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Inflammation Research

Anti-allergic effects of natural tetranortriterpenoids isolated from *Carapa guianensis* Aublet on allergen-induced vascular permeability and hyperalgesia

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Abstract. *Objective:* We investigated the anti-allergic and analgesic properties of an oil and a derived fraction of tetranortriterpenoids (TNTP) obtained from the seeds of *Carapa guianensis* Aublet.
Materials and methods: Pleurisy, paw and ear edema were induced in Swiss and C57/Bl10 mice mice, whereas thermal hyperalgesia was assessed in Wistar rats (n = 6–10 per group). Values of $p < 0.05$ were regarded as significant.
Results: C. guianensis oil (100 to 400 mg/kg, p.o.) and TNTP (12.5 to 100 mg/kg, p.o.) inhibited pleural exudation, paw and ear edema induced by ovalbumin (OVA) in sensitized mice. TNTP (12.5 to 100 mg/kg, p.o.) also inhibited paw edema induced by histamine, PAF and bradykinin. TNTP (100 mg/kg, p.o.) inhibited prostaglandin E_2 generation in the pleural cavity in response to antigenic challenge. Moreover, *C. guianensis* oil (100 to 400 mg/kg) and TNTP (12.5 to 100 mg/kg) decreased OVA- and histamine-induced hyperalgesia.
Conclusion: Taken together, these findings demonstrate the anti-edematogenic and analgesic effects of *C. guianensis* oil, and points out TNTP as the responsible bioactive compounds.

Key words: *Carapa guianensis* Aubl. – Allergy – Pleurisy – Edema – Hyperalgesia – Inflammatory mediators

Introduction

Natural compounds are broadly recognized for their great structural diversity as well as their wide range of pharmaceutical activities. *Carapa guianensis* Aublet is a member of the Meliaceae family widely used in folk medicine in Brazil and other countries encompassing the Amazon rainforest. This species contains triterpenes, tetraterpenes, alkaloids and limonoids, which are phytochemically characteristic of all members of the Meliaceae family [for review see 1]. While assessing the chemical composition of the extract of *C. guianensis* seeds, Pereira and coworkers [2] isolated and characterized six different tetranortriterpenoid compounds: 6a-acetoxygedunin, 7-deacetoxy-7-oxogedunin, andirobin, gedunin, methyl angolensate and 6α-acetoxyepoxyazadiradione (Fig.1).

Analgesic and anti-inflammatory activities are among the most remarkable properties attributed by ethnopharmacological research to the oil extracted from *C. guianensis* seeds, specially for rheumatic pain and arthritis [3, 4]. Although many different substances isolated from plants are known to display anti-inflammatory and analgesic properties, to date no scientific studies supporting the use of compounds isolated from *C. guianensis* in treating inflammatory conditions have been published.

Inflammatory mediators, including histamine, bradykinin, serotonin, platelet activating factor (PAF) and leukotrienes, play an important role in allergic responses and inflammation, by inducing increased microvascular permeability to plasma proteins (and hence edema), as well as cellular infiltration and hyperalgesia [5–7]. Indeed, different reports describe a rapid increase in histamine, bradykinin and leukotriene levels in bronchoalveolar and nasal lavage fluids of sensitized rodents after antigen challenge [8–10]. Moreover, histamine H_1 or H_2 receptor blockade by antagonists such as meclizine and cimetidine were shown to inhibit paw edema and plasma pleural exudation induced by antigen in both rats and mice [11, 12]. Selective PAF antagonists are able to inhibit innumerous parameters related with asthma in different in vivo models, such as lung-edema formation, bronchoconstriction and bronchial hyperactivity after antigen challenge [13, 14]. The involvement of bradykinin in vascular permeability during allergic responses has also been broadly shown. Bradykinin B_2 receptor antagonists, such as HOE 140, attenuate airway microvascular permeability and

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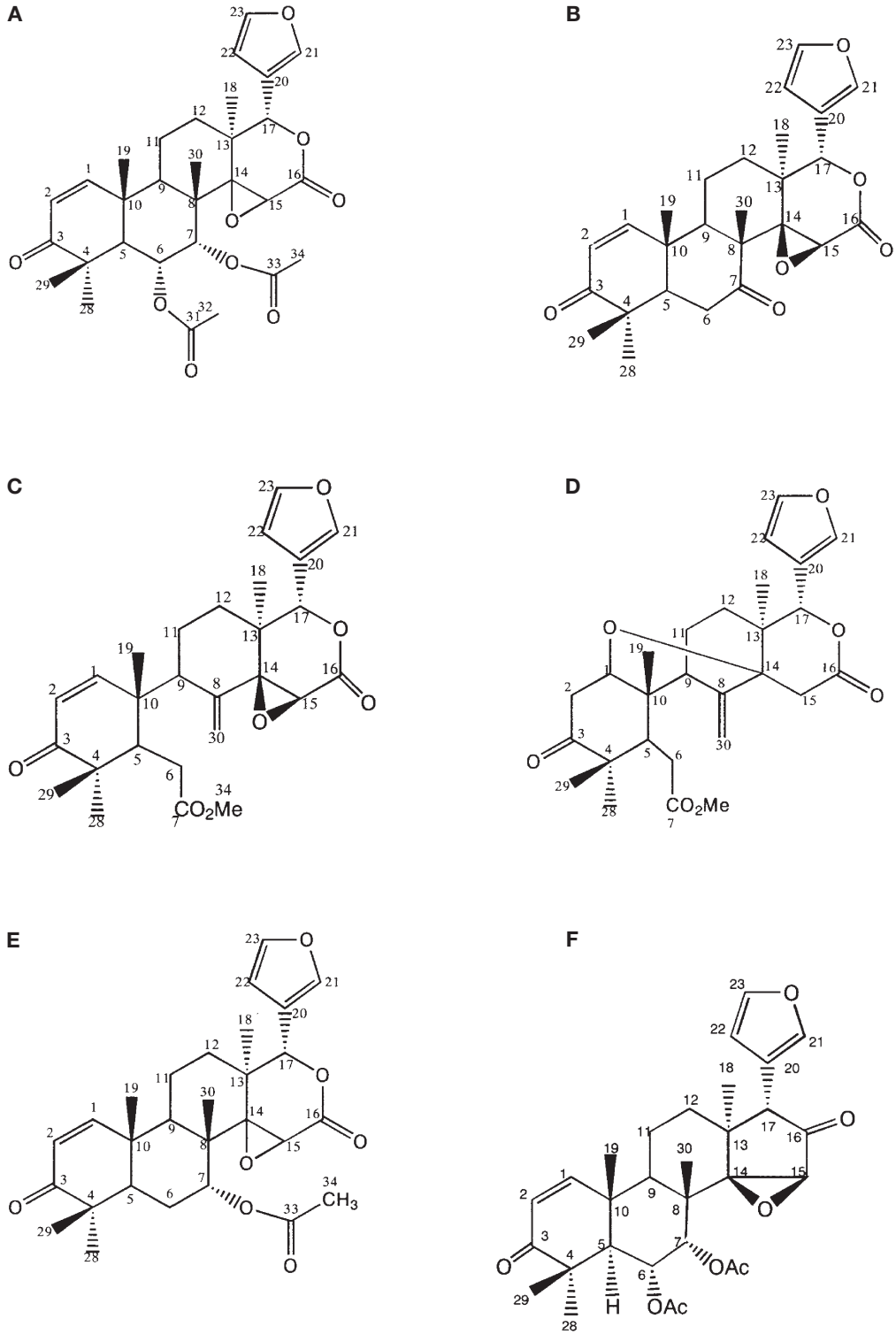

