Cocos nucifera Oil Decreases Edema and Mechanical Hypernociception Induced by Bothrops jararacussu Venom in Mice

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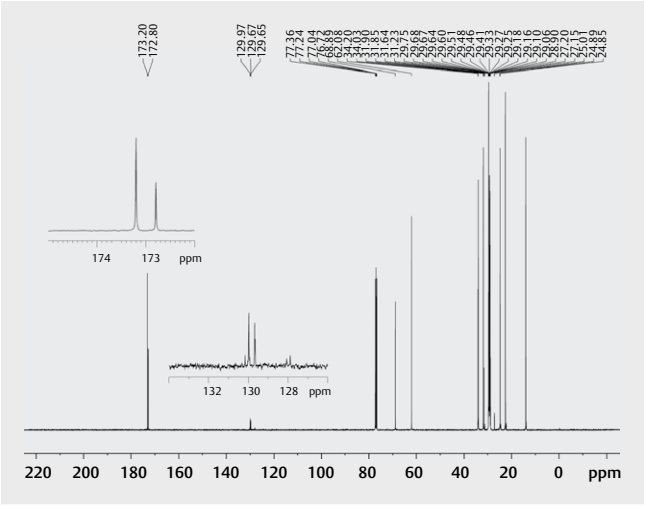
ABSTRACT

Bothropic venoms cause intense local damage, pain, edema, and myonecrosis. Cocos nucifera is the naturally most widespread fruit plant on Earth, and both the fruit and the plant have been used in folk medicine for the treatment of several inflammatory disorders. We evaluated the anti-inflammatory and analgesic effects of virgin coconut oil that was obtained from C. nucifera on paw lesions that were induced by venom from the Bothrops jararacussu snake in mice. Nuclear magnetic resonance spectroscopy was used to determine the chemical profile of virgin coconut oil. The analysis of the main components showed that saturated and unsaturated fatty acids were prominent components of the oil. Virgin coconut oil at doses of 100, 200, and 400 mg reduced local edema that was induced by B. jararacussu venom. The 200-mg dose of virgin coconut oil prevented edema that was induced by histamine, serotonin, and bradykinin. However, virgin coconut oil did not prevent edema that was induced by substance P or prostaglandin E_2. Virgin coconut oil also reduced peritoneal leukocyte infiltration that was induced by carrageenan and also decreased B. jararacussu venom-induced mechanical hypernociception of the paw. Virgin coconut oil exerted an anti-inflammatory effect on paw injury that was induced by B. jararacussu venom in mice, most likely by inhibiting leukocyte migration and reducing the action of the same inflammatory agents. The analgesic activity of virgin coconut oil appears to depend on opioid receptors.

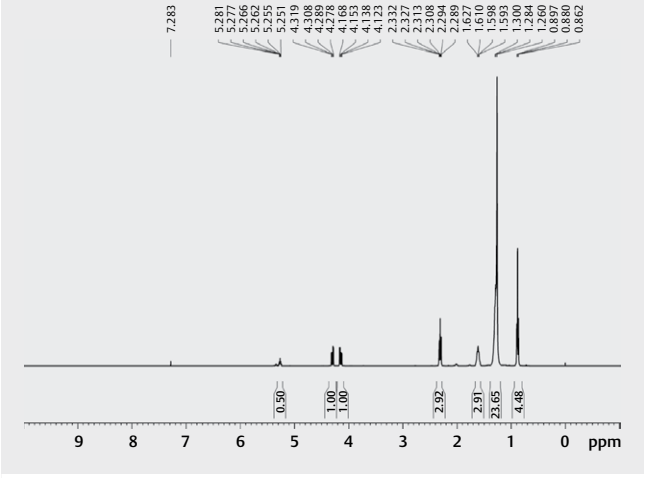
Introduction

Ophidic accidents are a concerning health problem, especially in tropical countries. In Brazil, most such accidents involve snakes of the Bothrops genus, the venom of which induces extensive local damage followed by marked edema, pain, erythema, ecchymosis, tissue necrosis, and extensive hemorrhage through alterations of platelet function [1, 2]. Several studies have shown that inflammatory mediators, such as histamine, bradykinin, and prostaglandin, may contribute to the amplification of local damage subsequent to an intraplantar injection of Bothrops venom, and commercial anti-venom does not effectively neutralize the inflammatory response [3–8].

