Study on the antipyretic activity of thyme (*Thymus broussonetii*) in experimental rats

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Abstract

Previous pharmacological essays had reported the anti-inflammatory and analgesic activities of *Thymus broussonetii* extracts. This current study extended these previous works and aimed at investigating the potential antipyretic effect of *T. broussonetii*. For that, we have tested the effect of aqueous, butanolic and ethyl acetate extracts of *T. broussonetii* on yeast- induced fever in rats. Our results showed that the three extracts (aqueous, butanolic and ethyl acetate), induced a significant reduction of rectal temperature in febrile rats indicating that *T. broussonetii* produce a marked antipyretic activity. However neither the thyme extracts, nor acetylsalicylic acid, did induce hypothermia in rats. This antipyretic effect may be related to the extract's analgesic and anti-inflammatory activities. On the other hand, no mortality and no signs of toxicity have been observed at the tested doses indicating that the three extracts of thyme present low toxicity. These findings provided strong arguments and validation of the popular use of this plant as analgesic, anti-inflammatory and antipyretic herb.

Key words: *T. broussonetii*, rectal temperature, febrile rats, antipyretic effect.

Introduction

Many species of *Thymus* (*Labiatae*) are widely used as aromatic and medicinal plants in the mediterranean area. As other *Thymus* species, *Thymus broussonetii* is widely distributed and found in several areas in Morocco (Jahandiez and Maire, 1934). The availability of thyme species and their relatively low cost had promoted their broad use as folk remedies. Traditional medicine practitioners treat digestive disorders, diarrhoea, fever, coughs, cold, painful events and infected wounds using powder or decoction of *Thymus* species (Bellakhdar, 1997; Sijelmassi, 2000).

The wide use of these herbs is done in absence of scientific base. This situation has incited many authors to study the pharmacological properties of thyme extracts. Previous pharmacological investigations had reported the antimicrobial (Lattaoui *et al.*, 1993; Lattaoui and Tantaoui-Elaraki, 1994), anxiolytic and immunostimulant (Elhabazi *et al.*, 2006b) effects of *T. broussonetii*. This species was also reported to have anti-inflammatory activity (Ismaili *et al.*,

2002). Moreover, in a previous paper, we have demonstrated that this species exerts a significant effect against pain in the four current nociceptive models in mice and rats: formalin, tail immersion, hot plate and writhing tests (Elhabazi *et al.*, 2006a). The obtained results revealed a more pronounced effect compared to that of the analgesic reference drug acetylsalicylic acid (ASA). The overall found results suggest that the species contains principles with both analgesic and anti-inflammatory activities.

It is well known that various anti-inflammatory and analgesic drugs have also antipyretic effect. The present study aims at investigating the potential antipyretic effect on yeast-induced fever in rats, using *T. broussonetii* aqueous, butanolic and ethyl acetate extracts. These extracts were also tested on normothermic rats to determine whether the antipyretic activity is related to a hypothermic effect.

Materials and methods

Animals

Experiments were conducted on male *Sprague-Dawley* rats (180-230g body weight). Animals were housed in standard cages with food and water ad-libitum. The air temperature was maintained at 22 ± 2 ºC in 12-hours light-dark cycle. The experiments were carried out in accordance with current guidelines for the care of laboratory animals and the ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1983).

Preparation of thyme extracts

The thyme extracts were obtained as previously described by Elhabazi *et al.*, (2006a). Briefly, leaves and their stem barks were dried at 40°C and triturated in order to obtain a powder. Then, 1 liter of ethanol was added to the thyme powder (200g) to obtain ethanolic extract using a Soxhlet apparatus. This ethanolic extract was concentrated under reduced pressure by using a rotary evaporator and then successively partitioned with solvents of increasing polarity (Hexane, Chloroform, Ethyl acetate and Butanol). The aqueous extract is the result of a filtered decoction prepared using 200g of thyme powder in 1liter of water. Aqueous extract, Ethyl acetate and butanolic fractions were made up to the appropriate volume with 0.9%NaCl just before use.

