

Phytochemical screening, antinociceptive and anti-inflammatory activities of *Chrysopogon zizanioides* essential oil

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Abstract: *Chrysopogon zizanioides* (L.) Roberty, Poaceae, is a plant widely used in northeast Brazil in folk medicine for the treatment of various pathological conditions, including inflammatory pain. The present study evaluated the antinociceptive and anti-inflammatory effects of *C. zizanioides* essential oil (EO) in rodents. EO was further characterized by GC/MS. The major components of EO were identified as khusimol (19.57%), *E*-isovalencenol (13.24%), α -vetivone (5.25%), β -vetivone (4.87%) and hydroxy-valencene (4.64%). Following intraperitoneal injection (*i.p.*), EO at 50 and 100 mg/kg significantly reduced the number of writhes (51.9 and 64.9%, respectively) and the number of paw licks during phase 2 (56.7 and 86.2%, respectively) of a formalin model when compared to control group animals. However, EO-treated mice were ineffective at all doses in hot-plate and rota-rod tests. The EO inhibited the carrageenan-induced leukocyte migration to the peritoneal cavity in a dose-dependent manner (34.7, 35.4, and 62.5% at doses of 25, 50 and 100 mg/kg, respectively). In the paw edema test, the EO (100 mg/kg) inhibited all three phases of the edema equally well, suggesting that the EO has a non-selective inhibitory effect on the release or actions of these mediators. Our results suggest possible antinociceptive and anti-inflammatory effects of the EO.

Introduction

Medicinal plants are known as an important source of new chemical substances with potential therapeutic effects (Almeida et al., 2001). Recently, essential oils extracted from various herbs and spices have been a subject of intensive research, partially due to the continuous discoveries of their multifunctional properties other than their classical roles as food additives and/or fragrances. For example, antibacterial, antifungal, anticonvulsant, antinociceptive and anti-inflammatory activities of many essential oils have been investigated (Elisabetsky et al., 1995; Hammer et al., 1999; Almeida et al., 2001; Quintans-Junior et al., 2008).

Chrysopogon zizanioides Nash, Poaceae, popularly known as vetiver and “grama-das-índias”, is

one of the most important raw materials in perfumery (Martinez et al., 2004). The genus *Vetiver* has recently been reclassified (Adams et al., 1998), and is now combined with *Chrysopogon* (Veldkamp, 1999), based on their overlapping genetic and morphological data. This led to the recognition of *C. zizanioides* (L.) Roberty as the correct classification for *Vetiveria zizanioides* (L.) Nash (Massardo et al., 2006). Except for termiticidal (Maistrello et al., 2001) and antimicrobial activities (Hammer et al., 1999), the oil has not been studied intensively concerning other biological functionalities, probably because of its complex constituents. Vetiver oil is composed of more than 170 compounds that are mainly sesquiterpenes and their derivatives (Nigam et al., 1968; Weyerstahl et al., 2000b).

Despite the traditional use as analgesic

Article

Received 16 May 2011

Accepted 18 Aug 2011

Available online 6 Jan 2012

Keywords:

anti-inflammatory effect
antinociceptive effect
Chrysopogon zizanioides
essential oil
phytochemical screening
vetiver

ISSN 0102-695X

<http://dx.doi.org/10.1590/S0102-695X2012005000005>

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and sedative in Brazil, no reports concerning the antinociceptive effects of vetiver are available. Besides, the properties of vetiver regarding free radicals and oxidative damage have never been studied. Considering the role of reactive oxygen species (ROS) in physiological and pathophysiological processes, and also considering that secondary metabolites have been observed to exert multiple roles in response to ROS-mediated insults when administered therapeutically or used in the diet, it is important to investigate redox-associated properties of compounds derived from natural sources. In this regard, we studied the antinociceptive and anti-inflammatory effects of essential oil of *C. zizanioides* in rodents, as well as some of its redox properties *in vitro*.

