J. Pharm. Pharmacol. 1998, 50: 1187–1193
Received November 24, 1997
Accepted May 19, 1998

© 1998 J. Pharm. Pharmacol.

Anti-hyperalgesic Effect of an Ethanolic Extract of Propolis in Mice and Rats

RAFAEL O. P. DE CAMPOS, NIRALDO PAULINO, CÉLIO H. M. DA SILVA, AMARILIS SCREMIN AND JOÃO B. CALIXTO

Department of Pharmacology, Universidade Federal de Santa Catarina, Florianópolis 88015-420, Brazil

Abstract

Propolis, or bee glue, which contains a complex mixture of secondary metabolites, has long been used in many countries for the management of several diseases. The purpose of this study was to evaluate, by means of several pharmacological models, the anti-hyperalgesic effect of propolis collected in the south of Brazil.

The abdominal constrictions induced in mice by intraperitoneal injection of acetic acid (0·6%), kaolin (50 mg kg^{-1}) or zymosan (40 mg kg^{-1}) were inhibited to different extents by an extract of propolis (1–60 mg kg^{-1}) administered intraperitoneally 30 min earlier; mean ID50 (concentrations resulting in 50% inhibition) values were 2·7, 10·8 and 10·7 mg kg^{-1}, respectively, and maximum inhibition was 58 ± 5, 57 ± 10 and 51 ± 5%, respectively. Given orally (25–200 mg kg^{-1}, 1 h previously) propolis also inhibited the abdominal constrictions induced by acetic acid (maximum inhibition 43 ± 5%). When injected intraperitoneally (3–60 mg kg^{-1}, 30 min previously), propolis attenuated both the neurogenic (first phase) and inflammatory (second phase) pain responses and paw oedema caused by intraplantar injection of formalin (2·5%); maximum inhibition was 32 ± 5, 43 ± 6 and 19 ± 2%, respectively. Oral administration of propolis (25–200 mg kg^{-1}, 1 h previously) inhibited both phases and reduced the oedema formation associated with the second phase of the formalin test (maximum inhibition 22 ± 5, 33 ± 6 and 26 ± 3%) and extract of propolis (3–30 mg kg^{-1} i.p. or 25–100 mg kg^{-1} p.o., respectively 30 min and 1 h previously) significantly inhibited capsaicin-induced pain with maximum inhibition of 39 ± 8 and 41 ± 8%, respectively. When assessed in the Randall–Sellito test of pain, the extract of propolis (3–30 mg kg^{-1}, i.p., 30 min previously) significantly reversed the hyperalgesia induced by intraplantar injection of bradykinin (3 nmol per paw) in rats ($P < 0.01$). In contrast with morphine the extract of propolis ($\leq$ 100 mg kg^{-1}, 30 min previously) was ineffective when assessed in the tail-flick and hot-plate thermal assays. Naloxone (5 mg kg^{-1} i.p.) reversed ($P < 0.01$) the effect of morphine (5 mg kg^{-1} s.c.) by 70 and 94% respectively in the first and second phases of the formalin test, but did not interfere with the analgesic effect of propolis (10 mg kg^{-1} i.p., 30 min previously).

These results show that ethanolic extract of propolis, given systemically, has significant anti-hyperalgesic action when assessed in chemical, but not thermal, models of nociception in mice and rats. Its analgesic action seems to be unrelated to release or activation of the opioid system.

Propolis, known also as bee glue, is a traditional remedy widely used in many countries for the management of numerous diseases, including airway affections and cutaneo-mucosal infections, mainly those of viral etiology (Amoros et al 1994). Phytochemical studies conducted with propolis extract revealed the existence of a mixture of different naturally occurring constituents, such as phenolic acids, terpenes, cinnamic acid, caffeic acid, several esters, and also flavonoids (Bankova et al 1995). In-vivo and in-vitro pharmacological studies of propolis extract have revealed a broad spectrum of action, including free-radical scavenging and antioxidant properties (Matsushige et al 1996), anticarcinogenic action (Jaiswal et al 1997),

Correspondence: J. B. Calixto, Department of Pharmacology, Universidade Federal de Santa Catarina, Rua Ferreira Lima, 82, 88015-420, Florianópolis SC, Brazil.

antiviral (Amoros et al 1994) and antibacterial (Bankova et al 1995) effects, antiprotozoan action against *Trypanosoma cruzi* (De Castro & Higashi 1995), immunomodulatory and anti-inflammatory activity (Ivanovska et al 1995) and liver-protective properties (Mahran et al 1996).