Antipyretic test

The effect of thyme extracts on the rectal temperature was tested using the method described by Reanmongkol et al., (1994). Briefly, male rats fasted overnight received a 20 % brew's yeast suspension (10 ml/kg body weight, intraperitonially). Seventeen hours after yeast injection, the rectal temperature of the tested animals was measured using a digital thermometer (Minitherm HI 8751, Hanna instruments). Only rats exhibiting at least 1º C increase in rectal temperature were selected for the study of the antipyretic effect. These rats (three groups of six animals in triplicate) were treated either with thyme extracts (200 mg/kg bw, ip.), 0.9 % saline solution (for control) or acetylsalicylic acid (ASA, 200 mg/kg bw., ip.) as a reference antipyretic drug.

After treatment, the rectal temperature was recorded every 15 min during the first hour and at 1.5, 2, 3 and 4 hours later.

On the other hand, the effect of thyme extracts on normothermia was tested without inducing fever. Rectal temperature in normal rats was

measured at 0.5, 1, 2, 3 and 4 hours after treatment.

Acute toxicity

Groups of six rats in triplicate received orally doses of 1, 2, 3, 4 and 5 g/kg of aqueous, butanolic and ethyl acetate extracts of *T. broussonetii*. The control group received orally distilled water. The groups were observed for 48 hours and during the following 7 days to note any signs of toxicity or mortality.

Data analysis

Data as mean± S.E.M from triplicate assays were analysed using the SPSS program. The statistical difference between groups was performed using one-way analysis of variance.

Results

Effect of *T. broussonetii* extracts on yeast-induced fever in rats

The experimental rats showed a mean increase of about 1 ºC in rectal temperature, 17 hours after yeast injection. The yeast-induced fever was significantly reversed (Table 1) by all the three extracts of thyme (200 mg/kg, ip.). The antipyretic effect was observed 15 min after the treatment and persists three hours for ethyl acetate extract and four hours for aqueous and butanolic extracts. These extracts reduced very significantly the fever on a critical period, between 30 and 180 min following the treatment. The reference drug, ASA (200 mg/kg, ip.) also reversed hyperthermia induced by yeast.

Effect of *T. broussonetii* extracts on rectal temperature in normal rats

There is no difference in the rectal temperature between control and rats treated by the three extracts (aqueous, butanolic and ethyl acetate) (Fig. 1). The reference drug, ASA did not affect the rectal temperature of normal rats.

Acute toxicity essay

During this essay, no mortality and no signs of toxicity have been observed at the tested doses indicating that the three extracts of *T. broussonetii* present low toxicity.

Discussion

The present study aimed at investigating a potential antipyretic activity of *T. broussonetii*. Knowing the reported anti-inflammatory (Ismaili *et al.*, 2002) and analgesic (Elhabazi *et al.*, 2006) activities of this species, we aimed to know if these two activities are combined with an eventual antipyretic effect. Thus, the three thyme extracts are tested at 200 mg/kg b.w. This dose was chosen because it was the most effective in inhibiting both inflammatory and central pains by thyme extracts in several nociceptive animal models (Elhabazi, 2007).

Our results showed that the three extracts: aqueous, butanolic and ethyl acetate induced a significant reduction in rectal temperature in febrile rats indicating that *T. broussonetii* produce a marked antipyretic activity. This effect was observed at the same dose producing analgesic effect. This let us to suppose that this antipyretic activity is related to the analgesic and anti-inflammatory effects. Thus, as similar to the classical non-steroid anti-inflammatory (NSAI) drugs, *T. broussonetii* possesses antipyretic, analgesic and anti-inflammatory activities. On the other hand and similar to the NSAI drugs, *T. broussonetii* extracts didn't induce hypothermia in normal rats. Besides, it was reported that thyme extracts inhibit the platelet aggregation as the same way of NSAI

Table 1. Effect of *T. broussonetii* extracts (200 mg/kg, ip.) on yeast-induced fever in rats.

