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(RESEARCH ARTICLE)

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Anti-inflammatory activity of hydroalcoholic extract of *Helychrisum faradifani* (Asteraceae) in mice

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Abstract

This study aimed to evaluate *Helychrisum faradifani* hydro alcoholic extract activity on the vascular and cellular phases of inflammation in mice. Carrageenan was injected on hind-paw to evaluate the extract effect on edema and acetic acid 0.6 % injected by intra peritoneal route to evaluate *H. faradifani* extract on vascular permeability. Cotton pellets imbibed with 1% carrageenan was inserted in the skin of the animals to investigate *H. faradifani* activity on granuloma formation. Indomethacin at the dose of 10 mg/kg was administered orally as positive control. The study of anti-inflammatory activity demonstrated that oral administration of the extract inhibited significantly inflammatory carrageenan-induced edema compared to the control. Control group paw edema is 0.61± 0.01 ml, versus 0.32± 0.02, 0.22± 0.01 and 0.15± 0.01ml respectively in groups treated with the extract, and 0.38± 0.02 for animals treated with indomethacin. It also reduces vascular permeability by 34.04 ± 0.001% and 67.30 ± 0.015% respectively compared to the control ($p < 0.05$). The weight of granuloma in the control animals is 53.85 ± 0.62 mg versus 33.32 ± 0.3 and 19.95 ± 0.38 mg for the rats treated with extract at 200, 400 and 800 mg/kg respectively ($p < 0.05$). Leucocytes number reduces from 119.55 ± 0.1in control group to 105.1 ± 0.1, 88.83 ± 0.1 and 70.48 ± 0.1 in groups treated with the extract and 85.52 ± 0.05 in animals treated with indomethacin. These results indicate that *H. faradifani* extract reduces the vascular and cellular phases of inflammation.

Keywords: *Helychrisum faradifani*; Anti-inflammatory; Vascular; Cellular phases; Mice

1. Introduction

Although synthetic drugs are dominating the market, herbal therapy is well established in Madagascar, and it is based on traditions and the way of life in almost rural areas population. It is also due to the geographical distance between them and the occidental health care center and the cost of pharmaceutical drugs. According to the ethnobotanical survey that we have conducted in Fianarantsoa, Madagascar, most of its population work in rice fields, and they are subject of back pain and small injuries. To release these pains, they drink the decoction of the entire plant of *Helychrisum faradifani* after work. Analysis of these data leads us to suggest that it might have an anti-inflammatory activity. *H. faradifani* (Asteraceae) is a perennial shrub growing in rocky and sandy places of Madagascar. It is rich in terpenoids which provide its fragrance and its insecticide activity [1]. This plant is rich in flavonoids and terpenoids, especially in α-pinene, 1,8-cineole and β-caryophyllene [2, 3].

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Chronic inflammatory diseases are one of the health problems of the world's population. Or, prolonged use of anti-inflammatory drugs may cause severe side effects especially gastrointestinal bleeding and peptic ulcers [4]. Meanwhile we noticed that these people don't complain of those problems, so we think this decoction is safe and effective anti-inflammatory.

Inflammation is a combination of heat, redness, swelling and pain in a local area. It occurs in tissues when external or internal agents cause damage to them [5]. In the inflammatory response there is an increase of permeability of endothelial lining cells and influxes of blood leukocytes into the interstitium, oxidative burst, and release of cytokines (interleukins [IL] and tumor necrosis factor-α [TNF-α]). At the same time, there is an induction of the activity of several enzymes (oxygenases, nitric oxide [NO] synthases, and peroxidases) and the arachidonic acid metabolism. In the inflammatory process, there is also the expression of cellular adhesion molecules, such as intercellular adhesion molecule and vascular cell adhesion molecule [6].

2. Materials and methods

2.1. Drugs and Chemicals

Indomethacin, carrageenan lambda and NaCl were purchased from SIGMA-ALDRICH CHEMIE Gmbh P.O. 1120, 89552 Steinheim, Germany 49-7329-970.

