykinin. The second phase (after 1 hour) is marked by the production of Prostaglandins (PGs) especially of the PGE2 isoform involved in the processes of pain and inflammation. Over time, a progressive decrease in edema is observed all the mice. In the control group, this decrease is undoubtedly due to the glucocorticoid hormone. This hormone is produced in the adrenal cortex and is a powerful anti-inflammatory. It inhibits the activation of Phospholipase A2 (PLA2) which is the enzyme responsible for the metabolism of membrane phospholipids into arachidonic acid and at the same time, inhibits the synthesis of prostaglandins and leukotrienes. In the group of mice that received indomethacin which is a Non-Steroidal Anti-Inflammatory Drug (NSAID), the anti-edematous activity observed is due to the inhibition of cyclooxygenases leading to an inhibition of the production of prostaglandins, thromboxane A2 as well as prostacyclins [12].

The results obtained demonstrate that the extract of *Rhopalocarpus similis* significantly ($p<0.05$) decreases the inflammatory edema. The effects of EB at 150 mg/kg and 300 mg/kg observed vary little with dose. On the other hand, more than 50% inhibition of inflammation (52.14%) was observed at a dose of 300 mg/kg, four hours after the injection of carrageenan. These results indicate that EB has an anti-edematous property, but this is not significant until the third hour after its administration. Therefore, it may be that EB acts mainly at the second phase of inflammation caused by carrageenan, mainly corresponding to the synthesis in large quantities of prostaglandins. Similar results were obtained by Riahi et al., studying the effects of different seaweed extracts on edema caused by carrageenan. Their extracts also inhibited the edema induced by carrageenan late. These extracts would probably contain substances which would seem to inhibit the production of prostaglandins (mainly that of PGE2) by blocking specific enzymes, cyclooxygenases (COX-1 and COX-2) involved in the inflammatory reaction and in the genesis of nociception [13].

For the study of analgesic activity, acetic acid and formalin tests are often used. Both methods cause pain which is mostly peripheral. With the first model, pain is caused by the intraperitoneal injection of acetic acid in mice. In this case, the pain is manifested by abdominal contortions which are the products of the release of different endogenous harmful mediators such as bradykinin, serotonin, histamine, substance P and prostaglandins. Inflammatory pain is caused by an increase in permeability, by an increased release of PGE2 and PGF2 at the peritoneal receptors. Our results demonstrate that there is a significant decrease ($p<0.05$) in the number of abdominal contortions in the mice of the groups treated with EB and with the reference indomethacin compared to the animals of the control group. The effects of EB at doses of 150 mg/kg and 300 mg/kg are similar to that of indomethacin (82.98% versus 81.91%) indicating that the extract would have analgesic activity with a mechanism of action comparable to that of this reference product. With the second model, the pain is caused by the injection of formalin into the paws of the mice. In this case, the analgesic test has two distinct mechanisms [14].

The early phase occurs the second formalin is injected. It corresponds to the initial neurogenic pain and lasts 5 minutes. It results from the chemical stimulation of peripheral TRPA-1 and the activation of the C fiber. The late phase corresponds to the inflammatory pain which lasts between 15 to 30 minutes after the injection of formalin and which is due to the release of histamine, serotonin, bradykinin and PGs. Our results demonstrate that there is a significant decrease ($p<0.01$) in pain in the mice of the treated groups compared to the animals of the control group. This is manifested by a decrease in the frequency of licking the paws of mice. Paracetamol has an inhibitory action on the synthesis of prostaglandins but exerts a weak anti-inflammatory activity. The inhibition of cyclooxygenases by paracetamol occurs mainly in the brain, which explains the few peripheral adverse effects observed unlike NSAIDs. Thus, the extract of *Rhopalocarpus similis* would indeed have anti-inflammatory properties and could intervene in the production of prostaglandins, by inhibition of COXs such as NSAIDs or by inhibition of PLA2 such as AIS or even by another mechanism of action [12-15].

CONCLUSION

This study made it possible to scientifically demonstrate that the crude methanolic extract of the leaves of *Rhopalocarpus similis* and used empirically in traditional Malagasy medicine indeed possesses anti-edematous and peripheral analgesic activities. The results obtained constitute a scientific basis which justifies the traditional use of this plant in the management of pathologies with an inflammatory component. Taking into account the difficulties of access of populations of developing countries including Madagascar, to pharmaceutical drugs, such research work makes it possible to better understand the use of medicinal plants in traditional medicine. However, for the case of *Rhopalocarpus similis*, more in-depth studies are still necessary to confirm the results obtained. Specifically, it is very important to isolate and identify the active molecule(s) and subsequently

elucidate their exact mechanisms of action and toxicity to finally make it into a phytomedicine or an anti-inflammatory drug at the reach of the majority of populations. Finally, this study contributes to improving the health of populations allowing better sustainable development.

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