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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.

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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

phosphate buffer (pH 7.4), blotted dry, and weighed. Isolated mice liver samples were homogenized in 3 volumes of 0.1 M potassium phosphate buffer (pH 7.4) containing 1.17% potassium chloride, using a Potter–Elvehjem homogenizer. After centrifugation of the homogenate fraction at 800 rpm for 15 min at 4 °C, the resultants supernatants were centrifuged at 9600 rpm for 45 min at 4 °C. The supernatants obtained were cytosolic fractions. The protein concentration was determined according to the method of Bradford (1976) using bovine serum albumin as the standard. The cytosolic fractions were stored at −80 °C until used.

2.8.2. Estimation of lipid peroxidation

Lipid peroxidation was assayed by the measurement of malondialdehyde (MDA) levels on the basis of reaction with thiobarbituric acid (TBA) (Okhawa et al., 1979). In this method, 1 ml of liver tissue was taken and to it 0.5 ml of 20% TCA was added, followed by 0.5 ml of 0.67% TBA reagent. The tubes were then covered with aluminum foil and kept in shaking water bath for 30 min at 80 °C. After 30 min tubes were taken out and kept in ice-cold water for 30 min. Four milliliters of *n*-butanol were then added and the tubes were centrifuged at 3000 rpm for 15 min. The optical density of the supernatant was recorded at 532 nm. A standard curve was obtained with a known amount of 1.1.3.3.-tetraethoxypropane, using the same assay procedure. The MDA was expressed in μmol/g of tissue.

2.8.3. Estimation of catalase activity (CAT)

Catalase activity was evaluated according to the method of Clairborne (1985). The estimation was measured in which supernatant (0.025 ml) was added to cuvette containing 1 ml of 0.1 M phosphate buffer (pH 7.4). Reaction was started by the addition of 0.950 ml of freshly prepared 0.019 M H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as units/mg protein according to the following formula:

$$\text{Units/mg protein} = (2.3033/T)\ \log(A_1/A_2)/\text{mg protein} \quad (3)$$

A_1 is the absorbance at time 0 min.
A_2 is the absorbance at time 1 min.
T is the time interval in minute.

2.8.4. Estimation of superoxide dismutase activity (SOD)

The activity of SOD was assayed according to the procedure of Beauchamp and Fridovich (1971). The reaction mixture was prepared by mixing of riboflavin (2×10^{-6} M), sodium cyanide (2×10^{-5} M), methionine (10^{-2} M), EDTA (6.6×10^{-3} M), and nitroblue tetrazolium (NBT 1.76×10^{-4} M). To 2 ml of this mixture, 5 μl of the cytosolic fraction were added. The cocktail was mixed and illuminated for 10 min in an aluminum foil coated wooden box containing two 20 W-Philips fluorescent lamps fitted parallel to each other. The change in absorbance was recorded at 560 nm using a UV spectrophotometer. The control was simultaneously prepared without liver homogenate. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50%. The specific activity was expressed in terms of units per milligram of proteins:

$$\%\text{Inhibition} = \frac{DO_{control} - DO_{sample}}{DO_{control}} \cdot 100 \quad (4)$$

$$\text{SOD Units/mg protein} = \%\text{inhibition} \times 6.35 \quad (5)$$

2.8.5. Estimation of glutathione (GSH)

The estimation of the reduced glutathione (GSH) level was done following the method of Ellman (1959). Three volumes of 5% TCA was added to 1 g of liver and centrifuged at 2000 rpm for 15 min. After centrifugation, 50 μl of the resultant supernatant was diluted in 10 ml phosphate buffer (0.1 M, pH = 8), and to this mixture was added 20 μl of 0.01 M DTNB. After incubation for 15 min, solutions were measured at 412 nm against blank. Absorbance values were compared with a standard curve generated from standard curve of known GSH level. The glutathione level in liver was calculated as mmol/g liver.

2.9. Statistical analysis

All values were expressed as mean ± standard deviation (SD). Data were compared on the basis of the mean values. Differences among means of variety groups were tested using a Tukey–Kramer HSD (Software JMP version 7.0) with a significance level of 0.05.

