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ANTI-INFLAMMATORY EFFECT OF *Myrtus nivellei* Batt & Trab (MYRTACEAE) METHANOLIC EXTRACT

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ABSTRACT

This work aims at evaluating the anti-inflammatory activity of an endemic species of the central sahara: *Myrtus nivellei* Batt & Trab. The methanolic extract of this plant was extracted by Soxhlet apparatus and concentrated under reduced pressure using a rotary evaporator.

In the carrageenan-induced paw edema test, five different groups of mice were established and the extract was administered orally in three different doses. The dose of 400 mg/kg was able to reduce significantly the paw edema with a comparable effect to that observed with Diclofenac (positive control). This is the first report to demonstrate a significant anti-inflammatory activity of the methanolic extract prepared from *Myrtus nivellei*.

Keywords: Anti-inflammatoy activity**;** *Myrtus nivellei* Batt & Trab; methanolic extract; paw edema.

1. INTRODUCTION

In recent years, there has been a considerable interest on the secondary metabolites obtained from many plants which gained a big scientific interest. Medicinal plants continue to be an important source of new chemical substances with potential therapeutic effects [1]. Commercially available anti-inflammatory drugs exert a wide range of side effects and are either too potent or too weak. Consequently, the search for new anti-inflammatory compounds has been a priority for the pharmaceutical industry.

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Many natural products have been tested in various animal models for the development of new anti-inflammatory agents [2].

In sight of this, our attention has been targeted to *Myrtus nivellei* Batt & Trab, an endemic Saharan species, belonging to the family of Myrtaceae, which grows in scattered populations, in rocky and sandy wades where subterranean water points exist, and generally at an altitude above 1800m [3]. It is a shrub up to 2 meters hight, with rough bark, leaves lanceolate, thick and linear (4 to 5 cm), five white petals, indeterminate stamens and the fruits are black berries [4,5]. It's known under the names of "*Tafeltest*" in Tamahaq and "*Raihane Essahara El Wousta*" in Arabic. Very little is known about the phytochemistry and biological activities of *Myrtus nivellei*. To our knowledge there is only few papers reporting the phenolic compounds and its antioxidant activity [6,7] and there is none about the potential activities of *Myrtus nivellei* extracts.

The purpose of the present study was to evaluate the anti-inflammatory activity of the methaolic extract using the carrageenan-induced paw edema test, for enduring safety and exploring the beneficial role of this plant to treat inflammatory diseases.

2. MATERIAL AND METHODS

2.1. PLANT MATERIAL

Aerial parts of *Myrtus nivellei* were collected in July 2013 during the flowering stage. near to Tamanrasset city (Hoggar massif, altitude: 1900 m, latitude: 22°38, longitude: 5°37). The plant was identified as *Myrtus nivellei* at the department of Botany INA-El Harrach (Algeria). The fresh leaves were washed thoroughly to remove dirt and dried under shade for a week. They were grounded into fine particles and stored until use.

2.2. EXTRACTION AND SAMPLE PREPARATION

Ground samples of *Myrtus nivellei* were weighted and extracted with methanol using a Soxhlet apparatus for 6 hours [8]. Then, the extract was concentrated to dryness under reduced pressure using a rotary evaporator to obtain a methanolic dry extract.

The dry extract was dissolved in a vehicle solution (1% Tween 80 in distillated water), and sterilized by filtration through sterile syringe filter with 0.2µm pore. Finally the filtered extract was stored in glass bottles at +4°C until use.

2.3. EXPERIMENTAL ANIMALS

Carrageenan-induced paw edema was carried out on male Swiss mice (25−30 g). Male animals were purchased from the laboratory of toxicology of Antibiotical Company (Medea, Algeria) and were housed in groups of six per standard cage, on a 12 h light/dark cycle with free access to food and water. They were acclimatized to laboratory conditions for at least 1 week before testing. The food was withdrawn on the day before the experiment, but free access to water was allowed. A minimum of six animals was used in each group.

2.4. DETERMINATION OF MEDIAN LETHAL DOSE (LD50)

LD_{50} of the extract was estimated in mice by using the method of Hilan et *al* [9]. In a preliminary test, animals in groups of three received 10, 100, or 1,000 mg/kg of dry methanolic extract suspended in the vehicle (1% v/v Tween 80) and sterilized by filtration through sterile syringe filter with 0.2µm pore. Animals were observed for 24 h for signs of toxicity and number of deaths. The LD_{50} was calculated as the geometric mean of the dose that resulted in 100% mortality and that which caused no deaths.

2.5. IN VIVO ANTI-INFLAMMATORY ASSAY: CARRAGEENAN-INDUCED PAW EDEMA IN MICE

The anti-inflammatory activity was evaluated by the carrageenan-induced paw edema test [10]. Paw edema was induced by injecting 0.1 ml of the carrageenan 1% suspension in isotonic saline (w/v) into the sub-plantar region of the left hind paw of the mouse.

