Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **27** (6): 865-70 (2008)

Original Article

Received: September 5, 2008
Accepted: September 23, 2008

Antinociceptive Activity of the Essential Oil and Fractions of *Pterodon emarginatus* Vogel Seeds

Rafael C. DUTRA *, Raphael TREVIZANI, Frederico PITTELLA & Nádia R. BARBOSA

Center for Identification and Quantification Analytical, Pharmacy and Biochemistry Faculty, Federal University of Juiz de Fora, Campus Universitário, Juiz de Fora, Minas Gerais, 36036-900, Brazil.

SUMMARY. **In the present study the antinociceptive property of *Pterodon emarginatus* Vog. (Leguminosae) seeds essential oil (EO) and fractions was investigated. The writhing test induced by acetic acid, paw licking induced by formalin and hot plate tests were performed in Swiss albino mice (n = 8-10/group), by oral route at doses of 100, 300 and 500 mg/kg. The EO, hexanic (HF) and buthanolic (BF) fractions reduced ($p < 0.01$) the abdominal contortions (100 mg/kg = 22.75 ± 4.03, 14.38 ± 3.41 and 24.6 ± 4.48; 300 mg/kg = 14.00 ± 5.81, 12.13 ± 3.19, and 25.88 ± 2.78; 500 mg/kg = 11.13 ± 3.59, 9.00 ± 2.09 and 17.75 ± 4.82), respectively when compared with control group. The EO and methanolic fraction (MF) reduced the paw licking in both phases ($p<0.05$): 1st phase (100 mg/kg = 31.63 ± 14.17 and 60.50 ± 11.61; 300 mg/kg = 28.38 ± 5.72 and 47.75 ± 6.13; 500 mg/kg = 28,25 ± 4.19 and 44.63 ± 6.33) and 2nd phase (100 mg/kg = 53.50 ± 11.96 and 24.13 ± 12.38; 300 mg/kg = 43.75 ± 11.91 and 35.13 ± 12.35; 500 mg/kg = 9.00 ± 4.41 and 23.50 ± 10.18), respectively. Only HF showed effect on the reaction time at hot plate were significant ($p<0.05$) after 60 min of treatment. These results suggest that *Pterodon emarginatus* could constitute a source of active substances with antinociceptive effect.**

INTRODUCTION

The 'sucupira branca', popular name of the specie *Pterodon emarginatus* Vog. (Leguminosae), is a native aromatic tree reaching 5-10 m in height [1] being easily found all over central Brazil. It is used in folk medicine for the treatment of rheumatism, sore throats, respiratory dysfunctions (bronchitis and amigdalytis), in addition to anti-inflammatory, analgesic, depurative and tonic activities [2]. In folk medicine, the seeds of *Pterodon emarginatus* are prepared as a hydroalcoholic solution using 50 g of seeds crushed in 250 ml of ethanol or alcoholic drink, such as brandy (concentration of 200 mg/ml)), under maceration for 24-48 h/room temperature. After preparation, the extract is orally consumed 40 ml/day, divided into 2 doses daily for 7 days.

Chemical studies on genus *Pterodon* have shown the presence of alkaloid compounds in the bark [3], isoflavone and some triterpenes in the wood [4] and diterpenes [2] and isoflavones in seed oil [5]. The diterpene 14,15-epoxygeranylgeraniol and some derivatives isolated from *Pterodon pubenscens* have been associated with protective activity against the penetration of cercarias of *Schistossoma mansoni,* a typical tropical disease [6]. The furane diterpene 6α-7β-dihydroxyvouacapan-17-oate sodium, isolated from the oil of the fruits of *Pterodon polygalaeflorus* Benth, presented anti-inflammatory activity on the test of edema induced by carrageenan in rats [7].

Because of the frequent use of *Pterodon emarginatus* seeds as a traditional medicine and the lack of reports concerning pharmacological studies of this species, this study attempts to evaluate the antinociceptive activity of the *Pterodon emarginatus* essential oil and fraction.

