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In vivo analgesic, anti-inflammatory and antioxidant potentials of *Achillea odorata* from north Algeria

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ABSTRACT

Objective: The current study aimed at evaluating in Albino mice the analgesic, anti-inflammatory, and antioxidant effects of *Achillea odorata*; a medicinal plant commonly used in Algerian folk medicine to provide scientific basis for its use.
Methods: The biological tests were performed on aqueous extracts. Antinociceptive activity was estimated using acetic acid that induces algesia in mice. Anti-inflammatory activity was investigated using carrageenan-induced paw edema method and the antioxidant effect was performed using MDA, GSH, CAT and SOD estimations.
Results: The study of the analgesic activity of the *A. odorata* polyphenolic extract showed a very potent analgesic activity with an ability to inhibit abdominal cramps similar to that of positive control. In addition, evaluation of edema inhibition percentage showed that plant extract has a significant anti-inflammatory activity. In addition, the extract has the ability to modulate the redox potential *in vivo*, induced by inflammation, which has been demonstrated by the determination of GSH, MDA, CAT and SOD.
Conclusions: Our results suggest that *A. odorata* possesses potential antinociceptive, anti-inflammatory and anti-oxidant compounds which could be tested as drug candidates against oxidative and inflammation-related pathological processes.

1. Introduction

Inflammation is one of the most important mechanisms of the body's defenses. Studies on inflammation have become one major focus of global scientific research. It is known that inflammation is correlated with oxidative process, mainly because they share similar pathways (Kunsch and Medford, 1999). An alteration of these mechanisms may evoke the development of various diseases. The oxidative damage caused by ROS may increase progression of various illnesses in the human body. These include aging, arthritis, cancer, inflammation, and heart diseases (Meerson et al., 1982; Busciglio and Yankner, 1995; Abe and Berk, 1998). Many anti-inflammatory, digestive, antinecrotic, neuroprotective, and hepatoprotective drugs have recently been found to have an antioxidant and/or radical scavenging mechanism as part of their activity (Perry et al., 1999; Lin and Huang, 2002; Repetto and Llesuy, 2002). Increased free radical level was found in many pathological conditions besides inflammation, such as cancer, ischemic disorders, and dementia (Harput et al., 2012). Thus free radicals are important mediators that initiate inflammatory processes and, consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation (Delaporte et al., 2002; Geronikaki and Gavalas, 2006). In the past few years, there has been a renewed interest in studying and quantifying the antioxidant and anti-inflammatory constituents of plants in terms of their potential health functionality through action against these various pathological processes (Menichini et al., 2009; Mueller et al., 2010). The use of plant products with known anti-inflammatory and/or antioxidant properties can be of great significance in therapeutic anti-inflammatory treatments. Phenolic compounds are known for their wide ranges of biological activities, including anticancer, antibacterial, antioxidant, antidiabetic and anti-inflammatory properties which could constitute an alternative in therapeutics. The study of the biological activities of plant extracts is therefore of great interest for the valorization of traditional medicine.

Achillea odorata, a herb from the family Asteraceae, is largely distributed throughout the Mediterranean regions (Bremer and Humphries, 1993). In Algeria, the leaves and flowers of *A. odorata* have been used for centuries for anti-inflammatory actions, such as rheumatism, skin inflammation and allergic rhinitis, wound healing and amelioration of diaphoresis and high blood pressure.

Despite the traditional use of this plant, there is no scientific record of the anti-inflammatory or analgesic activity of *A. odorata* in literature;

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hence, the aim of this work is to investigate the *in vivo* anti-inflammatory and analgesic properties of the plant with a view to validate its traditional use.

2. Materials and methods

2.1. Plant material and extraction

Leaves of *A. odorata* were collected in April 2015 from Jijel (Algeria). Fresh leaves of *A. odorata* were dried and ground in an electric grinder into a fine powder (less than 50 μm). About 100 g of this powder was extracted with 1000 ml of methanol (80%) for 48 h. The solvent was then filtered, defatted by hexane, and then evaporated using a rotary evaporator (Heidolph, LABOROT4003) at 40 °C. Concentrated extracts were stored in a refrigerator (4 °C) until used (Yu et al., 2005).