Material and Methods

Plant material and essential oil (EO) extraction

Roots were collected from the cultivation of *Chrysopogon zizanioides* (L.) Roberty, Poaceae, genotypes established at the Research Farm "Campus Rural da UFS" of the Federal University of Sergipe, Brazil, and a voucher sample has been deposited in the Herbarium of the Department of Biology, Federal University of Sergipe (ASE 13437). The roots of *C. zizanioides* were oven dried with air renewal and circulation (model MA-037/18) at 40 °C until complete dehydration has been achieved. The essential oil (EO) was obtained by hydrodistillation in a Clevenger-type apparatus using 250 g of dried roots. The essential oil (EO) obtained was dried over anhydrous sodium sulfate, producing yields of 4.15% (v/w).

Analysis of the essential oil by GC/MS

A Shimadzu system (Kyoto, Japan), consisting of a QP-5050A mass spectrometer equipped with a GC-17A gas chromatograph with a Shimadzu AOC 20i auto-injector and a split (ratio 1:83) injector was used for the identification and quantification of the EO studied. A fused silica column DB-5MS (30 m x 0.25 mm i.d, composed of 5% phenylmethylpolysiloxane), supplied by J&W Scientific (Folsom, CA, USA), was employed, with helium (99.999% purity) as carrier gas at a flow-rate of 1.2 mL/min. The column temperature was programmed as follows: 50 °C for 1.5 min, then directly to 200 °C at 4 °C/min, then 10 °C/min to 300 °C, ending with a 10 min isothermal at 300 °C. The injector port was maintained at 250 °C, ion-source temperature 280 °C and 0.5 L sample volumes were injected. The mass spectra were taken at 70 eV with scanning speed of 0.85 scan/s from 40 to 550 Da. The data were acquired and processed with a PC with Shimadzu Class-5000 software. The identification of the constituents was assigned on basis of comparison of their

relative retention indices (Van Den Dool & Kratz, 1963) to a *n*-alkane homologous series (nC9-nC18) obtained by co-injecting the oil sample with a linear hydrocarbon mixture, as well as, by computerized matching of the acquired mass spectra with those stored in NIST21 and NIST107 mass spectral libraries of the GC/MS data system and other published mass spectra (Adams, 2007; Adams et al., 2008).

Animals

Male Swiss mice weighing 25-35 g, maintained under standard environmental conditions, were used distributed in groups of ten (writhing, formalin, hot-plate and rota-rod tests) and six male Wistar rats weighing 150-180 g each (neutrophil migration activity and carrageenan-induced hind paw edema). The animals were randomly housed in appropriate cages at 22±1 °C on a 12 h light/dark cycle (lights on 6 am to 6 pm) with free access to food and water. Experimental protocols and procedures were approved by the Federal University of Sergipe Animal Care and Use Committee (CEPA/UFS 23/08).

Acetic acid-induced writhing

In this model (Koster et al., 1959), groups of mice were administered 0.85% acetic acid (10 mL/kg body wt., *i.p.*), and the number of abdominal contractions was registered over 20 min, starting 5 min after acetic acid injection. Animals (n=10, per group) were treated intraperitoneally (*i.p.*) with EO (25, 50, and 100 mg/kg), 30 min before acetic acid administration. Distilled water with one drop of tween 80 0.2% (vehicle) was used *i.p.* in control animals. The effect of pretreatment with naloxone (NAL, 1.5 mg/kg, *i.p.*) on the antinociception produced by EO (50 and 100 mg/kg) and morphine (MOR, 3.0 mg/kg, *i.p.*) was determined.

Formalin test

The formalin test was carried out as described by Hunskaar et al. (1985). The animals were separated into five groups (n=10, per group) and treated *i.p.* with vehicle (control), EO (25, 50, and 100 mg/kg) and aspirin (200 mg/kg). After 30 min, 20 µL of 2.5% formalin solution (0.92% formaldehyde in 0.9% saline) was injected into the subplantar area of the right hindpaw. The duration of paw licking was measured at 0-5 min (first phase) and 15-30 min (second phase) after formalin administration (Hunskaar & Hole, 1987).

