J Pharmacol Sci **93**, 307 – 313 (2003)

Journal of Pharmacological Sciences

Full Paper

Bulgarian Propolis Induces Analgesic and Anti-inflammatory Effects in Mice and Inhibits In Vitro Contraction of Airway Smooth Muscle

Niraldo Paulino[1], Andreia Pires Dantas[2], Vassya Bankova[3], Daniela Taggliari Longhi[1], Amarilis Scremin[1], Solange Lisboa de Castro[2,*], and João Batista Calixto[4]

[1]*Grupo de Pesquisa e Desenvolvimento de Biofármacos (BIOFAR), Universidade do Sul de Santa Catarina, Tubarão, 88704-900, SC, Brazil*
[2]*Departamento de Ultra-estrutura e Biologia Celular, Fundação Oswaldo Cruz, Rio de Janeiro, 21045-900, RJ, Brazil*
[3]*Institute of Organic Chemistry with Centre of Phytochemistry, Sofia, Bulgaria*
[4]*Departamento de Farmacologia, Universidade Federal de Santa Catarina, Florianópolis, 88015-420, SC, Brazil*

Received June 5, 2003; Accepted September 4, 2003

Abstract. Propolis is a bee product, which has long been used in folk medicine for the management of different diseases. In this study we evaluated the analgesic and anti-inflammatory effects of a standard ethanolic extract of Bulgarian propolis (Et-Blg) in mice and its in vitro effect on airway smooth muscle. Et-Blg inhibited acetic acid-induced abdominal contortions with an $ID_{50} = 7.4 \pm 0.7$ mg·kg^{-1}. In the formalin test, the extract caused a significant reduction in pain in mice treated with 100 mg·kg^{-1} Et-Blg during the neurogenic phase and for the inflammatory phase with all doses of the extract, with an $ID_{50} = 2.5 \pm 0.4$ mg·kg^{-1}. Et-Blg inhibited also the capsaicin-induced ear edema in mice; however, this extract was ineffective when assessed in the tail-flick and hot-plate thermal assays. The analgesic effect of Et-Blg was associated with the inhibition of inflammatory responses and not to a simple irritation of nervous terminals. In vitro, this extract inhibited the contraction of trachea smooth muscle induced by histamine ($IC_{50} = 50 \pm 5$ μg·mL^{-1}), capsaicin ($IC_{50} = 26.8 \pm 3$ μg·mL^{-1}), 80 mM KCl ($IC_{50} = 27.8 \pm 3$ μg·mL^{-1}), and carbachol ($IC_{50} = 54 \pm 2$ μg·mL^{-1}).

Keywords: propolis, anti-nociception agent, anti-inflammatory agent, guinea pig trachea, antispasmodic effect

Introduction

Propolis, also known as bee glue, is a traditional remedy widely used in many countries for the management of numerous diseases, including airway disorders and cutaneo-mucosal infections, mainly those of bacterial and viral etiologies. Chemical studies conducted with propolis extracts revealed the existence of a very complex mixture of different naturally-occurring constituents with more than 300 constituents identified to date (1 – 3), such as phenolic acid, terpenes, cinamic acid, caffeic acid, several esters, and also flavonoids. Propolis composition varies with the season and the geographic region; such extraordinary variability among samples from different sources leads to variation of the pharmacological properties of propolis. The high biodiversity of propolis has been discussed in a recent review (4). In temperate zones, the main constituents are flavonoids, while in tropical zones other classes of bioactive components have been described, such as aromatic acid derivatives, specific terpenoids and prenylated *p*-coumaric acids and acetophenones.

Propolis exhibits a variety of biological activities including bactericidal, antiviral, fungicidal, anti-tumoural, anti-oxidant, and anti-inflammatory properties (5, 6). Recently, we have shown that propolis can induce a relaxant effect in the guinea pig isolated trachea through the interaction of several mechanisms of action, such as nitric oxide, vasoactive intestinal peptide, and potassium channels modulators (7). We have previously demonstrated the anti-hyperalgesic action of an ethanol extract of propolis collected in the South of Brazil through

*Corresponding author. FAX: +55-21-2260 4434
E-mail: solange@ioc.fiocruz.br

acetic acid, kaolin, or zymosan models of nociception and also that this extract significantly inhibited capsaicin-induced pain and reverted the hyperalgesia induced by bradykinin (8).