2.2. Experimental animals

Healthy Swiss albino mice (22–30 g) of approximately the same age, from Pasteur institute (Algiers, Algeria) were used for the study. Animals were housed in polypropylene cages in a well maintained and clean environment at ambient temperature with an alternating 12 h light–dark cycle and were fed with standard chow diet and water *ad libitum*. Before starting the experiment, the animals were acclimatized to the laboratory environment for a period of one week. All animal experimentations were performed in compliance with the Directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purpose.

2.3. Pharmacological activities

2.3.1. Acetic acid writhing test

The test was performed as described by Koster et al. (1959). The mice were divided into five groups of six mice each and fasted overnight. The animals were pretreated orally (p.o.) with aqueous extracts of *A. odorata* (200, 400, and 600 mg/kg). A group of mice were treated with Aspirin (100 mg/kg, p.o.) used as reference drug. Control animals received a similar volume of saline solution. Nociception was induced by an intraperitoneal (i.p.) injection of acetic acid (0.6%, v/v in saline, 10 ml/kg, i.p.) 20 min after the above treatment was carried out. The number of abdominal writhes (abdominal constrictions and hind limbs stretchings) was counted for 25 min immediately after the acetic acid injection. The antinociceptive activity was expressed as percentage of inhibition of abdominal writhes.

$$\%\text{Inhibition} = \frac{[\text{number of writhes (control)} - \text{number of writhes (test)}}{\text{number of writhes (control)}} \times 100$$

2.3.2. Carrageenan-induced paw oedema

The acute anti-inflammatory effect was evaluated by carrageenan-induced mice paw edema according to the method of Winter et al. (1962). The animals were deprived of food overnight and were divided into: normal and control groups which received saline solution, standard group which received the reference drug, Ibuprofen (100 mg/kg) and the test groups which received the leaves extracts of *A. odorata* (200, 400 and 600 mg/kg). Each group contained six mice. Thirty minutes after oral administration of different substances, edema was induced by injection of 1% suspension of carrageenan in 0.9% sterile saline solution into the subplantar tissue of the right hind paw of the mice except the normal group. Paw volume was measured with Digital vernier caliper immediately before injection of the phlogistic agents (V_0) and at 30 min interval for 4 h (V_T). The percentages of inhibition were calculated according to the following formula:

$$\%\text{Inhibition} = \frac{(\text{VT}-\text{V0})\ \text{control} - (\text{VT}-\text{V0})\ \text{treated group}}{(\text{VT}-V0)\ \text{control}} \times 100$$

2.3.3. Assay of antioxidant activity in vivo

2.3.3.1. Preparation of liver homogenates. After the decapitation process, liver of each mice was removed and frozen immediately on dry ice. The frozen tissues were stored at −20 °C until further use. Liver homogenates were prepared by mincing and homogenizing 1 g of liver in three volumes phosphate buffer (0.1 M, pH 7.4) containing KCl 1.17% with a glass homogenizer. The homogenates were centrifuged at 800 rpm and 4 °C for 15 min and the pellets were discarded. The liver extracts (the total soluble liver protein) were obtained as 9600 rpm-supernatants for 45 min at 4 °C. The supernatants were collected and their protein contents were determined based on Bradford's method (Bradford, 1976), using BSA as a standard.

2.3.3.2. Malondialdehyde (MDA) assay. The lipid peroxidation level in mice liver homogenate was measured as Malondialdehyde (MDA) which is the end product of lipid peroxidation that reacts with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) to produce a pink colored complex which has peak absorbance at 530 nm according to Okhawa et al. (1979).

One g liver tissues were homogenized with three volumes cold 1.15% KCl. 0.5 ml of 20% trichloroacetic acid (TCA) and 1 ml of 0.67% TBA aqueous solution were added to 0.5 ml homogenate. The mixture was heated in a boiling water bath for 15 min. After cooling, 4 ml of *n*-butanol was added and mixed followed by centrifugation at 3000 rpm for 15 min. The absorbance of the supernatant was measured at 530 nm and a standard curve was obtained with a known amount of 1.1.3.3.-tetraethoxypropane, using the same assay procedure. The concentration of MDA was expressed as nmol/g.