Fig. 1. Structure of tetranortriterpenoids (TNTP) isolated from the seeds of *C. guianensis*. 6α-acetoxygedunin (**A**), 7-deacetoxy-7-oxogedunin (**B**), andirobin (**C**), methyl angolensate (**D**), gedunin (**E**) and 6α-acetoxyepoxyazadiradione (**F**).

plasma leakage into the pleural cavity, trachea, bronchi and nasal mucosa of antigen-challenged rats and guinea-pigs [10, 15–18]. Bradykinin has also shown to be involved in hyperalgesia, since B_1 and B_2 receptor antagonists inhibited hyperalgesia in different experimental models of inflammation and allergy [19, 20].

In the present study, we have assessed the anti-allergic and analgesic effects of a chemically characterized standard oil obtained from the seeds of *C. guianensis* and of its tetranortriterpenoid- enriched fraction (TNTP) in different in vivo models, including ear and paw edema, pleurisy and thermal hyperalgesia. We also investigated the ability of TNTP to

influence the effects of various mediators which contribute to inflammatory and allergic processes.

Materials and methods

Animals

Male Swiss and C57/Bl10 mice weighing 20 to 25 g and Wistar rats weighing 200 to 250 g, provided by Oswaldo Cruz Foundation breeding unit (Rio de Janeiro, Brazil), were used. Animals were caged with free access to food and fresh water in a room with temperature ranging from 22 to 24 °C and a 12 h light/dark cycle at the FarManguinhos experimental animal facility unit until used. Animals were treated with vermifuge (Mebendazol, 20 mg/1000 ml of water) *ad libitum* for 3 days, and were used after another 3-day period, to exclude the possibility of eosinophilia induced by intestinal parasites. All experimental procedures were performed according to the Committee on Ethical Use of Laboratory Animals of Fundação Oswaldo Cruz (Fiocruz, Brazil) and to the ethical guidelines of International Association for the Study of Pain [21].

Drugs and reagents

Ovalbumin, histamine, bradykinin, PAF, HOE 140, dexamethasone, phosphate buffered saline (PBS) and Tween 20 were purchased from Sigma Chemical Co. (St. Louis, MO). WEB 2170 was obtained from Boehringer-Ingelheim (England, UK). Evans blue dye and formamide were obtained from Merck (Darmstad, Germany). Promethazine hydrochloride (i.e. Fenergan®), a histamine H_1 receptor antagonist, was obtained from Aventis (São Paulo, Brazil). Aluminum hydroxide and mebendazol were purchased from EMS Sigma Pharma (São Paulo, Brazil). Diclofenac was obtained from FarManguinhos (Fiocruz, Brazil). *Carapa guianensis* oil was purchased from Brasmazon© (Pará, Brazil), and presented oleic (52%), palmitic (28%), stearic (7.8%) and arachidic (1.2%) acids, when analyzed by gas chromatography (Hewlett-Packard 6890, USA), after alkaline hydrolysis and methylation with methyl iodide. The concentration of total tetranortriterpenoids in the crude oil reached 2% (w/w), as previously reported [22]. These compounds were precipitated from the oil during the extraction process, to yield the TNTP, which presented the following composition: de 6α-acetoxygedunin [($C_{30}H_{36}O_9$), 7%], 7-deacetyl-7-oxogedunin [($C_{26}H_{30}O_6$), 7%)], 6α-acetoxy-epoxyazadiradione [($C_{30}H_{36}O_8$), 7%)], methyl angolensate [($C_{27}H_{34}O_7$), 6%], andirobin [($C_{27}H_{32}O_7$), 4%] and gedunin [($C_{28}H_{34}O_7$), 3%], when analyzed by HPLC (Shimadzu LC10 AD, Japan) [2]. PGE_2 EIA kit was purchased from Cayman Chemical (Ann Arbor, MI).