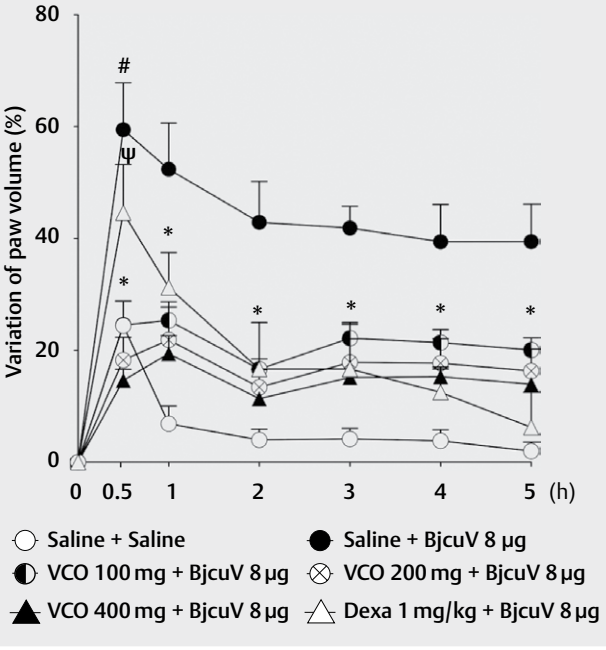
Nonsteroidal anti-inflammatory drugs (NSAIDs) can be used to treat inflammatory processes, including those that are elicited by snakebites [9]. However, the utility of NSAIDs is limited because of their side effects, such as gastric and duodenal ulceration and renal failure that occurs through the inhibition of prostaglandin synthesis [10]. Natural products with less toxic effects have been tested as alternative and complementary treatments for inflammatory responses that are related to ophidic accidents [11, 12].



▶ **Fig. 1** ^{13}C-NMR spectra of C. nucifera oil ($CDCl_3$, 100 MHz).



▶ **Fig. 2** ^{1}H-NMR spectra of C. nucifera oil ($CDCl_3$, 400 MHz).



▶ **Fig. 3** Effect of virgin coconut oil on BjcuV-induced paw edema in mice. The animals were orally pretreated with VCO (100, 200, or 400 mg), 0.15 M NaCl (0.2 ml), or dexamethasone (Dexa; 1 mg/kg, i.p.). Each point represents the mean ± SEM from 5 animals. * $P < 0.05$, significant difference from control group.

Plant extracts have been used in folk medicine to treat or attenuate several inflammatory conditions, including snakebites [12, 13]. The aqueous crude extract of husk fiber from Cocos nucifera L. (Arecaceae) is widely used in northeastern Brazilian folk medicine to treat diarrhea and arthritis, and several parts of the fruit and plant have been used by people in different countries for the treatment of several ailments [14, 15]. The crude extract and virgin coconut oil (VCO) from C. nucifera have been reported to have anti-inflammatory, analgesic, antimicrobial, and antiulcerogenic properties in experimental models in rodents [16–24].

