Phytochemical screening and in vivo antipyretic activity of the aqueous extracts of three Moroccan medicinal plants

Ghizlane Hajjaj¹*, Aziz Bahlouli², Karima Sayah¹, Mouna Tajani³, Yahia Cherrah¹, Amina Zellou¹

¹Laboratory of Pharmacology and Toxicology, Department of Drugs Sciences, Faculty of Medicine and Pharmacy, Mohammed V University, ERTP, BP 6203, Rabat Instituts, Agdal, Rabat, Morocco
²Laboratory of Biotechnology, Environment and Quality (LABEQ), ³Laboratory of Biological Testing, Department of Biology, Faculty of Sciences, Ibn Tofaïl University, BP 133; 14000 Kenitra, Morocco

*For correspondence
Dr. Ghizlane Hajjaj,
Laboratory of Pharmacology and Toxicology, Department of Drugs Sciences, Faculty of Medicine and Pharmacy, Mohammed V University, ERTP, BP 6203, Rabat Instituts, Agdal, Rabat, Morocco.
Email: hajjajghizlane1@gmail.com

ABSTRACT

Objective: In this study, the antipyretic effect of different extracts obtained from Matricaria chamomilla L. (MC), Ormenis mixta L. (OM) and Pistacia atlantica DESF. (PA) was investigated experimentally in rats.

Methods: Antipyretic activity of aqueous extracts was evaluated by yeast induced pyrexia method. The antipyretic effect was retained in all extracts tested and was comparable to that of paracetamol used as the standard drug negative control group (distilled water). The extracts were also phytochemically screened for alkaloids, tannins, saponins, flavonoids, terpenoids, quinones and anthraquinones.

Results: Treatment with aqueous extracts at dose of 400 mg/kg showed a significant (p≤0.05) reaction of pyrexia in rodents. The results suggest that this Moroccan medicinal plants possesses potent antipyretic activity. Phytochemical screening of the plants showed the presence of flavonoids, terpenoids, saponins, tannins, alkaloids and quinons which may responsible for this activity. However, anthraquinones were absent in all plants and alkaloids from PA.

Conclusions: This study might be the first formal report on antipyretic effects of these three plants in Morocco.

Keywords: Anti-pyretic activity, Pistacia atlantica DESF., Matricaria chamomilla L., Ormenis mixta L.

Introduction

Nature is a source of biologically active compounds and natural products have been used as medicines throughout the history of mankind. Although natural products include plant, animal and microbial sources, plants have been the origin and basis of pharmacy and pharmacology, constituting remedies in traditional medical systems and still being used as a source of bioactive compounds.
Exploring natural resources for drug discovery and development may represent advantages compared to some other approaches.\textsuperscript{1} Fever (also known as pyrexia) is when a human's body temperature goes above the normal range of 36.5 -37.5°C (98-100°F); it is a common medical sign. Fever is usually accompanied by different general symptoms, such as sweating, chills, sensation of cold and other subjective sensations. Missing of these symptoms during high temperature may be a sign of a serious illness.

Causes of fever include infections caused by parasites, viruses, bacteria, ricketsia, Chlamydia, immune reactions (including the defects in collagen, immunological abnormalities and acquired immunodeficiency). Other causes of fever are destruction of tissues, such as trauma, local necrosis (infarction), and inflammatory reaction in tissues and vessels (flebitis, arthritis), pulmonary infarction, and rhabdomyolysis.

An elevated body temperature (fever) is one of the ways our immune system attempts to combat an infection. Usually the rise in body temperature helps the individual resolve an infection. However, sometimes it may rise too high, in which case the fever can be serious and lead to complications.

A fever is usually accompanied by sickness behaviour, which consists of lethargy, depression, anorexia, sleepiness, hyperalgesia, and the inability to concentrate.\textsuperscript{2,3} The aim of this work was to carry out the Phytochemical screening and antipyretic activity of aqueous extract of \textit{Matricaria chamomilla} L., \textit{Ormenis mixta} L. and \textit{Pistacia atlantica} DESF. Moroccan medicinal plants to enhance its use in folk medicine.

**Materials and Methods**

**Plant material**

Aerial parts of \textit{Matricaria chamomilla} L., \textit{Ormenis mixta} L. and \textit{Pistacia atlantica} DESF. were collected in different regions of Morocco (Rabat, Sidi Yahya and wajda). The plants were identified at the Department of Plant Biology, Ibn Tofail University, Morocco. Voucher specimens (N° Rab78995, 101536 and 101537 respectively) of each are kept in the herbarium of Botany Department of Scientific Institute of Rabat.

**Preparation of the aqueous extract**

The aerial parts of \textit{M. chamomilla}, \textit{O. mixta} and \textit{P. atlantica} were separated and cleaned and washed with distilled water and dried under shade at temperature between 21-30 °C for 30 days. 50 g of powdered material of each plant was taken in beaker having 2 L capacities and 500ml of distilled water was added, soaked for 48 h with occasional shaking and stirring. The soaked material of plant was filtered through several layers of muslin cloth one by one for coarse filtration. The filtered extracts were concentrated under reduced pressure at 40°C, in rotary evaporator. The semi-solid mass was obtained and was weighed to calculate the yield, which was 16.76%, 7.84% and 15.3% (w/w) respectively and stored in a refrigerator (-8°C), until use.\textsuperscript{4,5}