Treatments

In designated experiments, dexamethasone was given intraperitoneally (i.p., 10 mg/kg) 1 h before stimulation, whereas HOE 140 (1 µg/paw, 50 µl) was administered into the paw immediately prior to stimulation. In another set of experiments, animals fasted overnight received either promethazine (10 or 30 mg/kg according to the stimulus or experimental model), WEB 2170 (16 mg/kg), diclofenac (100 mg/kg), *Carapa guianensis* oil (50–400 mg/kg) or TNTP (12.5–200 mg/kg) orally (p.o.) in a final volume of 200 or 400 µl (into mice or rats, respectively) 1 h prior stimulation. The control groups were similarly treated with the corresponding vehicle alone. It is noteworthy that *C. guianensis* oil (1000 mg/Kg) failed to induce toxicity as well as any alteration in peripheric and nervous system.

Ovalbumin sensitization

Active sensitization was achieved by a subcutaneous (s.c.) injection of 0.2 ml of a mixture of ovalbumin (OVA, 50 µg) and aluminum hydroxide (5 mg). Fourteen days later, animals were challenged by an intraplantar (i.pl.) injection of ovalbumin and used as described below. Sensitized mice challenged with vehicle alone were used as negative control group.

Induction of paw edema

In designed experiments, previously sensitized Swiss mice received an i.pl. injection of OVA (3 µg/paw), whereas naïve mice received histamine (100 µg/paw) or PAF (1 µg/paw) into one hind paw. In another set of experiments, Wistar rats were stimulated with i.pl. bradykinin (10 nmol/paw). In both instances, the final volumes were 50 µl/paw and the contralateral paw was injected with the same volume of the vehicle (PBS) and served as a control. The volumes of each hind paw were measured by using a plethysmograph (Ugo Basile, Italy) at different time points after stimulation, according to the stimulus. Paw edema is expressed in µl, as the difference between stimulated and non-stimulated paws.

Induction of ear edema

Ear edema was induced in naive Swiss mice by intradermal (i.d.) injection of histamine (10 µg/ear) into the upper side of mouse ear in a final volume of 25 µl, 24 h after intravenous (i.v.) treatment with Evans blue dye (25 mg/Kg in 50 µl). The opposite ear was injected with the same volume of vehicle alone. At 30 min after histamine injection, mice were killed by an excess of carbon dioxide and an 8-mm-diameter disc of tissue was punched from the center of each ear. Vascular permeability was estimated by Evans blue dye accumulation, measured as described below.

Vascular permeability measurements in mouse ear tissue

To extract Evans blue dye from the ear skin punch-outs, the tissues were minced and incubated in 500 µl of formamide at room temperature for 24 h. Evans blue in the supernatant was then quantified by measuring the absorbance of the formamide extracts at 600 nm with a spectrophotometer (Spectramax 190, Molecular Devices, CA).

Induction of pleurisy

Pleurisy was induced in Swiss mice by an intrathoracic (i.t.) injection of histamine (100 µg/cavity) diluted in sterile PBS to a final volume of 100 µl. The control group received an i.t. injection of 100 µl of sterile PBS. These animals received Evans Blue dye injection 24 h prior to stimulation as described above. At specific time points after i.t. injection, animals were killed by an excess of carbon dioxide and their thoracic cavities were rinsed with 1 ml of saline containing heparin (20 UI/ml). Total and differential cell counts were performed to monitor animals health conditions (data not shown). Alternatively, pleural washes recovered 1 h after i.t. histamine injection were processed for determination of Evans Blue content as described above.

Hot plate test

Hyperalgesia was evaluated in rats injected i.pl. with OVA (12.5 µg/paw) or histamine (100 µg/paw) in a final volume of 100 µl. The contralateral hind paw received the same volume of vehicle. One hour after stimulation, rats were individually placed on the center of a hot plate (Ugo Basile, Italy), confined in an acrylic cylinder. The temperature of the plate was previously adjusted to 56 ± 0.5 °C. The time elapsed until the animal displayed paw shaking, licking or jumping was

measured and considered as an index of nociceptive threshold. Hyperalgesia was calculated as the difference between the latencies to evoke reactions to noxious heat from stimulated and non-stimulated paws (Δ latency, in seconds). A cut off time of 20 s was used to minimize tissue damage.

PGE_2 assay

PGE2 levels were measured in cell-free pleural washes recovered from sensitized C57/Bl10 mice 16 h after OVA stimulation (12.5 µg/cavity). PGE_2 was assayed in duplicate by enzyme immunoabsorbant assay (EIA) according to the manufacturer's protocol (Cayman Chemical, Ann Arbor, MI).

Statistical analysis

Data are reported as the mean ± SEM and were analyzed statistically by means of analysis of variance (ANOVA) followed by Newman-Keuls-Student test or Student t test. Values of $p < 0.05$ were regarded as significant.

Results

Effect of Carapa guianensis oil and TNTP on OVA-induced paw edema

The i.pl. administration of OVA (3 µg/paw) into the hind paw of immunized mice triggered a significant paw edema 1 h after the injection, as shown in Figure 2 (A, B). Oral pre-treatment with *C. guianensis* oil inhibited edema formation at doses from 100 to 400 mg/kg (Fig. 2A), with a maximal inhibition of 48% achieved with 200 mg/kg. Pre-treatment with promethazine, an anti-histaminic compound, was also able to inhibit the paw edema at the dose of 30 mg/kg. Likewise, pre-treatment of mice with the TNTP from *C. guianensis* oil also inhibited OVA-induced paw edema at 12.5, 25, 50 and 100 mg/kg (Fig. 2B). Such results suggest that the compounds in TNTP contribute significantly to the anti-edematogenic effect of *C. guianensis* in allergen-induced paw swelling.

