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Antipsoriatic, anti-inflammatory, and analgesic effects of an extract of red propolis

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KEY WORDS psoriasis; propolis; indomethacin; keratosis; anti-inflammatory agents; analgesics

AIM: To study the antipsoriatic, anti-inflammatory, and analgesic effects of ethanolic extract of red propolis. **METHODS and RESULTS:** This extract induced the formation of granular layer in the mouse tail test used as a model of psoriasis. Propolis 50 mg·kg^{-1} ig showed anti-inflammatory activity in the cotton-pellet granuloma assay in rats, in croton oil-induced edema in mice at a dose of 25 % (2.5 μL), and in the peritoneal capillary permeability test in mice at a dose of 10 mg·kg^{-1}. The extract (25 mg·kg^{-1} ig) showed analgesic effect in the model of acetic acid-induced writhings, whereas 40 mg·kg^{-1} was effective in the hot plate test in mice. **CONCLUSION:** Anti-inflammatory, analgesic, and antipsoriatic properties of Cuban red propolis were evident.

Bees collect a resinous substance from the buds of trees, convert it to the bee-glue (called propolis), and use it to seal their hives. About 150 compounds, many of them flavonoids, have been identified in European propolis[1], and their pharmacological properties have been ascribed to them. However, propolis obtained in tropical countries do not contain flavonoid compounds[2]. We found antioxidative and hepatoprotective properties of red propolis collected in Cuba[3,4]. In the present study, we decided to determine the potential antipsoriatic, anti-inflammatory, and analgesic activities of extracts of Cuban red propolis.

MATERIALS AND METHODS

Preparation of propolis extract The propolis used in this study was collected from beehives in province of Pinar del Rio, Cuba. It was classified according to its separation by thin layer chromatography and named propolis red. Ethanol propolis extract was obtained[5], briefly: propolis 1 g was suspended in 95 % ethanol (vol/vol) in a mortar and the suspension decanted after 48 h at room temperature. The propolis extract had a concentration of 70 % (wt/vol) in ethanol and was kept at 4 ℃. Volatile constituents from this propolis were isolated and analyzed by gas chromatography-mass spectrometry (GC/MS), according to the conditions[6].

Cotton-pellet granuloma assay in rats Sprague-Dawley ♀ rats (220 ± 15 g) were treated with propolis extract, vehicle 10 mL·kg^{-1} (ethanol/water mixture), or indometacin 3 mg·kg^{-1} in 5 % $NaHCO_3$ used as positive control. The agents were given ig for 6 d from the day of cotton pellet insertion, which were removed on d 7[7].

Peritoneal capillary permeability Swiss albino ♀ mice (23 ± 3 g) were used. Food was withdrawn 6 h before ig propolis extract 10, 25, and 50 mg·kg^{-1}, indometacin (20 mg·kg^{-1}, in 5 % $NaHCO_3$), or ethanol/water mixture 10 mL·kg^{-1}. One hour later, each mouse received ip 0.6 % acetic acid 10 mL·kg^{-1} followed by 1 % Evans blue 10 mL·kg^{-1} injected into the rettoorbital plexus. Twenty-five minutes after dye injection, the mice were killed. Dye concentrations in the supernatant of peritoneal exudate were determined[8].

Croton oil-induced ear edema Croton oil at 3 % dissolved in acetone or propolis extract at 25 % (2.5 μL), 50 % (5 μL), and 75 % (7.5 μL) dissolved in croton oil/acetone was applied (10 μL) topically in the right ear pinna of ♀ OF1 mice (23 ± 2 g). The left pinna was control. The mice were killed 4 h later. Disks from the pinnae were taken with a punch 6 mm in diameter. The edema was expressed in terms of the weight difference between the inflamed and the control pinnae[9].

Acetic acid-induced writhing Swiss albino ♀ mice (21 ± 3 g) were given ig propolis extract 15, 25, and 50 mg·kg^{-1}, aspirin (68 mg·kg^{-1}) used as positive control, or vehicle 10 mL·kg^{-1} after 1 h writhing was induced by ip injection of 3 % acetic acid 300 mg·kg^{-1} and counted during 20 min from the time of injection[10].

Hot plate test The time interval between the moment of placing the swiss ♀ mice (22 ± 3 g) on a hot (56 ℃) plate and the first defence reaction (licking of the paws) was recorded 60 min after ig propolis extract 30, 40, and 50 mg·kg^{-1}, or vehicle 10 mL·kg^{-1}. Morphine sulfate 5 mg·kg^{-1} (positive control) was injected ip[10].

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Mouse tail keratosis test Swiss albino ♀ mice (23 ± 4 g) were used. Proximal tail was treated with 0.1 mL of propolis extract or ethanol/water mixture twice daily, 5 times per week for 3 wk. One day after the last treatment the mice were killed. Longitudinal sections (5 μm) of the tails were examined histologically for the presence of a granular layer or isolated granular cells induced in the previously parakeratotic skin areas (10 sequential scales per mouse). Quantitative values of orthokeratosis were obtained[11].

Statistical analysis Ten animals per group were used in all experiments. Paired t-test was used for the hot plate test. Nonparametric U-test was used for the tail keratosis test. For the other experiments, different groups were compared using a one-way ANOVA with completely randomized design and a Duncan's multiple comparison test.