3. Results

3.1. Determination of total phenolic and flavonoid contents

Total phenol compounds in the methanolic extract of *Mentha rotundifolia* L. leaves, as determined by the Folin–Ciocalteu method, are reported as Gallic Acid Equivalents (GAE) by reference to standard curve ($y = 0.0024x + 0.0886$, $r^2 = 0.9142$). From the results summarized in Table 1 we can easily conclude that *Mentha rotundifolia* L. leaves are rich in phenolic compounds. The total flavonoid contents were also higher in the extract with 79.44 ± 0.76 mg Quercetin Equivalent (QE)/g of crude extract(CE) by reference to standard curve ($y = 0.0087x + 0.1121$, $r^2 = 0.9608$).

3.2. Acute toxicity

Oral administration of *Mentha rotundifolia* L. extract at 5000 mg/kg did not produce any mortality. The extract did not produce significant changes in behavior during the time of observation.

3.3. In vivo anti-inflammatory effect

In vivo anti-inflammatory activity of *Mentha rotundifolia* L. extract at doses of 200, 400, and 600 mg/kg bw in carrageenan induced paw edema model is shown in Table 2. The extract caused dose dependent inhibition of increase in paw edema from 1 to 4 h. The peak inhibitory effect of the extract was recorded with a dose of 600 mg/kg (84.49%) at 4 h. Ibuprofen showed statistically a significant anti-inflammatory activity with the highest inhibition (92.45%) of paw edema at 4 h after carrageenan injection.

3.4. In vivo analgesic effect

The results in Table 3 shows that extract of *Mentha rotundifolia* L. at doses of 200, 400, and 600 mg/kg bw had significantly analgesic effects ($p \le 0.05$) and dose dependent with inhibition percentages from 79.05 to 85.29%. Aspirin induced protection 82% against the contractions induced by acetic acid. The analgesic effect of the extract (at 600 mg/kg bw) was greater than the Aspirin (150 mg/kg bw).

Table 1
Total phenols and flavonoid contents of methanolic extract of *Mentha rotundifolia* L. leaves.

Total phenols and flavonoid contents	Amount
Total phenolic content (mg GAE/g CE)	350.10 ± 0.96
Total flavonoid content (mg QE/g CE)	79.44 ± 0.76

Table 2
Effect of *Mentha rotundifolia* L. leaves extract on carrageenan-induced mice paw edema volume.

Treatment	Doses (mg/kg bw)	Right hind paw volume (% inhibition)			
		1 h	2 h	3 h	4 h
Control	–	2.75 ± 0.15	3.15 ± 0.04	3.30 ± 0.04	3.33 ± 0.025
Ibuprofen	200	1.01 ± 0.49^{bcde} (65.88%)	0.70 ± 0.22^{abcd} (77.28%)	0.47 ± 0.18^{ab} (85.47%)	0.25 ± 0.21^{a} (92.45%)
Extract	200	1.30 ± 0.08^{e} (55.86%)	0.98 ± 0.10^{bcde} (68.49%)	0.89 ± 0.11^{abcde} (72.87%)	0.72 ± 0.04^{abcd} (78.15%)
	400	1.23 ± 0.06^{de} (58.45%)	0.90 ± 0.17^{bcde} (71.06%)	0.68 ± 0.10^{abcd} (79.07%)	0.62 ± 0.11^{abc} (81.17%)
	600	1.17 ± 0.12^{cde} (60.36%)	0.79 ± 0.25^{abcde} (74.49%)	0.66 ± 0.21^{abc} (79.78%)	0.51 ± 0.19^{ab} (84.49%)

n = 5; Means followed by different letter are significantly different ($p < 0.05$).

3.5. In vivo antioxidant activity

3.5.1. Effect of extract on lipid peroxidation

Concentrations of MDA in liver samples of all experimental mice are shown in Fig. 1. Elevated MDA levels were observed for the inflammatory untreated group (inflammatory control) than for other samples, with the Ibuprofen pre-treatment showing the least MDA levels. Test sample of 600 mg/kg pre-treatment showed lower MDA levels comparable to Ibuprofen.

3.5.2. Effect of extract on catalase activity

CAT activities in the liver samples of mice for all experimental groups are shown in Fig. 2. CAT activity in the inflammatory group was considerably lower than for the normal control group. The CAT levels in liver homogenate of *Mentha rotundifolia* L. extract 600 mg/kg pretreated mice were comparable to Ibuprofen except for the 200 mg/kg pretreatment with significantly lower value than for Ibuprofen.