The biological activity of propolis is associated mainly with phenolic compounds such flavonoids and derivatives of hydroxycinnamic acids. Three derivatives of *p*-coumaric acid isolated from a Brazilian sample presented a relaxant effect on smooth muscle isolated from guinea pig trachea (9). In a previous study, we reported the chemical characterization of a standard ethanolic extract of a Bulgarian sample, named Et-Blg, that presented a high content of flavonoids and showed a strong inhibitory activity against *Staphylococcus aureus*, *Candida albicans*, and *Trypanosoma cruzi* (10).

In the present study, we sought to investigate the potential anti-hyperalgesic and anti-inflammatory properties and also the in vitro relaxant action in the guinea pig trachea of Et-Blg.

Materials and Methods

Preparation of the extract

The propolis sample was collected in Burgas (Southeast Bulgaria) by Dr. V.S. Bankova, stored in her laboratory, and the ethanolic extract was prepared as previously described (11). Briefly, the resin was cut into small pieces and extracted with 70% ethanol, 1:10 (1:10 w/v) at room temperature for 24 h. The extract was evaporated to dryness under vacuum (yield 62%), and a stock solution was prepared in dimethysulfoxide (DMSO) and subsequently named Et-Blg. Before use, the extract was diluted in phosphate-buffered solution (PBS) and the final concentration of DMSO in this solution did not exceed 1%, which had no effect per se on animal and in vitro tests.

Materials

Acetic acid, morphine hydrochloride, and formalin were supplied by Merck AG, Darmstadt, Germany. Capsaicin, histamine hydrochloride, and carbachol hydrochloride were purchased from Sigma Chemical Co., St. Louis, MO, USA. All other reagents were of a high purity grade. The drugs were dissolved in PBS just before use.

Animals

Swiss male mice (18 – 35 g), from UNISUL facilities, were housed at 22 ± 2°C under a 12-h light-dark cycle. Food and water were offered ad libitum. The animals were acclimatized to the laboratory for at least 1 h before testing and the experiments were carried out in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigations of experimental pain in conscious animals (12). In each experiment, the group treated with Et-Blg consisted of 5 mice. Guinea pigs of both sexes (250 – 400 g) were employed for the isolation of smooth muscle cells from trachea for in vitro studies.

Chemically-induced abdominal constrictions assay

The mice were treated with Et-Blg (3 to 100 $mg \cdot kg^{-1}$ body weight) by the intraperitoneal route (i.p.) 15 min before injection, i.p. of 0.6% acetic acid in PBS. Afterwards, the mice were placed in separate boxes and the number of abdominal constrictions was cumulatively counted, as previously described (8). Indomethacin (10 $mg \cdot kg^{-1}$, i.p.) was used as the positive control and PBS was used as the negative one.

Formalin-induced nociception assay

The mice were treated with Et-Blg (3 to 100 $mg \cdot kg^{-1}$ body weight, i.p.) 15 min before injection under the surface of the right hindpaw of 20 μL 2.5% formalin (0.92% formaldehyde) in PBS. Indomethacin (10 mg $\cdot kg^{-1}$, i.p.) was used as the positive control and PBS was used as the negative one. One mouse of each group was observed simultaneously from 0 to 30 min following formalin injection. The amount of time spent licking the injected paw, indicative of pain, was monitored. After the first 5 min, the licking time monitored the neurogenic phase and the following 10 min, the inflammatory phase (13). To corroborate the inflammatory process, at the end of the experiments, the animals were killed by cervical dislocation and the paws were cut at the knee joint and weighed on an analytical balance.

Capsaicin-induced edema assay

The animals were treated with Et-Blg (1 to 10 mg/kg body weight, i.p.) and after an adaptation period of 15 min, 10 μL of capsaicin (1.6 μg/ear) was applied under the skin of the surface of the right ear (14). After 15 min, the animals were observed individually and the increase of ear volume was considered indicative of edema. Indomethacin (10 $mg \cdot kg^{-1}$, i.p.) was used as the positive control and PBS was used as the negative one.

Hot plate test

The animals were treated with Et-Blg (10 or 100 $mg \cdot kg^{-1}$ body weight, i.p.) 15 min before the test. Morphine (10 $mg \cdot kg^{-1}$, i.p.) was used as positive control, and PBS as the negative one. The mice were placed into a heated surface of a glass cylinder (diameter of 24 cm), and the time until shaking or licking of the paws or jumping was recorded as response latency using a thermal analgesiometer hot plate (Hugo Basile,

Verese, Italy), according to the method described by Eddy and Leimback (15).

