Research Article

ANTI-INFLAMMATORY AND ANTINOCICEPTIVE ACTIVITIES OF THE ESSENTIAL OIL FROM ROSMARINUS OFFICINALIS L. (LAMIACEAE)

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ABSTRACT

Rosmarinus officinalis (Lamiaceae) has been used since ancient times and it is popularly known as rosemary. This study aimed the effect of the essential oil (REO) in inflammatory and nociceptive models. Administration of doses (50, 100, 200, 500, 1000 and 2000 mg/kg, *p.o.*) did not show toxicity in the LD_{50} assay. In the rat paw edema induced by carrageenan, administration of REO single doses (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mg/kg, *p.o.*) had inhibited this edema in a dose-dependent manner and the ED_{50} established as 300 mg/kg. Different doses of REO (10, 50, 100, 200, 300 and 400 mg/kg, *p.o.*) have produced a dose-dependent effect in the writhing test, and the ED_{50} established as 260 mg/kg. Daily administration of REO (6 days, 300 mg/kg, *p.o*) inhibited the granulomatous tissue formation by 59%. In the ear edema induced with oil of Croton, REO (300 mg/kg, *p.o.*) has inhibited the edema formation by 77%. In the vascular permeability assay, oral treatment with REO has inhibited the vascular permeability by 50%. The treatment with REO (300 mg/kg, *p.o.*) has produced an inhibition during both phase I (40%) and the phase II (48%) of the formalin test. REO has inhibited by 44% the number of gastric lesions induced by stress compared to the control group. According to the results, we suggest that REO has an anti-inflammatory activity on acute and chronic inflammatory processes as well as peripheral analgesic activity, and has shown to be harmless to the gastric mucosa.

Keywords: Anti-inflammatory, antinociceptive, Essential oil, Lamiaceae, *Rosmarinus officinalis*.

INTRODUCTION

The use of aromatic medicinal plants with essential oil in its composition has been growing during the years. Moreover, it has become a highly important economical activity in several countries. *Rosmarinus officinalis* has been a used for the treatment of several illnesses because of its essential oil. Popularly known as rosemary, during the year of 1992, it was estimated the use of 295 tons of this specie, especially in cosmetics and food companies[1,2].

R. officinalis belongs to Lamiaceae family, and it is a bush which may grow up to 2 meters high. It presents opposite, small, aromatic leaves and white or pink small flowers[1,3]. The essential oil is obtained from the whole plant, especially from the leaves. The oil has shown many pharmacological activities, described by research groups all over the world.

The essential oil composition has been described as a composition of several compounds, principally bornil acetate, borneol and linallol. Thirty compounds were identified in *R. officinalis* essential oil obtained by hydro distillation. The main constituents were alpha pinene, borneol, camphor and camphene[4].

The use of essential oils of rosemary in a study of cognitive performance and mood with 144 volunteers has shown a decrease in electroencephalogram (EEG) frontal alpha and beta power, suggesting increased alertness[5]. Rosemary enhanced memory performance but reduced memory reaction times. Other studies have similarly reported improved memory performance[6,7] and alertness[8] after olfactory exposure to different odors. This oil has shown to be able to increase memory and secondary factors related to memory in computerized cognitive assays[9].

The addition of essential oils from *Salvia fruticosa* and *R. officinalis*, compared to butylated hydroxytoluene (BHT) have shown that the addition of the essential oil reduced the lipidic peroxidation as well as BHT, and has also enriched the flavor[10]. The GC analyses have shown over 41 terpens, including alpha- pinene, beta- myrcene, caryophillene, linallol, camphor and 1,8-cineol. From this specie, 26 antioxidants phenolic compounds have been isolated[4]. The antioxidant effect of the essential oil was determined by in vitro assays, showing a higher activity than vitamin E, Trolox and Butyl 4-Hydroxyanisole (BHA)[11] and by the Briggs-Rasucher method[12].

Twelve different samples of rosemary were extracted using three different solvents. Moreover, different extraction procedures were performed as well. All of these obtained fractions have presented high antioxidant activity because of different concentrations of carnosic acid, related to the antioxidant activity and inhibition of linolenic acid oxidation. This study has demonstrated that the carnosoic acid is a potent antioxidant, but it is unstable, reacting to carnosol[13].

The presence of carnesol is also linked to the hepatic activity related to *R. officinalis*, which is able to regulate

plasmatic bilirrubin and hepatic parenquimal protection. Also, malondialdehyde (69%) and alanine amino transferases (50%) were found to be lower[14].

The inhalation of essential oil of rosemary does not produce a detectable analgesic effect. However, subjects' retrospective evaluations of aroma-induced changes in pain intensity and pain unpleasantness suggested that they perceived some benefit of the intervention with rosemary essential oil. The anti-inflammatory activity of the extract obtained from this plant in animal models was evaluated with in vivo assays[15]. This study has aimed the effect of the essential oil of *R. officinalis* L. in inflammatory and analgesic in vivo assays.