This study was, therefore, designed to examine whether an ethanolic extract of propolis had anti-hyperalgesic properties when assessed systemically in different chemical and thermal models of nociception in mice and rats.

Materials and Methods

Drugs

The drugs used were formalin, acetic acid, morphine hydrochloride (Merck, Darmstadt, Germany), bradykinin, kaolin, zymosan (Sigma, St Louis, MO) and naloxone hydrochloride (DuPont, Garden City, USA). Other reagents used were of a high grade of purity. All drugs were dissolved in 0·9% NaCl solution or in phosphate buffer just before use. The ethanolic extract of propolis was dissolved in phosphate buffer. The final concentration of ethanol did not exceed 5%; at this concentration it had no effect on animal tests.

Preparation of the extract

A commercial ethanolic extract of propolis (Apis Nativa Produtos Naturais, Araranguá, Brazil) was used in the study. The extract was concentrated as required by evaporation of the ethanol.

Pharmacological procedures

Animals. Experiments were performed on non-fasted Swiss male Wistar rats, 100–120 g, or on mice, 18–35 g, from our department, housed at 22 ± 2°C under a 12-h light–dark cycle. Food and water were freely available. The animals were acclimatized to the laboratory for at least 1 h before testing and were used once throughout the experiments, which were performed in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann 1983).

Chemically-induced abdominal constrictions in mice. Abdominal constrictions induced by intraperitoneal injection of acetic acid (0·6%), kaolin (50 mg kg^{-1}) or zymosan (40 mg kg^{-1}) were monitored by a procedure similar to that described elsewhere (De Campos et al 1996). The animals were pretreated with the propolis extract, intraperitoneally or orally, respectively 30 and 60 min before injection of the irritant. Control animals received a similar volume (10 mL kg^{-1}) of vehicle (5% ethanol solution prepared in phosphate buffer). After challenge pairs of mice were placed in separate boxes and the number of abdominal constrictions was counted.

Formalin-induced nociception in mice. The procedure used was similar to that described elsewhere (Corrêa & Calixto 1993). Animals were lightly anaesthetized with ether except when used to monitor the first phase of the formalin test. A solution of formalin (0·92% formaldehyde) was further diluted to 2·5% with phosphate buffer and 20 μL was injected under the surface of the right hind-paw. Two mice (control and treated) were observed simultaneously from 0 to 30 min after formalin injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of pain. Animals were pretreated intraperitoneally or orally with the ethanolic extract of propolis. Control animals received only the vehicle of the extract.

In an attempt to investigate participation of the opioid system in the anti-hyperalgesic effect of propolis separate groups of mice were treated with naloxone (5 mg kg^{-1} i.p.), which was injected 10 min before administration of propolis (10 mg kg^{-1} i.p.) or morphine (5 mg kg^{-1} s.c.; used as positive control).

To assess possible anti-oedematogenic activity, 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 nociception in mice. The procedure used was similar to that described elsewhere (Sakurada et al 1992; De Campos et al 1996). After a period of adaptation 20 μL capsaicin (1·6 μg per paw) was injected under the skin of the plantar surface of the right hind-paw by use of a microsyringe with a 26-gauge needle. The animals were observed individually for 5 min after capsaicin injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of pain. Animals were treated with the ethanolic extract of propolis, intraperitoneally or orally, before capsaicin injection. Control animals received a similar volume (10 mL kg^{-1}) of 5% ethanol solution prepared in phosphate buffer, either intraperitoneally or orally.

Hot-plate test on mice. The hot-plate test was used to measure response latencies according to the method described by Eddy & Leimback (1953). Animals were placed into a 24-cm diameter glass cylinder on the heated surface, and the time (s)

between placement and shaking or licking of the paws or jumping was recorded as response latency. The animals were treated either with the extract of propolis or with morphine (positive control) 30 min before experiments. Control animals received the same volume of vehicle (5% ethanol solution prepared in phosphate buffer; 10 mL kg^{-1} i.p.).

Tail-flick test in mice. A radiant heat tail-flick analgesiometer was used to measure response latencies as described by D'Amour & Smith (1941). Briefly, 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. The reaction time was recorded for control mice and for animals pretreated with morphine or with the extract of propolis. The animals were selected 24 h previously on the basis of their response in the model. A latency period of 20 s was defined as complete analysis. The animals were treated, 30 min before the experiments, with the extract of propolis or with morphine. Control animals received the same volume of vehicle (5% ethanol solution prepared in phosphate buffer; 10 mL kg^{-1} i.p.).