Tail flick test

The animals were treated with Et-Blg (10 or 100 $mg \cdot kg^{-1}$ body weight, i.p.) 15 min before the test. As the positive control, morphine (10 $mg \cdot kg^{-1}$, i.p.) was used and the negative control was injection of PBS. A radiant heat tail flick analgesiometer was used to measure response latencies (16). Briefly, the animals responded to a focused heat-stimulus (90 W) by flicking or removing their inflicted tail, exposing a photocell in the apparatus immediately below the tail. A latency period of 20 s was the cut-off point of the experiment.

Rota-rod test

The animals were treated with Et-Blg (3 to 100 $mg \cdot kg^{-1}$ body weight, i.p.) 15 min before the test. As positive control morphine (10 $mg \cdot kg^{-1}$, i.p.) was used and the negative control was injection of PBS. The apparatus used was a Rota-rod (Model-DS 37, Hugo Basile) and the bar was rotated at the constant speed of 22 rpm. The time during which the animals remained on the Rota-rod was measured with a chronometer, and the cut-off time was 60 s.

In vitro assays with trachea preparation

Guinea pigs were anesthetised and killed by cervical dislocation. The trachea was rapidly removed; and after being freed from connective tissue, each one was cut into six transverse rings (3 – 4-mm-wide), containing 3 cartilages as described previously (17 – 19). The rings were opened and suspended in individual 10-mL jacketed organ baths containing Krebs-Henseleit solution maintained at 37°C, pH 7.2, gassed with a mixture of 95% O_2 and 5% CO_2. Tissues were allowed to equilibrate for at least 60 min before drug addition, with the buffer solution being renewed every 15 min, under a resting tension of 1 g. Isometric responses were measured by means of TRI-201 force displacement transducers and were recorded on a polygraph (Letica Scientific Instruments, Barcelona, Spain). In all experiments, the epithelial layer of the trachea was gently removed with a cotton-tipped applicator and tested with bradykinin, as previously described (18, 19).

After equilibration of at least 60 min, the preparations were pre-incubated with Et-Blg (10 to 100 $\mu g \cdot mL^{-1}$) 15 min before the addtion of histamine (10^{-3} to 100 μM) or capsaicin (1 to 10 μM), which were added to the bath by the cumulative method. In another set of experiments, a single dose of 10 μM carbachol or a Krebs-Henseleit solution with high KCl concentration (80 mM) was used to induce the contraction 15 min after the addition of Et-Blg (10 to 100 $\mu g \cdot mL^{-1}$). Usually, two to four complete concentration-response curves were obtained for the propolis extract in each preparation, at 60-min intervals between the curves.

Statistical analyses

For the in vivo studies, the results are presented as the mean ± S.E.M., except for the ID_{50} values (i.e., the doses of extract necessary to reduce the response by 50% relative to the control value), expressed as the mean ± S.D. The difference between the experimental groups was evaluated using analysis of variance followed by Dunnett's multiple comparison test or by Student's *t*-test. When appropriate, the ID_{50} values were estimated from individual experiments by use of the least squares method. For the in vitro studies, the responses were expressed as absolute changes in the percent of tension and statistical analysis was carried out using unpaired Student's *t*-test. The IC_{50} values were determined from individual experiments for the complete concentration-response curves by graphical interpretation and were expressed as the mean ± S.D. *P* values less than 0.05 were considered significant.

Results

Et-Blg caused a significant inhibition of abdominal contrictions induced by acetic acid in mice, with an ID_{50} value of 7.4 ± 0.7 $mg \cdot kg^{-1}$; and at the dose of 100 $mg \cdot kg^{-1}$, such inhibition was higher than that observed in the indomethacin-treated group (Fig. 1). When assessed in the formalin test, the propolis extract caused a statistically significant reduction in pain monitored by the licking time of mice treated with 100 $mg \cdot kg^{-1}$ Et-Blg during the neurogenic phase and with all concentrations of the extract during the inflammatory one (Fig. 2: a and

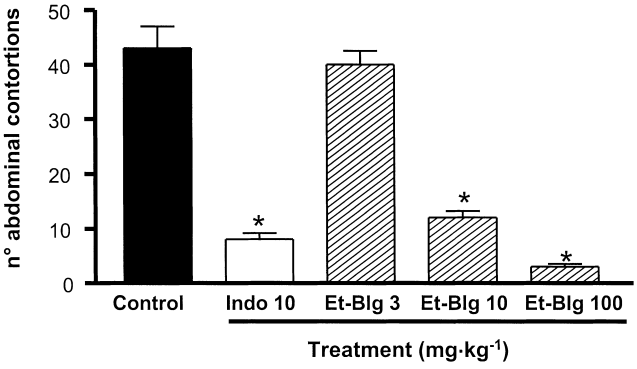

Fig. 1. Effect of Et-Blg in the acetic acid-induced pain model. Indomethacin (100 $mg \cdot kg^{-1}$) and the extract at 10 and 100 $mg \cdot kg^{-1}$ inhibited significantly (asterisks) the number abdominal contrictions in treated mice, $P<0.05$.