This study was designed to explore, in a controlled way, the effects of *Rosmarinus officinalis* essential oil on experimental models of acute and chronic inflammation in rats. The aim of this study was to verify the anti-inflammatory effect of rosemary oil in different conditions.

MATERIALS AND METHODS

Vegetal material and essential oil extraction

The vegetal material was obtained from Santos Flora Cia., São Paulo, sample ALER 04/02, from Turkey, with botanical authentication as *Rosmarinus officinalis* L. The specimen was storage at acclimatized room (15°C, low air humidity) during 2 months.

The essential oil from *R. officinalis* (REO) was obtained using hydro-distillation from the dried aerial parts (500 grams) using a Clevenger apparatus[16]. Briefly, the apparatus consists in a steam flow that passes through the dried plant carrying the volatile substances. The steam flow was kept during two hours. The process has furnished 7 g of REO (yielding 1.4% w/w).

Phytochemical analysis by combined gas chromatography–mass spectrometry (GC–MS)

The essential oil was submitted to quantitative analysis in a Hewlett-Packard 5890 Model II automated gas chromatograph mass spectrometer data system with selective mass detector Hewlett-Packard 5971. GC conditions: carrier gas, helium at flow rate of 1.0 ml min–1; sample size, 2 µl injected using the splitless injection technique; fused capillary silica column HP-S (25m × 0.20mm × 0.33 µm). Temperatures: injector = 220∘C, detector = 280∘C, column = 60∘C, 3 ∘Cmin^{-1}, 240 ∘C (7 min). The MS was taken at 70 eV. The main constituents were identified by comparison with the mass spectrums from Wiley and Nist 98 spectrum library.

The essential oil was also analyzed using a Hewlett-Packard 5890 Model II automated gas chromatograph with flame ionization detector. GC conditions: carrier gas, helium at flow rate of 1.0 ml min^{-1}; sample size, 2 µl injected using the splitless injection technique; fused capillary silica column HP-S (25m × 0.20mm × 0.33 µm). Temperatures: injector = 220 ∘C, detector = 280 ∘C,

column = 60∘C, 3∘Cmin^{-1}, 240∘C (7 min). The identification of the main constituents was made by comparison of the obtained retention times (*R*t) in comparison with previously injected known compounds.

Animals

Wistar male rats (*Rattus norvegicus*) and Swiss male albino mice (*Mus musculus*) weighing between 180g - 200g and 20g - 25g respectively, were used in the experiments for anti-inflammatory and analgesic activities. The animals were acquired from the Biotery of the University of Alfenas (UNIFENAS). The animals were kept in climatized environment (25 ± 3°C), with light/dark control each 12 hours (7 a.m. to 7 p.m.). The animals were placed in cages up to 5 rats or 10 mice. They were kept without any food 12 hours before the experiments, and water was *ad libitum*. The study was approved by the UNIFENAS Bioethical Committee (Number 05A/2002).

REO solution

The essential oil of *Rosmarinus officinalis* was solubilized in Tween 80 (no more than 3%) and then suspended to provide a 100 mg/ml solution in water to be administered orally to the animals.

Determination of ED$_{50}$

This assay intends to determine an effectiveness dose to use in the experiments during the study. Groups of rats (n = 6) were treated orally with REO (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mg/kg) 30 min before the injection of the stimulus (carrageenan 1000 µg/paw, 0.1 ml) into the right hind paw plantar surface. Sterile saline solution (0.9%, 0.1 ml) was injected into the left paw as the control reference for the tested paw. The inhibition of inflammation was calculated by measuring the volume difference between the right and left paws in comparison with the control group in the third hour (peak of edema). ED$_{50}$ was determined from the curve drawn for the percentage of edema inhibition as a function of the dose[17], using Graph Pad Instat® Software.

Determination of LD$_{50}$

The lethal acute dose has been determined as the smaller dose with no side or toxic effects associated to it. REO was orally administered as a single dose to groups of mice (n = 10) at different concentrations (50, 100, 200, 500, 1000 and 2000 mg/kg). These animals were observed for a 72-h period, and then for 7 days. The number of animal deaths was expressed as a percentile, and the LD$_{50}$ was determined by the probit test using the death percentage versus the dose's log[18], with Graph Pad Instat® Software.

Rats paw edema induced by carrageenan

The inflammatory agent (carrageenan 1000 µg/paw, 0.1 ml) was injected into the right hind paw planter surface of groups of rats (n = 6). Sterile saline solution (0.9%, 0.1 ml) was injected into the left paw as the control reference for the tested paw. The foot volumes of the animals were determined by plestimographic method[19]. This method

was used to evaluate the effectiveness of the REO (300 mg/kg, *p.o.*) in the inhibition of the inflammatory process by comparison with the control (0.9% saline solution, 0.5 ml, *p.o.*) and the standard drug, indomethacin (positive control, 10 mg/kg, *p.o.*). The results were obtained by measuring the volume difference between the right and the left paws.