2.3.3.3. Assay for reduced glutathione (GSH) activity. GSH levels were determined in the liver using Ellman's reagent (DTNB) (Ellman, 1959), with modifications. One g liver tissues were homogenized with three volumes of 5% TCA. The contents were mixed well and centrifuged at 2000 rpm for 15 min. An aliquot of clear supernatant (50 μl) was mixed with 10 ml of phosphate buffer (0.1 M, pH 8). 20 μl of DTNB was added to 3 ml of the above mixture and incubated at room temperature for 15 min. GSH solution was used as standard and absorbance was read at 412 nm against blank. The GSH value was expressed as mmol/g liver.

2.3.3.4. Assay for catalase activity. The assay of catalase was performed by following the method of Clairbone (1985). In brief, to a quartz cuvette, 1 ml phosphate buffer (pH 7.4, 0.1 M), and 25 μl enzymatic extract were added. The reaction was initiated by adding 0.950 ml hydrogen peroxide (H_2O_2). The decomposition of H_2O_2 was monitored at 240 nm every minute for 2 min. The CAT activity was calculated and expressed as enzyme units (U) per milligram of proteins (U/mg prot), where one unit of CAT activity was defined as the amount of enzyme that decomposes 1 nmol H_2O_2/min at 25 °C.

2.3.3.5. Assays for superoxide dismutase activity. Total cellular superoxide dismutase (SOD) activity was determined by the method of Beauchamp and Fridovich (1971). In brief, the reaction mix contained in 50 mM phosphate buffer (pH 7.8), 2×10^{-5} M sodium cyanide (NaCN), 1.76×10^{-4} M nitroblue tetrazolium, 10^{-2} M methionine, 6.6×10^{-3} M EDTA and 2×10^{-6} M riboflavin. 2 ml of the reaction mix was added to each tube, followed by addition of 5 μl of sample. Reaction was started placing the tubes under 15 W lamp for 10 min, then

Table 1
Effect of *A. odorata* leaves extract and aspirin on mouse writhing test in mice.

Treatment (N = 6)	Dose (mg/kg)	Number of writhings (within 30 min)	Percentage inhibition
Control (acetic acid)		125.33 ± 3.07	
Extract	200	44.50 ± 3.08***	64.49
Extract	400	32.83 ± 2.31***	73.80
Extract	600	20.66 ± 1.75***	83.51
Aspirin	100	22.16 ± 2.48***	82.31

Values are expressed as mean ± SD (n = 6).
*** $p < 0.001$ significant from control.

the reaction was stopped by switching off the light and putting the tubes into dark and the changes in absorbance was determined at 560 nm. The amount of enzyme required to inhibit the reduction of NBT by 50% under the specified conditions was defined as one unit of SOD activity. The result for SOD activity was expressed as U/mg protein.

2.4. Statistical analysis

Data were expressed as means ± S.E. Statistical analysis was performed by using Student's *t*-test. Differences were considered significant at $p < 0.05$ (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) *versus* control group.

3. Results

3.1. Acetic acid-induced writhing

The antinociceptive activity of *A. odorata* was evaluated by using the writhing test. Intraperitoneal injection of acetic acid (0.6%) induced an average of 125 writhes in a period of 25 min. Oral administration of the plant extract (200–600 mg/kg), 20 min before the acid injection, produced a significant ($p < 0.001$) and dose-related inhibition of acetic acid-induced abdominal constrictions in mice (Table 1). Doses of 200, 400 and 600 mg/kg inhibited writhing by 64.49, 73.80 and 83.51%, respectively. Aspirin (100 mg/kg), a standard used as positive control, also produced significant ($p < 0.001$) inhibition (82.31%) of acetic acid-induced writhing response in relation to control group (Table 1).

3.2. Carrageenan-induced paw oedema

Table 2 shows the effect of *A. odorata* on the carrageenin-induced mice paw edema test. After carrageenan induction there was a significant increase in edema formation in normal mice. The anti-inflammatory activity data indicated that all the test concentrations (200, 400 and 600 mg/kg) were able to significantly reduce the carrageenin-induced edema 30 min after the injection of the phlogistic agent, in comparison to control ($p < 0.001$) in a dose-dependent manner with increased activity at 1 h. Interestingly, the reduction of the edema by *A. odorata* at the dose of 600 mg/kg was similar to the standard used (Ibuprofen) throughout the entire period of observation.