3.5.3. Effect of extract on SOD activity

Fig. 3 shows the activity of superoxide dismutase (SOD) in the liver of normal control and experimental groups of mice. This activity was significantly lower in inflammatory control mice compared to control group of mice. The SOD levels in liver homogenate of *Mentha rotundifolia* L. extract 400 and 600 mg/kg pretreated mice were statistically comparable to Ibuprofen except for the 200 mg/kg pretreatment with significantly lower value than for Ibuprofen.

3.5.4. Effect of extract on GSH activity

The effect of extract on GSH activity for all experimental groups is shown in Fig. 4. GSH level in the inflammatory group was considerably lower than for the normal control group. The 200, 400, and 600 mg/kg *Mentha rotundifolia* L. extract pretreatment significantly enhanced the level of GSH when compared to the inflammatory group. The 600 mg/kg pre-treatment showed highest GSH levels comparable to that of Ibuprofen.

4. Discussion

In this study, we investigated the phytochemical analysis, anti-inflammatory, analgesic and antioxidant effects of *Mentha rotundifolia* L. leaves in animal model. Phytochemical results indicated that the extract was rich in phenolic compounds and presented higher flavonoid content.

Table 3
Effect of *Mentha rotundifolia* L. on acetic acid-induced writhing reflex in mice.

Treatment	Dose (mg/kg bw)	Number of writhes	Percentage of inhibition (%)
Mentha rotundifolia L.	200	7.33 ± 3.05^{b}	79.05
	400	5.66 ± 1.15^{b}	83.67
	600	5.00 ± 2.00^{b}	85.29
Aspirin	150	6.33 ± 3.21^{b}	82.00
Saline solution	10 (ml/kg)	35.00 ± 1.00^{a}	–

n = 5; Means followed by different letter are significantly different ($p < 0.05$).

The evaluation of the anti-inflammatory activity *in vivo* was conducted using the model of carrageenan-induced paw edema (Winter et al., 1962). Carrageenan-generated paw edema is an established well-known experimental model for acute inflammation. This model has significant predictive value for evaluation of antiedematogenic compounds acting by interfering with the inflammatory mediators (Taher, 2012). Carrageenan as a phlogistic agent is non-antigenic and is devoid of apparent systemic activity (Chakraborty et al., 2006). So far, several investigators have demonstrated that acute edema inflammation, due to carrageenan injection, has biphasic upshots. Histamine and serotonin trigger the first phase while, the later phase of inflammation in which the edema reaches its highest degree is elicited mostly by the release of prostaglandins (Vinegar et al., 1969; Crunkhorn and Meacock, 1971). It has been shown that prostaglandins encourage the formation of the inflammatory exudates during tenderness. This demagogic effect of prostaglandins is considerably attenuated by the use of non-steroidal anti-inflammatory drugs (NSAID). In the present investigation, oral pretreatment of 600 mg/kg *Mentha rotundifolia* L. extract significantly inhibited the paw edema. The anti-inflammatory activity of the extract could be due to its phytochemical compounds such as polyphenols. Several plant species rich in polyphenols in particular flavonoids are reported possessing important pharmacological actions, such as anti-oxidant, and anti-inflammatory actions (Meotti et al., 2006), and also it have been shown after systemic administration of flavonoid glycosides, it exerts CNS-mediated activities, and causes sedation, myorelaxation, analgesia, and antinociceptive effects (Fernandez et al., 2009). According to a number of other studies, flavonoids like rutin, quercetin, luteolin, hesperidin, and biflavonoids yielded important antinocieptive and/or anti-inflammatory activities (Ramesh et al., 1998; Bittar et al., 2009; Farahpour, 2014).

Also it cannot be excluded that abrogation of one of the two inflammatory mediators, histamine and serotonin, is responsible for the anti-inflammatory activity of *Mentha rotundifolia* L., the fact that the extract ameliorated edema formation during the late phase; 3 h after carrageenan injection, demonstrates that the effectiveness of *Mentha rotundifolia* L. extract is probably mediated through its interference with prostaglandins.