Hot-plate test

The hot-plate test measured response latencies according to the method described by Eddy & Leimback

(1953). Animals were placed on an Insight® hot-plate (Model EFF-361) maintained at 55 ± 1 °C and the time between placement of the animal on the hot-plate and the occurrence of either the licking of the hind paws, shaking or jump off from the surface was recorded as response latency. Mice with baseline latencies of more than 10 s were eliminated from the study 24 h later. The cut-off time for the hot plate latencies was set at 30 s. Animals ($n=10$, per group) were treated with EO (25, 50, and 100 mg/kg, *i.p.*) 30 min before the experiments. Animals control received the same treatment to abdominal constriction test.

Rota-rod test

Mice able to remain on the Rota-rod apparatus (AVS®, Brazil) longer than 180 s (9 rpm) were selected 24 h before the test according to Gonçalves et al. (2008). Then the selected animals were divided into five groups ($n=8$) and treated *i.p.* with vehicle (control), EO (25, 50, and 100 mg/kg), and diazepam (1.5 mg/kg). Thirty minutes later, each animal was tested on the Rota-rod and the time (s) they remained on the bar for up to 180 s was recorded.

Carrageenin-induced hind paw edema in rats

The acute hind paw edema was produced by injecting 0.1 mL of carrageenan (prepared as 1% suspension in sterile normal saline) locally into the plantar aponeurosis of the right hind paw of rats (Winter et al., 1962). EO (50, 100, and 200 mg/kg, *i.p.*) was administered to three different groups while the other two groups served as negative and positive controls and received vehicle and standard drug, Aspirin (300 mg/kg, *p.o.*), respectively. EO and aspirin were administered 1 h prior to the injection of carrageenan. The rat pedal volume up to the ankle joint was measured using plethysmometer (Model LE 7500 Panlab, Barcelona, Spain) at 0 (just before) and 3 h after the injection of carrageenan.

Leukocyte migration to the peritoneal cavity

The leukocyte migration was induced by injection of carrageenan (500 µg/cavity, *i.p.*, 500 µL) into the peritoneal cavity of rats 30 min after administration of EO (25, 50, and 100 mg/kg, *i.p.*) or dexamethasone (2 mg/kg, *s.c.*, $n=6$) by modification of the technique previously described by Bastos et al. (2007). The animals were euthanized by cervical dislocation 4 h after carrageenan injection. Shortly after, phosphate buffered saline (PBS) containing EDTA (1 mM, *i.p.*, 10 mL) was injected. Immediately a brief massage was done for further fluid collection, which was centrifuged (2000 rpm, 5 min) at room temperature. The supernatant was disposed of 1 mL of PBS was introduced to the precipitate. An aliquot

of 10 µL from this suspension was dissolved in 200 µL of Turk solution and the total cells were counted in a Neubauer chamber, under optic microscopy. The results were expressed as the number of neutrophils/mL. The percentage of the leukocyte inhibition = $(1-T/C) \times 100$, where T represents the treated groups leukocyte counts and C represents the control group leukocyte counts.

TBARS Assay

TBARS (thiobarbituric acid reactive species) assay was employed to measure the antioxidant capacity of EO using egg yolk homogenate as lipid rich substrate. Briefly, egg yolk was homogenized (1% w/v) in 20 mM phosphate buffer (pH 7.4), 1 mL of homogenate was sonicated (10 s in potency 4) and then homogenized with 0.1 mL of EO at different concentrations or controls in different concentrations prepared immediately before use. Lipid peroxidation was induced by addition of 0.1 mL of (2,2'-azobis[2-methylpropionamide]dihydrochloride) (AAPH, a free radical source) solution (0.12 M). Trolox was used as reference antioxidant molecule, (positive control); negative control was only EO vehicle (DMSO 10%). Reactions were carried out for 30 min at 37 °C. After cooling, samples (0.5 mL) were centrifuged with 0.5 mL of trichloroacetic acid (15%) at 1200 g for 10 min. An aliquot of 0.5 mL from supernatant was mixed with 0.5 mL TBA (0.67%) and heated at 95 °C for 30 min. After cooling, samples absorbance was measured using a spectrophotometer at 532 nm. The results were expressed as percentage of TBARS formed by AAPH alone (induced control) (Draper & Hadley, 1990).