2.2. Plant material

Aerial parts of *Helichrysum faradifani* were collected in September 2021 (dry season) at the region of Ambositra, Fianarantsoa, Madagascar. A specimen was identified at the Botany Department of Parc Botanique et Zoologique de Tsimbazaza (Antananarivo, Madagascar). They were dried under shade, in an aerated room, at ambient temperature, for 1 month. Dry material was ground, the powder was macerated in ethanol-water (60:40) for 72 hours. Macerate was filtered and centrifuge at 3 000 rpm. Supernatant was evaporated to dryness with rotative evaporator (Büchi). Dried extract was used in this work.

2.3. Animal of experimentation

Swiss mice, 7–8 weeks old were used in the work, they were breed at the animal house of Pharmacology Department, Faculty of Sciences, University of Antananarivo, Madagascar. The animals were maintained at a constant temperature, around 20°C, and a 12-h light/12-h dark cycle and were provided a standard diet with water ad libitum. They were fastened 12 hours prior tests.

2.4. Evaluation of *Helychrisum faradifani* extract on swelling

Helychrisum faradifani extract effect on swelling was evaluated on carrageenan induced edema. Animals were divided into 5 groups of 6 animals: 1 carrageenan control, 1 positive control treated with indomethacin (10 mg/kg) and 3 treated with the extract (100, 200 and 400 mg/kg). Indomethacin and the extract were administered orally. After 30 minutes, 50 µl of 1% carrageenan suspension in saline solution (NaCl 9‰) were injected into sub-plantar tissues on the right hind paw of each mouse. The paw volume of mice was measured by plethysmometer (Ugo Basile, Varese, Italy), before and after 1, 2, 3, 4, 5 and 6 h carrageenan injection [7].

The anti-inflammatory activity was calculated as percentage inhibition of edema in the animals treated with extract and indomethacin in comparison to the carrageenan control group.

The percentage (%) inhibition of edema is calculated using the formula:

$$\% \text{ inhibition} = (To - Tt)/To \times 100$$

Where:
To is the volume of paw edema of control group rats at a given time
Tt is the volume of paw edema of the tested groups rats at the same time
Volume of edema T = Vtc -Vti
Vtc: paw volume after carrageenan injection
Vti: paw volume before carrageenan injection

2.5. Evaluation of *Helychrisum faradifani* extract on vascular permeability

Anti-inflammatory effect of *H. faradifani* extract was measured by vascular permeability induced with acetic acid 0.6 % injected by intraperitoneal route [8].

Fastened animals were divided into 6 groups: 1 control and 3 have received the extract by oral route at doses 100, 200 and 400 mg/kg, 1 positive control treated with indomethacin (10 mg/kg). After 30 minutes of oral administration of the extract and indomethacin, 0.1 ml/kg of 0.6 % acetic acid was injected intraperitoneally to each animal. After 30 min of acetic acid injection, 0.2 ml of 4% Evans blue solution was injected into the tail vein of each mouse. After 20 min, mice were euthanized with an overdose of ether inhalation [8]; laparotomy was practiced, and 5 ml of saline solution was used to rinse the abdominal cavity. The washing solution was collected into test tubes and centrifuged for 10 min at 3000 rpm. The amount of Evans blue leakage into the abdominal cavity (vascular permeability) was determined by measuring the absorbance of the supernatant at 580 nm.

2.6. Evaluation of *Helychrisum faradifani* extract effect on inflammation tissular phase

Carrageenan-induced granuloma, which was induced by subcutaneous implantation of a cotton pellet imbibed with carrageenan, was used to investigate the effect of *H. faradifani* extract on inflammation tissular phase. Granuloma weight and the number of leucocytes in it were evaluated [9].

