In vivo anti-inflammatory and antiarthritic activities of aqueous extracts from Thymelaea hirsuta

Zora Azza, Mounia Oudghiri

Department of Biology, Faculty of Sciences Ain Chock, Laboratory of Physiology and Molecular Genetics, Immunology Unit, University Hassan II, B.P. 5366, Maarif, Casablanca, Morocco

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ABSTRACT

Background: The aerial parts of Thymelaea hirsuta (TH) are used as a decoction in the treatment of different pathologies in folk medicine in Morocco. Objective: The aqueous extracts were evaluated for its anti-inflammatory activity and in inhibition of adjuvant induction arthritis in male Wistar rats. Materials and Methods: The anti-inflammatory activity was carried out using carrageenan-induced rat paw edema model, and the antiarthritic activity was carried out using complete Freund’s adjuvant-induced arthritis model. Results: The plant extract (500 mg/kg body weight) exhibited significant activity in acute inflammation produced 60% of inhibition after 4 h as compared with that of the standard anti-inflammatory drug, the diclofenac (100 mg/kg) which showed 40% of inhibition. In arthritis model, the extract produced 85% inhibition after 18 days when compared with the diclofenac (10 mg/kg; 72%). Conclusion: These results indicate that the aqueous extract of TH had an anti-inflammatory activity and inhibited the induction of adjuvant arthritis in male Wistar rats.

Key words: Antiarthritis, Antinflammation, Medicinal plants, Thymelaea hirsuta, Wistar rats

INTRODUCTION

Traditional Moroccan medicine offers a variety of herbal products that have been used in the treatment of patients with different diseases for several decades. However, many of these products have not been validated experimentally for their composition and/or mechanism of action. Thymelaea hirsuta (TH) has been in use in Morocco for the treatment of patients with a variety of disorders, including diabetes.

We have previously reported that the aqueous extract of the aerial part of TH (5 g/kg body weight) can be considered safe as it did not cause either any lethality or adverse changes in the general behavior, histological or hematological and biochemical parameters in the acute and in sub-chronic toxicity studies in rats. We also reported that the extract has a potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats related to glutathione mediated the detoxification as well as free radical scavenging activity.

The phytochemical composition of different extracts of TH has been well-studied. This plant contains polyphenols with a substantial part of flavonoids, diterpenes that can explain its different pharmacological activities. Hirseins A and B, two daphnane diterpenoids, isolated from TH were found to inhibit melanogenesis in B16 murine melanoma cells.

Therefore, the aim of the present study was to explore in vivo others pharmacological potential of TH aqueous extracts; its anti-inflammatory activity and its inhibition action of adjuvant induction arthritis in male Wistar rats.

MATERIALS AND METHODS

Animals

Wistar rats weighing (220 ± 20 g) of either sex were purchased from the animal house of the Department of Biology, Faculty of Sciences, Rabat, Morocco. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines.
Plant material
The plant material was collected from the local market, identified and authenticated taxonomically at National Scientific Institute, Rabat, Kingdom of Morocco. A voucher specimen of the collected sample was deposited in the institutional herbarium for future reference.

Preparation of the aqueous extract of Thymelaea hirsuta
The aerial parts of the plant were prepared according to the traditional method used in Morocco (decoction): 50 g powdered of the whole plant mixed with 500 ml of distilled water was boiled for 15 min followed by cooling for 15 min. Thereafter, the aqueous extract was filtered and concentrated in rotary vacuum evaporator at a temperature below 40°C. The dried extracted material was stored at –20°C until use. For oral administration (gavages), the dried extract was dissolved in distilled water on the day of experimental studies and administered by gavages at 500 mg/kg of body weight (Azza et al. 2012).

Carrageenan-induced paw edema in rats
Edema in the right hind paw of rat was induced by an injection of 0.2 mL of 1% (w/v) of carrageenan (Sigma-Aldrich; St Louis, USA) in saline subcutaneously in the plantar side of the right hind paw of rat. The paw diameter was measured before the carrageenan injection and then each hour up to 5 times then after 24 and 48 h.

The rats were randomly divided into three groups (n = 6). The first group (control group) received normal saline (3 ml/kg body weight p.o.), while the second group received the standard antiinflammatory drug; the diclofenac (Troge; Germany) (100 mg/kg body weight p.o). The third group was treated with the extract of TH (500 mg/kg body weight p.o). The animals were pretreated 1 h before the administration of Carrageenan. Percentage of inhibition of edema was calculated using this formula:

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\% \text{ inhibition of edema} = \frac{\text{Mean edema increase in control group} - \text{Mean edema increase in treated group} \times 100}{\text{Mean edema increase in control group}}
\]

Adjuvant-induced chronic arthritis
Experimental arthritis was induced in the left footpad of each rat by a s.c. injection of 0.2 mL of complete Freund's Adjuvant (CFA, Difco Laboratories, Detroit, MI, USA). Rats in the test groups were treated with the extract of TH (500 mg/kg body weight p.o) every day for 21 days after the CFA challenge. The control group received normal saline (3 ml/kg body weight p.o), while the third group received the diclofenac (10 mg/kg body weight p.o).