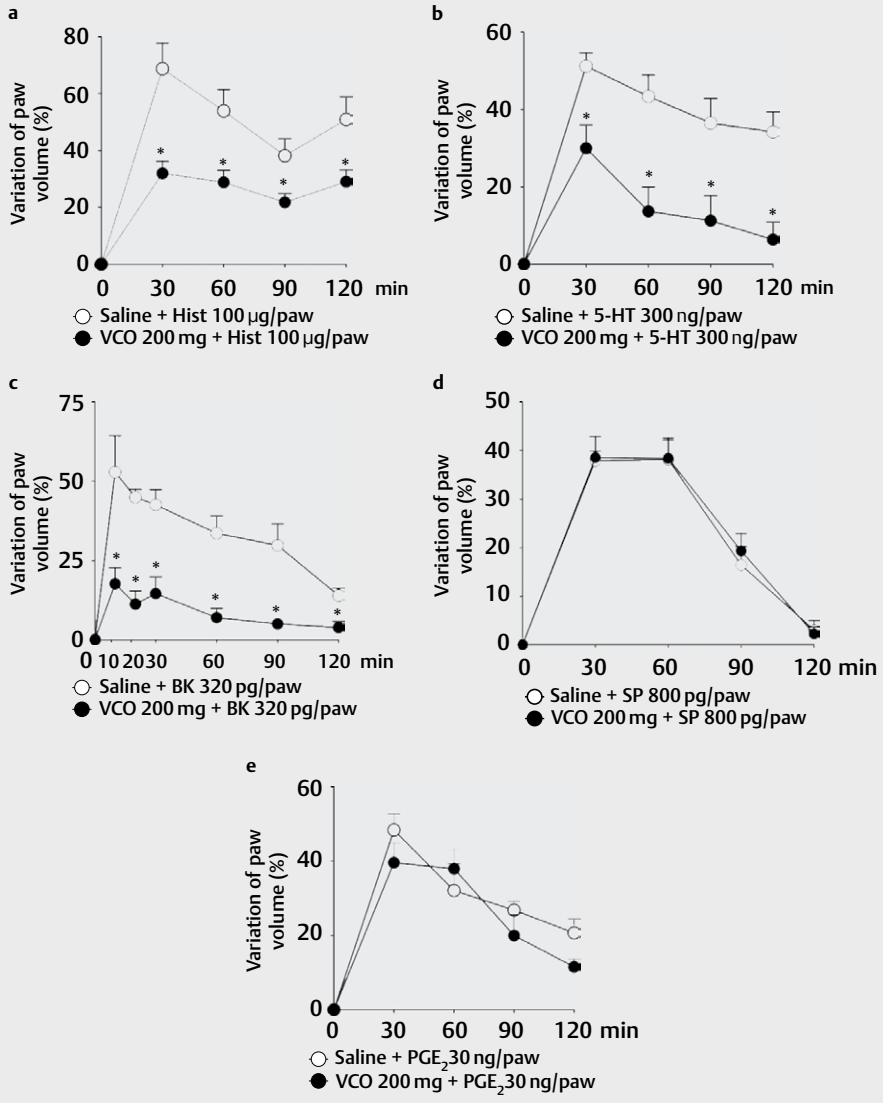
In the present study, we evaluated the possible anti-inflammatory and analgesic effects of VCO (C. nucifera) on paw injury that was induced by Bothrops jararacussu snake venom (BjcuV) and investigated the possible pharmacological mechanisms that underlie the effects of VCO.

Results and Discussion

The mixtures and composition of saturated and unsaturated fatty acids in the natural oils were identified by ^{13}C- and ^{1}H-NMR spectra [25]. The ^{13}C-NMR spectral data (▶ **Fig. 1**) indicated the presence of saturated fatty acids in VCO, with signals in the range of 14.00–34.00 ppm. In this region, there were many overlapping signals, and similar chemical shifts were observed for different fatty acids. The presence of unsaturated fatty acids was revealed by signals at 129.65 ppm (C_9) and 129.67 ppm (C_{10}), characteristic of the presence of oleic acid. Signals at 172.8 and 173.2 ppm were assigned to carbonyl groups of fatty acids [25]. The presence of olefinic protons of unsaturated fatty acids was confirmed by signals around 5.2 ppm in the ^{1}H-NMR spectrum (▶ **Fig. 2**).

This chemical composition of the VCO that we observed was consistent with the product packaging, in which the main fatty acids were caproic, caprilic, capric, lauric, meristic, palmitic, stearic, oleic, and linoleic acids. Some of these components were identified by gas chromatography of VCO that was collected from Chiang Rai province in Northern Thailand [18]. The presence of unsaturated acids (e. g., palmitic and stearic acids) and monounsaturated fatty acid (oleic acid) influences the membrane permeability of many organelles and suppresses enzyme activity (e. g., phospholipase A_2 [PLA_2]), leading to the suppression of inflammatory processes [18, 26].

These fatty acids from VCO may attenuate the inflammatory response that is elicited by BjcuV (▶ **Fig. 3**). The choice a bothropic venom as a phlogistic agent was based on a previous study that demonstrated that these snake venoms induce paw edema earlier than carrageenan, which has been widely used as a pharmacological agent to induce edema [2, 5, 7, 27]. Notably, neither mortality nor lethal toxicity occurred after VCO treatment or the BjcuV injection.