Writhing test

Groups of mice (*n* = 6) were treated with REO (10, 50, 100, 200, 300 and 400 mg/kg, *p.o.*), indomethacin (positive control, 10 mg/kg, *p.o.*) and control (0.9% saline solution, 0.25 ml, *p.o.*). The muscular contraction was induced by an intraperitoneal injection of 1.0% acetic acid solution (0.25 ml/animal) 30 min after the treatment. The number of muscular contractions was counted starting 5 min after the stimulus injection for a period of 20 min. Data represents the average of the total writhes observed[20].

Granulomatous tissue induction

Three groups of rats (*n* = 6) were used. Pellets weighing approximately 40 mg each were made with 5mm of dental cotton tampons. The pellets were sterilized and impregnated with 0.4 ml ampicillin water solution at the moment of implantation. Animals were anaesthetized, and the pellets were subcutaneously introduced through an abdominal skin incision. Each group was treated daily, for six consecutive days, with REO (300 mg/kg, *p.o.*), dexamethasone (positive control, 0.2 mg/kg, *p.o.*) or control (0.9% saline solution, 0.5 ml, *p.o.*). On the seventh day, the animals were sacrificed, the pellets dissected out and the granulomatous tissue dried at $60^{o}C$ overnight to determine the dried weight. The difference between the initial and final weights was considered as the weight of the tissues produced[21,22].

Ear edema induced by Croton oil

Groups of mice (*n* = 6) were used. The cutaneous inflammation was induced by Croton oil solution in acetone (10 mg/ml) on the right ear surface (0.1 ml, 1 mg/ear). The same volume of acetone was applied on the left ear. Thirty minutes after the stimulus, REO (300 mg/kg, *p.o.*), dexamethasone (positive control, 0.2 mg/kg, *p.o.*) and 0.9% saline solution (control, 0.25 ml, *p.o.*) were administered. After 6 hours, the animals were sacrificed and a sample (8 mm diameter) of each ear was obtained. The difference between the weigh of the stimulated ear (right) and the control ear (left) was the inflammation reference, expressed in mg[23].

Vascular permeability

The procedure is based on a spectrophotometric determination of a dye forced out to the interstitial space by the action of an inflammatory agent (histamine). The dye used in the assay was Evans blue. Groups of rats (*n* = 6) were treated with 0.9% saline solution (control, 0.5 ml, *p.o.*), cyproheptadine (positive control, 10 mg/kg, *p.o.*), and REO (300 mg/kg, *p.o.*). After 30 min the animals were

challenged with histamine (50 µg/animal). The Evans blue exudates were spectrophotometrically determined at 620 nm given a conversion factor calculated from calibration curves of the dye[24].

Formalin test in mice

Groups of mice (n = 6) have received REO (300 mg/kg, *p.o.*), indomethacin (positive control, 10 mg/kg. *p.o.*), morphine (positive control, 1 mg/kg, i.p.) and 0.9% saline solution (control, 0.25 ml, *p.o.*). These mice had injected 20 µL of a 2.5% formalin solution in their right footpad. After formalin administration, animals were isolated and observed for the first five minutes (early phase – neurogenic pain) and between 20 to 25 minutes (late phase – inflammatory pain)[25].

Hot plate test

Groups of mice (n = 6) had received REO (300 mg/kg, *p.o.*), indomethacin (positive control, 10 mg/kg. *p.o.*), morphine (positive control, 1 mg/kg, i.p.) and saline solution (control, 0.25 ml, *p.o.*). The hot plate apparatus (Socrel Mod. DS37, Ugo Basile, Italy) was maintained at $55 \pm 0.5^{o}C$. Animals were individual exposed and recorded the time they have spent to lick the footpad or any paw. The cut time used was 30 seconds. The measures were performed at 0, 30, 60, 90 and 120 minutes after treatments. The results were expressed as analgesia percentage, calculated by the formula $(t_1 - t_0) / (t_2 - t_0)$ x 100, were t_0 is control time, t_1 treated groups and t_2 the cut time[26].

Gastric ulcer induced by stress

Different groups of rats (*n* = 6) were treated with 0.9% saline solution (control, 0.5 ml, *p.o.*), cimetidine (positive control, 50 mg/kg, *p.o.*), REO (300 mg/kg, *p.o.*) and indomethacin (negative control, 10 mg/kg, *p.o.*). The number and severity of the lesions induced by water immersion stress were sorted out in three types: Type 1, presence of edema, hyperemia, and small hemorrhage; Type 2: presence of hemorrhage with small submucous erosions; and Type 3: presence of serious erosions with hemorrhagic boundaries and incursive lesions. A lesion index was determined following a matemathical formulae $IL = (N_3 \times 3) + (N_2 \times 2) + (N_1 \times 1) / N$ where N3, N2 and N1 are the number of lesions and its severity and N the number of animals used in each experiment[27].

Statistical analysis

The statistical analyses were done using Analysis of Variance (ANOVA) followed by the Tukey–Kramer multiple comparison test[28]. Results with $P < 0.05$ were considered to be significant. Data are expressed as mean ± S.E.M.