RESULTS

Propolis extract 50 mg · kg^{-1} inhibited the weight (mg) of the granuloma 0.101 ± 0.02 (17.5 % of inhibition) ($P < 0.05$) *vs* vehicle (0.120 ± 0.03), indometacin, 0.086 ± 0.02 (27.5 % of inhibition), as well as the peritoneal capillary permeability in mice (A at 290 nm), 0.11 ± 0.03 (62 % of inhibition) *vs* vehicle (0.29 ± 0.06), indometacin, 0.12 ± 0.02 (59 % of inhibition *vs* vehicle). Lower doses of propolis 25 mg·kg^{-1} did not induce significant inhibition in the granuloma model, although 10 mg · kg^{-1} (0.31 ± 0.05) induced inhibitory effect, 20.6 % ($P <$ 0.05) *vs* control in the capillary permeability test. In the model of croton oil-induced pinna edema, propolis 25 % (2.5 μL) (7.6 ± 0.3), 50 % (5 μL) (6.87 ± 0.13), and 75 % (7.5 μL) (5.7 ± 0.12) inhibited the edema (19 %, 27 %, and 39 %, respectively) *vs* control (9.42 ± 0.2).

In the acetic acid-induced writhing test and the hot plate test propolis 25 and 50 mg · kg^{-1} showed an analgesic activity, 20.4 ± 7.7 (32.6 %) and 22.2 ± 2.7 (29.7 % of inhibition), respectively *vs* vehicle (30.2 ± 2.4), aspirin, 14.7 ± 2.3 (47.7 %). In the hot plate test propolis 40 and 50 mg·kg^{-1} showed analgesic narcotic activities, 8.1 ± 0.4 (47 %) and 8.2 ± 1.1 (50 %), respectively *vs* vehicle (6.1 ± 0.8), morphine 10 ± 2.0 (58 %). Lower doses did not induce any significant change.

Propolis extract also induced orthokeratosis (66 % *vs* 27 % with ethanol/water) (Fig 1).

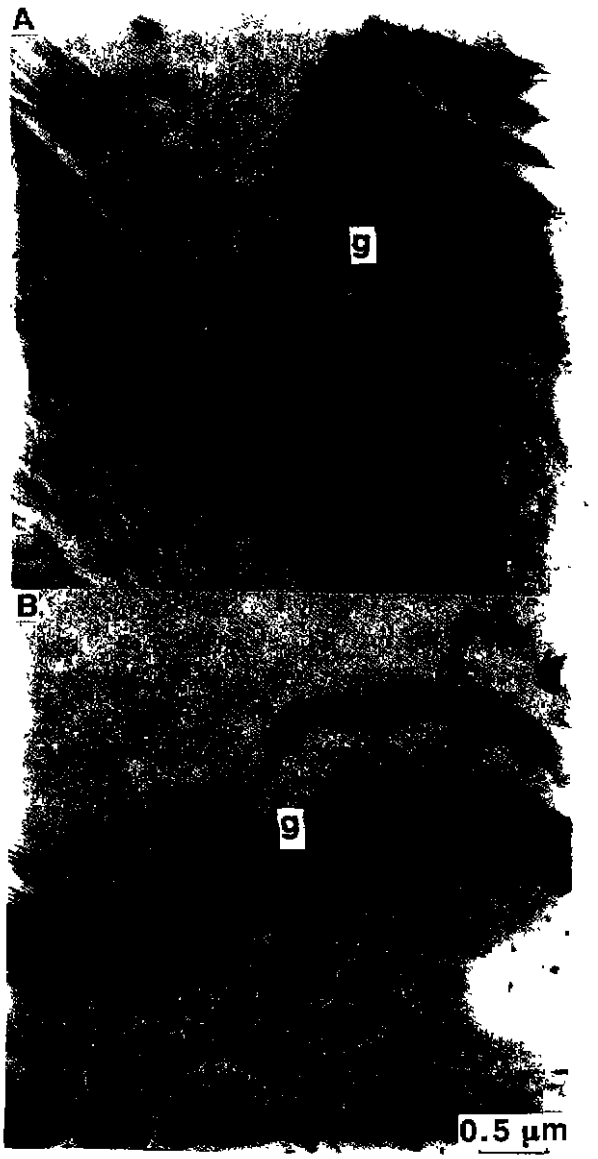

Fig 1. Keratosis tail of mouse. HE stain, ×200.
A) Untreated mouse as a normal characteristic, the granular layer (g) is only present at the level of the hair follicle.
B) Treated with propolis extract, the granular layer (g) is extended along the epidermis between 2 hair follicles.

DISCUSSION

Previously, other authors have reported anti-inflammatory and analgesic activities of propolis collected in other geographical areas with different climate and flora and they have ascribed to flavonoid compounds these pharmacological properties of propolis[12,13]. Research in our department about

chemical composition of this tropical red propolis collected in Cuba has not shown the presence of flavonoids in the tested samples by which it is conceivable that other compounds such as naphtoquinones (peaks 75, 80, and 81), anetol (peak 32), eugenol and methylisoeugenol (peaks 37 and 49), and elemicine (peak 56) identified in these samples of propolis by gas chromatography-mass spectrometry[6] might be able to induce the former pharmacological effects.

The finding of anti-inflammatory effects of propolis extract as well as results of a preliminary clinical trial in patients with psoriasis[14], encouraged us for elucidation of its potential antipsoriatic activity. Psoriasis is a chronic skin disease accompanied with an inflammatory reaction and itching that affects 1 % – 2 % of the general population[15]. Therefore, searching an animal model in which the classical drugs used in psoriasis such as dithranol and retinoic acid showed a good activity, we selected the modified mouse tail test[11], which is based on the induction of granular layer (orthokeratosis). In this model we found also a good antipsoriatic activity of red propolis extract.

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一种红蜂胶提取物治银屑病、消炎及镇痛作用

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关键词 银屑病; 蜂胶; 吲哚美辛; 角化病; 抗炎剂; 镇痛药

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