3.3. Assay of antioxidant activity in vivo

3.3.1. Effects of A. odorata on MDA level

In the present study, Carrageenan-induced acute inflammatory processes caused a significant ($p < 0.001$) increase in liver MDA levels compared to the normal control group (Fig. 1). However, administration of Ibuprofen (100 mg/kg) or *A. odorata* extract at the dose of 200, 400 and 600 mg/kg significantly decreased the MDA level compared with that of the Carrageenan control group ($p < 0.001$). None of the plant extract treatments showed significant differences ($p > 0.05$) in MDA levels compared to treatment with Ibuprofen, indicating that all tested doses of *A. odorata* extract inhibited lipid peroxidation in a manner that was comparable to that of Ibuprofen.

3.3.2. Effects of A. odorata on GSH level

In the present study, Carrageenan-induced acute inflammatory processes significantly decreased GSH levels in the liver compared to the normal control group ($p < 0.001$). As shown in Fig. 2, mice pretreatment with *A. odorata* extract resulted in a marked induction of GSH content at all concentrations (20,400 and 600 mg/kg). Similar observations were found in animals that were treated with Ibuprofen.

3.3.3. Effects of A. odorata on antioxidant enzyme activities

In the present study, catalase and SOD were measured as indices of the antioxidant status of tissues. Clearly, prominent oxidative stress in edema paw tissues was noticed in the carrageenan control group as shown by a significant ($p < 0.001$) decrease in CAT (Fig. 3) and SOD (Fig. 4) activities compared to the normal control group. There were significant increases ($p < 0.001$) in catalase and SOD activities in the plant extract-treated groups at doses of 200, 400 and 600 mg/kg compared to the carrageenan-treated group. *A. odorata* extract manifested a dose-dependent protective effects against carrageenan-induced decreases in antioxidant enzyme activities. A similar observation was found in animals that were treated with reference drug. When *A. odorata* at 600 mg/kg was compared to ibuprofen, there was no significant difference ($p > 0.05$) in the antioxidant enzyme activities, suggesting that the abilities of this dose of to restore the activity of antioxidant enzymes were comparable to that of positive control drug.

4. Discussion

This work represents the first attempt to provide pharmacological evidence of *in vivo* antinociceptive, anti-inflammatory and antioxidant effects of *Achillea odorata*.

Evaluation of the anti-inflammatory activity of *A. odorata* was carried out using carrageenan as a pro-inflammatory molecule. It has been reported that the carrageenan-induced mice paw edema is a suitable experimental model to study acute inflammation and to screen most of the anti-inflammatory natural products (Woldesellassie et al., 2011). Di Rosa (1972), have shown that the effects of carrageenan appear rapidly after injection and reach their maximum value 4 to 6 h later. For this reason, carrageenan is usually used to study acute inflammation. The edema produced by carrageenan injection remains located in the area of administration (Cicala et al., 2007) and is

Table 2
Effect of *A. odorata* extract on carrageenan-induced paw edema in mice.

Group (N = 6)	Dose	Mean increase in paw thickness (mm) ± SD (% inhibition)				
	mg/kg	0.5 h	1 h	2 h	3 h	4 h
Control		2.82 ± 0.19	2.96 ± 0.11	3.10 ± 0.16	3.28 ± 0.09	3.32 ± 0.03
Ibuprofen	100	1.26 ± 0.06*** (55.31)	1.31 ± 0.06*** (55.74)	1.09 ± 0.05*** (64.83)	0.72 ± 0.04*** (78.04)	0.32 ± 0.03*** (90.36)
A. odorata	200	1.47 ± 0.05*** (47.87)	1.53 ± 0.05*** (48.31)	1.42 ± 0.04*** (54.19)	1.12 ± 0.06*** (65.85)	0.53 ± 0.04*** (84.03)
A. odorata	400	1.32 ± 0.05*** (53.19)	1.48 ± 0.02*** (50)	1.28 ± 0.04*** (58.70)	0.83 ± 0.04*** (74.69)	0.38 ± 0.02*** (88.55)
A. odorata	600	1.27 ± 0.03*** (54.96)	1.35 ± 0.02*** (54.39)	1.11 ± 0.07*** (64.19)	0.75 ± 0.04*** (77.13)	0.34 ± 0.04*** (89.75)

Values are expressed as mean ± SD (n = 6).
*** $p < 0.001$ significant from control.