RESULTS

Phytochemical analysis by combined gas chromatography–mass spectrometry (GC–MS)

The extraction of the essential oil using a Clevenger apparatus yielded 1.4% (w/w). The chromatographic analysis of the REO showed seven major monoterpenic constituents, representing 76.1% of the REO components, including: 1,8-cineole (28.1%), α-pinene (17.4%), camphor (9.2%), 2-ethyl-4,5-dimethylphenol (8.5%), camphene (4.8%), β-pinene (4.6%), geranyl acetate (3.8%) and unidentified terpenes (23.9%).

Determination of LD_{50}

In the acute toxicity assay, no deaths were observed during the 72-h period after the administration of REO. At these doses (50, 100, 200, 500, 1000 and 2000 mg/kg), the animals showed no stereotypical symptoms associated with toxicity, such as convulsion, ataxy, diarrhea or increased diuresis. The median lethal dose was determined to be higher than the highest dose tested, 2.0 g/kg.

Determination of ED_{50}

The treatment with REO produced a reduction of the edema induced by carrageenan in a dose-dependent manner (correlation coefficient r = 0.9671 and linear regression y = 12.53 x – 16.53). ED_{50} was determined as 300 mg/kg (Fig. 1).
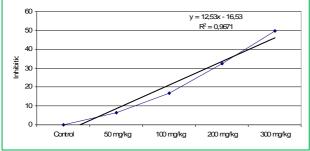

Figure 1: Determination of the effectiveness dose 50 (ED_{50}). Each point represents the average of eight animals, expressed as inhibition percentage. ED_{50} = 300 mg/kg (p.o.).
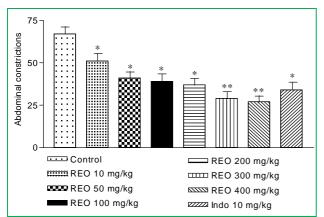

Figure 2: Effect of the oral administration of REO (10, 50, 100, 200, 300 and 400 mg/kg,) and indomethacin (10 mg/kg) on writhing induced in groups of mice by an intraperitoneal injection of 1.0% acetic acid. Data are expressed as mean ±

S.E.M. *$P < 0.05$, **$P < 0.01$, ANOVA followed by the Tukey–Kramer multiple comparison test.

Writhing test

In the writhing test, the treatment with different doses have produced a reduction of the writhes in a dependant manner as well (correlation coefficient r = 0.8891). The ED_{50} was determined as 261.0 mg/kg (Fig. 2).

Rats paw edema induced by carrageenan

The inhibitory activity shown by the essential oil of *Rosmarinus officinalis* (300 mg/kg) over a period of 4 hours in carrageenan-induced paw inflammation was quite similar to that exhibited by the group treated with indomethacin. REO has inhibited this edema formation over the four hours of experimentation ($P < 0.01$), with the best activity at the second hour (Fig. 3).
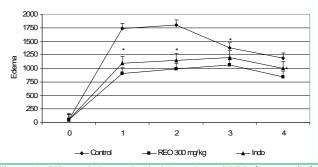

Figure 3: Effect of the oral administration of REO (300 mg/kg) and indomethacin (10 mg/kg) in rat paw edema induced by carrageenan. Data are expressed as mean ± S.E.M. *$P < 0.05$, **$P < 0.01$, ANOVA followed by the Tukey–Kramer multiple comparison test.

Granulomatous tissue induction

Daily administration of REO had inhibited the formation of granulomatous tissue by 59%, comparing to the control group ($P < 0.01$). Moreover, the orally administered dexamethasone reduced its formation by 78% ($P < 0.01$). The treated groups have shown statistically differences from the control but not between them (Fig. 4).
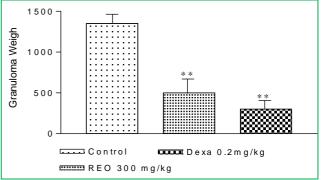

Figure 4: Effect of the oral administration of REO (300 mg/kg), dexamethasone (0.2 mg/kg) on cotton pellet-induced granuloma pouch in rats. Data are expressed as mean ± S.E.M. *$P < 0.05$, **$P < 0.01$, ANOVA followed by the Tukey–Kramer multiple comparison test.

Ear edema induced by Croton oil

The application of oil of Croton has induced an increasing edema over 6 hours. The treatment with REO has inhibited the edema formation by 77% when compared to the control group (P < 0.01). The group treated with dexamethasone has shown a similar inhibition (72%) to the group treated with REO (Fig. 5).
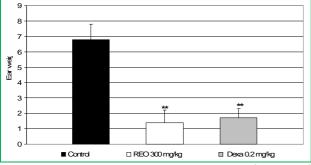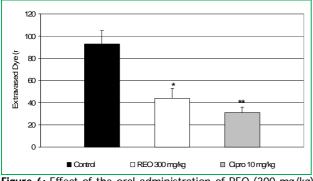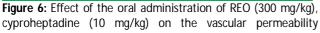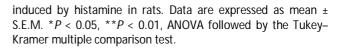

Figure 5: Effect of the oral administration of REO (300 mg/kg), dexamethasone (0.2 mg/kg) on ear edema induced by oil of Croton in mice. Data are expressed as mean ± S.E.M. *P < 0.05, **P < 0.01, ANOVA followed by the Tukey–Kramer multiple comparison test.