▶ **Fig. 4** Effects of virgin coconut oil on paw inflammation induced by different phlogistic agents. Edema was induced by histamine **a**, serotonin **b**, bradykinin **c**, substance P **d**, and prostaglandin E_2 (PGE_2) **e**. The animals were orally pretreated with VCO (200 mg) or 0.2 ml of 0.15 M NaCl. Each point represents the mean ± SEM from 5 animals. * $P < 0.05$, significant difference from control group.

▶ **Fig. 3** shows significant paw edema 30 min after the BjcuV injection, which was significantly decreased ($p < 0.05$) by 100, 200, and 400 mg VCO. The positive control dexamethasone decreased ($p < 0.05$) paw edema only 60 min after the BjcuV injection. These anti-inflammatory effects of VCO and dexamethasone were sustained for 5 h post-venom inoculation. The reduction of edema formation by pretreatment with the corticosteroid dexamethasone supports previous findings that the inflammatory response amplifies local tissue damage after bothropic inoculation [4, 7, 28].

The present results reinforce the potential therapeutic use of coconut products, which have been used as nutraceutical products in folk medicine for the treatment of various metabolic and inflammatory diseases [14, 15]. The use of C. nucifera in folk medicine has been supported by studies of the anti-inflammatory effects of a crude extract of C. nucifera in a rat model of carrageenan-induced paw edema [17, 20]. Similar anti-inflammatory activity of VCO (C. nucifera) has been observed, which may be associated with its polyphenol and fatty acid content [18, 19].

The significant suppressive effects of VCO on the first phase of carrageenan-induced paw edema formation in rats likely occur through an inhibitory action on the release or synthesis of early mediators of inflammation, such as histamine, serotonin (5-hydroxytryptamine [5-HT]), and bradykinin [18]. These mediators participate in the development of the early local inflammatory response to carrageenan. They act in vessels by inducing vasodilation and increasing vascular permeability, which are important triggers of edema formation [29, 30].

The 200-mg dose of VCO was used to investigate the possible mechanism of its anti-inflammatory effects. Rinald et al. [17] reported that a crude extract from husk fiber of C. nucifera decreased

paw edema 1 h after a histamine injection in rats. Oral VCO administration decreased ($p < 0.05$) acute inflammation that was induced by histamine 30 min after induction, possibly through H_1 receptor blockade (▶ **Fig. 4a**). This mechanism of action of VCO is different from dexamethasone, which modulates histamine by attenuating mast cell degranulation [31]. Previous studies showed that histamine and mast cells do not play a primary role in BjcuV-induced paw edema. The H_1 receptor antagonist loratadine and the compound 48/80 (a mast cell degranulator/depletor) did not prevent damage that was caused by BjcuV [7]. However, Landucci et al. [3] reported the significant participation of histamine, dependent from mast cell degranulation, in paw edema that was induced by 2 myotoxins (i. e., bothropstoxin-I and -II) that were isolated from BjcuV. These myotoxins have structures and activity that are similar to PLA_2. The present findings regarding the anti-histaminergic effect of VCO and the possible applicability of VCO in other experimental models of diseases (e. g., allergies and asthma) require further investigation.

Similar to histamine, serotonin is preformed in cytoplasmic granules of mast cells and platelets and has proinflammatory effects. Serotonin contributes to the sensitization of nerve fibers that is observed in inflammatory processes that are induced by Bothrops snakebites [5]. Furthermore, serotonin modulates the signal that is responsible for the chemotaxis of neutrophil migration during the innate immune response [32] and by modify the vascular permeability induce paw edema by 120 min later [33].

▶ **Fig. 4b** shows that VCO significantly decreased ($p < 0.05$) edema that was induced by serotonin. A similar result was reported for a crude extract from husk fiber of C. nucifera in rats [17]. The possible mechanisms of action of VCO may involve the modulation of 5-HT receptors. 5-HT receptors have been reported to participate in paw edema that is elicited by toxins from the venom of Bothrops jararacussu, Bothrops jararaca, and Bothrops lanceolatus [3–5]. The peripheral activity of serotonin that acts at 5-HT_1 and 5-HT_2 receptors modifies vascular permeability and indirectly allows the flow of other pronociceptive and proinflammatory factors, such as bradykinin and eicosanoids [34].