MATERIALS AND METHODS

Plant material

The seeds of *Pterodon emarginatus* were

KEY WORDS: Antinociceptive activity, Leguminosae, *Pterodon emarginatus*.

* Author to whom correspondence should be addressed. *E-mail:* rafaelcdutra@gmail.com

collected in September 2006 in the city of Três Marias (Minas Gerais, Brazil). The plant material was identified by Dr. Fátima Regina Gonçalves Salimena and a voucher specimen (Nº 48,077) were deposited at the Herbário CESJ of the Federal University of Juiz de Fora (Minas Gerais, Brazil).

Isolation of the essential oils

The seeds of *Pterodon emarginatus* were triturated (30 g) and hydrodistilled in a Clevenger-type apparatus. After 2 h-distillation, the essential oil (EO) was collected as a film floating on the surface of water. The samples were sealed and kept in dark glass vials in the refrigerator at 4 °C for further analysis.

Preparation of the fractions

Thirty grams of seeds were triturated with the aid of pruning shears and submitted to extraction using a Soxhlet extraction with hexane, buthanol and methanol until reaching the exhaustion of solvent. The fractions obtained were evaporated until completed elimination of the solvent. Three fractions of different polarities were obtained: hexanic (HF), buthanolic (BF) and methanolic (MF) fractions.

Animals

Male Swiss mice weighing 25-30 g were obtained from the Reproduction Biology Center of the Federal University of Juiz de Fora (Minas Gerais, Brazil) and used in the experiments for the assessment of the antinociceptive activity. The animals were housed in groups of five in standard cages at room temperature (25 ± 3 °C), 12 h:12 h light-dark cycle, with both food and water *ad libitum*. Twelve hours before each experiment, animals received only water in order to avoid food interference with substances absorption. This study was conducted in accordance with guidelines set forth by the Brazilian Association for Laboratory Animal Science (COBEA) and has been approved by the Research Commission for Ethics and Animal Experimentation of UFJF (protocol number 59-64/2006). In all tests described below, the EO and the fractions obtained from the seeds of *Pterodon emarginatus* were dissolved in a solution of 1% DMSO: Tween 80 (1:2, v/v) in saline.

Antinociceptive activity

Acetic acid-induced abdominal writhing

The animals (n = 8/group) were pre-treated orally (p.o.) with EO or fractions (100-500 mg/kg) or vehicle [saline + 1% DMSO: Tween 80 (1:2, v/v)], 60 min before acetic acid 0.6% injection (0.3 ml, i.p.). A writhe is defined as a sequence of events beginning with the arching of the back, contraction of the abdomen, twisting of the trunk and/or pelvis and usually ending with the extension of the hind limbs. After the challenge (acetic acid injection), mice were placed in a clear plastic box and the number of writhes/mice was counted during 20 min, starting 10 min after the administration of the acetic acid solution. The positive control group received the reference drug indomethacin (5 mg/kg, p.o.) [8].

Formalin test

Animals received 20 µl of 2.5% formalin, dissolved in saline, into the dorsal surface of the left hind paw, after which, the animals were immediately individualized in an observation chamber. The amount of time the animal spent licking the injected paw was measured during the first 5 min (phase 1: neurogenic) and 15–30 min after formalin injection (phase 2: inflammatory). The animals (n=8/group) were pre-treated with an oral dose of EO or fractions (100-500 mg/kg) or morphine (5 mg/kg, s.c.) or vehicle [saline + 1% DMSO: Tween 80 (1:2, v/v)], 60 min before administration of formalin [9].