The doses of 100, 200, or 400 mg/kg and vehicle (0.2% Tween 80 in 0.9% NaCl) were administrated orally (per os) 30 min before injection of the edematogenic agent to different groups of mice for each treatment (n=6 per group). Diclofenac sodium dissolved in 0.9% NaCl (50 mg/kg, oral) was used as a reference drug. Paw thickness was measured before the application of the inflammatory substance and every 30 min for 4 h after induction of inflammation. The difference in footpad thickness was measured by a gauge calliper (Facom, Paris, France).

Mean values of treated groups were compared with those of control group (vehicle) and analyzed statistically. The data obtained for the various groups are reported as means±standard deviation (SD) and expressed in mm.

The percentage inhibition of the inflammatory reaction was determined for each animal by comparison to the controls and calculated by the formula:

$$I(\%) = \left[1 - \frac{\Delta(PV)t}{\Delta(PV)c}\right] x100$$

Where I (%)=percentage inhibition of edema, $(\Delta PV)_t$=the change in paw volume in the treated mice, and $(\Delta PV)_c$=the change in paw volume in the control mice.

2.6. STATISTICAL ANALYSIS

Results of the paw edema of the mice are reported as mean±SD. Comparison between groups was made by one-way analysis of variance (ANOVA). Differences with $P<0.05$ were considered statistically significant. Statistical data analysis was determined using XLStats 2013 statistical software (Addinsoft, France).

3. RESULTS AND DISCUSSION

3.1. ACUTE TOXICITY RESULTS

The methanolic extract of Myrtus nivellei Batt & Trab did not cause any mortality in the mice in doses up to 1,000 mg/kg. Therefore, we suggest that oral LD_{50} of the tested doses of the extract is higher than 1,000 mg/kg. Thus, this extract can be considered as highly safe. The traditional pharmacopeia doesn't report any toxicity to the use of this plant [11].

3.2. ANTI-INFLAMMATORY ACTIVITY RESULTS

The anti-inflammatory effect of the methanolic extract was evaluated in carrageenan-induced paw edema in mice, an animal model widely employed to assess the anti-edematogenic effect of natural products. Carrageenan is commonly used as a phlogistic (inflammation-inducing) agent. The resulting signs and symptoms of inflammation can be measured as an increase in paw thickness due to the edema.

The anti-inflammatory effect of the methanolic extract at different concentrations (100, 200 and 400 mg/kg) was evaluated in the paw edema model (n=6 per group). As shown in Table 1, the oral administration of this extract at doses of 100, 200 and 400 mg/kg resulted in approximately 29.76, 36.16 and 80.41% reduction in paw edema respectively. Furthermore, the inhibition of paw edema resulting from a 400-mg/kg extract dose was not significantly different from that of positive control (50 mg/kg) (80.41% vs 80.76%). This is the first demonstration that oral administration of the methanolic extract produces significant anti-inflammatory effects.

Table 1: Effect of methanolic extract on carrageenan-induced paw edema in mice (n=6)

Treatment	Dose (mg/kg)	Thickness of the left hind paw (mm), mean±SD	Inhibition of paw edema (%)
Negative control	20	3.3±0.2	–
Methanolic extract	400	1.83±0.16	80.41
	200	2.78±0.30	36.16
	100	4.0±0.25	29.76
Positive control (Diclofenac)	50	2.76±0.12	80.76

Groups of mice were pre-treated with vehicle (control group, 20 mg/kg, *n*=6), Diclofenac (50 mg/kg) or *Myrtus nivellei* extract (at doses of 100, 200 and 400 mg/Kg *n*=6/group) 30 min before carrageenan-induced paw edema.

Some plant constituents, particularly phenolics have been reported to be useful in the management of inflammatory processes [12]. We had reported in a previous work that the methanolic extract of this plant is particularly rich in total phenolics (348 µg/mg dry extract), flavonoids (152.25µg/mg dry extract), tannins (155.27µg/mg dry extract) compared to the *Myrtus communis* L. growing wild in North of Algeria [3]. We suggest that there is an important relationship between the richness of the extract with phenolics and its strong anti-inflammatory effect.

4. CONCLUSION

This paper reported for the first time the anti-inflammatory activity of the methanolic extract prepared from *Myrtus nivellei* growing wild in Hoggar mountains. These results demonstrate the efficacy and safety of *Myrtus nivellei* and support its use by the Touaregs in Saharan traditional medicine and give strong impulsion to consider the methanolic extract as a potentially useful anti-inflammatory substance to preclude inflammatory diseases.

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