Rota-rod test in mice. The apparatus used was a Ugo Basile model-DS 37. The bar rotated at a constant speed of 22 rev min^{-1}. Animals were treated intraperitoneally, 30 min previously, with the vehicle (5% ethanol solution prepared in phosphate buffer; 10 mL kg^{-1}) or with the ethanolic extract of propolis (up to 100 mg kg^{-1}). The time (s) during which animals remained on the rota-rod was recorded. The cut-off time was 60 s.

Bradykinin-induced hyperalgesia in the rat paw. The procedures used were similar to those described elsewhere (De Campos et al 1996). Animals were pretreated with the angiotensin-converting enzyme-inhibitor, captopril (5 mg kg^{-1} s.c.) 1 h before the experiments to prevent bradykinin degradation (Corrêa & Calixto 1993). The animals were pretreated intraperitoneally with propolis 30 min before injection of bradykinin (3 nmol) or of phosphate buffer (0·1 mL) into the right hind-paw; the anti-hyperalgesic action was assessed 30 min later. The hyperalgesic threshold (squeak response or paw withdrawal) was assessed by applying increasing pressure to the dorsal site of the injected paws, using a modified Basile analgesia meter (Ugo Basile, Milan, Italy) according to the method of Randall & Sellito (1957), with minor modifications. The weight on the analgesia meter ranged from 0 to 750 g, and the threshold was expressed as tolerated load (g).

Statistical analysis

Results are presented as means ± s.e.m., except for ID50 values (the doses of extract necessary to reduce the response by 50% relative to the control value) which are reported as geometric means with their respective 95% confidence limits. The statistical significance of differences between groups was determined by analysis of variance then Dunnett's multiple comparison test or Student's *t*-test. P values < 0.05 were considered to be indicative of significance. When appropriate, values for ID50 (amount of drug affording 50% protection against pain) were estimated from individual experiments by use of the least-squares method.

Results

The ethanolic extract of propolis (1–60 mg kg^{-1} i.p.) significantly and dose-dependently inhibited the abdominal-constriction response induced by intraperitoneal injection of acetic acid (0·6%), kaolin (50 mg kg^{-1}) or zymosan (40 mg kg^{-1}) in mice (Tables 1 and 2). The calculated mean ID50 values (and 95% confidence limits) for these effects were: 2·7 (1·8–4·1), 10·8 (5·3–22·0) and 10·7 (4·9–23·5) mg kg^{-1}, respectively. Oral administration of

Table 1. Effect of the ethanolic extract of propolis on acetic acid-induced abdominal constrictions in mice.

Treatment	Intraperitoneal dose (mg kg^{-1})	Number of abdominal constrictions	Oral dose (mg kg^{-1})	Number of abdominal constrictions
Propolis	0	45·9 ± 1·6	0	47·6 ± 3·3
	1	28·8 ± 3·4†	25	29·2 ± 2·3†
	3	19·5 ± 2·1†	50	27·2 ± 2·4†
	10	24·0 ± 1·9†	100	33·4 ± 4·0*
	30	25·1 ± 3·5†	200	33·9 ± 4·3*
	60	25·7 ± 1·5†		
Maximum inhibition (%)		58 ± 5		43 ± 5
Dose reducing pain by 50%‡		2·7 (1·8–4·1)		–

Data are means ± s.e.m. of results from 6 to 10 animals. * $P < 0.05$, † $P < 0.01$, significantly different from respective control values (Dunnett's multiple comparison test). ‡ mg kg^{-1} with 95% confidence limits.

Table 2. Effect of intraperitoneal administration of the ethanolic extract of propolis on kaolin- or zymosan-induced abdominal constrictions in mice.