Hot plate test

For the hot plate test, animals were placed on a hot-plate set at 55 ± 0.5 °C. When the animals licked their fore and hind paws or jumped, reaction time was recorded at different times (30, 60, 90 and 120 min) after oral administration of EO or fractions (100-500 mg/kg) or morphine (5 mg/kg, s.c.) or vehicle [saline + 1% DMSO: Tween 80 (1:2, v/v)]. Animals which did not react after 30 s were taken off the plate to avoid tissue damage, which could jeopardize further evaluations. Baseline was considered as the mean of reaction time obtained at 30 min the administration of EO, fractions, morphine or vehicle [saline + 1% DMSO: Tween 80 (1:2, v/v)] and was defined as a normal reaction of the animal to temperature. Increase in baseline (in %) was calculated by the formula: [(reaction time x 100)/baseline] -100 [10].

Statistical analysis

The results are shown as the mean ± standard error of mean (S.E.M.) of eight animals per group. Statistical significance between groups was performed by the application of analysis of variance ANOVA followed by Bonferroni's test. *P* values less than 0.05 were used as the significance level.

RESULTS

The extraction procedures accomplished for EO and fractions yielded in 3.90 % of EO, 7.26 % of HF, 3.52 % of BF and 4.83 % (w/w) of MF.

Acetic acid-induced abdominal writhing

Intraperitoneal injection of acetic acid (0.6%) induced 36.23 ± 4.61 writhings on group pre-treated with saline. When mice were pre-treated with increasing doses (100-500 mg/kg) of EO and HF a significant inhibition of number of writhing was observed (Figure 1A and 1B). Indomethacin caused inhibition of 70.80% in the number the abdominal writhings, proving its efficacy as an analgesic agent. The BF demonstrated activity only at the dose of 500 mg/kg (Figure 1C) and the MF did not demonstrate antinociceptive activity in the abdominal writhing model in the concentrations tested (Figure 1D).

Formalin test

When EO and fractions were tested on the formalin test, it was observed that both EO and MF presented a biphasic licking response (Figure 2A and 2D). On the other hand, the HF and BF only presented antinociceptive activity in the first phase, corresponding to acute neurogenic pain (Figure 2B and 2C). A group of mice treated with morphine in order to compare the