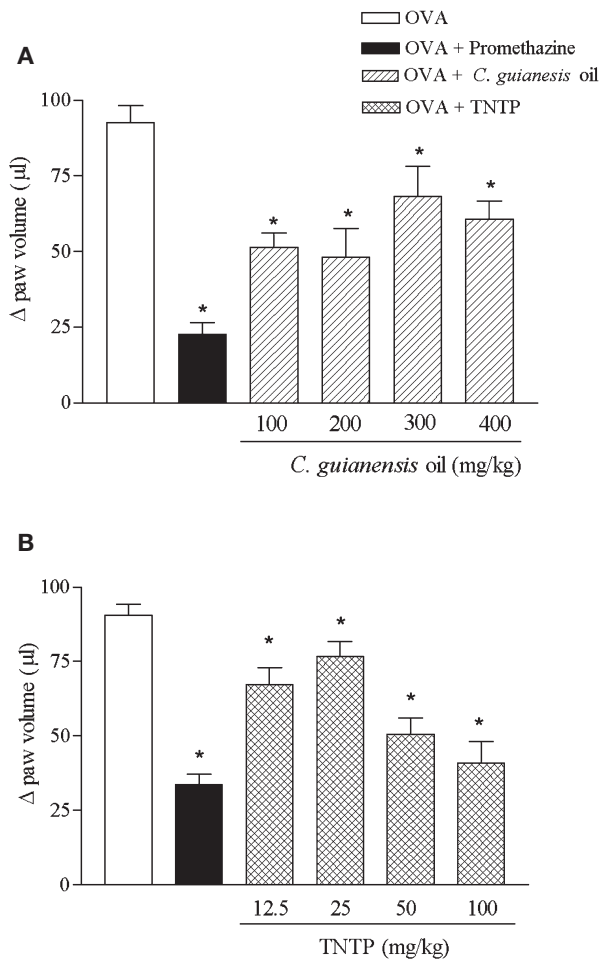

Fig. 2. Effect of *C. guianensis* oil (A) or TNTP (B) oral pre-treatment on OVA (3 µg/paw, i.pl.)-induced paw edema in Swiss mice. Promethazine (30 mg/Kg, p.o.) was used as reference inhibitor. Animals were treated 1 h before stimulation. Analysis was performed 1 h after stimulation. Results are expressed as the mean ± S.E.M. from at least 6 animals per group. Statistically significant differences ($p \leq 0.05$) between treated and non-treated groups are indicated by an asterisk.

Effect of Carapa guianensis oil and TNTP on histamine-induced paw edema

Histamine is an important mediator involved in allergic reactions, eliciting features such as vascular permeability and edema. As observed in Figure 3 (A, B), the i.pl. administration of histamine (100 µg/paw) into the hind paw of naive mice induced a significant paw edema 30 min after the injection, which was inhibited by promethazine administration (10 mg/kg, p.o.). The oral pre-treatment with *C. guianensis* oil inhibited the edema at doses ranging from 100 to 400 mg/kg, as shown in figure 3A, with a maximal inhibition of 71% (at 400 mg/kg). Histamine-induced paw edema was also significantly reduced following oral pre-treatment with TNTP at the doses of 12.5, 25, 50, 100 and 200 mg/kg (Fig. 3B; maximal inhibition of 81% at 12.5 mg/kg). Thus, it seems that the anti-edematogenic actions of *C. guianensis* oil and TNTP are largely due to an anti-histaminic effect. It is also noteworthy that topical administration of a cream formulation containing 31% of *C. guianensis* oil to the hind paw surface, 30 min prior to histamine stimulation, inhibited paw swelling to the same extent as promethazine cream (Fenergan©; 57.7% inhibition by promethazine and 54.5% inhibition by *C. guianensis* cream).

Effect of Carapa guianensis oil and TNTP on histamine-induced ear edema and pleural plasma exudation

As observed in Figure 4, histamine administration induced a significant ear edema and plasmatic protein exudation into the pleural cavity in naive mice, at 30 min or 1 h after stimulation, respectively. Oral administration of *C. guianensis* oil 1 h before histamine stimulation was able to inhibit significantly protein accumulation into ear (Fig. 4A) and pleural cavity (Fig. 4C), to the same extent as that seen following promethazine treatment. Pre-treatment with TNTP was also effective in inhibiting histamine-induced ear edema and plasma exudation at all doses tested (Fig. 4B and 4D).