Treatment	Dose ($mg\,kg^{-1}$)	Number of abdominal constrictions	
		Kaolin ($50\,mg\,kg^{-1}$)	Zymosan ($40\,mg\,kg^{-1}$)
Propolis	0	26·4 ± 4·1	25·4 ± 0·8
	3	17·0 ± 1·0	16·0 ± 0·7†
	10	11·3 ± 2·6†	12·5 ± 1·4†
	30	14·7 ± 1·4*	17·8 ± 2·0†
Maximum inhibition (%)		57 ± 10	51 ± 5
Dose reducing pain by 50%‡		10·8 (5·3–22·0)	10·7 (4·9–23·5)

Data are means ± s.e.m. of results from 6 to 10 animals. * $P < 0.05$, † $P < 0.01$, significantly different from respective control values (Dunnett's multiple comparison test). ‡ $mg\,kg^{-1}$ with 95% confidence limits.

Table 3. Time-course of the anti-hyperalgesic effect of the ethanolic extract of propolis, given intraperitoneally or orally, against acetic acid-induced abdominal constrictions in mice.

Time (h)	Number of abdominal constrictions	
	Intraperitoneal ($3\,mg\,kg^{-1}$)	Oral ($50\,mg\,kg^{-1}$)
0	41·4 ± 2·1	52·8 ± 2·9
0·5	20·2 ± 1·1*	–
1·0	22·0 ± 2·7*	30·6 ± 2·2*
2·0	25·6 ± 2·2*	37·0 ± 1·0*
3·0	29·0 ± 4·2*	–
4·0	37·3 ± 0·6	45·5 ± 1·7
6·0	–	46·0 ± 2·0

Data are means ± s.e.m. of results from 8 to 12 animals. * $P < 0.01$, significantly different from respective control values (Dunnett's multiple comparison test).

the ethanolic extract of propolis ($25–200\,mg\,kg^{-1}$) significantly inhibited the abdominal constrictions induced by acetic acid (Table 1) but was less potent and efficacious than when given intraperitoneally. After intraperitoneal or oral administration the anti-hyperalgesic action of propolis lasted 2 to 3 h (Table 3).

When assessed in the formalin test, the extract of propolis administered intraperitoneally ($3–60\,mg\,kg^{-1}$, 30 min previously) or orally ($25–200\,mg\,kg^{-1}$, 1 h previously) significantly inhibited both phases (neurogenic and inflammatory) of formalin-induced nociception (Table 4). The maximum inhibition observed was 32 ± 5 or 22 ± 5% for the first phase and 43 ± 6 or 33 ± 6% for the second phase, respectively, when the extract was given intraperitoneally or orally (Table 4). In addition, the extract of propolis also significantly reduced paw oedema associated with the second phase of the formalin test, with maximum inhibition of 19 ± 2 or 26 ± 3%, respectively, after intraperitoneal or oral administration (Table 4).

The pain reaction induced by intraplantar injection of capsaicin was significantly inhibited by intraperitoneal ($3–30\,mg\,kg^{-1}$) or oral ($25–100\,mg\,kg^{-1}$) administration of the ethanolic extract of propolis (Table 5). The maximum inhibition observed was 39 ± 8 or 41 ± 8%, respectively, for intraperitoneal or oral administration.

When assessed by the Randall–Sellito model the ethanolic extract of propolis ($3–30\,mg\,kg^{-1}$) significantly reversed the hyperalgesic effect of intraplantar injection of bradykinin (3 nmol per paw) when administered intraperitoneally 30 min previously (Table 6). The maximum effect was 28 ± 8% ($P < 0.01$). In contrast, the extract of propolis (up to $100\,mg\,kg^{-1}$ i.p.) was largely ineffective when assessed in the tail-flick and hot-plate thermal assays, under conditions when morphine ($10\,mg\,kg^{-1}$ s.c.) led to a marked increase in the pain latency in both tests (Table 7).

The analgesic effect of morphine ($5\,mg\,kg^{-1}$ s.c.), but not that of the ethanolic extract of propolis ($10\,mg\,kg^{-1}$ i.p.), was significantly reversed by previous treatment of the animals with naloxone ($5\,mg\,kg^{-1}$ i.p.), when analysed against both phases of formalin test. The amounts of licking in the first and second phases for mice treated with propolis only were, respectively, 41·8 ± 3·1 and 112·0 ± 15·8 s; for those treated with naloxone and propolis the values were 42·0 ± 3·4 and 112·5 ± 12·8 s. The ethanolic extract of propolis (up to $100\,mg\,kg^{-1}$ i.p.) had no significant effect on the motor coordination of animals when assessed by means of the rota-rod model (data not shown).