Hydroxyl scavenging activity

The formation of ·OH (hydroxyl radical) from Fenton reaction was quantified using 2-deoxyribose oxidative degradation. The principle of the assay is the quantification of the 2-deoxyribose degradation product, Malondialdehyde (MDA), by its condensation with 2-thiobarbituric acid (TBA). Briefly, typical reactions were started by the addition of Fe^{2+} (6m M final concentration) to solutions containing 5 mM 2-deoxyribose, 100 mM H_2O_2 and 20 mM phosphate buffer (pH 7.2). To measure EO antioxidant activity against hydroxyl radical, different concentrations of EO were added to the system before Fe^{2+} addition. Reactions were carried out for 15 min at room temperature and were stopped by the addition of 4% phosphoric acid (v/v) followed by 1% TBA (w/v, in 50 mM NaOH). Solutions were boiled for 15 min at 95 °C, then cooled at room temperature. The absorbance was measured at 532 nm and results were expressed as MDA equivalents formed by Fe^{2+} and H_2O_2 (Payá et al., 1994).

Statistical analysis

The data obtained were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's or Fisher's tests. Differences were considered to be statistically significant when $p < 0.05$. The percent of antinociceptive inhibition was evaluated for each experimental group using the following formula (Reanmongkol et al., 1994): Inhibition % = $100 \times (\text{control-experiment})/\text{control}$.

Results

Analysis of the essential oil by GC/MS

GC-MS analysis showed a mixture of compounds, with khusimol (19.57%), *E*-isovalencenol (13.24%), α -vetivone (5.25%), vetiselinenol (5.08%), α -cadinol (5.01%), α -vetivone (4.87%) and hydroxy-valencene (4.64%) as the major compounds in the EO (Table 1).

The analysis of the vetiver oil showed the presence mainly the tricyclic sesquiterpene khusimol, and the bicyclic sesquiterpenes (*E*)-isovalencenol. Some species showed variable oil composition, these differences often separate them into several chemotypes. The biosynthesis of secondary metabolites, although controlled genetically, is strongly affected by environmental, harvest and post-harvest factors. Agricultural factors have a critical effect on quantitative and qualitative characteristics of some Poaceae (such as citronella or vetiver oils), which affect plant growth and yield. Kim et al. (2005) and Massardo et al. (2006) found a large variation on volatile oil yield and content depending on seasonal changes and harvesting time. This variability of metabolism causes changes in the biological effect of essential oil and in concentration of the mainly compounds (Oliveira et al., 2009). Massardo reported that the major components in vetiver essential oil were identified as (*E*)-isovalencenol (19.5 to 25.0%), khusimol (11.1 to 12.9%) and zizanoic acid (4.4 to 5.7%). These authors reported that vetiver essential oil production is closely related to the metabolism of plant, which is affected by changes in environmental temperatures.

Pharmacological assays

In the acetic acid-induced writhing test, the antinociceptive effect, represented by writhes reduction, elicited by 50 and 100 mg/kg of EO (6.3±1.6 and 4.6±2.0) in mice was similar to that of morphine 3 mg/kg (0.7±0.2), a standard opioid drug, when both groups were compared with control (13.1±2.1). EO in dose of 25 mg/kg did not produce any significant effect (Table 2).

It was observed that naloxone (1.5 mg/kg, *i.p.*) antagonized the antinociceptive response of morphine

from 0.7±0.2 (with vehicle only) to 11.8±2.4 (with vehicle plus naloxone) writhes in the acetic acid-induced writhing test. Naloxone (1.5 mg/kg, *i.p.*) partially reversed the antinociceptive effect of the EO (100 mg/kg, *i.p.*) (Table 2).

Table 1. Essential oil composition of *Chrysopogon zizanioides*.