Five groups of Swiss mice (n = 6) were used in the study: one served as carrageenan control, one received 10 mg/kg of indomethacin as positive control; and 3 groups were treated with the extract at doses of 100, 200 and 400 mg/kg, respectively. 30 minutes after administration of drugs and vehicle, the animals were lightly anesthetized by inhalation of ether; and a sterile cotton pellet weighing (10 ± 1 mg) saturated with 1% carrageenan suspension in saline solution (NaCl 9‰) was implanted subcutaneously bilaterally below the axilla. Treatments were continued for a duration of seven days [10].

On the 8th day, animals were anesthetized by inhalation of ether and cotton pellets were collected. A sample of the exudate was sprayed on Mallasez cell and observed under optic microscope at 1000X, to count the white blood cells in the sample. The cotton pellets were weighed and dried overnight at 60°C until their weight was constant. The difference between the wet and dry weight was considered to be the weight of the granuloma tissue.

3. Results

3.1. *Helychrisum faradifani* extract effect on swelling

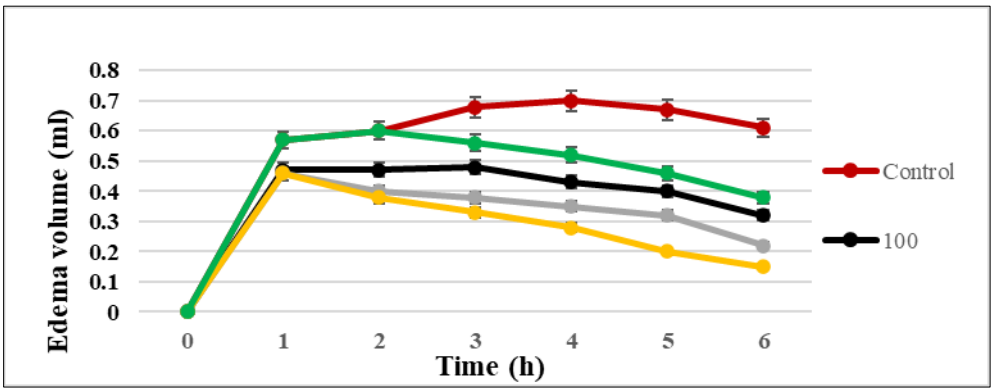

Figure 1 Carrageenan-induced plantar edema volume in control group and animals treated with the extract, administered orally, at the doses 100 , 200 and 400 mg/kg and indomethacin , administered orally at 10 mg/kg ($\bar{x} \pm \bar{\sigma}$; n = 6; p<0.05)

Intraplantar injection of carrageenan in mice resulted in a time-dependent increase in paw volume, an indication of induced inflammation. However, treatment with *H. faradifani* extract and indomethacin reduces the paw edema in a dose-dependent manner. Paw edema of control group animal increases up till 4 hours after carrageenan injection, while edema start decreasing after 2 hours for animal treated with indomethacin, and after 1 hour for animal treated with the extract at doses 200 and 400 mg/kg (Figure 1). In addition, the maximal volume of edema in treated animals is inferior to the volume of edema in the 2 control groups; it is equal to 0.46 ± 0.01 ml in the 3 groups treated with the extract 1

hour after carrageenan injection, versus 0.6 ± 0.01 ml in the positive group 2 hours after carrageenan injection and 0.7 ± 0.02ml in the carrageenan group 4 hours after carrageenan injection.

Six hours after carrageenan injection, paw edema volume of control group is 0.61 ± 0.01 ml, versus 0.38 ± 0.02 ml for animals treated with indomethacin and 0.32 ± 0.02, 0.22 ± 0.01 and 0.15 ± 0.01ml respectively for animals treated with the extract at doses 100, 200 and 400 mg/ml ($p<0.05$). The anti-inflammatory activity was found to be dose dependent in carrageenan-induced paw edema model. The extract has shown significant ($P < 0.05$) inhibition of paw oedema, 49.52±2.6, 62.22 ±3.3 and 76.82 ±2.1 % on 6th hour with the doses of 100, 200 and 400 mg/kg respectively, versus 40.95± 1.5 % for the animals treated with Indomethacin at 10 mg/kg (Table I).