Vascular permeability

The vascular permeability has been decreased in the group treated with REO by 50% when compared to the control group (P<0.05). The group treated with cyproheptadine, a H_1 antagonist, has decreased the vascular permeability by 70%. This result is statistically different from both the control group (P < 0.01) and the treatment with REO (P < 0.05). These results are shown at Figure 6.

Figure 6: Effect of the oral administration of REO (300 mg/kg), cyproheptadine (10 mg/kg) on the vascular permeability

induced by histamine in rats. Data are expressed as mean ± S.E.M. *P < 0.05, **P < 0.01, ANOVA followed by the Tukey–Kramer multiple comparison test.

Formalin test in mice

The treatment with REO has inhibited the phase I in the formalin test by 40% and the phase II by 48% (P < 0.05) when compared to the control group. The treatment with morphine has produced an inhibition by 83% and 92% respectively (P < 0.01). All groups have shown statistically significant differences (Figure 7).
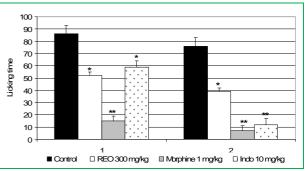

Figure 7: Effect of the administration of REO (300 mg/kg, *p.o.*), morphine (1.0 mg/kg, i.p.) and indomethacin (10 mg/kg, *p.o.*) in the formalin test in mice. Data are expressed as mean ± S.E.M. *P < 0.05, **P < 0.01, ANOVA followed by the Tukey–Kramer multiple comparison test.

Hot plate test

During the hot-plate test, the group treated with REO has shown a longer latency time (before the treatment) but this difference was not significant (data not shown). Only the group treated with morphine has increased the latency time (P < 0.01).

Gastric ulcer induced by stress

The treatment with REO in the gastric lesions induced by stress has decreased the lesions by 44% when compared to the control group (P < 0.05) and by 64% when compared to the group treated with indomethacin (P < 0.01). The group treated with cimetidine has shown a better effect when compared to the control group, decreasing the lesions by 79% (P < 0.01) when compared to REO (P < 0.05). These results are presented at Table 1.

Table 1: Effect of the administration of REO (300 mg/kg, p.o.), indomethacin (10 mg/kg, p.o.) and cimetidine (10 mg/kg, p.o.) on the gastric mucosa in rats submitted to stress.

Treatment	Lesion severity			Ulcer index
	1+	2+	3+	
Saline solution (0.5 ml, *p.o.*)	42 ± 1.6	46 ± 1.7	34 ± 1	47.2
REO (300 mg/kg, *p.o.*)	18 ± 0.8	22 ± 1.5	23 ± 1.9	26.2*
Indomethacin (10 mg/kg, *p.o.*)	84 ± 2.4	62 ± 2.4	53 ± 1.7	73.4
Cimetidine (10 mg/kg, *p.o.*)	26 ± 0.97	7 ± 1.4	3 ± 0.6	9.8**

Data are expressed as mean ± S.E.M. *P < 0.05, **P < 0.01, ANOVA followed by the Tukey–Kramer multiple comparison test.

DISCUSSION

Essential oils are complex mixtures of volatile, liquid, lipophilic and odoriferous substances. The composition of an essential oil is genetically determinate, specific for a tissue or a characteristic of its development stage. These facts also corroborates to its concentration in the plant[2].

Rosmarinus officinalis is an aromatic plant which names means "marine rock", since it has been endemic in coasts with rocks. This plant presents about 0.48% to 1.75% of essential oil[9]. The sample used in this study has yielded 1.4% in agreement with literature data.

In this study, we evaluated the safety and efficacy of the essential oil obtained from *R. officinalis*. ED_{50} of REO was established as 300 mg/kg by the carrageenan-induced paw edema in rats and 261 mg/kg for the writhing test in mice. Using the acute toxicity assay, the median lethal dose (LD_{50}) was determined to be higher than 2.0 g/kg. In this assay, neither deaths nor symptoms associated with toxicity, such as convulsion, ataxia, diarrhea or increased diuresis occurred during the 72-h observation period. These results indicate the effectiveness and relative safety of REO for the treatment of conditions associated with inflammation.

Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1–2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissues surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages[29]. There was a significant reduction ($P < 0.05$) in this assay at 300 mg/kg dose of REO and at 10 mg/kg of indomethacin. Both results are statistically significant compared to the control, but not between them. Since the 300 mg/kg dose has presented the best performance and is quite similar to the determined ED_{50}, it had been used during the study.