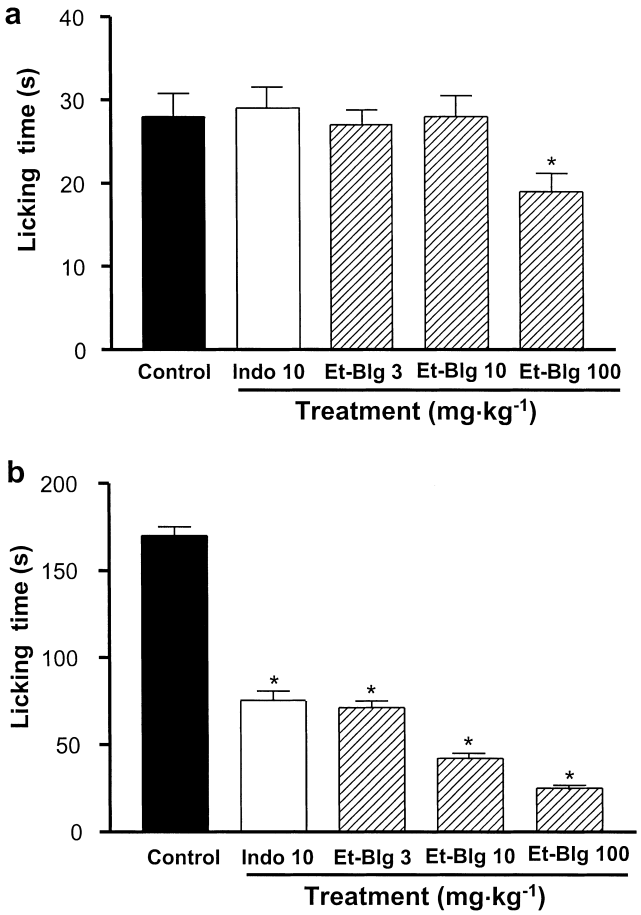

Fig. 2. Effect Et-Blg in the formalin-induced nociception model measured by the licking time: neurogenic phase (a) and inflammatory phase (b). Asterisks indicate significant inhibition in relation to the untreated group, $P<0.05$.

b). In this second phase, the anti-hyperalgesic effect had an ID_{50} value of $2.5 \pm 0.4\ mg \cdot kg^{-1}$, indicating that the effect observed by administration of $3\ mg \cdot kg^{-1}$ Et-Blg was similar to that of $10\ mg \cdot kg^{-1}$ indomethacin (Fig. 2b), moreover; and the inhibition of the paw edema was of $64 \pm 4\%$ at this dose of the extract (data not shown). The edema induced by ear application of capsaicin was significantly inhibited following the administration of Et-Blg with values between 34 – 40% for doses 1 to $10\ mg \cdot kg^{-1}$ of the extract and of 64.7% for indomethacin (Fig. 3). It is important to mention that Et-Blg up to $100\ mg \cdot kg^{-1}$ did not cause any significant effect on the motor co-ordination of the animals assessed in the Rota-rod test (data not shown).

When the hot-plate and the tail-flick thermal assays were performed, while morphine caused a increase of 2.6- and 8.3-fold increase, respectively, in the time of latency, administration of 10 and $100\ mg \cdot kg^{-1}$ Et-Blg led to no significant effect (Fig. 4: a and b).

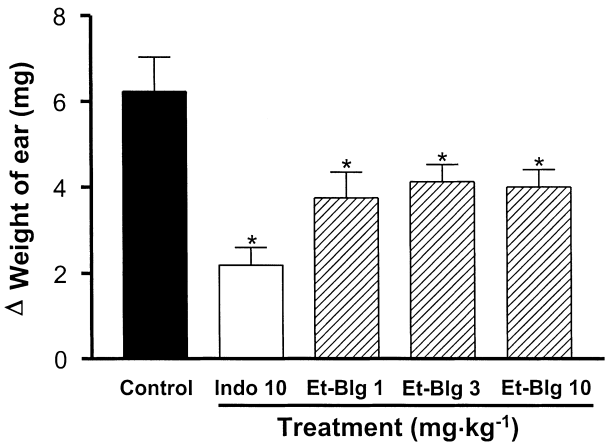

Fig. 3. Effect of Et-Blg in capsaicin-induced ear edema measured by the increase in ear weight. Asterisks indicate significant inhibition in relation to the untreated group, $P<0.05$.