Groups	Rectal temperature									
	Initial	17 h after yeast injection	Time after treatment (min)							
			15	30	45	60	90	120	150	180	240
Control	37.48 ±0.20	38.65 ±0.30	39.05 ±0.20	39.00 ±0.20	38.90 ±0.20	38.92 ±0.20	38.87 ±0.2	38.80 ±0.30	38.85 ±0.20	38.77 ±0.20	38.60 ±0.30
Aqueous extract	37.52 ±0.30	38.88 ±0.30	38.32* ±0.30	31.18** ±0.20	37.88** ±0.20	37.76** ±0.10	37.74** ±0.20	37.62** ±0.30	37.72** ±0.30	37.84* ±0.30	37.46* ±0.50
Butanolic extract	37.37 ±0.30	38.41 ±0.10	38.10** ±0.20	37.93** ±0.30	37.86** ±0.50	37.88** ±0.40	37.71** ±0.40	37.70** ±0.40	37.53** ±0.41	37.51* ±0.23	37.43* ±0.21
Et. Acetate extract	37.56 ±0.20	38.86 ±0.40	38.40* ±0.40	38.30 ±0.30	38.13* ±0.30	37.93** ±0.20	37.66** ±0.30	37.46** ±0.40	37.38** ±0.40	37.65* ±0.40	38.00 ±0.40
A.S.A.	37.40 ±0.10	38.48 ±0.20	38.30* ±0.30	38.36* ±0.40	38.26* ±0.40	36.06** ±0.40	37.74** ±0.30	37.78* ±0.30	37.90* ±0.30	37.90 ±0.50	38.26 ±0.30

Rectal temperature recorded at different times on control (0.9% saline solution 200mg/Kg bw. ip.) and on treated rats (aqueous, butanolic, ethyl acetate extracts or ASA, 200mg/Kg bw. Ip), as described in material and methods. Each group contains 6 animals and essay is in triplicate. Significant difference at p=1% (******) and at p= 5% (*).

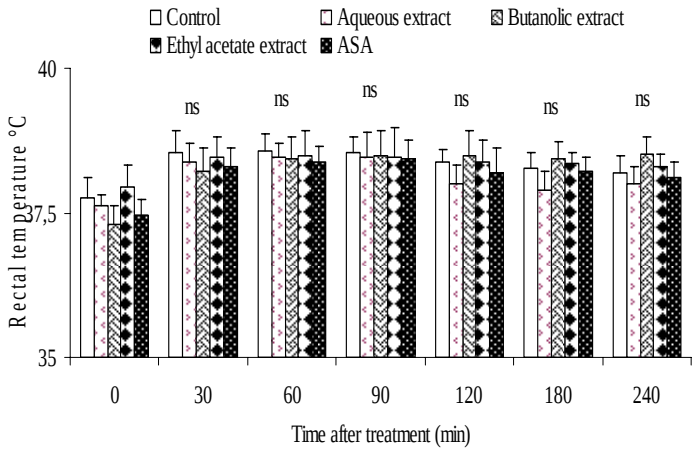

Figure 1. Effect of *T. broussonetii* extracts (200 mg/kg, ip.) in normothermic rats.

drugs (Okazaki *et al.*, 2002). Considering all these findings, we can suggest that the mechanism of action of thyme extracts on fever is comparable with that of NSAI drugs. It is well known that most of the anti-inflammatory analgesic drugs produce their antipyretic action thought inhibition of prostaglandins synthesis within the hypothalamus (Lullmann *et al.*, 1991). Although, there is no direct evidence of *T. broussonetii* to interfere with prostaglandin synthesis but it can be supported by a previous study demonstrating the ability of *T. broussonetii* to inhibit the inflammatory process (Ismaili *et al.*, 2002). Therefore, it could be suggested that the antipyretic action of *T. broussonetii* may be related to the inhibition of prostaglandins synthesis in the hypothalamus.

T. broussonetii extracts contain many kinds of compounds such as flavonoids, tannins, saponins and triterpens (Ismaili, 2004; Elhabazi, 2007). So, it's difficult to predict which class is responsible for the antipyretic activity. Interestingly, previous phytochemical and pharmacological investigation allowed identification of the triterpens ursolic and oleanolic acids as the two anti-inflammatory principals in *T. broussonetii*. Also, it was reported that flavonoids such as apigenin and luteolin possess topical anti-inflammatory activity (Della Loggia *et al.*, 1986; Tubaro *et al.*, 1989). Containing such compounds, they may give a significant contribution to the antipyretic activity of *T. broussonetii*. Moreover, Swain *et al.*, (1985) have reported that thyme extracts contain several salicylates. This let us to suppose that these salicylates may be responsible for the analgesic, antipyretic and anti-inflammatory effects. It could be suggested that the antipyretic activity of thyme extracts may be the result of action of three kinds of compounds, triterpenes, flavonoids and salicylates.