The intraperitoneal administration of agents that irritate serous membranes provokes a stereotypical behavior in mice and rats which is characterized by abdominal contractions, movements of the body as a whole, twisting of dorsoabdominal muscles, and a reduction in motor activity and coordination[29]. The quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats obtained after the intraperitoneal injection of acetic acid demonstrated high levels of prostaglandins $PGE_{2\alpha}$ and $PGF_{2\alpha}$ during 30 min after stimulus[30,31]. It should be taken into consideration that the mechanism involved in the genesis of the carrageenan-induced edema may cause the release of prostaglandins and kinins, among other substances[11,30]. The writhing test has shown results similar to that obtained in the edematogenic assay using carrageenan. All REO doses tested in this assay have produced a significant inhibition in a dose dependant manner.

The ear edema induced with topical application of oil of Croton has been used to evaluate anti-inflammatory activity of topical agents. This oil is a vascular irritant, leading to infiltration of leukocytes and other defense cells, causing intracellular edema and topic dermatitis[32]. This process may be inhibited by the use of corticoids[33]. The group treated with REO has inhibited the edema by 77%, just as dexamethasone (72%). Both results are different from the control group but not between them. Since the inhibition of both treatments is similar, probably the REO has inhibited the formation of cyclo-oxygenases products generated by the topic application of oil of Croton[34,35].

The anti-inflammatory and antinociceptive effects of 1,8-cineol, presented in large quantity in essential oils obtained from *Rosmarinus officinalis* have been described[36]. Since this compound acts on prostaglandins models for evaluation of anti-inflammatory drugs, it has an important hole on the inhibition of the inflammatory process.

The acute inflammation causes an increase in the vascular permeability, releasing compounds related to the inflammatory response. Histamine and serotonin have a role in this process. The treatment with REO has antagonized this process by 50%, and cyproheptadine, an H_1 antagonist, has shown the best activity, inhibiting this process by 70%.

The granulomatous tissue induction has been a widely used method for the assessment of anti-inflammatory substances[21,22]. It may be divided in phases, occurring at the same time. The liberation of plasmatic mediators and the increase of the vascular permeability happen at first. Then, there is a phase characterized by cellular and plasmatic exudates. Afterwards, there is a degenerative and necrotic phase and the last one characterized by cell proliferation to repair the damaged tissue[37]. The treatment with REO had inhibited the granulomatous tissue formation by 59%, as well as dexamethasone, which had inhibited the granulomatous tissue formation by 78%.

The formalin test has been used to study central analgesia, during the first phase and inflammation in the second phase. In terms of fibre size/conduction velocity, there are two main groups of nociceptive somatic primary afferent neurons, those with C-fibres and those with A-fibres. The stimulation from the external environment mechanonociceptive neurons (Type A nociceptive nerve fibres) gives rise to a well-localized sharp sensation, while stimulation of nociceptive neurons (Type C nociceptive nerve fibres) is said to give rise to a poorly localized unpleasant sensation[38]. The activation of afferent Type C nociceptive nerve fibres may be more effective in causing reflex changes such as cardiovascular or respiratory changes[39]. Also, the release of excitatory amino acid neurotransmitters in the CNS facilitates acetylcholine secretion at synapses[40]. Adrenocorticotrophic hormone

release from the pituitary gland after intracerebroventricular injection was also described[41].

The intraplantar injection of formalin stimulates the Type C nociceptive nerve fibres inducing a neurogenic pain during a 5 to 10 minutes period. After 30 minutes, the late phase is caused by inflammation and spinal cord stimulus[42]. The genesis of pain by formalin involves pos-synaptic NMDA receptors, corresponding to the delta Type A or Type C nociceptive nerve fibers. These receptors have been associated to memory and learning process as well as acute and chronic painful syndromes. If the stimulus is repetitive, there is an increase of the receptor fields and the nociceptive spinal neurons, releasing glutamate and Substance P that stimulates NMDA receptors. This complex activates the protein C kinases through the inositol pathway, synthesizing nitric oxide and prostaglandins, leading to the secondary hyperalgesia[43]. The group treated with REO has shown a significant inhibition both in the first and second phase compared to the control group (P < 0.01). The effect was similar to the group treated with indomethacin, confirming the peripheral effect of REO and suggesting an inhibition of prostaglandin synthesis, once it was effective in the writing test as well. The hot-plate test did not show significant results, only for the group treated with morphine, suggesting a NSAID effect of this extract since it did not inhibit the central pain but inhibited the abdominal constrictions and has also demonstrated an anti-inflammatory effect.

The hydroethanolic extract from *Rosmarinus officinalis* aerial parts has decreased the ulcerative gastric lesions induced by indomethacin, ethanol and reserpine in rats because of the presence of antioxidant compounds[44]. Also, it has been shown that natural compounds with antioxidant activity protect against several physiopathological illness, preventing or diminishing the oxidative stress[45].

The group treated with REO has shown less hazardous effects in the gastric mucosa, with a lesion index smaller compared to the group treated with indomethacin (P < 0.05). It had shown no difference with the group treated with cimetidine, the standard drug used in this assay, suggesting a protective action[44]. Another fact that must be considered is the antioxidant potential of the essential oil which could prevent the formation of gastric lesions induced by stress.