Leukocytes migration assay
Dorsal subcutaneous air pouches (20 ml sterile air) were formed as described previously in four groups of rats (n = 6). Three days later, 0.5 ml of carrageenan (1%) was injected in the formed cavity of three groups and NaCl 0.9% in one group. Rats in the test groups were treated with the extract of TH (500 mg/kg body weight p.o) every day for 4 days after the air pouches treatment. The control group received normal saline (3 ml/kg body weight p.o) while the third group received the diclofenac (100 mg/kg body weight p.o). At the end of the experimentation, 5 ml of ice cold NaCl0.9% were injected in the formed cavity and then collected for the evaluation of the number of leukocytes.

Statistical analysis
The data were expressed as mean ± standard error of the mean. The test followed by Dennett's test P < 0.05 was considered to be statistically significant.

RESULTS
Reduction of carrageenan-induced paw edema in rats
The paw edema was increased and reached maximally 5 h after carrageenan treatment.

The aqueous extracts of TH produced statistically significant (P < 0.01) decreases in the carrageenan-induced rat paw edema after 2 h as shown in Table 1. The percentage of inhibition of edema was 55% after 3 h for the group treated with the TH extracts compared to the control group treated with the diclofenac (100 mg/kg) with 35% of edema inhibition as shown in Table 1.

Reduction of adjuvant arthritis-induced paw edema
Oral administration of TH for 21 days after adjuvant injection showed the reduction of paw edema in rats compared to the vehicle-treated control group [Figure 1]. The extracts of TH produced 85% inhibition of edema after 18 days when compared with that of the standard drug diclofenac (72%) as shown in Figure 1.

Leukocyte migration
The number of leukocyte attracted to the air induced cavity after the carrageenan injection alone was (5 × 10⁷ cells/ml) higher than the number of leukocytes found in the group treated with the anti-inflammatory drug; the diclofenac (3.5 × 10⁶ cells/ml) or in the group treated with the plant extract (2.5 × 10⁵ cells/ml).

DISCUSSION
The results observed in the rat paw edema assays showed a significant inhibitory activity of the plant
The aqueous extract of the plant was given daily by the oral route to groups of Wistar rats (n=6 per group) 0.5 g/kg, for 30 days. Data are expressed as mean ± standard error of the mean; *P<0.05; **P<0.01 versus the control group. Edema in the right hind paw of the rat was induced by an injection of 0.2 mL of 1% (w/v) of carrageenan. The animals were pretreated 1 h before the administration of Carrageenan. The paw diameter was measured before the carrageenan injection and then each hour up to 5 times then after 24 and 48 h. TH=Thymelaea hirsuta

Figure 1: Inhibitory effect of *Thymelaea hirsuta* (TH) extracts on carrageenan-induced paw edema in rats. The in vivo anti-inflammatory activity observed in these experiments±standard error of the mean; *P<0.05; **P<0.01

Table 1: Effect of TH extracts in carrageenan-induced rat hind paw edema

| Treatments and doses | 0 h | 1 h | 2 h | 3 h | 4 h | 5 h | 24 h | 48 h |
|----------------------|-----|-----|-----|-----|-----|-----|------|------|
| Carrageenan 1%       | 7.33±0.57 | 10.66±0.57 | 11.33±0.57 | 10.66±0.57 | 11.33±0.57 | 14.00±0.00 | 12.00±0.00 | 11.33±0.57 |
| Percentage of inhibition | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TH 500 mg/ml          | 8.00±0.00 | 10.66±0.57 | 11.00±0.00 | 11.00±0.00 | 11.00±0.00 | 10.33±0.57 | 10.00±0.00 | 9.00±0.00 |
| Percentage of inhibition | 0 | 44 | 55 | 55 | 59 | 59 | 68 | 65 |

The adjuvant arthritis model has extensively been used for studies regarding the pathogenesis of autoimmune arthritis as well as for the testing of new natural therapeutic products.[9] A variety of herbal products have been shown to attenuate the severity of the disease in the rat adjuvant arthritis model.[9] The suppression of inflammation and arthritic processes has been also attributed to their antioxidant activity.[9,10] In our earlier studies, we tested the toxicity, the hepatoprotective and the antioxidant activity of the aqueous extract of TH.

*Thymelaea hirsuta* appears to be a promising candidate for further preclinical and clinical trials in inflammation and arthritis.

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