Table I Reduction of carrageenan-induced edema on mice paw

Groups	Control	100 mg/kg	200 mg/kg	400 mg/kg	Indomethacin (10 mg/kg)
Edema inhibition (%)		49.52±2.6	62.22 ±3.3	76.82 ±2.1	40.95± 1.5

3.2. *Helychrisum faradifani* extract effect on vascular permeability

Intraperitonal injection of acetic acid in mice resulted in increased extravasation of Evans blue. It is materialized with the absorbance of the washing solution absorbance. However, treatment with *H. faradifani* extract reduces the absorbance of intraperitoneal fluid in a dose-dependent manner (Figure 2). Control group intraperitoneal absorbance is equal to 2.30 ±0.15, versus 1.65± 0.08, 1.40± 0.01 and 0.59± 0.01 respectively in group treated with the extract at doses 100, 200 and 400 mg/kg , and 1.49± 0.1 for animals treated with indomethacin 10 mg/kg ($p<0.05$). These results show a dose dependent inhibition of Evans blue extravasation, which reflects vascular permeability reduction.

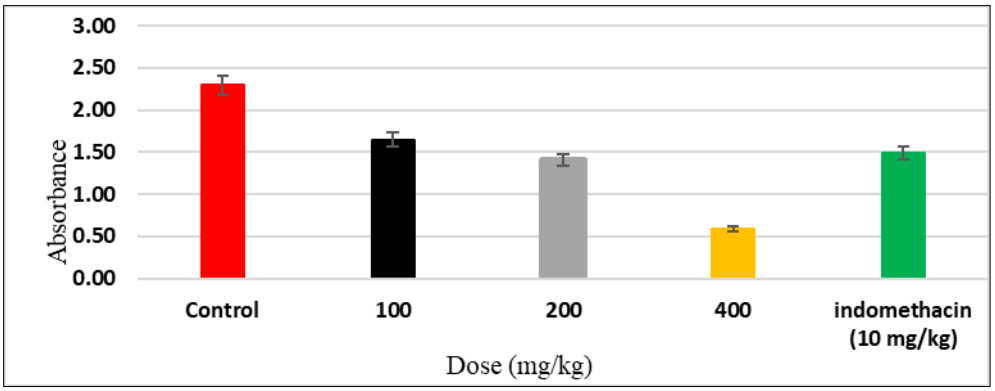

Figure 2 Absorbance of Evans blue leaked in abdominal cavity of control group animals and treated with the extract, administered orally, at the doses 100, 200 and 400 mg/kg and indomethacin, administered orally at 10 mg/kg, after injection of acetic acid intraperitoneally ($\bar{x} \pm \bar{\sigma}$; n = 6; $p<0.05$)

3.3. *Helychrisum faradifani* extract effect of on induced-carrageenan granuloma

After 8 days cotton pellets insertion, there is a granuloma formation. The treatment with the extract and indomethacin reduces the granulomatous tissue weight. This reduction is dose dependent manner. It is 5 ±0.2 in control group, versus 4.32± 0.2, 3.4± 0.1, and 2.25± 0.08 mg in the groups treated with *H. faradifani* extract and 3.25 ±0.1 mg in the positive control group (Figure 3). These results show the positive effect of the extract on the cellular phase of inflammation. This effect is confirmed with the reduction of leucocytes number in the cotton pellets (Figure 4).

The exudate analysis revealed that in *H. faradifani* and indomethacin pre-treatment animals, the number of leucocytes in the granuloma decreases in a dose dependent manner. The control group granuloma contains 119.55 ± 0.1, versus 105.1 ± 0.1, 88.83 ± 0.1 and 70.48 ± 0.1 in the treated animals granuloma and 85.52 ± 0.05 in the positive control group's granuloma ($p<0.05$) (Figure 4). These results indicate the reduction of leucocytes migration into the cotton pellets.