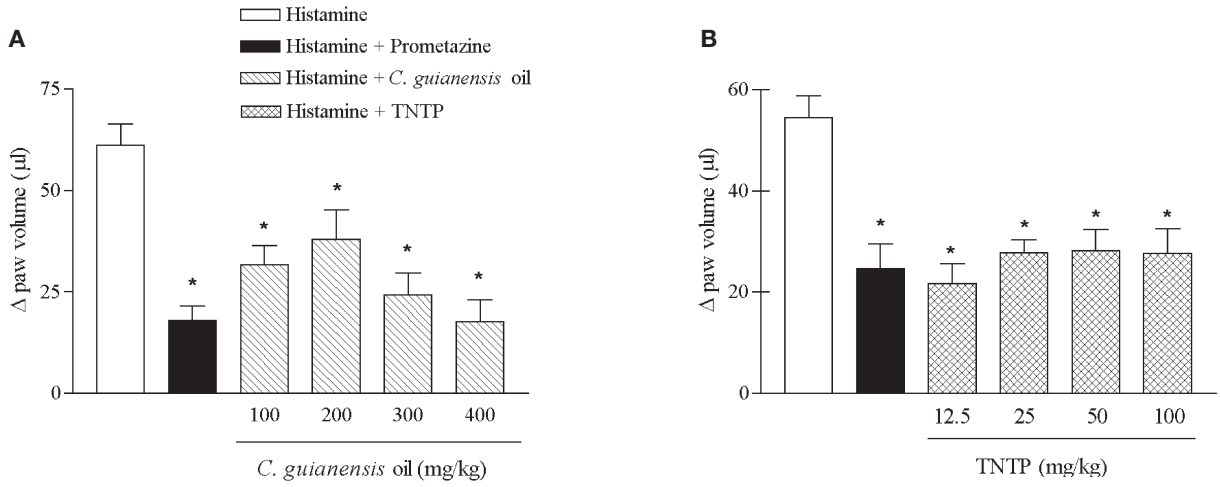

Fig 3. Effect of *C. guianensis* oil (**A**) or TNTP (**B**) oral pre-treatment on mice paw edema induced by histamine injection (100 µg/paw, i.pl.). Promethazine (10 mg/Kg, p.o.) was used as reference inhibitor. Animals were treated 1 h before stimulation. Analysis was performed 30 min after stimulation. Data represent the mean ± S.E.M. from at least 6 animals per group. Statistically significant differences ($p \leq 0.05$) between treated and non-treated groups are indicated by an asterisk.

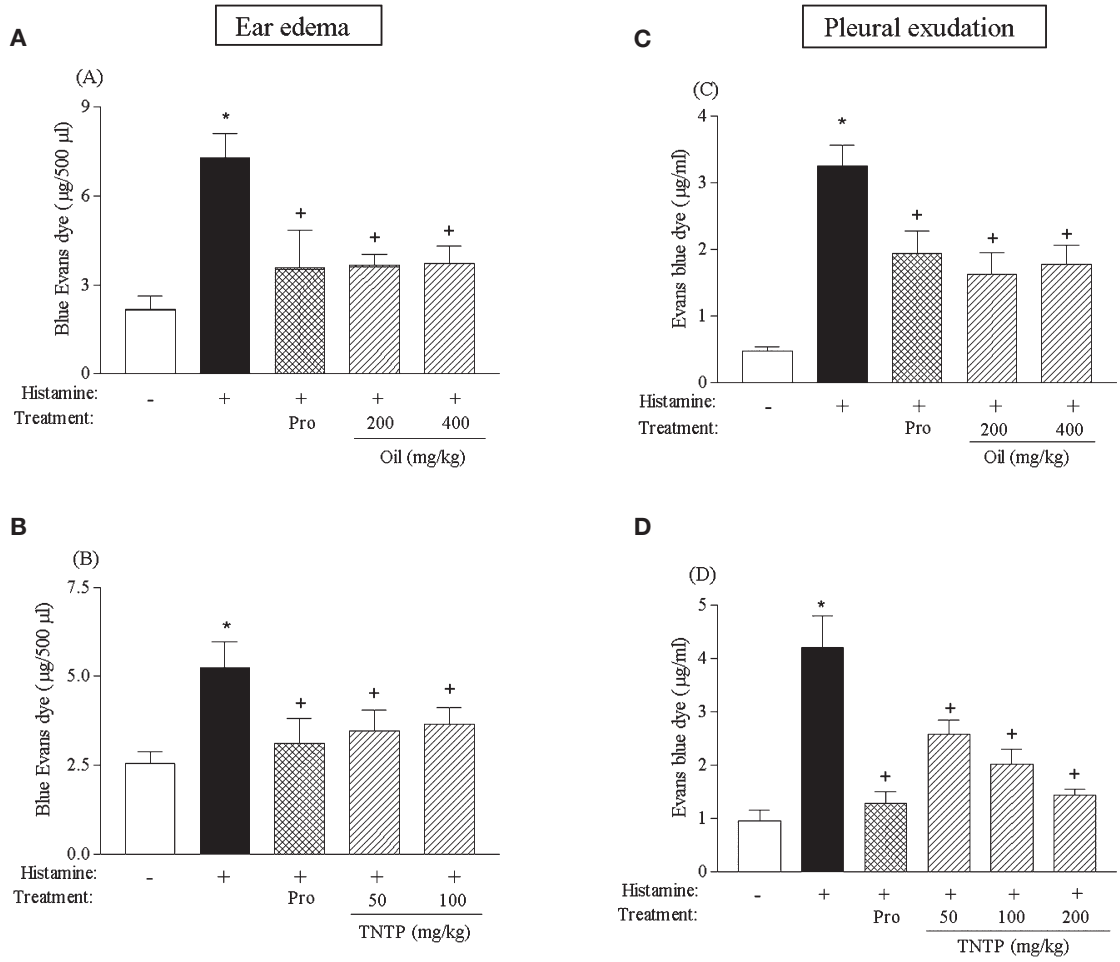

Fig 4. Effect of *C. guianensis* oil (**A**, **C**) or TNTP (**B**, **D**) oral pre-treatment on histamine-induced ear edema (10 µg/ear, i.d.; **A**, **B**) or pleural exsudation (100 µg/cavity, i.t.; **C**, **D**) in Swiss mice. Promethazine was used as reference inhibitor at 10 or 30 mg/Kg, p.o., for ear edema or pleurisy, respectively. Animals were treated p.o. 1 h before stimulation. Analysis was performed 30 min (ear edema) or 1 h (pleural exudation) after stimulation. Results express the mean ± S.E.M. performed with at least 6 animals per group. Statistically significant differences ($p \leq 0.05$) between agonist stimulated and non-stimulated values are indicated by an asterisk, whereas + indicates significant differences between treated and non-treated groups.

Inhibition by TNTP of paw edema induced by PAF and bradykinin

As shown in Figure 5 A, the i.pl. injection of 1 µg of PAF into the hindpaw of naive mice triggered paw swelling within 30 min, that was significantly inhibited by the PAF antagonist WEB 2170 (16 mg/kg, p.o., 1 h before stimulation). The administration of TNTP at 12.5, 25, 50 and 100 mg/kg was also able to significantly inhibit PAF-induced paw edema, with maximal inhibition of 55%, achieved with 25 mg/kg. The hindpaw edema seen 30 min after i.pl. administration of bradykinin (10 nmol/paw) in rats, which was significantly inhibited by simultaneous i.pl. HOE 140 (1 µg/paw) injection, was also sensitive to inhibition by oral administration of TNTP (Fig. 5B).