The analgesic activity was assessed by acetic-acid-induced abdominal constriction, as a model of visceral pain. It is a highly sensitive method able to reveal the antinociceptive potential of drugs at doses that might appear inactive in other pain reliever patterns. So, this model is commonly used for the assessment of the peripheral pathway of analgesic drugs. Previous studies have demonstrated that acetic acid stimulates the pain nerve endings and induces contraction of abdominal muscle *via* the sensitization of the nociceptive receptor to the peripherally released prostaglandins, in particular PGE2α and PGF2α (Deraedt et al., 1980). It has been shown that inhibition of prostaglandins by aspirin and other related NSAIDs is involved in the protection against induction of pain (Inger et al., 2010). Therefore, although the mechanism of *Mentha rotundifolia* L. leaves in this study is not clear, it seems likely that inhibition of prostaglandins contributes to its effectiveness in protecting mice from visceral pain. Thus, this and the observation

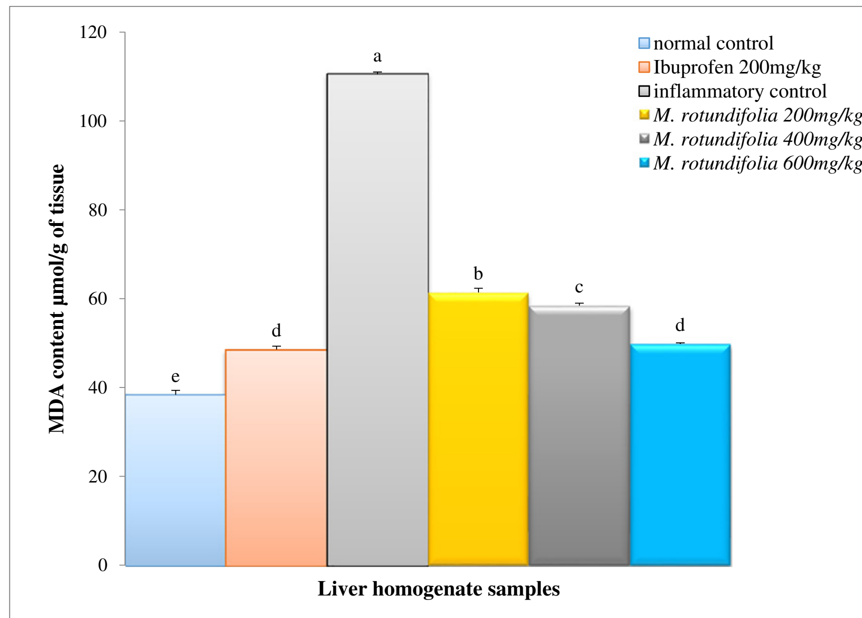

Fig. 1. Effect of 200, 400, and 600 mg/kg *Mentha rotundifolia* L. extract and controls on the MDA levels. Values are mean ± SD. n = 5 in each group. Different letters indicate that samples are significantly different ($p < 0.05$).

that the extract, when given before carrageenan, appeared to inhibit the late phase of inflammation, indicate that inhibition of prostaglandins peripherally is a mechanism by which the extract induces its pharmacological effect.

Numerous pathological events such as the inflammation process and aging phenomena are associated with the generation of reactive oxygen species (ROS) and the induction of lipid peroxidation. Antioxidants (free radical scavengers) are chemicals that interact with and neutralize free radicals, thus preventing them from causing cellular damage in the biological system (Diplock et al., 1998). The body makes some of the antioxidants uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body also relies on external (exogenous) sources, primarily the diet, to obtain the rest of the antioxidants it needs (Valk et al., 2007). These exogenous antioxidants are commonly called dietary antioxidants. Fruits, vegetables, and grains are rich sources of dietary antioxidants (Bouayed and Bohn, 2010).