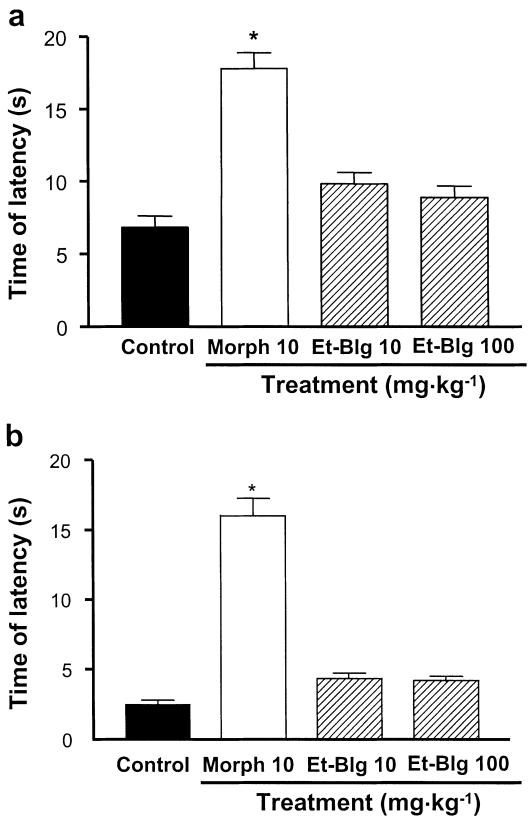

Fig. 4. Effect of Et-Blg in thermal model tests: hot plate (a) and tail flick radiant heat (b). The propolis extract caused no significant increase of the time of latency, $P<0.05$.

In experiments with isolated guinea pig trachea, incubation with Et-Blg induced a concentration-dependent and non-competitive inhibition of contraction induced by histamine ($IC_{50} = 50 \pm 5\ \mu g \cdot mL^{-1}$) or capsaicin ($IC_{50} = 26.8 \pm 3\ \mu g \cdot mL^{-1}$) (Fig. 5: a and b). In addition,

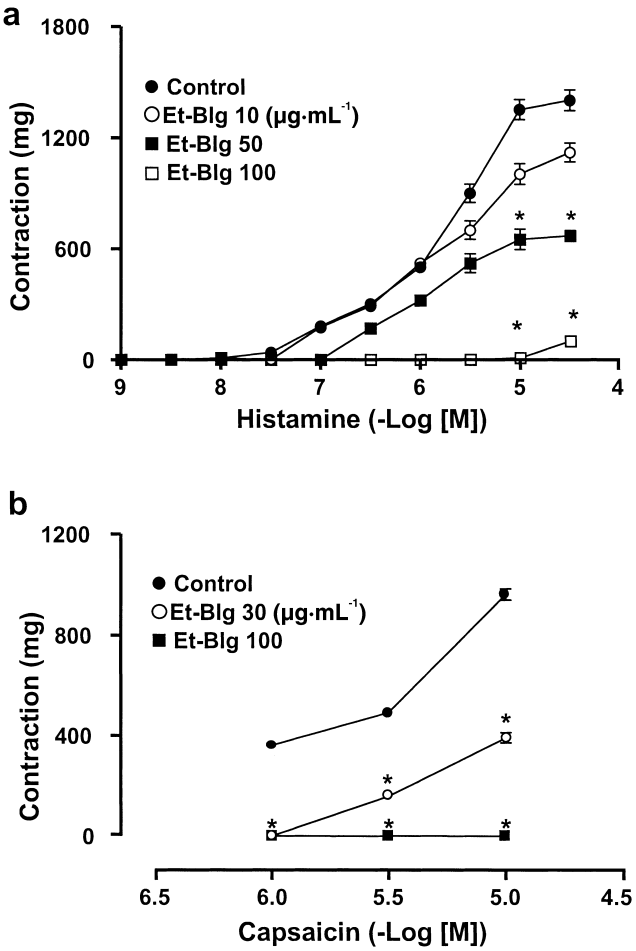

Fig. 5. Effect of Et-Blg in the contraction of guinea pig trachea induced by: 1 nM to 30 μM histamine (a) and 1 to 10 μM capsaicin (b). Asterisks indicate significant inhibition in relation to the untreated group, $P<0.05$.