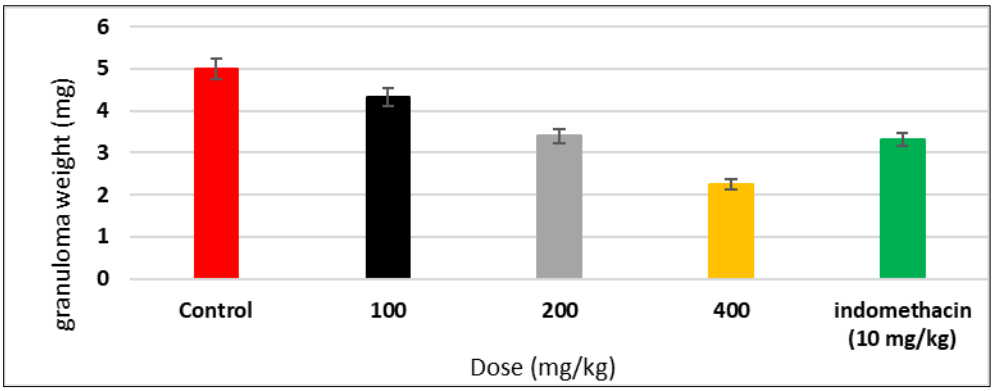

Figure 3 Granuloma weight in the control group animals and treated with the extract, administered orally, at the doses 100, 200 and 400 mg/kg and indomethacin, administered orally at 10 mg/kg, after insertion of cotton pellets imbibed of carrageenan in their skin ($\bar{x} \pm \bar{\sigma}$; n = 6; p<0.05)

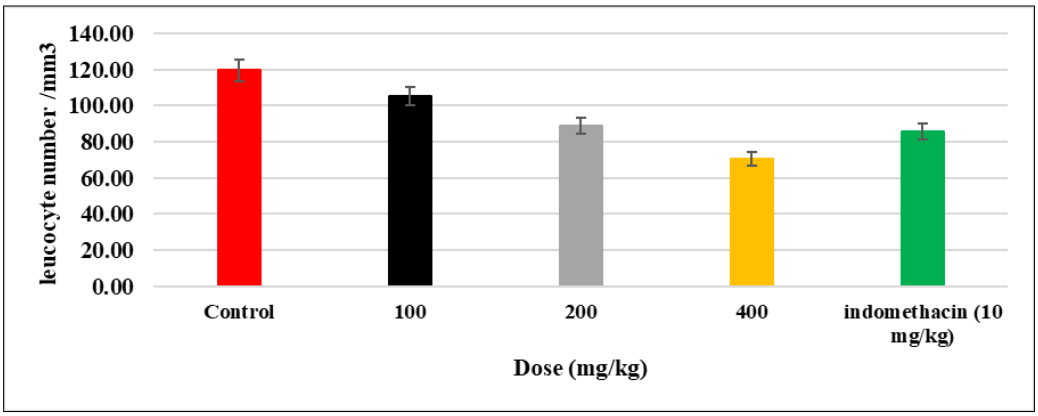

Figure 4 Leucocyte number in the control group animals and treated with the extract, administered orally, at the doses 100, 200 and 400 mg/kg and indomethacin, administered orally at 10 mg/kg, after insertion of cotton pellets imbibed of carrageenan in their skin ($\bar{x} \pm \bar{\sigma}$; n = 6; p<0.05)

4. Discussion

Despite of the existence of pharmaceutical drugs, a couple of people in Madagascar still use medicinal plants. This work aimed to investigate the anti-inflammatory action of *H. faradifani*. The results of the tests that we have carried out is relevant, hydroalcoholic extract of this plant reduces carrageenan induced-swelling, vascular permeability and granuloma weight.

Carrageenan and acetic acid are used as phlogogenic agents in animal experimental models to investigate the anti-inflammatory activity of drugs. Edema developed in the murine paw after carrageenan injection is a biphasic event. Histamine, an important inflammatory mediator and a potent vasodilator is released in the initial phase during the first hour of injection, and prostaglandin is released in the second phase of edema [11]. While intraperitoneal injection of acetic acid stimulates production of PGE2 and some other endogenous substances [12].