Conclusion

In conclusion, the present results suggest that *T. broussonetii* extracts possess antipyretic activity. This effect may be related to the extract's analgesic and anti-inflammatory activity. There is evidence to believe that this plant contains compounds acting as NSAI drugs. These findings provided strong arguments and validation of the popular use of this plant as analgesic, anti-inflammatory and antipyretic herb.

References

Bellakhdar J (1997) La pharmacopée marocaine traditionnelle. Edition Ibiss Press; Paris. p 358.

Della Loggia R, Tubaro A. Dri P, Zilli C, Del Negro P (1986) The role of flavonoids in the anti-inflammatory activity of *Chamomilla recutita*. *Progr. Clin. Biol. Res*. **213**: 481-484.

Elhabazi K, Aboufatima R, Benharref A, Zyad A, Chait A, Dalal A (2006a) Study on the antinociceptive effects of *Thymus broussonetii* Boiss. extracts in mice and rats. *J. Ethnopharmacol.* **107**: 406-411.

Elhabazi K, Dicko A, Desor F, Dalal A, Younos C, Soulimani R (2006b) Preliminary study on immunological and behavioural effects of *Thymus broussonetii* Boiss., an endemic species in Morocco. *J. Ethnopharmacol.* **103:** 413–419.

Elhabazi K (2007) Contribution à l'étude pharmacologique et comportementale de certaines espèces de thym marocain. Thèse nationale. Université Cadi Ayyad. Marrakech. Maroc.

Ismaili H (2004) Valorisation du genre *Thymus* du Maroc : Étude phytochimique et pharmacologique des extraits non-volatiles de trois espèces de thym : *T. willdenowii Boiss, T. broussonetii* Boiss, et *T. satureioides* Coss. Thèse d'état. Université Mohamed V, Rabat. Maroc.

Ismaili H, Sosa S, Brkic D, Fkih-Tetouani S, Ilidrissi A, Touati D, Aquino R, Tubaro A (2002) Topical anti-inflammatory activity of extracts and compounds from *Thymus broussonetii*. *J. Pharm. Pharmacol*. **54**: 1137-1140.

Jahandiez E, Maire R (1934) Catalogue des plantes du Maroc. Imp. Minerva Alger. pp.653.

Lattaoui N, Tantaoui-Elaraki A, Errifi A (1993) Composition and antimicrobial activity of the essential oils of *Thymus broussonetii, T. zygis* and *T. satureioides*. *J. Essential Oil Res*. 5: 45-53.

Lattaoui N, Tantaoui-Elaraki A (1994) Comparative kinetics of microbial destruction by the essential oils of *Thymus broussonetii, T. zygis* and *T. satureioides*. *J. Essential Oil Res*. **6**: 165-171.

Lullmann H, Mohr K, Ziegler A (1991) Atlas de Poche de Pharmacologie (2ème édition). Flammarion ed. Paris. p: 32.

Okazaki K, Kawazoe K, Takaichi Y (2002) Human platelet aggregation inhibitors from thyme (*Thymus vulgaris* L.). *Phytother. Res*. **4**: 398-9.

Reanmongkol W, Matsumoto K, Watanabe H, Subhadhirasakul S, Shin-Ishiro S (1994) Antinociceptive and antipyretic effects of alkaloids extracted from the stem bark of *Hunteria zeylanica*. *Biol. Pharm. Bull.* **17**: 1345-1350.

Sijelmassi A (2000) Les plantes médicinales du Maroc. Le fennec Ed. Casablanca Maroc. pp:223.

Swain AR, Dutton SP, Truswell AS (1985) Salicylates in foods. *J. Am. Diet. Assoc*. **85**: 950-960.

Tubaro A, Del Negro P, Bianchi P, Romassi G, Della Loggia R (1989) Topical anti-inflammatory activity of a new acylated flavonoid. *Agents acrulis.* **26:** 229-230.

Zimmermann M (1983) Ethical guidelines for investigation of experimental pain in conscious animals. *Pain.* **16**: 109–110.