Discussion

These results indicate that the extract of propolis, administered either intraperitoneally or orally, has dose-dependent and significant anti-hyperalgesic action when assessed in several models of

Table 4. Effect of the ethanolic extract of propolis against the first phase, 0 to 5 min, and the second phase, 15 to 30 min, in the formalin test on mice.

Ethanolic extract	Dose (mg kg^{-1})	Amount of licking (s)		ΔPaw weight (mg)
		0–5 min	15–30 min	
Intraperitoneal	0	61·9 ± 2·1	202·3 ± 13·1	70·5 ± 3·1
	3	47·3 ± 3·0†	161·1 ± 12·4	65·9 ± 2·7
	10	42·9 ± 2·2†	115·3 ± 12·6†	57·0 ± 1·7†
	30	41·8 ± 3·4†	121·1 ± 12·2†	61·1 ± 3·9
	60	48·0 ± 5·5*	145·2 ± 9·8*	62·2 ± 3·2
Maximum inhibition (%)		32 ± 5	43 ± 6	19 ± 2
Oral	0	57·7 ± 1·6	181·3 ± 18·8	70·9 ± 4·2
	25	44·8 ± 5·5	121·8 ± 12·5	64·0 ± 2·2
	50	44·8 ± 2·7*	120·7 ± 10·6*	53·2 ± 1·9†
	100	45·4 ± 5·0*	139·3 ± 18·8	59·8 ± 4·0*
	200	51·0 ± 2·4	189·8 ± 16·5	65·8 ± 5·2
Maximum inhibition (%)		22 ± 5	33 ± 6	26 ± 3

Data are means ± s.e.m. of results from 8 to 12 animals. * $P < 0.05$, † $P < 0.01$, significantly different from respective control values (Dunnett's multiple comparison test).

Table 5. Effect of the ethanolic extract of propolis on capsaicin-induced neurogenic pain in mice.

Treatment	Intraperitoneal dose (mg kg^{-1})	Amount of licking (s)	Oral dose (mg kg^{-1})	Amount of licking (s)
Propolis	0	37·7 ± 1·7	0	39·5 ± 1·8
	3	26·8 ± 3·9*	25	24·6 ± 3·0†
	10	23·0 ± 3·0†	50	22·2 ± 3·0†
	30	25·4 ± 1·3†	100	23·6 ± 2·8†
Maximum inhibition (%)		39 ± 8		41 ± 8

Data are means ± s.e.m. of results from 6 to 8 animals. * $P < 0.05$, † $P < 0.01$, significantly different from respective control values (Dunnett's multiple comparison test).

Table 6. Anti-hyperalgesic properties of the ethanolic extract of propolis on bradykinin (3 nmol)-induced hyperalgesia in the rat paw.

Treatment	Dose of propolis (mg kg^{-1})	Load tolerated (g)
Vehicle (intraperitoneal) + saline (intraplantar)	–	405·0 ± 35·7
Vehicle (intraperitoneal) + bradykinin (intraplantar)	–	165·0 ± 6·7
Propolis (intraperitoneal) + bradykinin (intraplantar)	3	213·7 ± 7·2*
	10	210·8 ± 17·7
	30	231·0 ± 18·7†
Maximum reversion (%)		28 ± 8

Data are means ± s.e.m. of results from 5 or 6 animals. * $P < 0.05$, † $P < 0.01$, significantly different from results for vehicle + bradykinin group (Dunnett's multiple comparison test).

Table 7. Effect of morphine and the ethanolic extract of propolis in the hot-plate and tail-flick tests in mice.

Treatment	Dose (mg kg^{-1})	Latency (s)	
		Tail-flick	Hot-plate
Control	0	4·98 ± 0·60	4·50 ± 0·43
Morphine	10	16·08 ± 3·91*	16·25 ± 3·48*
Propolis	3	–	4·66 ± 0·80
	10	4·60 ± 0·36	4·56 ± 0·30
	30	–	5·13 ± 0·65
	100	5·65 ± 1·39	6·46 ± 0·86

Data are means ± s.e.m. of results from 10 animals. * $P < 0.01$, significantly different from respective control values (Dunnett's multiple comparison test).

chemically-induced nociception, namely acetic acid, kaolin and zymozan-induced abdominal constriction, both neurogenic (early phase) and persistent (late phase) of the formalin test, the neurogenic pain caused by subplantar injection of capsaicin, and bradykinin-induced hyperalgesia in the rat paw. In contrast with the potent anti-hyperalgesic action of morphine, used as reference drug, the ethanolic extract of propolis was largely ineffective in thermal models of nociception, such as the tail-flick and hot-plate assays.