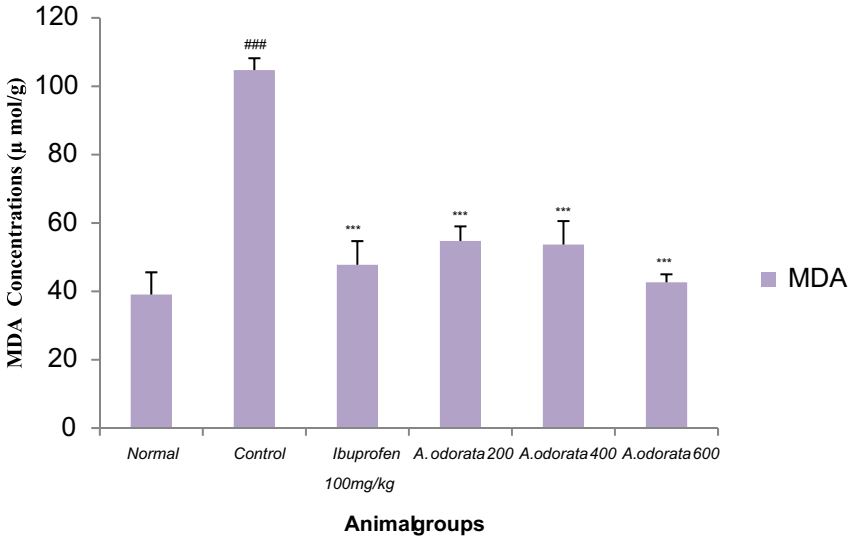

Fig 1. Effects of *A. odorata* on liver MDA. Values are the mean ± SD for six mice; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared with carrageenan control group, $###p < 0.001$ compared with normal group.

characterized by biphasic response which involves the release of several mediators, cell migration and plasma exudation (Loram et al., 2007). Initial stage, during the first hour, which is attributed to the action mediators like histamine, serotonin, kinin and bradykinin (Moncada and Higgs, 1993) and a last phase (1.5–4 h) attributed to the synthesis of prostaglandins after the induction of COX-2 and NO release after iNOS expression in activated leukocytes infiltrated the edema (Salvemini et al., 1996). In the present study, the subcutaneous administration of carrageenan provokes an acute and progressive increase in the mouse paw volume in response to the production of diverse inflammatory mediators. The edema volume, which is proportional to the intensity of the response to inflammation, is a useful indicator of anti-inflammatory activity. In our screening, the results obtained were compared with a reference anti-inflammatory, ibuprofen 100 mg/kg. In this investigation, *A. odorata* at doses of 200, 400, and 600 mg/kg showed considerable reduction of mice paw edema and attenuated both early and delayed phases of carrageenan-induced inflammation. In this test, the extract inhibited the induction of paw oedema in all phases, thus demonstrating its anti-oedematogenic activity. The early response suggests that the extract plays an important role as a protective factor against carrageenan-induced acute inflammation and possibly has an inhibiting effect on the release or actions of these inflammatory mediators, such as histamine and serotonin. The late response may be due to the inhibition of biosynthesis of prostaglandins (Niemegeers et al., 1964). The anti-inflammatory activity recorded in this study can be explained by the presence of secondary metabolites since the phytochemical screening revealed the presence of polyphenols and flavonoids in the extract of this plant (Boutennoun et al., 2017). In addition, recent research has shown that flavonoids decrease the production of NO by the inhibition of proteins inducing the synthesis of iNOS (inducible synthesis of NO) in particular by inhibition of the activation of NF-kB (Mladinka et al., 2010).

In this study, the analgesic activity of *A. odorata* was carried using the mouse writhing test, a widely used inflammatory pain model for its high sensitivity (Koster et al., 1959). This test represents a model of peripheral nociception (Souza et al., 2009). An IP injection of an algogenic product: acetic acid, into the peritoneal cavity of animals causes a tissue damage responsible for the release of a number of chemical mediators such as bradykinin, histamine, serotonin, acetylcholine and prostaglandins. These latter sensitize nociceptors to painful stimuli and cause late and diffuse pain (Collier et al., 1968; Raj, 1996; Le Bars et al., 2001). This pain is manifested in mice by abdominal contractions, stretching movements of the body and hind legs, torsion of the dorso-abdominal muscles associated with decreased activity and motor in coordination.