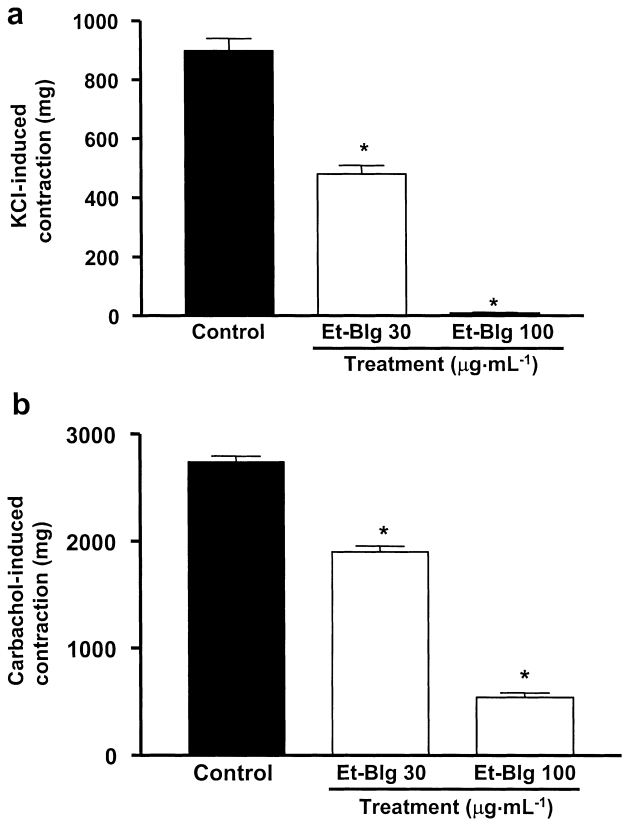

Fig. 6. Effect of Et-Blg in the contraction of guinea pig trachea induced by: 80 mM KCl (a) and 10 μM carbachol (b). Asterisks indicate significant inhibition in relation to the untreated group, $P<0.05$.

Et-Blg at 30 and 100 $\mu g \cdot mL^{-1}$ inhibited, respectively, $54 \pm 4\%$ and 100% of the 80 mM KCl-induced contraction of the trachea preparation (Fig. 6a). The corresponding values of inhibition for the carbachol-mediated tonic contraction were $54 \pm 5\%$ and $84 \pm 4\%$, respectively (Fig. 6b). Pre-incubation with the voltage-gated calcium channel antagonist nicardipine caused an inhibition similar to the propolis extract of the KCl-mediated contraction (data not shown).

Discussion

In some countries, propolis is used for the treatment of different diseases, such as odontological, dermatological, and gynaecological disorders, in which inflammation and pain are important components (5, 20). Scavenging of free radicals, generated by neutrophils in inflammatory processes, is the principal mechanism of conventional anti-inflammatory drugs, and is also a known property of propolis (21 – 23). The inflammation process involves production and/or release of mediators from neurons or damaged tissues, which are responsible for different responses including pain. Kinins, serotonin, excitatory amino acids, and prostanoids are involved in paw and ear edema formation in mice induced by formalin and capsaicin, respectively (13, 24 – 28).

Et-Blg showed a potent analgesic effect in models that induce pain by acetic acid (abdominal contortions) or formalin (licking time during the inflammatory phase), while on the other hand, this extract showed activity in the first phase of the formalin test only at the highest dose and no effect in the two thermal assays, hot plate and tail flick tests, that evaluate analgesy in neurogenic conditions. These results suggest that the analgesic effect of Et-Blg is associated with the inhibition of inflammatory responses, but not to a simple irritation of nervous terminals (neurogenic effect). Since voltage-gated Ca^{2+} channel blockers reduce the first and second phases of pain in mice (29, 30), the observed analgesic effect could involve blockage of these Ca^{2+}

channels.

The anti-inflammatory (31 – 34) and analgesic (8, 35, 36) properties of propolis have been previously reported in animal models; however, in none of these studies was the composition of the extract known. By high temperature high resolution gas chromatography coupled to mass spectrometry, we previously determined that the chemical composition of Et-Blg contains a high level of flavonoids (42.0%) (pinostrobin, pinocembrin, chrysin, and a series of pinobanskins), in addition to caffeic and ferulic acids and esters and phenylethyl caffeate (10). Krol et al. (37) have shown that propolis components such as some flavonoids and phenethyl caffeate are able to scavenge free radicals, and Mirzoeva and Calder (38) reported that among some propolis components, phenylethyl caffeate was the most effective in modulating eicosanoid production by mouse macrophages. Taken together these results, we can tentatively correlate the observed in vivo anti-inflammatory and analgesic activities of Et-Blg with the synthesis and/or liberation of inflammatory mediators and the high content of phenolic components in the extract.

