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Anti-inflammatory, analgesic and antioxidant effects of phenolic compound from Algerian *Mentha rotundifolia* L. leaves on experimental animals

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ABSTRACT

The existence of numerous side effects following the use of anti-inflammatory drugs has led to the present study about *Mentha rotundifolia* L. The plant is prescribed in folk medicine treatment of inflammatory diseases to discover biomolecules that have substantial beneficial effects with the least adverse effects. In this study, the anti-inflammatory and analgesic effects of polyphenols from *Mentha rotundifolia* L. leaves extract were evaluated, using carrageenan-induced mice paw edema model and acetic acid induced writhing method. The effects on oxidative stress of plant extract were also evaluated after sacrifice of the experimental mice. The extract showed a dose dependent effect on inflammation inhibition. The highest percentage of edema inhibition was 84.49% after 4 h at dose of 600 mg/kg. The extract showed a significant ($p < 0.05$) dose dependent increase in reaction time in mice in writhing method at doses of 200, 400, and 600 mg/kg. The result revealed also significant increases ($p < 0.05$) in the activities of catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and significant decreases in the malondialdehyde (MDA) level activity in the liver homogenate after Carrageenan injection, in comparison with the inflammatory group. The results suggest that the polyphenolic extract of *Mentha rotundifolia* L. possesses anti-inflammatory and analgesic activities. It possesses also *in vivo* antioxidant activity and can be employed in protecting tissue from oxidative stress.

1. Introduction

Inflammation is a physiological defense mechanism resulting from an attack to the body to isolate and repair the tissue damage. It plays a protective role by participating in the process of innate defense of the body and manifests itself clinically by four cardinal signs such as redness, heat, pain, and edema.

The inflammatory process involves the release of pro-inflammatory cytokines, prostaglandins, and the formation of reactive oxygen species (ROS). Excessive inflammatory mediators lead to maintain inflammation and induce a chronic inflammation (Mouhibatou et al., 2016). Treating inflammation with the analgesic, nonsteroidal anti-inflammatory drugs (NSAIDs), and corticosteroids makes us face a new era of people presenting symptoms of analgesic abuse and their hostile effects like gastric discomfort, gastric erosion, and hypersensitivity reactions (Santangelo et al., 2007). With the progress of more and more synthetic drugs which have adverse effects, it is time to consider indigenous herbal plants as possible remedies. This has sped up the global effort to collect those medicinal plants that have substantial beneficial effects with the least adverse effects. *Lamiaceae* is one of the most widely used families as a source of biomolecules with high antioxidant effect. In this family, the *Mentha* genus includes several species such as *Mentha rotundifolia* L. which is widely distributed around the Mediterranean basin, in America and in occidental Asia (Mailhebiau, 1994; Bezanger-Beauquesne and Pinkas, 1980). In Algeria and northern Africa, this aromatic plant is well known such as "timarssad". It has been applied in the traditional medicine for a wide range of actions including tonic, stomachic, carminative, analgesic, antispasmodic, anti-inflammatory, sedative, hypotensive, and insecticidal potentials (Bremnes, 2002). *Mentha rotundifolia* L. total phenolic content was considered in literature but there are very few reports studying the *in vivo* anti-inflammatory, analgesic and antioxidant activities of *Mentha rotundifolia* L. leaves, especially in Algeria, where no published reports concerning these effects were found. Thus, the present study was aimed to investigate the role of administration of polyphenolic extract from *Mentha rotundifolia* L. leaves in alleviating inflammation of carrageenan-induced mice paw edema and its analgesic activity. Also, attempts have been made to explore its effects on oxidative stress.

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2. Materials and methods

2.1. Plant material and extraction

The leaves of *Mentha rotundifolia* L. were collected in September 2015 from Taher at Jijel, in the North East of Algeria. Voucher specimens were deposited in the Herbarium of the Agronomic Institute of the Hassiba Ben Bouali, University of Chief in Algeria. The plant material was stored at room temperature in a dry place until used. Air-dried leaves were ground using an electric grinder (Sayona model: Sy-601, China) in order to get a fine powder (Awika et al., 2005). The sieving was achieved with a sifter (Retsch, Germany) with a pore diameter of 50 μm. The plant powder was then kept in small bottles of tinted glass to avoid the oxidization of their compounds. Polyphenols extraction was carried out by maceration at ambient temperature for 48 h in methanol–water solvent mixture (80:20, v/v) at a solid–liquid ratio of 1:10 (w/v) with continuous stirring. The hydro-methanolic extract was filtrated by No.1 Whatman Millipore filter paper. The resultant hydro-methanolic filtrate was refluxed with hexane for delipidation as described (Yu et al., 2005). Then the filtrate was concentrated in a rotary evaporator to have a crude dried methanol extract. The extract was dissolved in normal saline for realization of the *in vivo* studies.