RT (min) ^a	Compounds ^b	(%)	IK ^c
25.017	prezizaene	0.15	1444
25.167	khusimene	0.19	1455
25.925	γ -gurjunene	0.66	1478
26.242	β -vetispiorene	0.32	1488
26.350	γ -amorphene	0.11	1491
28.125	elemol	0.18	1547
28.283	β -vetivenene	0.13	1552
28.583	maaliol	1.60	1562
29.525	viridiflorol	0.34	1592
29.900	khusimone	1.09	1605
30.000	β -atlantol	0.46	1608
30.417	junenol	3.58	1622
30.708	10- <i>epi</i> - γ -eudesmol	0.54	1632
30.758	cubenol	0.77	1634
31.042	1,7-diepi- α -cedrenal	0.38	1643
31.375	α -cadinol	5.01	1654
31.825	mustakone	2.75	1669
32.050	2- <i>epi</i> -ziza-6(13)-em-3-ol	2.79	1677
32.658	junicedranol	1.90	1697
33.083	nootkatol	0.53	1712
33.383	vetiselinenol	5.08	1723
33.567	(<i>Z</i>)- β -curcumen-12-ol	1.50	1730
33.875	hydroxy-valencene	4.64	1740
34.058	khusimol	19.57	1747
35.150	(<i>E</i>)-isovalencenol	13.24	1786
35.267	spirovetiva-3,7-(11)-dien-12-ol	2.06	1790
35.608	nootkatone	1.57	1801
35.642	vetivenic acid	1.64	1803
35.858	β -vetivone	4.87	1808
36.558	α -vetivone	5.25	1825
	Total identified	82.90	

^aRetention time; ^bCompounds listed in order of elution from an DB-5MS column; ^cKovats indices were calculated against *n*-alkanes (C₉-C₁₈) on a DB-5MS column; NI: Not Identified

EO inhibited the licking response to the injected paw when 50 mg/kg (62.3±23.7 s) and 100 mg/kg (19.9±7.4 s) were administered *i.p.* in mice compared with the control group (144.0±20.2 s) in the second phase of the formalin test. However, EO or aspirin did not inhibit the licking response in first phase of the formalin test (Table 3).

Moreover, the hot plate test evaluated a possible

central antinociceptive effect of the EO since this is considered a specific test for central pain analysis. EO did not interfere with nociception in this test (data not shown).

In the rota-rod test, EO-treated mice did not show any significant motor performance alterations with the doses of 50, 100, or 200 mg/kg (data not shown). As might be expected, the CNS depressant diazepam (1.5 mg/kg) reduced the time of treated animals on the Rota-rod after 30 min (54.8±19.0) of treatment with this standard drug.

Table 4 shows that EO, in higher dose, inhibited all three phases (52.8, 39.1, and 39.7% at dose 100 mg/kg) of the carrageenan-induced rat paw edema equally well, suggesting that the EO has a non-selective inhibitory effect on the release or actions of these mediators.

Table 2. Effect of EO or morphine on writhing induced by acetic acid.

Treatment	Dose (mg/kg)	Number of writhings ^a	Inhibition (%)
Vehicle	-	13.1±2.1	-
EO	25	11.3±1.9	13.7
EO	50	6.3±1.6 ^b	51.9 ^c
EO	100	4.6±2.0 ^c	64.9 ^c
EO+NAL	100+1.5	10.7±2.8	18.3
MOR	3	0.7±0.2 ^d	94.7 ^f
MOR+NAL	3+1.5	11.8±2.4	9.9

NAL: naloxone; MOR: morphine; n=10; ^a Values represent mean±SEM. ^bp<0.05, ^cp<0.01 and ^dp<0.001 (ANOVA and Dunnett's test), significantly different from control; ^ep<0.01 and ^fp<0.001 (Fisher's test), significantly different from control.

Table 3. Effect of EO or aspirin on formalin-induced pain.