For this, we have also evaluated the *in vivo* antioxidant potential of our extract using superoxide dismutase (SOD), catalase activity (CAT), malondialdehyde (MDA), and glutathione (GSH) level assays. Our experimental data indicated that the extract increased the activity of SOD, CAT, and GSH, and decreased the level of MDA in liver cytosolic fraction. Catalase is a ubiquitous enzyme that catalyzes the decomposition of hydrogen peroxide, a reactive oxygen species, which is a toxic product of both normal aerobic metabolism and pathogenic ROS production (Kohen and Nyska, 2002; Maritim et al., 2003). CAT measured in tissues is indicative of the degree of damage that tissues are undergoing or the degree of protection offered by the protective enzymatic agents against ROS (Omonhinmin and Agbara, 2013). The SOD catalyzes the

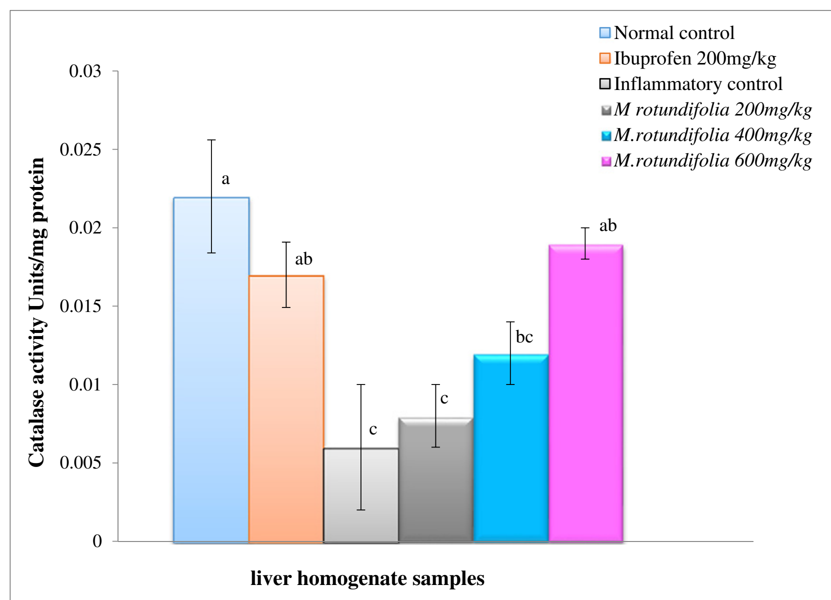

Fig. 2. Effect of 200 mg/kg, 400 mg/kg, and 600 mg/kg *Mentha rotundifolia* L. extract and controls on the CAT levels. Values are mean ± SD. n = 5 in each group. Different letters indicate that samples are significantly different ($p < 0.05$).

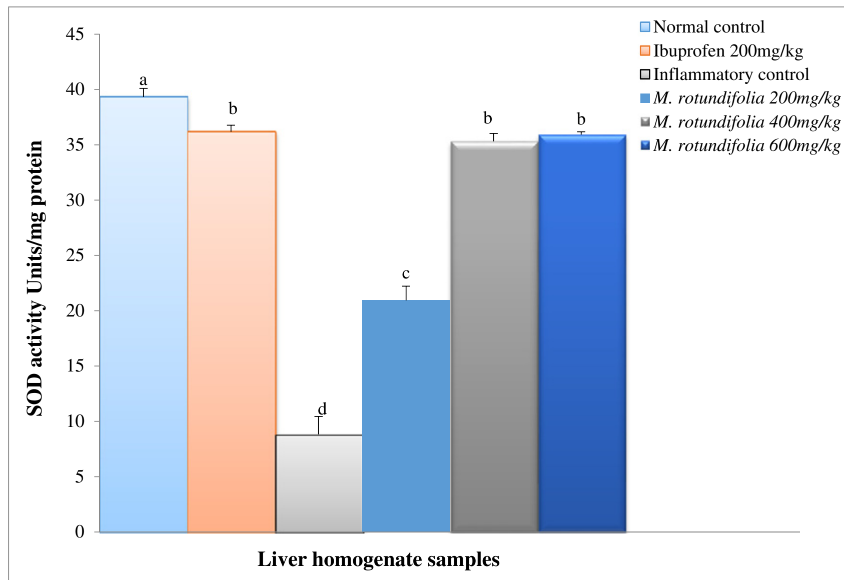

Fig. 3. Effect of 200 mg/kg, 400 mg/kg, and 600 mg/kg *Mentha rotundifolia* L. extract and controls on the SOD levels. Values are mean ± SD. n = 5 in each group. Different letters indicate that samples are significantly different ($p < 0.05$).