Using smooth muscle preparations from guinea pig trachea, Et-Blg was able to block the depolarization induced by KCl (similarly to nicardipine) and to inhibit the contraction induced by histamine, capsaicin, and carbachol. Since KCl and histamine mediate the opening of calcium channels and carbachol, the intracellular release of this ion, the observed effect of the propolis extract was associated with the control of calcium mobilization. We have previously reported that the ethanol extract of a Brazilian sample and isolated compounds induced a relaxant effect in this model (7, 9). Although this Brazilian ethanol extract and Et-Blg, prepared by the same standard procedure, have totally distinct chemical compositions (7, 10), they exhibit similar in vitro activity in guinea pig trachea preparations. Previous comparison between propolis samples from temperate and tropical zones showed that despite the huge differences in their chemical composition, they shared several biological activities (39). To the best of our knowledge, the present work represents the first study demonstrating that a standardized propolis extract, with known chemical composition, causes, at low concentrations, a complete and well-reproducible inhibition of the contractile response induced by different agents.

Using smooth muscle preparations from guinea pig trachea, Et-Blg was able to block the depolarization induced by KCl (similarly to nicardipine) and to inhibit the contraction induced by histamine, capsaicin, and carbachol. Since KCl and histamine mediate the opening of calcium channels and carbachol, the intracellular release of this ion, the observed effect of the propolis extract could be associated with the control of calcium mobilization. We have previously reported that three *p*-coumaric acid derivatives isolated from a Brazilian propolis sample induced a relaxant effect in this model (9). To the best of our knowledge, the present work represents the first study demonstrating that a standardized propolis extract, with known chemical composition, causes, at low concentrations, a complete and well-reproducible inhibition of contractile response induced by different agents.

Acknowledgments

This study was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) e Fundação de Amparo e Pesquisa Carlos Chagas Filho do Rio de Janeiro (FAPERJ) and Grupo de Pesquisa e Desenvolvimento de Biofármacos (BIOFAR), Universidade do Sul de Santa Catarina, Brazil.

References

1 Ghisalberti EL. Propolis: a review. Bee World. 1979;60:59–84.
2 Greenaway W, Scaysbrook T, Whatley FR. The analysis of bud exudate of Populus x euroamercicana and propolis, by gas chromatography mass-espectrometry. Proc Roy Soc London. 1987;Series B 232:249–272.
3 Marcucci MC. Propolis: chemical composition, biological properties and therapeutic activity. Apidologie. 1995;26:83–99.
4 Bankova VS, De Castro SL, Marcucci MC. Propolis: recent advances in the chemistry and plant origin. Apidologie. 2000;31: 3–15.
5 De Castro SL. Propolis: biological and pharmacological activities. Therapeutic uses of this bee-product. Annu Rev Biol Sci. 2001;3:49–83.
6 Banskota AH, Tezuka Y, Kadota S. Recent progress in pharmacological research of propolis. Phytother Res. 2001;15:561–571.
7 Paulino N, Scremin FM, Raichaski LB, Marcucci MC, Scremin A, Calixto JB. Mechanisms involved in the relaxant action of the ethanolic extract of propolis in the guinea pig trachea in vitro. J Pharm Pharmacol. 2002;54:845–852.
8 De Campos RO, Paulino N, Da Silva CH, Scremin A, Calixto JB. Anti-hyperalgesic effect of an ethanolic extract of propolis in mice and rats. J Pharm Pharmacol. 1998;50:1187–1193.
9 Marcucci MC, Ferreres F, Garcia-Viguera C, et al. Phenolic compounds from Brazilian propolis with pharmacological activities. J Ethnopharmacol. 2001;74:105–112.
10 Prytzyk E, Dantas AP, Salomão K, et al. Flavonoids and trypanocidal activity of Bulgarian propolis. J Ethnopharmacol. 2003;88:189–193.
11 Bankova VS, Boudourova-Krasteva G, Sforcin JM, et al. Phytochemical evidence for the plant origin of Brazilian propolis from São Paulo State. Z Naturforsch. 1999;54C:401–405.