Bradykinins participate in the genesis of local edema and act as important pain mediators in the pathophysiological process of Bothrops snakebite accidents [35]. In the present study, to reproduce the effects of bradykinin, the animals were first intraperitoneally pretreated with 5 mg/kg captopril 30 min before the injection of bradykinin to inhibit the activity of the angiotensin-converting enzyme, which is involved in the biological degradation of bradykinin and blocked by Bothrops toxins [35]. Thus, the bradykinin injection resulted in edema formation, which peaked at 10 min and was attenuated ($p < 0.05$) by VCO treatment (▶ **Fig. 4c**).

The participation of bradykinin in the process of BjcuV-induced edema formation is still uncertain. Wanderley et al. [7] reported that pretreatment with the bradykinin receptor antagonist HOE140 did not prevent edema that was induced by BjcuV. Rioli et al. [6] reported the presence of a bradykinin-potentiating peptide in B. jararacussu toxin. Such compounds increase the recruitment and adherence of leukocytes and vasodilation.

In the present study, substance P induced paw edema, and this effect was not prevented by VCO pretreatment ($p > 0.05$; ▶ **Fig. 4d**). The desensitization of afferent C-fibers by capsaicin did not affect the formation of edema or migration of neutrophils to the site of injury that was caused by BjcuV [7]. Thus, we speculate that substance P does not participate in the inflammatory response that is induced by BjcuV. Our results indicate that VCO does not modulate substance P in this experimental mouse model of inflammation.

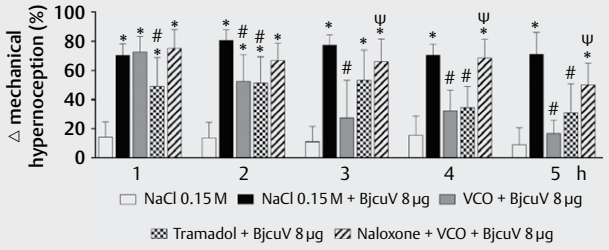
According to Zakaria et al. [19], VCO inhibits phlogistic mediators, such as autacoids. Prostaglandins, unlike many autacoids, are not stored in vesicles or other organic compartments, and they require synthesis that depends on the enzymatic activity of PLA_2 that is present in phospholipids within cell membranes. The product of this reaction is arachidonic acid, which is then acted upon by cyclooxygenase (COX), producing the final synthesis of prostaglandins. BjcuV increases the activity of COX_2, and prostaglandins are major inflammatory mediators that are involved in paw edema that is induced by BjcuV in mice [7]. We further investigated the effect of VCO on inflammation that was elicited by exogenous prostaglandin E_2. Pretreatment with VCO did not prevent ($p > 0.05$) edema that was triggered by prostaglandin E_2 (▶ **Fig. 4e**). We cannot exclude the possibility that the anti-inflammatory effect of VCO occurs through the attenuation of COX_2 activation that is elicited by BjcuV and not through the direct blockade of prostaglandin receptors.

Notably, BjcuV stimulates the migration of neutrophils to the paw in mice [7]. In a previous study, an aqueous crude extract of C. nucifera inhibited the inflammatory process that was induced by a subcutaneous injection of carrageenan by reducing leukocytes and protein extravasation [21]. In the present study, carrageenan increased the number of leukocytes in the peritoneal exudate ($7.74 \pm 0.23 \times 10^6$ cells/ml vs. $14.23 \pm 1.85 \times 10^6$ cells/ml), which was prevented by 200 mg VCO ($6.57 \pm 0.5 \times 10^6$ cells/ml) and dexamethasone ($6.20 \pm 0.68 \times 10^6$ cells/ml). These results suggest that VCO exerts anti-inflammatory effects at least partially through interactions with several of these pathways that are involved in leukocyte migration. Including the VCO to neutralize reactive oxygen species (ROS) that are produced by neutrophils in the inflammatory process [21, 26].