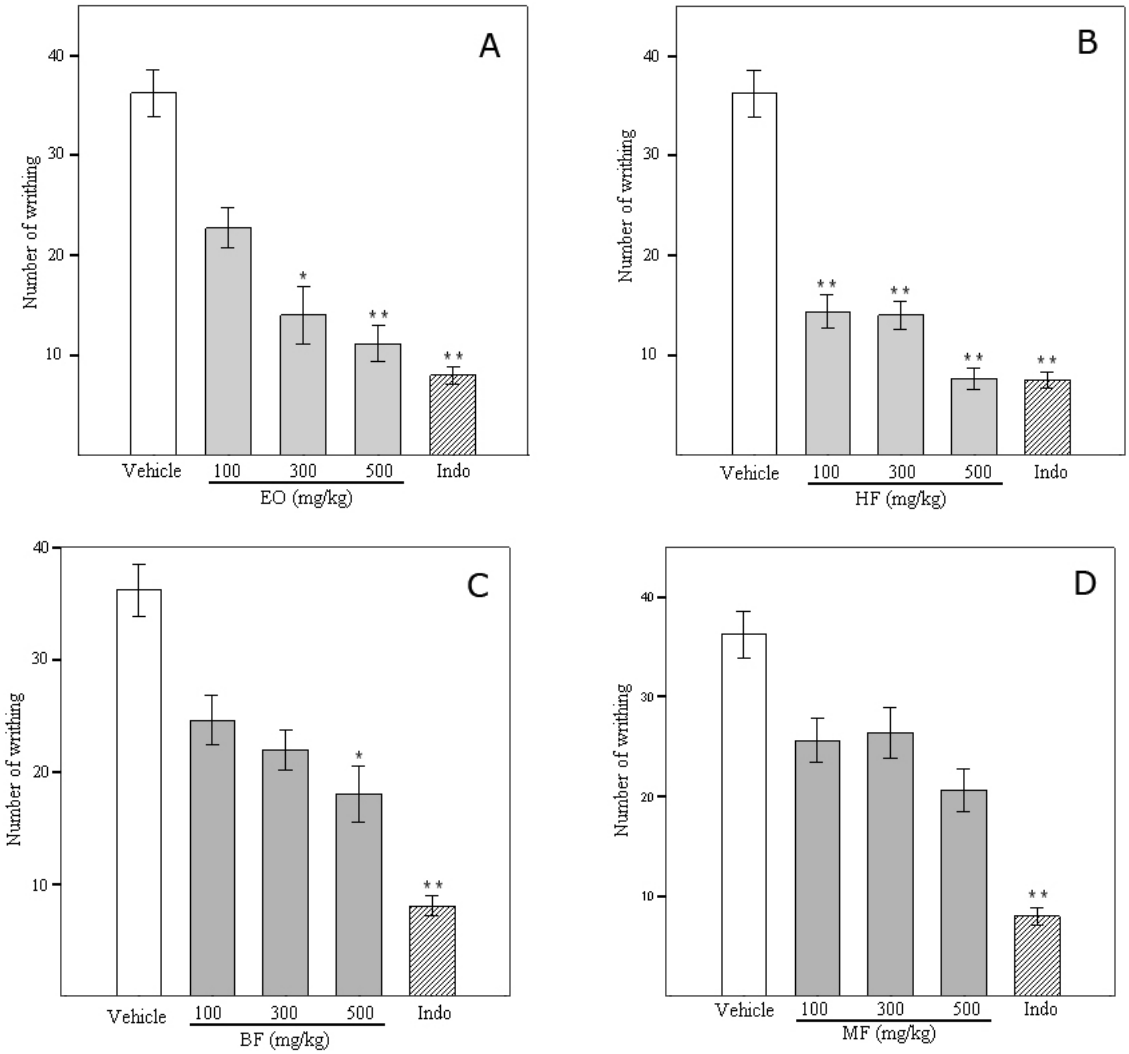

Figure 1. Effect of essential oil (EO: 1A), hexanic fraction (HF: 1B), buthanolic fraction (BF: 1C), and methanolic fraction (MF: 1D) of seeds from *Pterodon emarginatus* on the writhing response induced by acetic acid in mice. Indomethacin (Indo: 5 mg/kg). Data are expressed as means ± S.E.M. (n=8/group). Statistical significance was calculated by ANOVA followed by Bonferroni's test. * $p < 0.05$ and ** $p < 0.001$ when compared with vehicle-treated mice.