The results of the tests that we have conducted indicates that *H. faradifani* extract reduces carrageenan-induced edema and intraperitoneal vascular permeability induced by acetic acid. In the control group, maximum edema volume was observed in the first hour following carrageenan injection and this persisted until the 4th hour. This edema reduces slowly showing immune response to abate inflammation. The inflammation reduces faster in the groups treated with the extract and indomethacin. At high dose *H. faradifani* extract has stronger effect compared to indomethacin. In addition, the extract is effective in the first hour of inflammation prosses induced by intraplantar injection of carrageenan, meanwhile indomethacin is effective from the second hour. This means that the extract is also effective in the first part of carrageenan-induced swelling which is due to the release of histamine. Herein, we can speculate that *H.*

faradifani extract might reduce histamine release [13]. Our results also demonstrated that extract decreases the Evans blue concentration in acetic acid-induced vascular permeability test. Therefore, we speculate that the anti-inflammatory effects of the extract might be related to the inhibition, synthesis, and release of inflammatory mediators. Since prostaglandins are involved in these two processes, we advance a hypothesis that the extract at the doses used in this work inhibits either the release or the synthesis of this mediator.

Granuloma-induced cotton pellets was used to evaluate cellular phase activity of *H. faradifani* extract. Granuloma is the consequence of exposure to foreign object which is the cotton pellets. It is an inflammatory response characterized by collections of macrophages, epithelioid cells and multinucleated giant cells in the lesion [14]. The results of this test show a distinct inhibition of cotton-induced granuloma formation. These results suggest that *H. faradifani* extract may act through a selective suppression on the cellular immune response involved in inflammations. This is confirmed by the decrease of leucocytes number in the granuloma of animals treated with the extract as compared to control group. In inflammatory response, there is an endothelial permeability increases which allows the blood leukocytes migration into the interstitium, there is also an oxidative burst, a release of cytokines (interleukins and tumor necrosis factor-α), an induction of arachidonic acid metabolism, oxygenases, peroxidases and nitric oxide synthase [15].

The reduction of leucocytes number in granuloma means that the extract reduces leucocytes migration into the cotton pellets. It might be due to decrease of pro inflammatory cytokines release. Scientific evidences demonstrated that flavonoids, polyphenols, and triterpenoids possess anti-inflammatory effects in animal models [16]. Recent studies have also demonstrated that flavonoids can inhibit enzymes responsible in mediators synthesis involved in inflammation. They are also known as potent antioxidants which attenuate tissue damage [17]. It is also reported that terpenes inhibited the production of prostaglandin E2, the modulation of arachidonic acid (AA) metabolism and the reduction of nitric oxide (NO) production [18,19,20].

Analysis of *Helichrysum faradifani* essential oil from Madagascar led to the identification of fenchene, curcumene, caryophyllene and limonene [21]. Referring to those evidence, and since *H. faradifani* extract contains terpenes and flavonoids, one can also suggest that *H. faradifani* extract decreases nitric acid synthesis and the oxidative burst, like the crude extract of *Myrciaria dubia* seeds suppresses the formation of paw edema by inhibiting localized NO production in carrageenan-treated mice [22].

5. Conclusion

Our study showed that *Helichrysum faradifani* inhibit carrageenan-induced paw edema in the first hour after carrageenan and in the second phase of the inflammation. It also reduces leucocytes migration into the interstitial tissue. These results suggest that *H. faradifani* extract may act as a therapeutic agent of inflammatory diseases through a selective suppression on the cellular immune response involved in inflammations as well as through a direct anti-inflammatory mechanism including inhibiting histamine release or PGE2 and NO production, it can also reduce the oxidative burst. This study therefore provides a scientific justification for the use of this plants in herbal medicinal preparations targeted at diseases which involve inflammation, such as back pain and wound healing.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interests.

Statement of ethical approval

The experimental protocols were approved by the Sciences Faculty Animal Care Ethics Committee (Ref: FacSc/CE013/22).

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