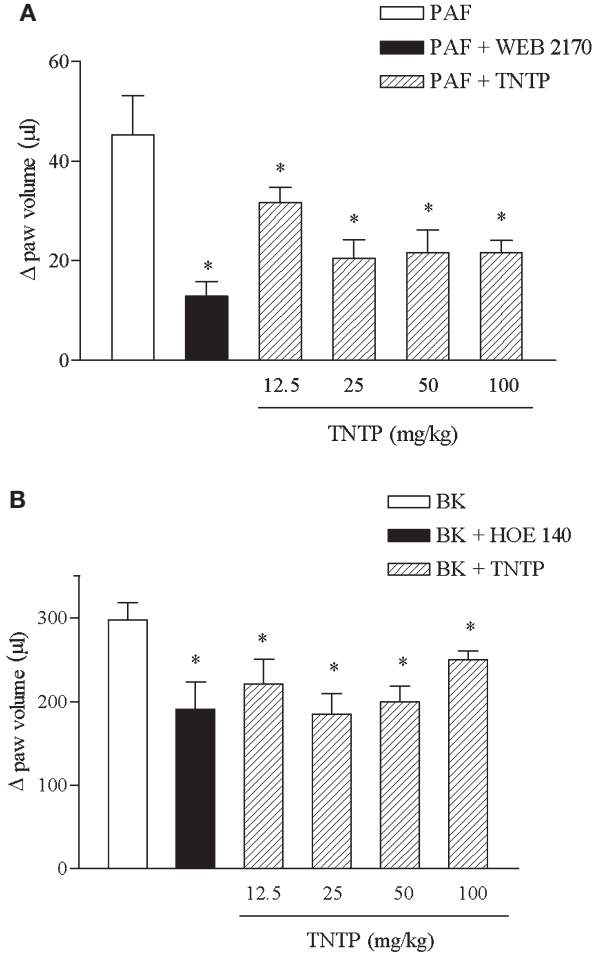

Fig 5. Effect of TNTP oral pre-treatment on paw edema induced by PAF (A, 1 µg/paw, i.pl.) or bradykinin (B, 10 nmol/paw, i.pl.) in Swiss mice or Wistar rats, respectively. WEB 2170 (16 mg/Kg, p.o.) was used as PAF antagonist, and HOE 140 (1 ≤ µ/paw, i.pl.) was used as bradykinin (B2) antagonist. Analysis was performed 30 min after stimulation. Asterisk indicates statistically significant differences ($p \leq 0.05$) between non-treated and treated groups. Data represents the mean ± S.E.M. from at least 8 animals.

Inhibition of hyperalgesia induced by OVA and histamine by Carapa guianensis oil and TNTP in rats

The i.pl. administration of OVA (12.5 µg/paw) induced thermal hyperalgesia in sensitized rats, as observed by the increase in Δ latency tin the hot plate test within 1 h of challenge (Fig. 6). Diclofenac (100 mg/kg, p.o.), a non-steroidal anti-inflammatory drug (NSAID), abolished the hyperalgesic effect of OVA (inset in figure 6), whereas promethazine (30 mg/kg, p.o.) failed to affect this response to OVA stimulation. *C. guianensis* oil exhibited a dose-dependent inhibition of OVA-induced hyperalgesia, with maximal inhibition of 91% observed with the dose of 400 mg/kg (Fig. 6).

As shown in figure 7 (A, B), histamine administration (100 µg/paw) also induced hyperalgesia in rats within 30 min. This hyperalgesic response to histamine was abolished by prior treatment with promethazine (30 mg/kg, p.o.; inset) and significantly inhibited by pre-treatment with *C. guianensis* oil (at 200 and 400 mg/kg, p.o.; full inhibition with highest dose) or TNTP (all doses tested, p.o.; maximal inhibition of 68%, observed with 50 mg/kg).

Effect of TNTP on PGE_2 generation in mice pleural cavity

The effect of TNTP pre-treatment on PGE_2 generation during the allergic response was investigated in C57/Bl10 mice pleural washes. Pleural washes were recovered 16 h after

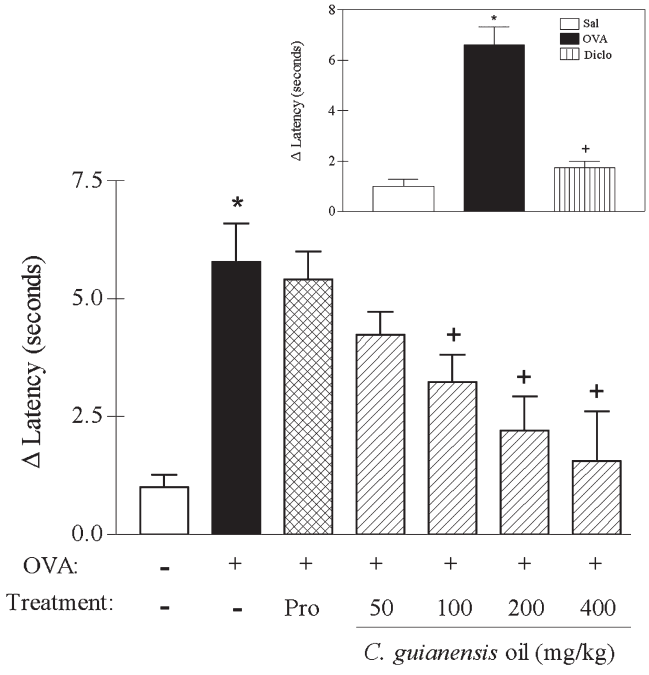

Fig 6. Effect of *C. guianensis* oil oral pre-treatment on hyperalgesia induced by i.pl. injection of OVA (A, 12.5 µg/paw) in Wistar rats. Promethazine (30 mg/Kg, p.o.) and diclofenac (100 mg/Kg, p.o.; inset) were used as reference inhibitors. Analysis was performed 1 h after stimulation. Results are expressed as the mean ± S.E.M. from at least 5 animals. Asterisk indicates statistically significant differences ($p \leq 0.05$) when compared with control values, whereas + indicates differences between stimulated and treated groups.