12 Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 1983;6:109–110.
13 Corrêa CR, Calixto JB. Evidence for participation of B_1 and B_2 kinin receptors in formalin-induced nociceptive response in the mouse. Br J Pharmacol. 1993;110:193–198.
14 Da Cunha FM, Frode TS, Mendes GL, et al. Additional evidence for the anti-inflammatory and anti-allergic properties of the sesquiterpene polygodial. Life Sci. 2001;70:159–169.
15 Eddy NB, Leimback D. Synthetic analgesics. II – Dithienylbutenyl and dithienylbutylamines. J Pharmacol Exp Ther. 1953; 107:385–393.
16 D'Amour FE, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther. 1941;72:74–79.
17 Schlemper V, Calixto JB. Nitric oxide pathway-mediated relaxant effect of bradykinin in the guinea pig isolated trachea. Br J Pharmacol. 1994;11:83–88.
18 Schlemper V, Calixto JB. Mechanisms involved in the relaxant response of bradikinin in epithelium intact strips of the guinea-pig trachea. Eur J Pharmacol. 1995;282:177–184.
19 Paulino N, Pizollati MG, Yunes RA, Filho VC, Creczynski-Pasa TB, Calixto JB. The mechanisms underlying the relxant effect of methyl and ethyl gallate in the guinea pig in vitro: contribution of potassium channels. Naunyn Schmiedebergs Arch Pharmacol. 1999;360:331–336.
20 Castaldo S, Capasso F. Propolis, an old remedy used in modern medicine. Fitoterapia. 2002;73 Suppl 1:S1–S6.
21 Pascual C, Gonzalez R, Torricella RG. Scavenging action of propolis extract against oxygen radicals. J Ethnopharmacol. 1994;41:9–13.
22 Moreno MIN, Isla MI, Sampietro AR, Vattuone MA. Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. J Ethnopharmacol. 2000;71:109–114.
23 Ichikawa H, Satoh K, Tobe T, et al. Free radical scavenging activity of propolis. Redox Rep. 2002;7:347–350.
24 De Campos ROP, Alves RV, Kyle DJ, Chakravarty S, Mavunkel BJ, Calixto JB. Antioedematogenic and antinociceptive actions of NPC 18521, a novel bradykinin B_2 receptor antagonist. Eur J Pharmacol. 1996;16:277–286.
25 Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain. 1987; 30:103–114.
26 Chapman V, Dickenson AH. The spinal and peripheral roles of bradykinin and prostaglandins in nociceptive processing in the rat. Eur J Pharmacol. 1992;219:427–433.
27 Malmberg AB, Yaksh TL. Cyclooxygenase inhibition and the spinal release of prostaglandin E_2 and amino acids evoked by paw formalin injection: a microdialysis study in unanesthetized rats. J Neurosci. 1995;15:2768–2776.
28 Doak GJ, Sawynok J. Formalin-induced nociceptive behavior and edema: involvement of multiple peripheral 5-hydroxytryptamine receptor subtypes. Neuroscience. 1997;80:939–949.
29 Venegas H, Schaible HG. Effects of antagonists to high-threshold calcium channels upon spinal mechanisms of pain, hyperagesia and allodynia. Pain. 2000;85:9–18.
30 Li SW, Westwick J, Poll CT. Receptor-operated Ca^{2+} influx channels in leukocytes: a therapeutic target? Trends Pharmacol Sci. 2002;23:63–70.
31 Scheller S, Gazda G, Krol W, et al. The ability of ethanolic extract of propolis to protect mice against γ-irradiation. Z Naturforsch. 1989;44C:1049–1052.
32 Ivanovska ND, Dimov VB, Bankova VS, Popov SS. Immunomodulatory action of propolis. VI. Influence of a water soluble derivative on complement activity in vivo. J Ethnopharmacol. 1995;47:145–147.
33 Hepsen IF, Er H, Cekic O. Topically applied water extract of propolis to suppress corneal neovascularization in rabbits. Ophthalmic Res. 1999;31:426–431.
34 Ozturk F, Kurt E, Cerci M, et al. The effect of propolis extract in experimental chemical corneal injury. Ophthalmic Res. 2000; 32:13–18.
35 Prokopovich NN. Propolis a new anesthetic. Vrach Delo. 1957; 10:1077–1080.
36 Park EH, Kahng JH. Suppressive effect of propolis in rat adjuvant arthritis. Arch Pharm Res. 1999;22:554–558.
37 Krol W, Scheller S, Czuba Z, et al. Inhibition of neutrophils chemiluminescence by ethanolic extract of propolis (EEP) and its phenolic components. J Ethnopharmacol. 1996;55:19–25.
38 Mirzoeva OK, Calder PC. The effect of propolis and its components on eicosanoid production during the inflammatory response. Prostaglandins Leukot Essent Fatty Acids. 1996;55: 441–449.
39 Bankova VS, Chirstov R, Kujumgiev A, Marcucci MC, Popov S. Chemical composition and antibactericidal activity of Brazilian propolis. Z Naturforsch. 1995;50C:167–172.