The ethanolic extract of propolis, at doses which resulted in consistent anti-hyperalgesic action in the chemical and inflammatory pain models, resulted in significant attenuation of paw oedema formation associated with the late phase of formalin-induced pain. Previous studies by our group (Corrêa & Calixto 1993; De Campos et al 1996) and others (Hunskaar & Hole 1987; Chapman & Dickenson 1992; Malmberg & Yaksh 1995; Doak & Sawynok 1997) have demonstrated that several mediators involved in the inflammatory processes, such as kinins, 5-hydroxytryptamine, excitatory amino acids and prostanoids, but not tachykinins (Santos & Calixto 1997), seem to be involved in paw oedema formation in response to subplantar injection of formalin in mice. It therefore seems likely that the anti-hyperalgesic action of the active principle(s) present in the ethanolic extract of propolis are secondary to its anti-inflammatory activity, probably because of interaction with the synthesis or action, or both, of kinins or prostanoids, or both. In support of this hypothesis Mirzoeva & Calder (1996) have shown that the ethanolic extract of propolis, and some of its constituents, suppress prostaglandin and leukotriene generation by murine peritoneal macrophages in-vitro and during zymosan-induced acute peritoneal inflammation in-vivo. Interestingly, and in agreement with this, in the current study propolis elicited dose-dependent reduction of zymosan-induced abdominal constrictions.

That the systemic treatment of animals with propolis extract also consistently prevented the hyperalgesic effect of subplantar injection of bradykinin into the rat paw implies possible interaction of the active constituent(s) present in this extract with the inflammatory and hyperalgesic actions elicited by bradykinin, widely reported as a powerful pro-inflammatory and algesic endogenous peptide (review by Bhoola et al 1992). In accordance with this possibility it has been convincingly demonstrated that the abdominal constrictions induced by kaolin, which involve activation of the kinin cascade (Fujiyoshi et al 1989), action which is powerfully inhibited by bradykinin-receptor antagonists (De Campos et al 1996), are also consistently attenuated by the ethanolic extract of propolis.

The finding that intraperitoneal or oral pretreatment of animals with the ethanolic extract of propolis consistently prevented the neurogenic pain response to formalin (early phase) and capsaicin are relevant, because the majority of the non-steroidal anti-inflammatory drugs tested, including indomethacin, paracetamol, diclofenac and naproxen, are all largely ineffective in producing nociception in such models of pain (Hunskaar & Hole 1987; Vaz et al 1996). Morphine, and to a lesser extent dipyrone, given systemically (orally or intraperitoneally), intracerebroventricularly or intrathecally, resulted in pronounced anti-hyperalgesia when tested against both formalin (first phase) and capsaicin-induced nociception (Hunskaar & Hole 1987; Corrêa & Calixto 1993; Vaz et al (1996) Beirith et al 1998). The lack of anti-hyperalgesic properties of the ethanolic extract of propolis when assessed in thermal models of pain, which are very sensitive to the action of opioids (Vaz et al (1996) and these results) suggests that interaction of the extract with the opioid system does not account for the analgesic action of the active principle(s) present in the extract of propolis. The lack of reversion of the anti-hyperalgesic action of the extract of propolis by naloxone, under conditions where this effect of morphine was significantly reversed, gives further support to the idea that the opioid system does not participate in the mechanism of anti-hyperalgesia of propolis. The lack of any detectable non-specific action of the ethanolic extract on motor coordination in animals indicates that the anti-hyperalgesia caused by the propolis extract is not the consequence of a non-specific central or peripheral depressant effect.

Acknowledgements

This study was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior, Brazil. R.O.P. de Campos is an undergraduate medical student receiving a grant from CNPq.

References

Amoros, M., Lurton, E., Boustie, J., Girre, L. (1994) Comparison of the anti-herpes simplex virus activities of propolis and 3-methyl-but-2-enyl caffeate. J. Nat. Prod. 57: 644–647

Bankova, V., Christov, R., Kujumgiev, A., Marcucci, M. C., Popov, S. (1995) Chemical composition and antibacterial activity of Brazilian propolis. Z. Naturforsch. 50: 167–172

Beirith, A., Santos, A. R. S., Rodrigues, A. L. S., Pasa, T. B. C., Calixto, J. B. (1998) Spinal and supraspinal anti-nociceptive action of dipyrone in formalin, capsaicin and

glutamate tests. Study of the mechanism of action. Eur. J. Pharmacol. 345: 233–245.