dismutation of superoxide to hydrogenperoxide and oxygen, thereby reducing the likelihood of superoxide anion reacting with nitric oxide to form reactive peroxynitrite (Maritim et al., 2003). The increased levels of catalase and SOD as observed in this study suggest that the extract has an *in vivo* antioxidant activity and is capable of ameliorating the effect of ROS in biologic system (Manonmani et al., 2005; Bakirel et al., 2008). ROS, also react with all biological substances; however, the most susceptible ones are polyunsaturated fatty acids. MDA is the major oxidation product of peroxidized poly-unsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation and ultimately tissue damage by series of chain reactions. The model of inflammation induced by carrageenan used in the study and by some authors was accompanied by an increased production of peroxidized lipids. (Tanas et al., 2010). In addition, lipid peroxidation was reported as complicating the inflammatory process (Uzkeser et al., 2012). In our study, the level of MDA in the extract treated groups decreased in a dose dependent manner when compared to normal control group. Glutathione (GSH), a tripeptide present in all the cells is an important antioxidant (Lu, 1999) and also functions as free radical scavenger in the repair of biological damage caused by radicals (Meister, 1984). In the present study it is observed that inflammation depletes GSH concentration in the mice livers. The extract and Ibuprofen treatment reverses this effect, which may be due to *de novo* GSH synthesis or GSH regeneration. These results suggest that the plant could possess antioxidant and anti-inflammatory properties. The phytochemical constituents of the extract such as flavonoids may be responsible for the antioxidant activity as demonstrated in our study. Numerous studies have shown that flavonoids possess potent antioxidant activities capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals (Amić et al., 2007). Shahidi and Wanasundara (1992) attributed the

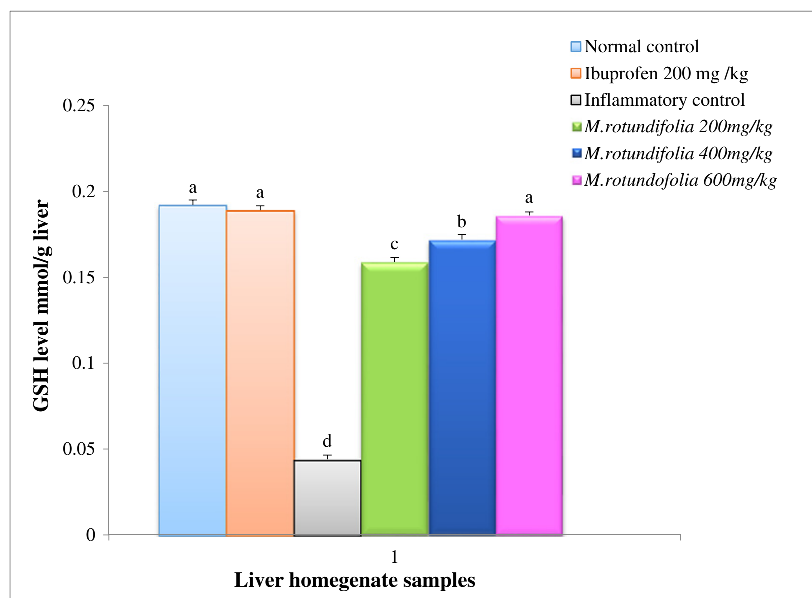

Fig. 4. Effect of 200, 400, and 600 mg/kg *Mentha rotundifolia* L. extract and controls on the GSH levels. Values are mean ± SD. n = 5 in each group. Different letters indicate that samples are significantly different ($p < 0.05$).

pharmacological activities (anti-inflammatory, antiviral, antibacterial, antiulcer, antiosteoporotic, antiallergic, and antihepatotoxic actions) of flavonoids to their potent antioxidant activity.

5. Conclusion

Mentha rotundifolia L. is widely used in Algerian traditional medicine as analgesic, antispasmodic, and anti-inflammatory agent. According to the obtained results, it may be concluded that pain and acute inflammation induced experimentally in mice were considerably ameliorated by the use of the polyphenolic extracts. Moreover, in our investigation on *Mentha rotundifolia* L. the enzymatic oxidants such as catalase, SOD, MDA, and GSH were improved in drug treated groups as compared to control. Based on this we conclude that the extract possesses *in vivo* antioxidant activity and may be employed in protecting tissues from oxidative stress. Further investigations are required to identify all the active compounds present in *Mentha rotundifolia* L. and their precise mechanisms of action.

Conflict of interest

The authors declare that there are no conflicts of interest.

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