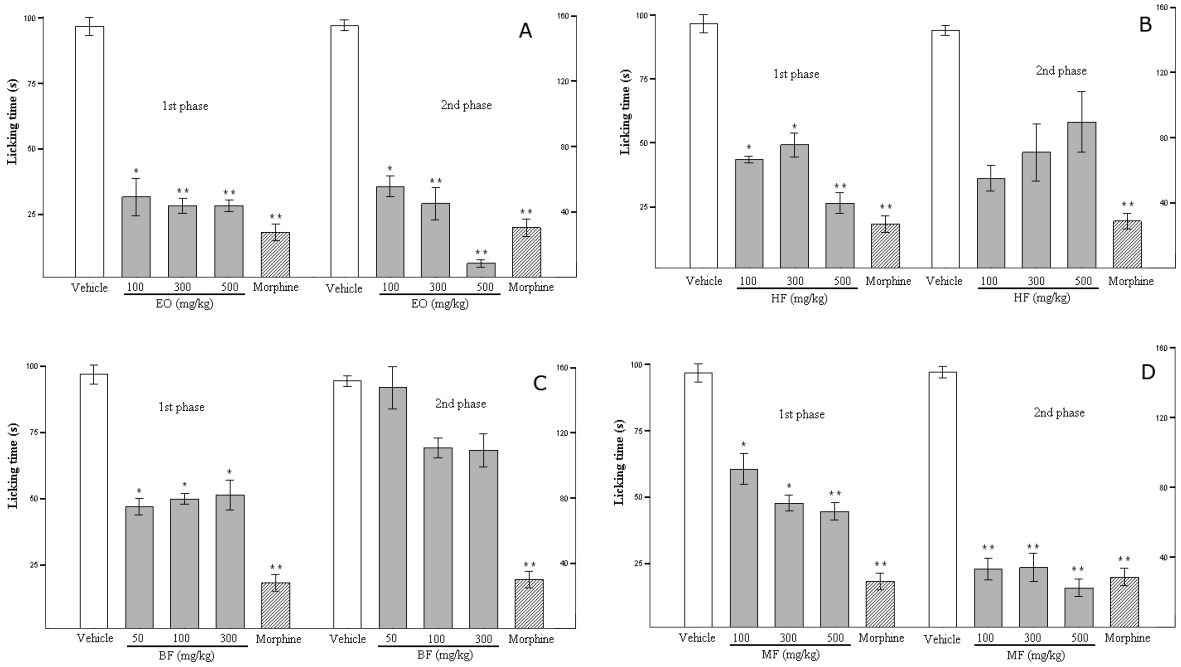

Figure 2. Effect of essential oil (EO: 2A), hexanic fraction (HF: 2B), buthanolic fraction (BF: 2C) and methanolic fraction (MF: 2D) of seeds from *Pterodon emarginatus* on formalin test in mice. Morphine (5 mg/kg). Data are expressed as means ± S.E.M. (n=8/group). Statistical significance was calculated by ANOVA followed by Bonferroni's test. * $p < 0.05$ and ** $p < 0.001$ when compared with vehicle-treated mice.

antinociceptive efficacy of EO and fractions. This opioid significantly inhibited the total licking of both phases after formalin injection (Figure 2).

Hot plate test

The Figure 3 (B and C) shows that HF and BF at the tested doses showed antinociceptive effects in the hot plate model. The HF and BF induced an increase in baseline at the time of 90 and 120 minutes after treatment, in the 300 and 100 mg/kg doses, respectively. The EO and MF did not demonstrate antinociceptive activity in this model in the concentrations tested (Figure 3 A and D).

DISCUSSION

The oral treatment of animals with EO and fractions produced antinociception when assessed in the acetic acid induced contortions, a useful method to screen both peripherally and centrally acting analgesic activities.

The mouse writhing model involves different nociceptive mechanisms, such as sympathetic system (biogenic amines released), cyclooxygenases (COX) and their metabolites [11] and opioid mechanisms [12]. Thus, EO and fractions of these seeds could be inducing antinociception maybe by blocking the receptor or the release of endogenous substances that excite pain nerve endings [9] or reducing the release of those inflammatory mediators. Nonsteroidal antiinflammatory drugs (NSAIDs), such as indomethacin, inhibit COX in peripheral tissues, reducing therefore prostaglandin E_2 (PGE_2) synthesis and interfering with the mechanism of transduction in primary afferent nociceptors [13]. Another possibility could be the blockage in the eicosanoid system. In this situation, chemical constituents of *Pterodon emarginatus* could act inhibiting phospholipase A_2 or directly blocking cyclooxygenases (COX-1 and/or COX-2).

