Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
19(1A): 46-50, Jan./Mar. 2009
Received 18 October 2008; Accepted 15 February 2009

Artigo

Antinociceptive effects of *Abarema cochliacarpos* (B.A. Gomes) Barneby & J.W.Grimes (Mimosaceae)

Nina C. B. Silva,*[1,2] Maria A. Esquibel,[1] Iura M. Alves,[3] Eudes S. Velozo,[3] Mara Z. Almeida,[4] Jaci E. S. Santos,[5] Fátima de Campos-Buzzi,[6] Aleandra V. Meira,[6] Valdir Cechinel-Filho[6]

[1]Programa de Pós-Graduação em Biotecnologia Vegetal, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21900-000 Rio de Janeiro-RJ, Brazil,
[2,]Departamento de Produção Vegetal, Universidade Federal do Espírito Santo, Alto Universitário s/n, Cx. Postal 16, 29500-000, Alegre-ES, Brazil,*
[3]Laboratório de Pesquisas em Matéria Médica, Universidade Federal da Bahia, Faculdade de Farmácia, 40170-240 Salvador-BA, Brazil,
[4]Programa Farmácia da Terra, Faculdade de Farmácia, Universidade Federal da Bahia, Av Barão de Jeremoabo s/n, sala 231, 40170-240 Salvador-BA, Brazil,
[5]Associação Comunitária da Barra II, 44859-000 Morro do Chapéu-BA, Brazil,
[6]Núcleo de Investigações Químico-Farmacêuticas, Universidade do Vale do Itajaí, Rua Uruguai 458, Cx.Postal 360, 88302-202 Itajaí-SC, Brazil

RESUMO: "Efeito antinociceptivo de *Abarema cochliacarpos* (B.A. Gomes) Barneby & J.W.Grimes (Mimosaceae)". No presente trabalho foram avaliados a atividade antinociceptiva e o perfil fitoquímico dos extratos aquosos e metanólico produzidos com a casca do caule de *Abarema cochliacarpos*, uma espécie de Mata Atlântica com diversas indicações populares. Todos os extratos apresentaram atividade analgésica quando avaliados pelo teste das contorções abdominais induzidas pelo ácido acético via intraperitonial, apresentando respostas superiores às drogas usadas como referência, bem como no modelo da dor induzida por capsaicina. A avaliação fitoquímica demonstrou a presença de saponinas, catequinas, taninos, fenóis e antraquinonas.

Unitermos: *Abarema cochliacarpos,* Mimosaceae, atividade analgésica, plantas medicinais, perfil fitoquímico.

ABSTRACT: In this study, we investigated the analgesic activity of crude aqueous and methanol extracts obtained from *Abarema cochliacarpos* bark in mice, and analyzed its phytochemical profile. All the extracts exhibited analgesic properties against the writhing test in mice, but the aqueous and methanol extracts were more active, and more potent than two known analgesic and anti-inflammatory drugs used as reference. They were also active against the capsaicin-model, but inactive when evaluated in the hot-plate test. Phytochemical studies revealed the presence of saponins, catechins, tannins, phenols and anthraquinones.

Keywords: *Abarema cochliacarpos,* analgesic effects, medicinal plant, phytochemical profile.

INTRODUCTION

A*barema cochliacarpos* (B.A. Gomes) Barneby & J.W.Grimes (Mimosaceae) is a Brazilian native plant, occurring mainly in the Atlantic Forest (IUCN, 2004). Popularly known as "barbatimão", the decoction of stem bark is frequently used in traditional medicine for wound-healing, as analgesic, anti-inflammatory, antiseptic and to treat leucorrhea, among other uses (Silva, 2006; Agra et al., 2008). To the best of our knowledge, the pharmacological properties of this plant have not yet been extensively investigated, and only a few studies have been reported. The aqueous extract reduced alcohol gastric lesions (Silva, 2003) and the hydroalcoholic extract bark showed antimicrobial activity (Araujo et al., 2002; Santos et al., 2007). Regarding the chemical composition, the presence of tannins, triterpenoids and cathechins has been suggested (Mendonça, 2000).