**Preliminary phytochemical screenings**

Phytochemical properties of \textit{Matricaria chamomilla} L., \textit{Ormenis mixta} L. and \textit{pistacia atlantica} DESF. aqueous extracts were tested according to the method described by Trease and Evans using the following chemicals and reagents: Alkaloids with Mayer and Dragendoff’s reagents, Saponins (fothing test) and tannins (FeC\textsubscript{3}), flavonoids (Nacl and HC\textsubscript{1}), terpenoids (Salkowski test), anthraquinones (H\textsubscript{2}SO\textsubscript{4}).\textsuperscript{6,7}

**Animals**

Female swiss mice (25-30 g) and Wistar rats (180-220 g) procured from the animal centre of Mohammed V University, Medicine and Pharmacy Faculty, Rabat, Morocco. The animals were group-housed in polypropylene cages and kept under standard environmental conditions (23±1°C, and 12h/12h dark/light cycle) and maintained on balanced ration with free access to clean drinking water. The experiments were performed following the guidelines set for the international association for the study of pain and the national institute of health (NIH...
publication no. 85-23, 1985) regarding the care and treatment of experimental animals.8,9

**Lethal dose 50% determination**

The acute toxicity study was conducted in accordance with Organization for Economic Co-operation and Development (OECD) guidelines 423.10 Mice were treated with different doses 300 and 2000 mg/kg, p.o. of extracts. After single dose administration, rodents were observed for death or any other deformities during 14 days.

**Antipyretic activity**

The antipyretic activity of the tested extracts was screened in adult Wistar rats by using yeast-induced hyperpyrexia method.11 Pyrexia was induced by subcutaneous injection of 1 ml of 20% brewer's yeast suspension in saline solution. After 18 h of yeast injection, rats which showed a rise in temperature of at least 0.6 °C were taken for the study. Hyperthermic rats were divided into seven groups. The 1st group was kept as a control (received the vehicle) while the 2nd one was given paracetamol in a dose of 100 mg/kg (standard). The 3rd–5th groups were orally given the aqueous extract of *M. chamomilla* L. (AEMC), *O. mixta* L. (AEOM) and *P. atlantica* Desf. (AEPA) plants, respectively. Each extract was given in dose of 400 mg/kg. Rectal temperature of each rat was then recorded at 1 h interval after administration for 4 h.

**Statistical analysis**

Statistical analysis was carried out using one way ANOVA followed by student test. A value of *p*≤0.05 was considered a statistically significant difference between analyzed groups.

**Results and Discussion**

Since, *M. chamomilla*, *O. mixta* and *P. atlantica* showed significant anti-inflammatory and analgesic activities in our previous studies, it is worthwhile to evaluate their effect on antipyretic activity.4,5 Therefore, in the present study we investigated antipyretic activity of those plants aqueous extracts using experimental animal models.

Phytochemical screening is one of the necessary steps to find out the chemical constituents which lead the isolation of compounds. The phytochemical screening performed in this study showed that aerial parts of MC, OM and PA are rich in Tannins, Saponins, Terpenoids, flavonoids and alkaloids and does not contain Anthraquinones of all this plants and does not contain alkaloids just from PA (Table 1).

In acute oral toxicity study AEMC, AEOM and AEPA found safe at all tested doses (up to 2000 mg/kg) and did not show any noxious symptom in mice like convulsions, diarrhea, and irritation. During the 14 days of observation, no mortality was found. So, it suggested that LD$_{50}$ is more than 2 gm/kg.

![Figure 1: Antipyretic effect of *M. chamomilla*, *O. mixta* and *P. atlantica* in rats; -18 = Temperature just before yeast injection; 0 = Temperature just before drug administration.](image)

The subcutaneous injection of 20% brewer's yeast suspension substantially increased the rectal temperature of the rats 18 h after administration. The aqueous extracts treatment of MC, OM and PA at 400 mg/kg does significantly reduced the rectal temperature of the animals from the 1st hour until the 4th hours after administration, reaching peak antipyretic effect in the 2nd hour (36.36±0.09°C, *p*≤0.05) for AEMC, (36.40±0.10°C, *p*≤0.05) for AEOM and (36.47±0.14°C *p*≤0.05) for AEPA compared to control (38.32±0.11°C) (Figure 1). Paracetamol treatment (100 mg/kg) caused
significant reduction in rectal temperature at all periods of time, reaching peak antipyretic effect in the 2nd hour among all groups (36.90± 0.08°C, p≤0.05) compared to control group (38.32±0.11°C) (Table 2).

Antipyretic fever may be a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection, or other disease states. Antipyretic are drugs, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature.\textsuperscript{12}

The Brewer’s yeast-induced pyrexia test is of great relevance, as most NSAIDs inhibit the hyperthermal response. The subcutaneous injection of 20% Brewer’s yeast suspension causes release of pro-inflammatory cytokines which, upon reaching circulation, stimulate the synthesis of PGE\textsubscript{2} in the surroundings of the hypothalamic thermoregulator centers.\textsuperscript{13} AEMC, AEOM and AEPA reduced the yeast-induced hyperthermia.

The aqueous extracts of MC, OM and PA possesses a significant antipyretic effect in yeast-induced elevation of body temperature in rats and this may be due to anti-inflammatory
and analgesic effects based on the results of our previous studies and may be due to saponins, which according Gepdiremen et al. are potent inhibitors of prostaglandins.  

**Conclusions**

Based on these results, it can be concluded that water extracts of *Matricaria chamomilla* L., *Ormenis mixta* L. and *Pistacia atlantica* DESF. contain principles with antipyretic properties. It is now left for the therapeutic dosage to be determined for clinical applications. However, it needs isolation, structural elucidation, and screening of any of the abovementioned active principle/s to pin point activity of drug.

**Acknowledgements**

The authors wish to thank all the individuals and institutions who made this study possible.

Funding: No funding sources
Conflict of interest: None declared

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