Intraplantar injections of B. jararaca venom caused hyperalgesia in rodents [36, 37]. Crude extracts and VCO of C. nucifera have been reported to have antinociceptive effects in different rodent models of pain [17–21]. We investigated the possible analgesic effects of VCO on hyperalgesia that was induced by BjcuV. Virgin coconut oil at a dose of 200 mg significantly decreased ($p < 0.05$) mechanical hypernociception that was induced by BjcuV 2 h after pain stimulation, and this analgesic effect persisted throughout the study. The positive control tramadol significantly decreased ($p < 0.05$) mechanical hypernociception 1, 2, 4, and 5 h after the BjcuV injection (▶ **Fig. 5**).

To elucidate the mechanism of the antinociceptive effect of VCO, the animals were pretreated with the opioid receptor antagonist naloxone. ▶ **Fig. 5** shows that naloxone blunted the antinociceptive effect of VCO on BjcuV-induced mechanical hypernociception 3 h after the BjcuV injection. A previous study also reported that the analgesic effects of C. nucifera occurred through opioid receptors [17].

In summary, in the present study VCO decreased paw edema that was induced by BjcuV and inflammatory mediators, including histamine, serotonin, and bradykinin. Virgin coconut oil also inhibited leukocyte migration to the inflammatory focus and decreased BjcuV-induced mechanical hypernociception. The



▶ **Fig. 5** Antinociceptive effect of virgin coconut oil on BjcuV-induced mechanical hyperalgesia in mice. The data represent variations in mechanical hypernociception that was evaluated for 5 h in mice that were orally treated with 0.2 ml of 0.15 M NaCl and then received an intraplantar injection of saline (30 µl, negative control) and in mice that were treated with 0.2 ml of 0.15 M NaCl (p.o., control), virgin coconut oil (VCO; 200 mg, p.o.), or tramadol (40 mg/kg, i.p., positive control) and then received an intraplantar injection of BjcuV (8 µg/paw) 60 min later. Other animals received VCO (p.o.) and then BjcuV, followed by naloxone. The data are expressed as the mean ± SEM from 5 animals per group. * $P < 0.05$, *vs*. negative control group; # $p < 0.05$, *vs*. control group; Ψ $p < 0.05$, *vs*. VCO group.

antinociceptive effects of VCO appeared to occur through opioid receptors. The present results support the possible therapeutic use of coconut products, which have been used a nutraceutical products in animals and humans for the treatment of inflammatory diseases.

Materials and Methods

Drugs and venom

Virgin coconut oil (Batch no. 91026716) was purchased from Copra Sul Natural Products. It was obtained from the fruit endosperm of C. nucifera. Lyophilized BjcuV was obtained from the Butantan Institute, São Paulo, Brazil. The venom was maintained at 20 °C and diluted in 0.9 % sterile saline. Dexamethasone (Decadron®, batch no. 1113428) was purchased from Aché Pharmaceutical Laboratories SA. Carrageenan, histamine, serotonin, bradykinin, substance P, and prostaglandin E_2 were purchased from Sigma. Tramadol (purity 99.7 %, batch no. AW 022/14) was obtained from Hipolabor. Naloxone (purity > 95 %, batch no. 08129231) was obtained from Cristália.

Nuclear magnetic resonance spectra

^{1}H- and ^{13}C-NMR spectra were acquired in $CDCl_3$ at 301 K using a BrukerAVANCE III 400 NMR spectrometer that operated at 9.4 Tesla, observing ^{1}H and ^{13}C at 400 and 100 MHz, respectively. The spectrometer was equipped with a 5-mm multinuclear inverse detection probe with a z-gradient. All of the ^{1}H- and ^{13}C-NMR chemical shifts are presented in ppm and were related to the TMS signal at 0.00 ppm as an internal reference. The coupling constants (J) are presented in Hz.

Animals

Female Swiss mice (n = 125; 25–30 g body weight) were housed at a temperature of 25 °C ± 2 °C under a 12 h/12 h light/dark cycle (lights on at 6:00 AM) with food and water available ad libitum (Purina Lab). All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA) and were approved by the Ethics Committee in Research of the Federal University of São Francisco Valley (protocol no. 12081038).