In Brazil, besides *A. cochliacarpos*, there are other four different species which are known by the same popular name (*Stryphnodendron adstringens*, *S. polyphylum*, *S. obovatum* and *Dimorphandra mollis*) and used for the same therapeutic purposes (Migliatti, 2003). These species present some medicinal properties

* *E-mail: ninacbs@gmail.com, Tel./Fax +55-28-3552-8935*

ISSN 0102-695X

which have been confirmed by the scientific literature. Studies on stem-bark extracts of *Stryphnodendron adstringens* (Mart.) Coville have demonstrated significant wound-healing, anti-inflammatory (Lima et al.,1998), antiseptic (Souza et al., 2007) and anti-ulcerogenic properties (Martins et al., 2002; Audi et al., 1999). Stem bark of *Stryphnodendron adstringens* (Mart.) Coville, *Stryphnodendron obovatum* Benth. and *Stryphnodendron polyphyllum* Mart. has been used for the treatment of wounds, burns and other cutaneous injuries (Lopes et al., 2005).

The present work reports the evaluation of a possible antinociceptive activity of aqueous and methanol extracts obtained from the bark of *Abarema cochliacarpos* using different models of pain in mice, and describes the phytochemical profile of this plant.

MATERIAL AND METHODS

Plant material

The botanical material was collected in a private property in Vila Sauípe, State of Bahia, Brazil, in August 2003. The plant was classified by MSc Érika von Sohsten de Souza Medeiros (Departamento de Botânica Sistemática, Jardim Botânico do Rio de Janeiro) and a voucher specimen was deposited in the Herbarium under number RB365914. Steam bark was collected at the same place from different trees.

Preparation of extracts and phytochemical analysis

Three extracts were prepared using the bark of *A. cochliacarpos*, dried at 50 ºC for one week, and powdered to a fine grade using a laboratory mill. Hot aqueous extracts (AH) were prepared by boiling sterile distilled water (1.5 L) with powdered bark (128 g) for five minutes in a microwave oven. To prepare the cold aqueous extract (AC), 256 g of powdered bark was extracted with sterile distilled water (1.5 L), at room temperature, for three days. Both extracts were filtered and lyophilized to produce the crude dry extracts. These aqueous extracts were tested because in popular medicine *A. cochliacarpos* is used as a tea, prepared by decoction or infusion in water. To obtain the methanol extract (ME), powdered stem bark (908 g) was extracted with methanol, by maceration at room temperature for three weeks, in darkness. The resulting extract was filtered and evaporated until dry, under reduced pressure. The preliminary analysis of the residue of all the extracts was performed by the phytochemical group test for saponins, catechins, tannins, steroids and triterpenoids, alkaloids, phenols and anthraquinones (Matos, 1997).

Pharmacological assays

Abdominal constriction response caused by intraperitoneal injection of diluted acetic acid

Abdominal constriction was induced by intraperitoneal injection of acetic acid (0.6%), according to the procedure described previously (Collier et al., 1968) with minor modifications. Male Swiss mice (25-30 g) were pre-treated with extracts (3-20 mg/kg), intraperitoneally (i.p.), or by the oral route (100 mg/kg), 30 and 60 min before the acetic acid injection, respectively (six to eight animals in each group). The control animals received a similar volume of 0.9% NaCl (10 ml/kg, i.p.). All the experiments were carried out at 23 ± 2 °C. After the challenge, pairs of mice were placed in separate glass funnels and the number of contractions of the abdominal muscles, together with stretching, were counted cumulatively over a period of 20 min. Antinociceptive activity was expressed as the reduction in the number of abdominal contractions between the control animals and the mice pretreated with the test materials.

Capsaicin-induced pain

The procedure used was similar to that described previously (Sakurada et al., 1992). The animals (n = 6-8) were placed individually in transparent glass cylinders. Following the adaptation period, 20 µl of capsaicin (1.6 µg/paw) was injected under the skin of the plantar surface of the right hindpaw, using a microsyringe. The animals were observed individually for 5 minutes following capsaicin injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of nociception. The animals were intraperitoneally treated with extracts at 10 mg/kg or saline (10 mL/kg, i.p.) 1 hour before administration of capsaicin. The control animals received a similar volume of 0.9 % NaCl (10 mL/kg, i.p.).

Hot-plate test

The hot-plate was used to estimate the latency of responses according to the method described by Eddy and Leimback (1953) with minor modifications. The temperature of the hot-plate was maintained at 56 ± 3 °C. The animals (n = 6) were placed on glass funnels in the heated surface and the time between placing the animals and the beginning of licking the paws or jumping were recorded as latency of response in untreated saline (10 ml/kg, i.p.) or extracts (10 mg/kg, i.p.) animals.