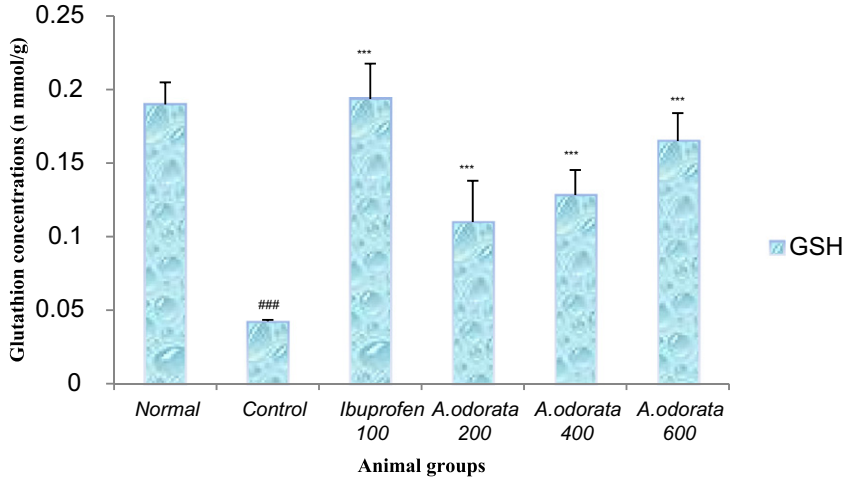

Fig 2. Effects of *A. odorata* on liver glutathione. Values are the mean ± SD for six mice; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared with carrageenan control group, $###p < 0.001$ compared with normal group.

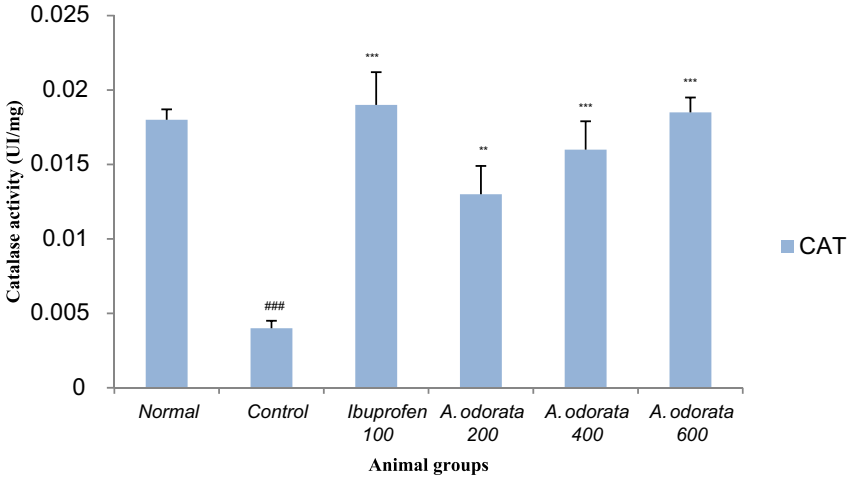

Fig. 3. Effects of *A. odorata* on liver catalase. Values are the mean ± SD for six mice; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared with carrageenan control group, ###$p < 0.001$ compared with normal group.

These behaviors are considered as reflex responses, testifying to visceral pain. From the results obtained, the polyphenolic extract revealed an important analgesic effect. A dose-dependent inhibition of writhing by the extract was observed in this study. The potential analgesic activity might be due to the presence of high polyphenol content of plant especially flavonoids and tannins (Handa et al., 1992; Orhan et al., 2007). Flavonoids have been shown to exert analgesic effect on acetic acid-induced writhing test (Calixto et al., 2000; Ahmed et al., 2007). The mechanism of the analgesic effect of polyphenolic extracts may be related to the process involved in preventing sensitization of nociceptors, regulating sensitized nociceptors and/or blocking nociceptors at the peripheral and/or central level (Ferreira et al., 1990). Flavonoids are known to prevent the synthesis of prostaglandins. Biochemical investigations on the mechanism of action of flavonoids have shown that these compounds can inhibit a wide variety of enzymes. The release of arachidonic acid is closely related to the cyclooxygenase and 5-lipoxygenase enzyme systems (Williams et al., 1995; Middleton et al., 2000). The polyphenols present in this plant may be responsible for the observed analgesic activities.