It has been reported that formalin-induced persistent pain in mice paw produces a distinct biphasic nociception. The earlier phase (0-5 min after formalin injection) characterized by intense neurogenic pain, starts immediately after the injection, and seems to be caused predominantly by activation of C-fibers subsequent to peripheral stimulation (direct stimulation of nociceptors). Then, there is a 10 min period of reduced nociceptive activity. The late phase of moderate pain (inflammatory pain) starts about 20 min after the formalin injection and lasts about 40 min. This phase seems to be caused by tissues and functional changes in the dorsal horn of the spinal cord and is accompanied by the release of inflammatory mediators [9]. It has been well documented that inflammatory mediators such as

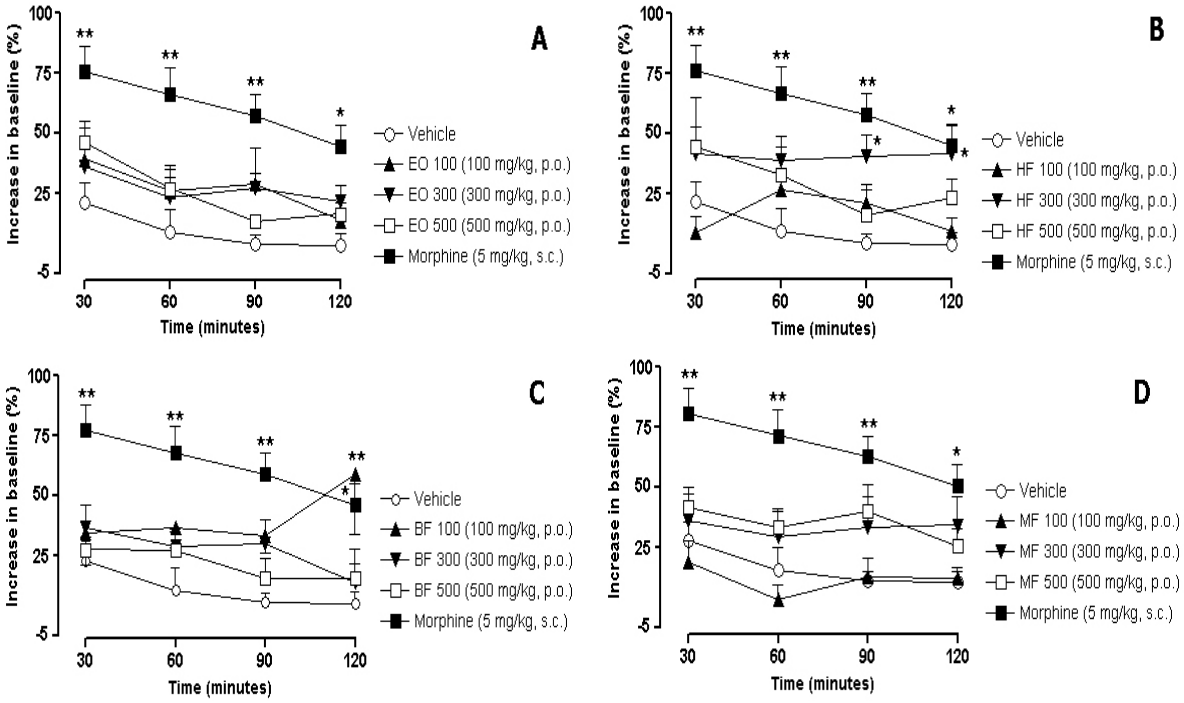

Figure 3. Effect of essential oil (EO: 3A), hexanic fraction (HF: 3B), buthanolic fraction (BF: 3C) and methanolic fraction (MF: 3D) of seeds from *Pterodon emarginatus* on hot plate test in mice. Morphine (5 mg/kg). Data are expressed as means ± S.E.M. (n=8/group). Statistical significance was calculated by ANOVA followed by Bonferroni's test. * $p<0.05$ and ** $p<0.001$ when compared with vehicle-treated mice.

substance P participate in the manifestation of the first phase response, while prostaglandins [9,14], serotonin, histamine [15], and kinins are involved in the second phase response in the formalin test [16]. Although the mechanisms underlying the analgesic effects of EO and fractions remain somehow unknown, we can suggest that their activities might be interfering with the action or release of inflammatory mediators.