Statistical analysis

The results are presented as mean ± S.E.M., and the statistical significance between the groups was

analyzed by means of the t test or analysis of variance followed by Dunnett´s multiple comparison test. P values of less than 0.05 were considered significant. Where appropriate, the ID_{50} values (the dose of the extract that reduced acid-induced pain by 50% relative to control) were estimated by graphical interpolation from individual experiments.

RESULTS

The extracts presented the following yields: 19.5% (AC), 17.7% (AH) and 12.8% (ME). The phytochemical analysis revealed that all the extracts gave positive results for saponins, catechins, tannins, phenols and anthraquinones. Alkaloids and steroids/ triterpenoids were absent.

The pharmacological results indicate that all the tested extracts exhibited pronounced antinociceptive activity against the writhing test (10 mg/kg, i.p.), causing inhibition of 73, 68 and 39 %, for AC, AH and ME, respectively, whereas the reference drugs acetaminophen and acetyl salicylic acid caused inhibition of 38.0 ± 1 % and 35.0 ± 2 % respectively, in the same model and dose. We therefore selected AC and ME for more detailed studies.

When administered intraperitoneally, both extracts caused graded dose-dependent inhibition of abdominal constrictions (Figures 1 and 2). ME presented a calculated ID_{50} value of 12.8 (11.0 - 14.9) mg/kg, whereas the AC was more potent with ID_{50} less than 3 mg/kg, both extracts being more active than the reference drugs, which presented ID_{50} of approximately 25 mg/kg. On the other hand, when administered by the oral route, ME caused 47% inhibition, whereas AC inhibited abdominal constrictions by 33%, at 100 mg/ kg (Figure 3), showing a similar profile of that of the reference drugs (Vaz et al., 1996).

ME also reduced the licking/biting response to intraplantar capsaicin, with 62% inhibition, which provided more direct evidence of the analgesic effect of this extract on neurogenic pain. AC was less active in this model, with only slight inhibition of 31% (Figure 4). When evaluated by the hot plate test, both extracts AC and ME, at 10 mg/kg, given intraperitoneally, proved to be inactive (results not shown).

DISCUSSION

We initially prepared three crude extracts from A. *cochliacarpos* barks: aqueous could (AC), aqueous hot (AH) and methanol (ME) extracts in order to determine the best solvent for extraction of the possible active principles. Based on pharmacological results, we therefore selected AC and ME for more detailed studies. The results obtained against writhing test suggest that the more polar compounds act as analgesic when administered intraperitoneally. On the other hand,

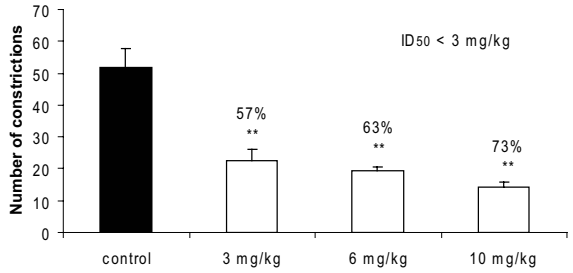

Figure 1. Effect on acetic acid-induced pain in mice test of extract AC, administrated intraperitoneally at 3, 6 and 10 mg/ kg. Each column represents mean ± s.e.m. of six experimental values. **p<0.01 compared with corresponding control values.

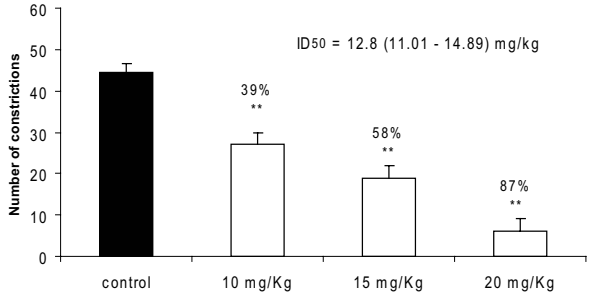

Figure 2. Effect on acetic acid-induced pain in mice test of extract ME, administrated intraperitoneally at 10, 15 and 20 mg/ kg. Each column represents mean ± s.e.m. of six experimental values. **p<0.01 compared with corresponding control values.