2.2. Determination of total phenolic content

The total phenols content was determined by Folin–Ciocalteu method (Othman et al., 2007). An aliquot of 0.2 ml of sample was dissolved in 1.5 ml diluted Folin–Ciocalteu reagent (1/10 dilution factor). The obtained solutions were mixed and incubated at room temperature for 5 min. A volume of 1.5 ml of 7.5% (w/v) sodium carbonate (Na_2CO_3) solution was then added. After 90 min, the absorbance was measured at 725 nm. Gallic acid was used as standard for the calibration curve. Results were expressed as Gallic Acid Equivalents (GAE) per g of crude extract. All samples were analyzed three times and the mean value was calculated.

2.3. Determination of total flavonoid content

The extracts flavonoid content was determined by a Shimadzu UV mini 1240 spectrophotometer according to the method of Djeridane et al. (2006). This method is based on the formation of a flavonoid–aluminum complex that has a maximum absorbance at 430 nm. Quercetin was used to make the calibration curve. A 1.5 ml of diluted sample was mixed with 1.5 ml of 2% (w/v) aluminum chloride solution. After incubation at room temperature in the dark for 30 min, the absorbance of the reaction mixture was measured at 430 nm. The flavonoids content was expressed as Quercetin Equivalents (QE) per g of crude extract. The test is carried out in three replicates.

2.4. Experimental animals

All the experiments were carried out using Swiss albino mice (25–30 g) of either sex obtained from Pasteur institute. Animals were housed at a temperature of 24 ± 2 °C and relative humidity of 60–70%. A 12-h light/12-h dark cycle was followed. All animals were allowed to free access to water and fed with standard commercial mice chaw pallets. All the experimental procedures were conducted in accordance with the ethical guidelines for the care and use of laboratory animals.

2.5. Acute toxicity

The acute toxicity test was carried out for *Mentha rotundifolia* L. to evaluate any possible toxicity. Mice (n = 5) of either sex were treated with a single oral dose of the extract (5000 mg/kg), while the control group received saline (10 ml/kg). The mice were observed for any gross effect and mortality for 1, 4, and 24 h after treatment. Animals were further observed for up to seven days for any signs of delayed toxicity and mortality.

2.6. Carrageenan-induced mice paw edema

The method of Winter et al. (1962) was used to assess the anti-inflammatory effect of polyphenols extract of *Mentha rotundifolia* L. leaves. Thirty mice were randomly divided into six groups (five mice in each group) and treated as follows:

Group 1: normal control mice given distilled water (vehicle).
Group 2: inflammatory control mice given distilled water.
Group 3: inflammatory mice given *Mentha rotundifolia* L. leaves extract at a dose of 200 mg/kg bw (bw = body weight).
Group 4: inflammatory mice given *Mentha rotundifolia* L. leaves extract at a dose of 400 mg/kg bw.
Group 5: inflammatory mice given *Mentha rotundifolia* L. leaves extract at a dose of 600 mg/kg bw.
Group 6: inflammatory mice given Ibuprofen (standard) at a dose of 200 mg/kg bw.

A volume of 50 μl of a 1% carrageenan solution (0.9% NaCl) was injected into the foot pad of the right hind paw of mice, 1 h after substance administration (polyphenols extract, sterile distilled water, and Ibuprofen). The volume of edema was measured 1 h prior to, and 1, 2, 3 and 4 h after carrageenan injection with calibrated digital thickness gauge (Shanghai, China). The anti-edema effect was evaluated by using the following formula:

$$\%\text{Inhibition} = \frac{(P_t - P_0)}{P_0} \cdot 100 \quad (1)$$

P_t represents the volume of the right hind paw after carrageenan treatment.
P_0 represents the volume of the right hind paw before carrageenan treatment.

At the end of the experiment, the animals were sacrificed by cervical dislocation and the livers were collected for cytosolic fraction preparation for evaluation of *in vivo* antioxidant studies.

2.7. Analgesic activity

The method of Koster et al. (1959) was used for this activity. The mice were divided into five groups of five mice each and fasted overnight. The animals were treated with Aspirin (150 mg/kg, p.o.), saline solution (10 ml/kg, p.o.) and *Mentha rotundifolia* L. (200, 400 and 600 mg/kg, p.o.). The mice were treated with acetic acid (0.6%, v/v in saline, 10 ml/kg, i.p.) 1 h after the above treatment was carried out. The number of abdominal writhes (full extension of both hind paws) was cumulatively counted every 5 min over a period of 25 min immediately after the acetic acid injection. The analgesic effect was expressed as reduction percentage of contortions by using the following formula:

$$\%\text{Inhibition} = \frac{(N_{te} - N_t)}{N_{te}} \cdot 100 \quad (2)$$

N_{te} number of contortion of the negative control.
N_t number of contortion batch test or the positive control.

2.8. In vivo antioxidant activity

2.8.1. Preparation of mice liver cytosolic fraction

Mice livers were removed immediately after sacrifice and rinsed with ice-cooled distilled water followed by ice-cooled 0.1 M potassium