Recent report indicates that antioxidants are able to reduce pain and inflammation induced by chemical and thermal stimulus (Hacimuftuoglu et al., 2006). The oxidant–antioxidant system is in equilibrium in normal condition. However, an imbalance in the oxidant–antioxidant status causes tissue damage known as oxidant injury (Karaca et al., 2006). In this study, the protective role of *A. odorata* polyphenolic extract against oxidative stress was evaluated by the estimation of its parameters (MDA, GSH, CAT and SOD). Reactive oxygen species (ROS) attacking to membrane lipids may result in lipid peroxidation which is one of the most important mechanisms contributing to oxidative stress. Exposure of membrane lipids to ROS in the presence of iron salts stimulates the process of lipid peroxidation (Ozgocmen et al., 2004). Hence, the measurement of lipid peroxidation is an important indicator in the assessment of antioxidant potential. We calculated the amount of MDA, which is the end product of lipid peroxidation, the level of MDA indicates the degree of lipid peroxidation (Okhawa et al., 1979; Akyol et al., 2002) and its level is increased under stress conditions (Liu et al., 2009). ROS such as $O_2^{\bullet -}$ and endogenous H_2O_2 can be overproduced under stress conditions and will thereby increase MDA content (Alscher et al., 2002). In the present study, carrageenan caused a significant increase in liver MDA levels compared to the control group. This increase is due to excessive production of free radicals, in particular H_2O_2. The latter was described in the mucosa of inflamed rats (Shi et al., 2011) and also in the plasma of an azoxymethane (AOM) model in mice (Ashokkumar and Sudhandiran, 2008). However, treatment with *A.*

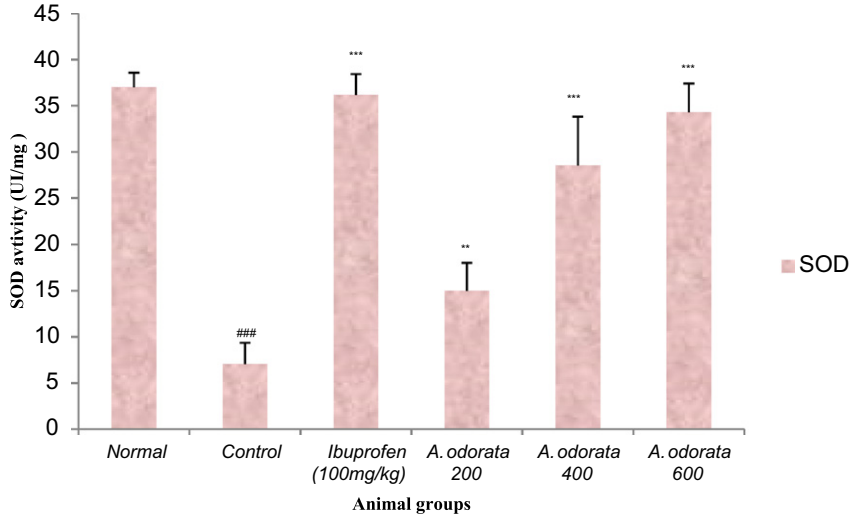

Fig. 4. Effects of *A. odorata* on liver SOD. Values are the mean ± SD for six mice; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared with carrageenan control group, ###$p < 0.001$ compared with normal group.

odorata extract significantly reversed these changes. Cheng et al. (2003) reported that phenolic compounds afford their protective actions in lipid peroxidation by scavenging the lipid-derived radicals (R•, RO• or ROO•) in a heterogeneous lipid phase. Babu et al. (2006) reported that green tea polyphenols can inhibit the formation of EORs by inhibiting the enzyme xanthine oxidase. They also reported that polyphenols exert antioxidant activity by chelating the transition metals which can contribute to the formation of free radicals *via* the fenton reaction. Polyphenols have an ideal chemical structure for capturing free radicals, so several studies have demonstrated the ability to capture free radicals by phenolic compounds (Santos-Gomes et al., 2002; Babu et al., 2006).