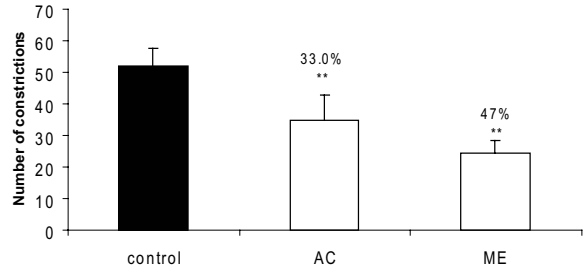

Figure 3. Effect on acetic acid-induced pain in mice test of extracts AC and ME, administrated orally at 100 mg/kg. Each column represents mean ± s.e.m. of six experimental values. ** $p < 0.01$, compared with corresponding control values.

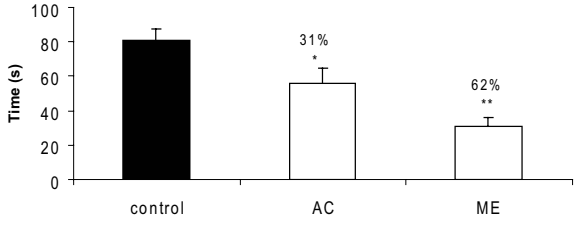

Figure 4. Effect of extracts AC and ME, administrated intraperitoneally at 10 mg/kg, on licking/biting response induced by intraplantar injection of capsaicin in mice. Each group represents the mean of six experiments. *p<0.05 and **p<0.01, compared with corresponding control values.

when evaluated by oral route, the ME caused a most pronounced effect. This means that there is a significant difference in relation to the qualitative or quantitative aspects concerning the chemical composition of this plant, suggesting a need for further phytochemical studies.

The writhing test is considered a non-specific model (e.g. anticholinergic and antihistaminic and other agents show activity in this assay), but it is frequently used for analgesic screening and involves local peritoneal receptors (cholinergic and histamine receptor) and acetylcholine and histamine mediators (Calixto et al., 2000; Choi et al., 2003). The acetic acid model is generally used to evaluate the action of peripheral drugs, acting indirectly by inducing the release of endogenous mediators, which stimulate nociceptive neurons that are sensitive to non-steroidal anti-inflammatory drugs and/or opioids (Collier et al., 1968).

Another interesting result was the action of ME in inhibited capsaicin-induced stimulation of primary afferents, suggesting that it acts on neurogenic pain and may be involved with the antagonism of vanilloid receptor (Caterina et al., 1997).

The results obtained on hot plate test, which is a technique that presents selectivity for opioid-derived analgesics (Abbott & Franklin, 1986), suggest that it is unrelated to the activation of the opioid receptors.

In conclusion, the results of the present study demonstrate, for the first time, that the extracts obtained from *A. cochliacarpos* bark possess active substances, which exert marked antinociceptive properties in mice. Such pharmacological effects confirm and justify, at least in part, the popular use of this plant to treat dolorous processes. Phytochemical studies are currently underway to elucidate the active principles acting as analgesic in this plant.

ACKNOWLEDGEMENTS

The authors are grateful to Érika von Sohsten de Souza Medeiros for the botanical identification, to CNPq, FAPESC-SC and ProPPEC/UNIVALI for their financial support and to all members of the community of Barra II, Bahia, for their contribution to the ethnobotanic work.

REFERENCES

Abbott FV, Franklin KBJ 1986. Noncompetitive antagonism of morphine analgesia by diazepam in the formalin test. *Pharmacol Biochem Behav 24*: 319-321.

Agra MF, Silva KN, Basílio IJLD, França PF, Barbosa-Filho JM 2008. Survey of medicinal plants used in the region Northeast of Brazil. *Rev Bras Farmacogn 18*: 472-508.

Araújo CW, Peixoto Neto PAS, Campos NV, Porfírio Z, Caetano LC 2002. Antimicrobial activity of *Pithecolobium avaremotermo* bark. *Fitoterapia 73*: 698-700.

Audi EA, Toledo DP, Peres PG, Kimura E, Pereira WKV, Mello JCP, Nakamura CV, Alves-do-Prado W, Cuman RKN, Bersani-Amando CA 1999. Gastric antiulcerogenic effects of *Stryphnodendron adstringes* in rats. *Phytother Res 13*: 246-266.