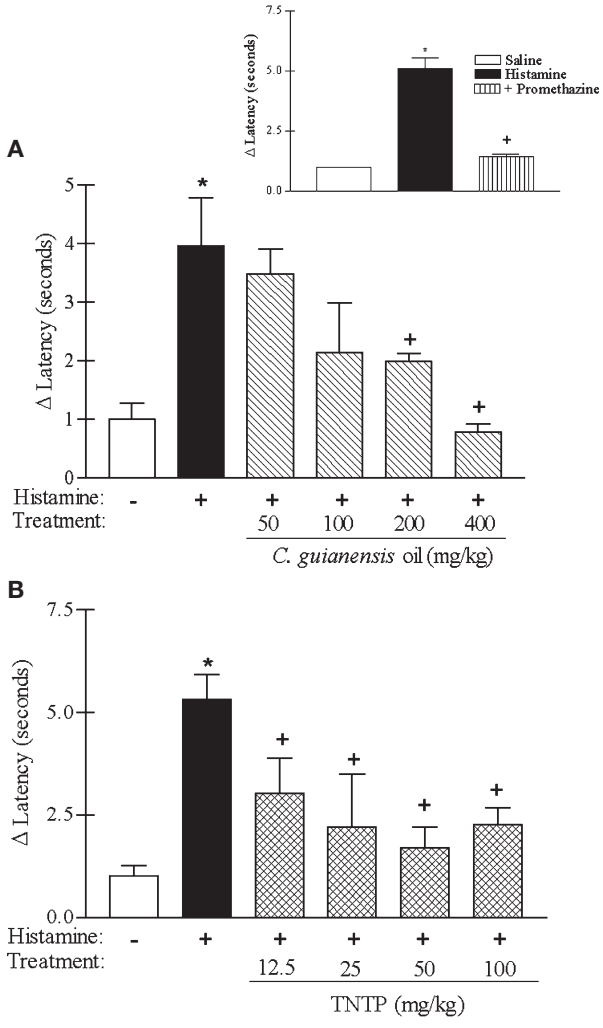

Fig 7. Effect of *C. guianensis* oil (**A**) and TNTP (**B**) oral pre-treatment on histamine (100 µg/paw)-induced hyperalgesia in Wistar rats. Promethazine (10 mg/Kg, p.o.) was used as reference inhibitor (inset). Analysis was performed 30 min after stimulation Results are expressed as the mean ± S.E.M. from at least 5 animals. Asterisk indicates statistically significant differences ($p \leq 0.05$) between non-treated and treated groups, whereas + indicates differences between stimulated and treated groups.

saline or OVA (12.5 µg/cavity) stimulation. As shown in Figure 8, the i.t. injection of OVA into previously sensitized mice induced pronounced generation of PGE_2 in the mice pleural cavity. TNTP pre-treatment (at 100 mg/kg, p.o.) suppressed PGE_2 production, thus closely mimicking the effect of dexamethasone (10 mg/kg, p.o., 1 h before stimulation).

Discussion

The proposed medicinal properties of *C. guianensis* have been attributed to the presence of limonoids, which are tetranortriterpenoids [1]. The results of the current study demonstrate that the oil obtained from the seeds of *C. guianensis* and also TNTP, i.e. the fraction containing the pool of tetranortriterpenoids, both display marked anti-allergic and anti-hyperalgesic properties in rodents.

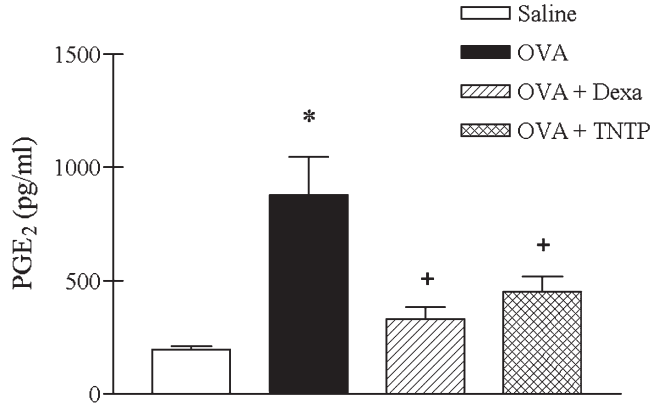

Fig 8. Effect of TNTP oral pre-treatment (100 mg/Kg) on PGE_2 levels on pleural washes recovered from saline- or OVA (12.5 µg/cavity, i.t.)-stimulated C57/Bl10 mice. Dexamethasone (10 mg/Kg, i.p.) was used as reference inhibitor. Analysis was performed by EIA in pleural washes recovered from 10 mice per group 16 h after stimulation. Asterisk indicates statistically significant differences ($p \leq 0.05$) when compared with control values, whereas + indicates differences between stimulated and treated groups.