The hot plate test is a model of supra-spinal nociception and is regarded as one of the most suitable models for determining the involvement of central antinociceptive mechanism [17]. The exposure of animal paws to thermal stimuli in the hot plate test leads to the development of non-inflammatory, acute nociceptive response and the ability of the HF (100 and 300 mg/kg doses in the time of 60, 90 and 120 min) to inhibit the thermal-induced nociceptive response was observed.

The inhibitory effect observed with EO and fractions on the models of analgesia could also be due to a direct decrease of the activity evoked by C-fibers in ascendant axons or to a decrease in the production of prostaglandins responsible for C-fibers stimulation [18]. The observed activity can be attributed to the overall effects of the EO and fraction constituents or to the compounds having action similar to non-steroidal anti-inflammatory or opioid drugs, in a complementary manner.

The majority constituents presented in EO of *Pterodon emarginatus* seeds are trans-caryophyllene (36.00%), beta-elemene (15.30%) and alpha-humulene (6.80%). These constituents, mainly the sesquiterpenes, were evaluated by several authors and showed antinociceptive and anti-inflammatory activities [19-21]. For this reason, the activity showed by the EO in this work can be explained, at least in part, by the presence of these constituents, not excluding the possibility of synergism between other constituents present in the oil.

Duarte *et al.* [22] showed for the first time the presence of acid 6α-7β-dihydroxyvouacapan-17-oic in the HF obtained from seeds of *P. polygalaeflorus* through alkaline hydrolysis with sodium salt and purified by technique of crystallography. After its isolation, it was demonstrated that this compound showed antinociceptive activity in the writhing model induced by acetic acid in mice. Then, in this work the authors believe that the antinociceptive activity showed by the HF, might be due to this constituent, since both species are very similar taxonomically. However, further studies more specific are needed for the identification of others pharmacological activities of this compound.

The BF and MF could have shown antinociceptive activity due to the concentration of

flavonoids present in these samples, since Dutra *et al.* [23] demonstrated the presence of these constituents in these fractions. Flavonoids have been responsible for several pharmacological activities, including analgesic and anti-inflammatory, as proposed by several authors [24,25]. For this reason, these fractions could be presenting antinociceptive activity due to flavonic constituents in these samples.

In spite of the fact that the doses used for EO and fractions were higher than morphine and indomethacin, we must take into account that the EO and fractions are not pure drugs and are not synthetic. They have different composition and different concentration of several constituents. Another important observation is that the EO and fractions were all administered orally. Influence of pH from stomach and liposolubility may interfere with their absorption by the gastrointestinal tract limiting the amount that reaches the blood and the tissues. Even so, with all these additional factors, an important antinociceptive activity was observed.

CONCLUSION

Our results demonstrated for the first time that EO and fractions of seeds from *Pterodon emarginatus* develop peripheral and central antinociceptive activities in these models in the concentrations tested. Nevertheless, it is necessary to clarify the mechanism of action of this plant and design less expensive therapies with minor adverse effects in treating analgesic processes, reinforcing the use of *Pterodon emarginatus* as a phytomedicine.

Acknowledgements. This study was supported by grants from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil. R.C.D. is an MSc student and holds a grant from CAPES. We are grateful to Dr Fátima Regina Gonçalves Salimena Pires for the plant identification. The authors would like to thank Juliana Fabris Lima-Garcia for her assistence in English corrections this manuscript.