GSH, one of the most potent biological molecules, affects scavenging of all functions including free radical reactions. GSH acts as a nonenzymatic antioxidant in the detoxification pathway that reduces H_2O_2, hydroperoxide and xenobiotic toxicity. GSH is readily oxidized to glutathione disulfide (GSSG) upon reaction with xenobiotic compounds, which may then cause a decrease in GSH levels. GSSG is either rapidly reduced by GSH-Rd and NADPH or utilized in the endoplasmic reticulum to aid protein folding processes. Eventually, GSSG is recycled by protein disulfide isomerase to form GSH. Because of these recycling mechanisms, GSH is an extremely efficient intracellular buffer for oxidative stress (Ting et al., 2011). Therefore, it appears that GSH conjugation is essential to decrease the toxic effects of carrageenan. In the present study, carrageenan treatment significantly decreased GSH levels in the liver compared to the control group. However, the GSH levels significantly increase in *A. odorata* extract-treated groups. Depletion of GSH may be due to its consumption during reactions of conjugation with carrageenan metabolites, with products of lipid peroxidation and with hydrogen peroxide which is produced during inflammation responsible for tissue damage (Mulier et al., 1998). Many Studies have shown that tissue lesions induced by different stimuli are coupled with significant depletion of GSH (Paller and Patten, 1992; Sener et al., 2003). Oz et al. (2005) reported that in inflamed mice a decrease in GSH and GSH/GSSG ratio was observed in the plasma, thus showing a systemic oxidative state. Phenolic compounds of the extract administered have a scavenger effect; the free radicals will be captured by these compounds and not by GSH (Quinlan and Gutteridge, 1988). On the other hand, polyphenolic compounds are known for their ability to trap free radicals so it can directly participate in reducing the use of GSH (Babu et al., 2006).

For the purpose of evaluating *in vivo* antioxidant potential, we measured the activities of SOD and CAT. It is reported that SOD and CAT, the major endogenous antioxidant enzymes, are regarded as the first line of defense against ROS generated *in vivo* during oxidative stress (Ke et al., 2009). They act by converting the active oxygen molecules into nontoxic compounds (Ames et al., 1993). SOD dismutates superoxide radicals into hydrogen peroxide and molecular oxygen. CAT further detoxifies hydrogen peroxide into water (Sogut et al., 2003). Hence, these enzymes act mutually and compose the enzymatic antioxidant capacity against ROS. This study showed significant decrease in the cytosolic SOD in the carrageenan group. The increase in SOD activity in the groups treated with the polyphenolic extract suggests that this oxidative defense could be reactivated by active ingredients present in the extract, which may have increased the capacity of detoxification by improving the capture of free radicals. Any compound, natural or synthetic, with antioxidant properties, could contribute to the partial or total attenuation of damage that can be caused by EORs. The superoxide anion and the hydroxyl radicals induce various lesions in the organs and can play an important role in certain clinical alterations. Therefore, suppression of O_2• and •OH is probably one of the most effective means of disease control (Pari and Latha, 2005). However, this process can lead to lipid peroxidation if H_2O_2 is not decomposed immediately (Taleb-Senouci et al., 2009). Catalase (CAT) is a hemoprotein that catalyzes the reduction of hydrogen peroxides to H_2O and oxygen and protects tissues from hydroxyl radicals that are highly reactive (Sathishsekar and Subramanian, 2005). In the present study, we found a significant decrease in catalase activity in the group receiving only carrageenan compared to the negative control group. However, most authors favor the hypothesis that the decrease in catalase levels is due to an increase in lipid peroxidation (Ramakrishna et al., 2006). On the other hand, the activity of the catalase is increased in significant way in the groups receiving the polyphenolic extracts. This elevation may be due to an adaptive response to the generation of free radicals (Akhgari et al., 2003). Studies have shown positive correlations between CAT and the presence of flavonoids which possess powerful antioxidant properties (Cai et al., 2006).

5. Conclusion

In conclusion, the methanolic extract of *A. odorata* was found for the first time to exhibit significant anti-inflammatory activity. The extract also showed important peripheral analgesic activity. Additionally, the observed antioxidant activity of *A. odorata* extract could be, in part, responsible for their anti-inflammatory effects. The phenolics content may also play a role in the observed anti-inflammatory and analgesic activities. The results obtained in this work confirm the potential interest in *A. odorata* as a medicinal plant and justify its use in the treatment of inflammation-related diseases traditionally.

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