Treatment	Dose (mg/kg)	Number of licks (s)			
		0-5 min		15-30 min	
		Score of pain ^a	Inhibition (%)	Score of pain ^a	Inhibition (%)
Vehicle	-	48.4±4.3	-	144.0±20.2	-
EO	25	40.9±3.9	15.5	98.6±25.6 ^b	31.5 ^d
EO	50	37.1±4.6	23.3	62.3±23.7 ^b	56.7 ^e
EO	100	38.9±6.7	19.6	19.9±7.4 ^c	86.2 ^f
Aspirin	200	41.4±9.7	14.5	45.9±29.6 ^b	68.1 ^e

n=10; ^aValues represent mean±SEM; ^bp<0.05 and ^cp<0.001 (one-way ANOVA and Dunnett's test), significantly different from control; ^dp<0.05, ^ep<0.01 and ^fp<0.001 (Fisher's test), significantly different from control.

Table 4. Inhibitory effect of EO or aspirin on carrageenan-induced paw edema in rats.

Treatment	Dose (mg/kg)	Paw volume increase (mL) ^a			Inhibition (%)		
		1 h	2 h	3 h	1 h	2 h	3 h
Vehicle	-	0.36±0.07	0.69±0.11	0.73±0.10	-	-	-
EO	25	0.29±0.10	0.71±0.07	0.65±0.09	14.4	-2.9	11.0
EO	50	0.31±0.08	0.59±0.15	0.58±0.09	13.9	14.5	20.6
EO	100	0.17±0.03 ^b	0.42±0.04 ^b	0.44±0.02 ^c	52.8 ^c	39.1 ^d	39.7 ^d
Aspirin	300	0.09±0.03 ^c	0.28±0.02 ^c	0.39±0.03 ^c	75.0 ^e	59.4 ^e	46.6 ^d

n=6, per group; ^aValues represent mean±SEM; ^bp<0.01 and ^cp<0.001 (one-way ANOVA and Dunnett's test), significantly different from control; ^dp<0.05 and ^ep<0.01 (Fisher's test), significantly different from control.

Carrageenan (500 µg/cavity) induced leukocyte migration to the peritoneal cavity 4 h after stimulus. Figure 1 shows the inhibitory effect of EO on carrageenan-induced responses in a dose-dependent manner (34.7, 35.4, and 62.5% at doses of 25, 50, and 100 mg/kg, respectively, p<0.05).

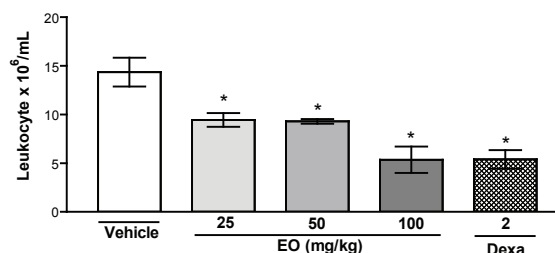


Figure 1. Effect of EO on leukocyte migration into the peritoneal cavity induced by carrageenan in rats. Groups of rats were pre-treated with vehicle (C, control group, 10 mL/kg, *i.p.*), dexamethasone (Dexa, 2 mg/kg, *s.c.*), or EO in concentrations of 25, 50, and 100 mg/kg (*i.p.*) 30 min before carrageenan (500 µg/cavity, 500 µL, *i.p.*)-induced peritonitis. Cell counts were performed at the time 4 h after the injection of carrageenan. Each value represents the mean±S.E.M. Asterisks denote statistical significance, *p<0.01 related to control group. ANOVA followed by Dunnett's test (n=6, per group).

The ability of EO to prevent lipid peroxidation was analyzed (omitting Figure). We observed that all concentrations of EO were not able to prevent AAPH-induced lipoperoxidation as assessed by the *in vitro* TBARS assay. Besides, we observed that EO was did

not prevent 2-deoxyribose degradation by a hydroxyl-generating system at any tested doses. However, at the concentration of 1 mg/mL, EO enhanced hydroxyl-mediated 2-deoxyribose degradation.

Discussion

Chrysopogon zizanioides (L.) Roberty, Poaceae, is one of the most important raw materials in perfumery and has been used in folk medicine for the treatment of inflammatory pain in Brazilian Northeast (oral communication) (Lima, 2010). The main goal of this study was to evaluate the phytochemical screening, antinociceptive and anti-inflammatory activities of *C. zizanioides* essential oil (EO) in rodents.