REFERENCES

1. Lorenzi, H. & F.J.A. Matos (2002) *"Plantas medicinais no Brasil: nativas e exóticas"*, Instituto Plantarum ed., Nova Odessa, págs 60-3.
2. Arriaga, A.M.C., M.A.B. Castro, E.R. Silveira & R. Braz-Filho (2000) *J. Braz. Chem. Soc.* **11**: 187-90.
3. Torrenegra, R., P. Bauereib & H. Achenbach (1989) *Phytochemistry* **28**: 2219-21.
4. Marques, D.D., M.I.L. Machado, M.G. Carvalho, L.A.C. Meleira & R. Braz-Filho (1998) *J. Braz. Chem. Soc.* **9**: 295-301.
5. Braz Filho, R., O.R. Gottlieb & R.M.V. Assumpção (1971) *Phytochemistry* **10**: 2835-6.
6. Dos Santos Filho, D., W. Vichnewski, P.M. Baker & B. Gilbert (1972) *An. Acad. Bras. Cienc.* **44**: 45-9.
7. Silva, M.C.C., C.R.M. Gayer, C.S. Lopes, N.O. Calixto, P.A. Reis, C.P.B. Passaes, M.C. Paes, S.R. Dalmau, K.C.C. Sabino, A.R. Tedeschini & M.G.P. Coelho (2004) *J. Pharm. Pharmacol.* **55**: 135-41.
8. Matheus, M.E., L.F. Berrondo, E.C. Vieitas, F.S. Menezes & P.D. Fernandes (2005) *J. Ethnopharmacol.* **102**: 377-81.
9. Hunskaar, S. & K. Hole (1987) *Pain* **30**: 103–4.
10. Franzotti, E.M., C.V. Santos, H.M. Rodrigues, R.H. Mourão, M.R. Andrade & A.R. Antoniolli (2000) *J. Ethnopharmacol.* **72**: 273–7.
11. Duarte, I.D.G., M. Nakamura-Craig & D.L. Ferreira-Alves (1988) *Braz. J. Med. Biol. Res.* **21**: 341-3.
12. Collier, H.O.J., L.C. Dinneen, C.A. Johnson & C. Scheneider (1968) *Br. J. Pharmacol.* **32**: 295-310.
13. Dionne, R.A., A.A. Khan & S.M. Gordon (2001) *Clin. Exp. Rheumatol.* **19**: 63-70.
14. Shibata, M., T. Ohkubo, H. Takahashi & R.Inoki(1989) *Pain* **38**: 347-52.
15. Parada, C.A., C.H. Tambeli, F.Q. Cunha & S.H. Ferreira (2001) *Neuroscience* **102**: 937-44.
16. Tjolsen, A., O.G. Berge, S. Hunskaar, J.H. Rosland & K. Hole (1992) *Pain* **51**: 5-17.
17. Pini, L.A., M. Sandrini & G. Vitale (1997) *Eur. J. Pharmacol.* **308**: 31-40.
18. Rossi, G.C., G.W. Pasternak & R.J. Bodnar (1993) *Brain Res.* **8**: 171–180.
19. Fernandes, E.S., G.F. Passos, M.M. Campos, J.G. Araújo, J.L. Pesquero, M.C. Avelllar, M.M. Teixeira & J.B. Calixto (2003) *Br. J. Pharmacol.* **139**: 973–81.
20. Medeiros, R., G.F. Passos, C.E. Vitor, J. Koepp, T.L. Mazzuco, L.F. Pianowski, M.M. Campos & J.B. Calixto (2007) *Br. J. Pharmacol.* **151**: 618-27.
21. Passos, G.F., E.S. Fernandes, F.M. Da Cunha, J. Ferreira, L.F. Pianowski, M.M. Campos & J.B. Calixto (2007) *J. Ethnopharmacol.* **110**: 323–33.
22. Duarte, I.D.G., D.L. Ferreira-Alves, D.P. Veloso & M. Nakamura-Craig (1996) *J. Ethnopharmacol.* **55**: 13-8.
23. Dutra, R.C., M.N. Leite & N.R. Barbosa (2008) *Int. J. Mol. Sci.* **9**: 606-14.
24. Goulart, S., M.I.G. Moritz, K.L. Lang, R. Liz, E.P. Schenkel & T.S. Frode (2007) *J. Ethnopharmacol.* **113**: 346-53.
25. Montanher, A.B., S.M. Zucolotto, E.P. Schenkel & T.S. Frode (2007) *J. Ethnopharmacol.* **109**: 281-8.