The marked anti-edematogenic effect of *C. guianensis* oil pre-treatment in allergen-induced paw edema in mice was already maximal at the lowest dose tested (i.e. 100 mg/kg). In addition, the tetranortriterpenoids contribute substantially to the anti-allergic properties of *C. guianensis* seed oil, as TNTP was active in this model at a dose as low as 12.5 mg/kg, and at 100 mg/kg was eqüieffective to 30 mg/kg of promethazine, a well established anti-allergic compound. Since histamine is an important mediator of allergy, evoking features such as plasma leakage and edema, the anti-histaminic effects of *C. guianensis* seed oil and TNTP were evaluated in mice against histamine-induced hindpaw (and ear) edema and plasma protein extravazation into ear skin and pleural cavity [5, 14]. Indeed, pre-treatment with *C. guianensis* seed oil or TNTP each inhibited all these effects of histamine very effectively. It is especially noteworthy that the extent and potency of inhibition provided by TNTP against histamine-induced paw and ear edema as well as pleural exudation was similar to that of promethazine, the reference anti-histaminic compound tested.

Nonetheless, the mechanisms whereby *C. guianensis* seed oil and TNTP inhibit histamine-induced edema and leakage of plasma proteins might well differ from those underlying the actions of promethazine (i.e. histamine H_1 receptor blockade) [23], as TNTP was also effectively inhibited paw edema induced by PAF in mice and bradykinin in rats. It is well established that PAF induces important events associated with allergic inflammatory responses, such as vascular permeability enhancement [24–26], cellular influx [27, 14], expression of adhesion molecules and bronchial hyperresponsiveness [28, 29]. Also, it has been shown that allergen-induced paw edema in mice and plasma exudation into the pleural cavity of rats are inhibited by the PAF receptor antagonist WEB 2170 [13, 27]. Increased vascular permeability is one of the important features induced by bradykinin during allergic processes, and OVA-challenge induces an increase in bradykinin concentrations in bronchoalveolar lavage fluids, as well as the up-regulation of bradykinin

receptors in experimental models of airway inflammation [10, 30, 31]. Moreover, treatment with bradykinin antagonist, HOE 140, inhibited vascular leakage in the airways after OVA inhalation [10]. In light of such considerations, it appears that the anti-allergic properties of TNTP (and perhaps the *C. guianensis* seed oil from which it is derived) can be ascribed to inhibition of pro-inflammatory mechanisms triggered via histamine H_1, PAF and bradykinin B_2 receptors, rather than to blockade of these receptors themselves. However, further studies have to be carried out to fully elucidate the anti-allergic mechanisms of TNTP and *C. guianensis* seed oil.

Allergic reactions are frequently accompanied by the onset of pain (or nociception) and hyperalgesia (i.e. sensitization to noxious stimuli) [20, 32]. Indeed, Lavich and coworkers [20] showed that allergen-induced thermal hyperalgesia in rats is mediated by the synergistic effects of bradykinin, serotonin and histamine. In this regard, the present study also demonstrates that *C. guianensis* seed oil effectively caused graded inhibitions of OVA-induced thermal hyperalgesia in the rat paw. It is important to note that a 21-day treatment with *C. guianensis* oil at 1000 mg/kg failed to induce any alteration in peripheric and central nervous systems, somatic motor activity or behavior (data not shown). Such results suggest that the analgesia induced by *C. guianensis* was not a secondary effect of lethargy or other alteration of physical motility, but it is indeed very likely that it relies on the blockade of pro-inflammatory mediators. Despite the fact that Lavich et al. [20] reported that the antigen-induced hyperalgesia was amenable to inhibition by the selective H_1 receptor blocker meclizine, we failed to observe any antagonism by promethazine. This apparent discrepancy might be due to the differences between both models of sensitization to antigen. Nevertheless, we also found that thermal hyperalgesia triggered in the paw by histamine in non-sensitized rats was markedly attenuated by prior treatment with either *C. guianensis* seed oil or TNTP. In addition, it is possible to speculate that the suppressive actions of *C. guianensis* seed oil on antigen-induced hyperalgesia might involve the blockade of the sensitizing bradykinin and PAF, as we found that HOE 140 treatment inhibited OVA-induced hyperalgesia in rats (results not shown), and PAF causes hyperalgesia associated to a wheal and flare response in man [33]. Moreover, bradykinin induces hyperalgesia in the rat paw and joints [20, 34], and blockade or gene disruption of its receptors reduces hyperalgesia in inflammatory and allergic processes [19, 20, 35, 36].

It is well established that prostaglandins contribute most significantly to inflammatory and allergic reactions, playing key roles in generation of edema and hyperalgesia [for review see 37]. Indeed, it has been previously demonstrated that during allergic responses, cyclooxygenase expression and prostaglandin production are increased in rats, guinea pigs and human lungs [38–42]. Moreover, histamine [43], bradykinin [31, 44–46] and PAF [38] are potent stimulators of prostaglandin production. In this regard, we found that i.t. OVA challenge, in sensitized animals, markedly increased PGE_2 levels in the pleural exudate. In addition, the thermal hyperalgesia in response to i.pl. OVA was prevented by non-selective cyclooxygenase blocker diclofenac, a finding similar to the blockade by indomethacin of mechanical hyperalgesia induced by allergen reported by Cunha and coworkers [47]. The present demonstration that prior treatment with TNTP from *C. guianensis* seed oil, like the glucocorticoid dexamethasone, markedly attenuated the production of PGE_2 in the pleural cavity triggered by OVA strongly suggests that the anti-allergic effects of these compounds could be related to blockade of eicosanoid generation. However, it remains to be shown if this inhibition occurs at the level of expression or activity of phospholipase A_2 and/or cyclo-oxygenase(s), and/or to an additional mechanism(s).

In conclusion, the current results reveal that *C. guianensis* oil presents remarkable anti-allergic and anti-hyperalgesic activities, which are closely mimicked by its constituents, TNTP. The effects of this oil and derived TNTP are dependent on blockade of signaling mechanisms triggered by histamine, bradykinin and PAF, which stimulate prostaglandin E_2 formation. The precise molecular mechanisms underlying the anti-allergic and anti-hyperalgesic activities of *C. guianensis* oil and its tetranotriterpenoid compounds remain to be elucidated.

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