GC-MS analysis of *C. zizanioides* EO exhibited a mixture of compounds similarly to a previous study of Weyerstahl et al. (2000a,b), Kim et al. (2005) and Adams et al. (2008). Among the odorous components in vetiver oils from different sources, khusimol, *E*-isovalencenol, α -vetivone, vetiselinol, α -cadinol and are the major constituents, and their presence is often considered as the fingerprint of the oil.

The first test used for pharmacological evaluation was the writhing test. Past studies have postulated that the acetic acid acts indirectly by inducing the release of endogenous mediators which stimulate the nociceptive neurons sensitive to nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids (Fischer et al., 2008). Although abdominal writhing induced by acetic acid represents a peripheral nociception model (Wei et al., 1986) and is normally used for screening synthetic and natural compounds, this is not a specific model, since several compounds, including opioid analgesics (Gomes et al., 2005) tricyclic antidepressants (Carter & Sullivan, 2002) and antihistaminic compounds (Koster et al., 1959) inhibit acetic acid induced writhing. Based in our result, it can be assumed that the mode of action of this activity might involve a peripheral mechanism.

The formalin test has an advantage over other frequently used tests as it involves a biphasic response with an early and a late phase representing neurogenic and inflammatory pain and agents can be screened for activity in these two models of pain (Hunskar and Hole, 1987). Neither EO or aspirin (200 mg/kg) decreased the paw licking time during the neurogenic (0-5 min) phase 1. However, the inflammatory phase (late phase) was significantly inhibited by aspirin and by EO. Moreover, the hot plate test checked a possible central antinociceptive effect of the EO since this is considered a specific test for central pain analysis. EO did not interfere with nociception in this test (data not shown).

Previous studies suggested that the CNS depression and the nonspecific muscle relaxation effect can reduce the response of motor coordination and

consequently might invalidate the nociceptive behavioral tests results (Gonçalves et al., 2008). We did not see any interference with the motor coordination of the animals in the rota-rod test, therefore eliminating a nonspecific muscle relaxation effect of EO at the doses used. Various mediators are released by carrageenan in the rat paw. Thus, while the initial phase may be due to the release of histamine and serotonin, kinins may play a role in the middle phase (Di Rosa and Sorrentino, 1968) and prostaglandins could be the most important mediators in the final 3-5 h post-carrageenan response (Vinegar et al. 1969).

Corroborating the relevant anti-inflammatory activity of EO, we observed that the leukocyte migration to peritoneal cavity was strongly reduced by the all doses of EO. According to Bradley et al. (1982) the cell migration inhibition in models of inflammation has been considered a convincing indicator of anti-inflammatory activity. The ability of the EO, in this study, to suppress abdominal writhes, inhibit the second phase of formalin induced pain as well as suppress the carrageenan- induced inflammation confirms the analgesic and anti-inflammatory activities of the EO.

The lipoperoxidation and hydroxyl-scavenging activity assays showed that EO does not act as an antioxidant, since EO was not able to prevent the lipoperoxidation damage to an lipid enriched system where a free radical source (AAPH) is added; besides, EO was also ineffective in prevent the degradation of 2-deoxyribose by hydroxyl radicals generated *in vitro* through Fenton reaction. As a matter of fact, the highest concentration of EO tested further enhanced the damage caused by hydroxyl. Altogether these results indicate that the effects of EO observed in this and other studies are nor mediated by free radicals or by a possible antioxidant activity.

Based on our results it is possible to conclude that EO has potential antinociceptive and anti-inflammatory activities, which might involve a peripheral mechanism, such as, inhibiting the synthesis or action of prostaglandins. However, further studies will enable as to understand the precise action mechanisms and toxicity of this plant.

Furthermore, some studies attribute the anti-inflammatory activity of vetiver oil to its secondary metabolites, such as khusimol, *E*-isovalencenol, α -vetivone, vetiselinol, α -cadinol and β -vetivone (Hammer et al., 1999; Güllüce et al., 2003).

Acknowledgements

We thank Mr Rangel R. Bonfim for the technical support. This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico/Brazil) and the Fundação de Amparo à Pesquisa do Estado de Sergipe.

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