Bhoola, K. D., Figueroa, C. D., Worthy, K. (1992) Bioregulation of kinins: kallikreins, kininogens, and kininases. Pharmacol. Rev. 44: 1–80

Chapman, V., Dickenson, A. H. (1992) The spinal and peripheral roles of bradykinin and prostaglandins in nociceptive processing in the rat. Eur. J. Pharmacol. 219: 427–433

Corrêa, C. R., Calixto, J. B. (1993) Evidence for participation of B_1 and B_2 kinin receptors in formalin-induced nociceptive response in the mouse. Br. J. Pharmacol. 110: 193–198

D'Amour, F. E., Smith, D. L. (1941) A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72: 74–79

De Campos, R. O. P., Alves, R. V., Kyle, D. J., Chakravarty, S., Mavunkel, B. J., Calixto, J. B. (1996) Antioedematogenic and antinociceptive actions of NPC 18521, a novel bradykinin B_2 receptor antagonist. Eur. J. Pharmacol. 316: 277–286

De Castro, S. L., Higashi, K. O. (1995) Effect of different formulations of propolis on mice infected with *Trypanosoma cruzi*. J. Ethnopharmacol. 46: 55–58

Doak, G. J., Sawynok, J. (1997) Formalin-induced nociceptive behavior and edema: involvement of multiple peripheral 5-hydroxytryptamine receptor subtypes. Neuroscience 80: 939–949

Eddy, N. B., Leimback, D. (1953) Synthetic analgesics II. Dithienylbutenyl and dithienylbutylamines. J. Pharmacol. Exp. Ther. 107: 385–393

Fujiyoshi, T., Hayashi, I., Oh-Ishi, S. (1989) Kaolin-induced writhing response in mice: activation of the plasma kallikrein-kinin system by kaolin. J. Pharmacobiodyn. 12: 483–487

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

Ivanovska, N. D., Dimov, V. B., Bankova, V. S., Popov, S. S. (1995) Immunomodulatory action of propolis. VI. Influence of a water soluble derivative on complement activity in vivo. J. Ethnopharmacol. 47: 145–147

Jaiswal, A. K., Venugopal, R., Mucha, J., Carothers, A. M., Grunberger, D. (1997) Caffeic acid phenethyl ester stimulates human antioxidant response element-mediated expression of the NAD(P)H: quinone oxidoreductase (NQO1) gene. Cancer Res. 57: 440–446

Mahran, L. G., el-Khatib, A. S., Agha, A. M., Khayyal, M. T. (1996) The protective effect of aqueous propolis extract on isolated rat hepatocytes against carbon tetrachloride toxicity. Drugs Exp. Clin. Res. 22: 309–316

Malmberg, A. B., Yaksh, T. L. (1995) Cyclooxygenase inhibition and the spinal release of prostaglandin E2 and amino acids evoked by paw formalin injection: a microdialysis study in unanesthetized rats. J. Neurosci. 15: 2768–2776

Matsushige, K., Basnet, P., Hase, K., Kadota, S., Tanaka, K., Namba, T. (1996) Propolis protects pancreatic beta-cells against the toxicity of streptozotocin (STZ). Phytomedicine 3: 203–209.

Mirzoeva, O. K., Calder, P. C. (1996) The effect of propolis and its components on eicosanoid production during the inflammatory response. Prostaglandins Leukot. Essent. Fatty Acids 55: 441–449

Randall, L. O., Sellito, J. J. (1957) A method for measurement of analgesic activity on inflamed tissue. Arch. Int. Pharmacodyn. Ther. 111: 409–419

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. Neuropharmacology 31: 1279–1285

Santos, A. R. S., Calixto, J. B. (1997) Further evidence for the involvement of tachykinin receptor subtypes in formalin and capsaicin models of pain in mice. Neuropeptides 31: 381–389

Vaz, Z. R., Cechinel Filho, V., Yunes, R. A., Calixto, J. B. (1996) Antinociceptive action of 2-(4-bromobenzoyl)-3-methyl-4,6-dimethoxy benzofuran, a novel xanthoxyline derivative on chemical and thermal models of nociception in mice. J. Pharmacol. Exp. Ther. 278: 304–312

Zimmermann, M. (1983) Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16: 109–110