The anti-inflammatory and analgesic activities of the aerial parts crude extract from *Rosmarinus officinalis in vivo* assays have been previously described[15]. This study corroborates the anti-inflammatory and analgesic effects regarding this medicinal plant. Therefore, the essential oil has inhibited both acute and chronic inflammatory process, similar to COX inhibitors, but it did not induce gastric lesions. It is strongly suggested that the pharmacological activity of this plant is probably because of terpenic compounds, such as camphor and 1,-8-cineol[36,46], from the essential oil.

REFERENCES

1. Lorenzi H, Matos FJA. Labiatae (Lamiaceae). In: Plantas Medicinais no Brasil: nativas e exóticas cultivadas no Brasil. *Instituto Plantarum de Estados da Flora LTDA*, Nova Odessa, São Paulo, Beazil, p. 261. 2002.

2. Simões CMO, Gosman G, Schenkel EP, Mello JCP, Mentz LA, Petrovick PP. Farmacognosia: da planta ao medicamento. 5. ed. rev. Porto Alegre/Florianópolis: Editora da UFRGS/Editora da UFSC, Brazil, p.48-64, p.486-490, p.664. 2003.

3. Beckett KA. *Labiadas. Hierbas Aromáticas*. Ediciones Folio, S. A., Barcelona, Spanish, p. 76. 1988.

4. Nakatani N. Phenolic antioxidants from herbs and spices. Biofactors,13, 2000,141-6.

5. Moss M, Cook J, Wesnes K, Duckett P. Aromas of rosemary and lavender essential oils differentially affect cognition and mood in healthy adults. Int. J. Neurosci., 113, 2003, 15–38.

6. Ehrlichman H, Halpern JN. Affect and memory: effects of pleasant and unpleasant odors on retrieval of happy and unhappy memories. J. Pers. Soc. Psychol., 55, 1988, 769 – 79.

7. Degel J, Koster EP. Odors: implicit memory and performance effects. Chem. Senses, 24, 1999, 317–25.

8. Gedney JJ, Psyd TL, Glover M, Fillingim RB. Sensory and affective pain discrimination after inhalation of essential oils. Psychosom. Med., 66, 2004, 599–606.

9. Angioni A, Barrsa A, Cereti E, Barile D, Coisson JD. Chemical composition, plant genetic differences, antimicrobial and activity investigation of the essential oil of *Rosmarinus officinalis* L. J Agric Food Chem., 11, 2004, 3530-5.

10. Estevez M, Ventanas S, Ramirez R, Cava R. Analysis of volatiles in porcine liver with added sage and rosemary essential oils by using SPME-GC-MS. *J Agric Food Chem.*, 16, 2004, 5168-74.

11. Stahenko EE, Puertas MA, Martinez JR. SPME determination of volatile aldehydes for evaluation of in - vitro antioxidant activity. Anal Bional Chem., 373, 2002,70-74.

12. Cervellati, R., Rezulli, C., Guerra, M.C., Speroni, E. (2002) Evaluation of antioxidant activity of some natural poliphenolic compounds using the Briggs-Rauscher reaction method. J Agric Food Chem., 26, 7504-9.

13. Wellwood CR, Cole RA. Relevance of carnosic acid concentrations to the selection of rosemary, *Rosmarinus officinalis* L., accessions for optimization of antioxidant yield. J Agric Food Chem., 20, 2004, 6101-6107.

14. Sotelo-Felix JI, Martinez-Fong D, Muriel de la Torre P. Protective effect of carnesol on CCL(4) - induced acute liver damage in rats. Eur J Gastroenterol Hepatol., 14, 2002, 1001-6.

15. Carvalho JCT, Hinsberger A, Cardoso LGV, Fonseca YM, Sales WP, Faria LRD. Untersuchung der antiinflammatorischen und analgetischen Aktivität des hydroalkoholischen Rohextraktes von Rosmarin. Ärztezeitschrift für Naturheilverfahren, 45, 2004, 11-15.

16. Farmacopéia Brasileira. 1ª Edição. Estados Unidos do Brasil,

Ed. Atheneu. 1988.

17. Perazzo FF, Souza GHB, Lopes W, Cardoso LGV, Carvalho JCT, Nanayakkara DNP, Bastos JK. Anti-inflammatory and analgesic properties of water–ethanolic extract from *Pothomorphe umbellata* (Piperaceae) aerial parts. J Ethnopharmacol., 99, 2005, 215–220.

18. Thompson WR, Weil CS. On the construction of tables for moving average interpolations. Biometrics, 8, 1952, 51–54.

19. Carvalho JC, Sertié JA, Barbosa MV, Patrício KC, Caputo LR, Sarti SJ, Ferreira LP, Bastos JK. Anti-inflammatory activity of the crude extract from the fruits of *Pterodon emarginatus* Vog. J Ethnopharmacol. 64, 1999, 127-33.

20. Koster R, Anderson M, DeBeer EJ. Acetic acid analgesic screening. Fed Proc., 18, 1959, 418–420.

21. Swingle KF, Shideman FE. Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their modification by certain anti-inflammatory agents. J Pharmacol Exp Ther., 183, 1972, 226–234.