Calixto JB, Beirith A, Ferreira J, Santos AR, Cechinel-Filho V, Yunes RA 2000. Naturally occurring antinociceptive substances from plants. *Phytother Res 14*: 401-418.

Caterina MLJ, Schumacher MA, Tominaga M, Rose TA, Levine JD, Julius D 1997. The capsaicin receptor: a heat-activated in channel in the pain pathway. *Nature 389*: 816-824.

Choi J, Lee KT, Ha J, Yun SY, Ko CD, Jung HJ, Park HJ 2003. Antinociceptive and anti-inflammatory effects of niga-ichigoside F1 and 23-hydroxytormentic acid obtained from *Rubus coreanus*. *Biol Pharm Bull 10*: 1436-1441.

Collier HDJ, Dinnin LC, Johnson CA, Schneider C 1968. The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Brit J Pharmacol Chemother32*: 295-310.

Eddy NB, Leimback D 1953. Synthetic analgesics: Il dithienylbutenyl and dithienylbutylamines. *J Pharmacol Exp Ther 107*: 385-393.

IUCN 2004. Red List of Threatened Species, http://www.redlist.org, access in august 2004.

Lima JCS, Martins DTO, de Souza Jr PT 1998. Experimental evaluation of stem bark of *Stryphnodendron adstringens* (Mart) Coville for antiinflammatory activity. *Phytother Res 12*: 218-220.

Lopes GC, Sanches AC, Nakamura CV, Dias Filho BP, Hernandes L, de Mello JC 2005. Influence of extracts of *Stryphnodendron polyphyllum* Mart. and *Stryphnodendron obovatum* Benth. on the cicatrisation of cutaneous wounds in rats. *J Ethnopharmacol 99*: 265-272.

Martins DTO, Lima JCS, Rao VSN 2002).The acetone soluble fraction from bark extract of *Stryphnodendron adstringens* (Mart.) Coville inhibits gastric acid secretion and experimental gastric ulceration in rats. *Phytother Res 16*: 427-431.

Matos FJA 1997. *Introdução à fitoquímica experimental*. Fortaleza: UFC.

Mendonça, RPC 2000. *Estudo da atividade biológica de Pithecolobium avaremotemo Mart. e de alguns compostos obtidos comercialmente*. Maceió, 123p MSc. Thesis - CPGQB, Universidade Federal de Alagoas.

Migliatti JFZ 2003. Barbatimão (*Stryphnodendron* spp., Leguminosae). *J Bras Fitomed 1*: 32-35.

Sakurada T, Katsumata K, Tan-No K, Sakurada S, Kisara K 1992. The capsaicin test in mice for evaluating tachykinin antagonists in the spinal cord. *Neuropharmacol 31*: 1279-1285.

Santos SC, Ferreira FS, Rossi-Alva JC, Fernandez LG 2007. Atividade antimicrobiana *in vitro* do extrato de *Abarema cochliocarpos* (Gomes) Barneby & Grimes. *Rev Bras Farmacogn 17*: 215-219.

Silva MS 2003. *Avaliação farmacológica de plantas medicinais de uso popular contra distúrbios do trato gastrintestinal no Povoado Colônia Treze*

em Lagarto/SE. Sergipe, 118 p. MSc. Thesis - Universidade Federal de Sergipe.

Silva NCB 2006. *Potencial biotecnológico de plantas medicinais: estudo etnofarmacológico em uma comunidade quilombola da Chapada Diamantina - BA*. Rio de Janeiro, 227 p. Ph.D. Thesis - Universidade Federal do Rio de Janeiro.

Souza TM, Moreira RRD, Pietro RCLR, Isaac VLB 2007. Avaliação da atividade anti-séptica de extrato seco de *Stryphnodendron adstringens* (Mart.) Coville e de preparação cosmética contendo este extrato. *Rev Bras Farmacogn 17*: 71-75.

Vaz ZR, Cechinel-Filho V, Yunes RA, Calixto JB 1996. Antinociceptive action of 2-(4-bromobenzoyl)-3-methyl-4,6-dimethoxybenzofuran, a novel xanthoxyline derivative on chemical and thermal models of nociception in mice. *J Pharmacol Exp Ther 278*: 304-312.