Effect of virgin coconut oil on paw edema induced by B. jararacussu venom

To investigate the local effects of BjcuV, the mice were divided into groups of 5 animals each. They received injections of the following in the subplantar region of the right hindpaw: 30 µl of 0.15 M NaCl (control group) or BjcuV (8 µg/paw) plus oral treatment with 100, 200, or 400 mg VCO in oil solution or 0.2 ml of 0.15 M NaCl. The VCO was weighed, and respective volumes were administered by gavage 1 h before the injection of BjcuV. In other experimental groups, dexamethasone (1 mg/kg, positive control) was administered intraperitoneally 1 h before the injection of BjcuV.

Paw edema was measured by plethysmography (PanLab 7500 water plethysmometer) immediately before (basal volume) and then hourly for 5 h after the BjcuV or saline injection. The results are expressed as the difference between the final and basal paw volumes (% variation of paw volume) [38].

Effect of virgin coconut oil on paw edema induced by different phlogistic agents

To investigate the possible mechanisms that are involved in the anti-inflammatory activity of VCO, additional groups of 5 animals each were orally pretreated with 0.2 ml of 0.15 M NaCl (control) or 200 mg VCO. One hour after pretreatment, paw edema was induced by an intraplantar injection of serotonin (300 ng/paw), histamine (100 µg/paw), prostaglandin E_2 (30 ng/paw), substance P (800 pg/paw), or bradykinin (320 pg/paw) in the right hindpaw [34]. Paw volume was measured immediately before (basal volume) and 30, 60, 90, and 120 min after the histamine, serotonin, prostaglandin E_2, and substance P injections. For bradykinin, paw edema was evaluated 10, 20, 30, 60, 90, and 120 min after the injection. The edema response was measure as described previously [38].

Effect of virgin coconut oil on carrageenan-induced peritonitis

To determine neutrophil migration to the peritoneal cavity, the mice were treated with 0.2 ml of 0.15 M NaCl solution (p.o.), 200 mg VCO (p.o.), or 1 mg/kg dexamethasone (i.p.). Each group consisted of 5 mice each. One hour later, 250 µl of carrageenan was administered (500 µg/cavity, i.p.). The mice were euthanized 4 h later, and the peritoneal cavity was washed with 1.5 ml of heparinized phosphate-buffered saline to harvest peritoneal cells. The recovered volumes were similar in all experimental groups and equivalent to ~95 % of the injected volume. Total cell counts were performed in a Neubauer chamber. The results are presented as the total number of leucocytes per milliliter of peritoneal exudate as previously described [39].

Effect of virgin coconut oil on B. jararacussu venom-induced mechanical hypernociception

The animals were first fasted for 18 h and then orally treated with 0.2 ml of 0.15 M NaCl (p.o., negative control), 200 mg VCO (p.o.),

or 40 mg/kg tramadol (i.p., positive control). One hour later, they received an intraplantar injection of 30 µl of 0.15 M NaCl or BjcuV (8 µg/paw) in the right hindpaw. Mechanical hypernociception was then assessed for 5 h. The mechanical nociceptive threshold was assessed by stimulating the hindpaws with a pressure meter that consisted of a handheld force transducer that was fitted with a 0.5 mm^2 polypropylene tip (electronic von Frey Digital Analgesymeter; Insight Instruments). In a quiet room, the mice were placed in acrylic cages (12 cm × 20 cm × 17 cm) with wire grid floors 1 h before the test. A tilted mirror was placed under the grid to provide a clear view of the hindpaw. The investigator was trained to apply the tip perpendicularly to the central area of the hindpaw using a gradual increase in pressure. The stimulus was discontinued and its intensity recorded when the paw was withdrawn. The end-point was characterized by removal of the paw in a clear flinch response after paw withdrawal. The difference in mechanical nociceptive thresholds before and after the BjcuV injection was calculated [40]. To assess the possible analgesic mechanism of action of VCO, the animals were treated with 1 mg/kg naloxone (i.p). 15 min later, they received 200 mg VCO (p.o.) to evaluate mechanical hypernociception that was induced by BjcuV.

Statistical analysis

The data are expressed as mean ± standard error of the mean (SEM). The data were analyzed using 2-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test, as appropriate. Values of $p < 0.05$ were considered statistically significant.

Acknowledgements

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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