22. Niemegeers CJE. The activity of suprofen on nystatin-induced paw oedema in rats. Arzneimittel-Forschung., 23, 1975, 1516–1519.

23. Tubaro A, Dri O, Zilli C, Loggia RD. The croton oil ear test revisited. Agents and Actions, 17, 1985, 347-349.

24. Lykbe AMJ, Cummings R. Inflammation in healing: 1. Timecourse and medition of exudation in wound healing of the rat. Brit J Exp Pathol., 50, 1969, 309–318.

25. Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain, 30, 1987, 103-114.

26. Eddy NB, Leimbach D. Synthetic analgesic. II. dithienylbutenyl and dithienylbutylamines. J Pharmacol Exp Ther., 107, 1953, 385-393.

27. Basile AC, Sertie JAA, Panizza S, Oshiro TT, Azzolini CA. Preventive anti-ulcer activity and toxicity of the leaf crude extract from *Casearia sylvestris*. J Ethnopharmacol., 30, 1990, 185–187.

28. Sokal RR, Rohlf FJ. Biometry: The Principles and Practice of Statistics in Biologial Research, 2nd ed. (Freeman) Campbell, R.C., pp. 175–205; 404–486. 1995.

29. Bars D, Gozariu M, Cadden SW. Animal models of nociception. Pharmacol Rev., 53, 2001, 597-652.

30. Garcia-González A, Morales-Hernandes RC, Porta-Gándara MA, Rubio-Cerda E, Ochoa JL. Superoxide dismutase and naproxen in the very late phase of carragenin induced edema in rats. Ver Invest Clin., 52, 2000, 56-160.

31. Deraedt R, Jouquey S, Delevallee F, Flauhaut M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur J Pharmacol., 61, 1980, 17–24.

32. Montello MSAG, Martins RABL. Efeito da terapia com laser de baixa potência na dermatite induzida por óleo de croton em orelha de camundongo. São José dos Campos, São Paulo, Brazil, UNIVAP, 67p., 2002.

33. Galey GI, Ziboh VA, Marcelo CI, Voorhees JJ. Modulation of phospholipid metabolism in murine keratinocytes by tumor promoter, 12-O-tetradecanoyl-13-acetate. J Invest Dermatol., 85, 1985, 319-323.

34. Inoue H, Mori T, Shibata Y. Modulation by glycerrhetinic acid derivates of TPA-induced mouse ear oedema. Brit J Pharmacol., 96, 1989, 204-210.

35. Young JM, De Young LM. Cutaneous models of inflammation for the evaluation of topical and sistemic pharmacological agents. In: Pharmacological Methods in the control of inflammation, Alan R. Liss, New York, USA, pp.215-231. 1989.

36. Santos FA, Rao VS. Anti-inflammatory and antinociceptive effects of 1,8-cineole a terpenoid oxide present in many plant essential oils. Phytother Res., 14, 2000, 240-4.

37. Corrêa L, Novelli MD. Patologia Geral: Inflamação. Faculdade de Odontologia, USP, Available at www.fo.usp.br/lido/patoartegeral, 2000.

38. Torebjork HE, Ochoa JL. New method to identify nociceptor units innervating glabrous skin of the human hand. Exp. Brain Res., 81, 1990, 509–514.

39. Clark D, Hughes J, Gasser HS. Afferent function in the group of nerve fibres of slowest conduction velocity. Am. J. Physiol., 114, 1935,69–76.

40. Ogura A, Myojo Y, Higashida H. Bradykinin evoked acetylcholine release via inositol triphosphate- dependent elevation in free calcium in neuroblastoma X glioma hybrid NG108-15 cells. J. Biol. Chem., 265, 1990, 3577–3584.

41. Madeddu P, Glorioso N, Varoni MV, Demontis MP, Fattaccio MC, Anania V. Cardiovascular effects of brain kinin receptor blockade in spontaneously hypertensive rats. Hypertension, 23, 1994, 1189–1192.

42. Pini LA, Vitae E, Ottani A, Sandrini M. Reversible antinociception by paracetamol in the rat. J Pharmacol Exp Ther., 280, 1997, 934-940.

43. Wei F, Guo-Du W, Geoffrey AK, Susan JK, Hai-Ming X, Zhou-Feng C, Min Z. Genetic enhancement of inflammatory pain by forebrain. NBR2B overexpression. Nature Neurosci., 4, 2001, 164-169.

44. Dias PC, Foglio MA, Possenti A, Carvalho JE. Antiulcerogenic activity of crude hydroalcoholic extract of *Rosmarinus officinallis* L. J Ethnopharmacol., 69, 2000, 57-62.

45. Repetto MG, Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz J Med Biol Res., 35, 2002, 523-534.

46. Perazzo FF, Carvalho JCT, Carvalho JE, Rehder VLG. Central properties of the essential oil and the crude ethanol extract from aerial parts of *Artemisia annua* L. Pharmacol Res., 48, 2003, 497–502.

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