In vivo Antinociceptive and Anti-inflammatory Activities of Dried and Fermented Processed Virgin Coconut Oil

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Key Words
Virgin coconut oil · Antinociceptive activity · Anti-inflammatory activity

Abstract
Objective: The present study was carried out to investigate the antinociceptive and anti-inflammatory activities of virgin coconut oil (VCO) produced by the Malaysian Agriculture Research and Development Institute (MARDI) using various in vivo models. Materials and Methods: Two types of VCOs, produced via standard drying (VCOA) and fermentation (VCOB) processes were used in this study. Both VCOA and VCOB were serially diluted using 1% Tween 80 to concentrations (v/v) of 10, 50 and 100%. Antinociceptive and anti-inflammatory activities of both VCOs were examined using various in vivo model systems. The antinociceptive activity of the VCOs were compared to those of 1% Tween 80 (used as a negative control), morphine (5 mg/kg) and/or acetylsalicylic acid (100 mg/kg). Results: Both VCOA and VCOB exhibited significant (p < 0.05) dose-dependent antinociceptive activity in the acetic acid-induced writhing test. Both VCOs also exerted significant (p < 0.05) antinociceptive activity in both phases of the formalin and hot-plate tests. Interestingly, the VCOs exhibited anti-inflammatory activity in an acute (carrageenan-induced paw edema test), but not in a chronic (cotton-pellet-induced granuloma test) model of inflammation. Conclusion: The MARDI-produced VCOs possessed antinociceptive and anti-inflammatory activities. Further studies are needed to confirm these observations.

Introduction
In addition to providing food, drink, and a key constituent of numerous cosmetic products, all of which have imparted wealth to various people throughout the world, \textit{Cocos nucifera} L., of the family \textit{Palmae}, more familiarly known as coconut, is also used in Malay traditional medicine to treat ailments such as fever, headaches, stomach upset and diarrhea. We have previously reported on the anti-inflammatory, antipyretic and wound healing properties of the fresh juice and kernel extracts of \textit{C. nucifera} [1].

Recently, the focus of research on \textit{C. nucifera} has shifted towards virgin coconut oil (VCO) and its benefits in the pharmaceutical, nutraceutical, cosmetic and industrial fields. The processes used to obtain VCO, which involve the use of controlled temperature, are believed to result in a preparation that has more beneficial effects than copra oil due to the greater retention of its active components, such as squalene, tocopherols and sterols [2]. Copra oil, on the other hand, is prepared by subject-
ing the copra’s crude oil to high temperatures up to 200°C to deodorize the oil. The advantage of using VCO over copra oil has been further supported by studies wherein VCO has been reported to have reduced total cholesterol, triacylglycerols, phospholipids, low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) cholesterol and to have increased high-density lipoprotein levels in serum and tissues compared to copra oil [3, 4]. Moreover, it has been reported that VCO is capable of increasing the activity of antioxidant enzymes and of reducing lipid peroxide levels and also has greater anti-thrombotic activity than copra oil [4, 5].

Despite such claims for C. nucifera, there have been no studies on the pharmacological properties of VCOs produced by the Malaysian Agricultural Research and Development Institute (MARDI) using animal models. Therefore, the aim of the present study was to investigate the potential role of MARDI-produced VCO as an antinociceptive and anti-inflammatory agent using various established in vivo animal models.

Materials and Methods

Samples
MARDI-produced VCOs, labeled as VCOA and VCOB, were donated by Dr. Kamariah Long from the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Malaysia. Briefly, VCOA was prepared using a standard drying method while VCOB was prepared via a fermentation process.

Preparation of VCO Concentrations
The freshly prepared VCOs, thought to have a concentration strength of 100%, were serially diluted in 1% Tween 80 (v/v) to 10% and 50% concentration levels. The 10%, 50% and 100% concentrations of each VCO were then used in the present study.

Preparation of Drugs
Acetylsalicylic acid (ASA) (100 mg/kg; Bayer, Singapore) and morphine (5 mg/kg; Sigma, Taufkirchen, Germany), used for the purpose of comparison, were prepared by dissolving them in distilled water (dH2O).

Experimental Animals
Male Balb-C mice (25–30 g; 5–7 weeks old) and Sprague-Dawley rats (180–200 g; 8–10 weeks old), obtained from the Animal Source Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia, were used in this study. All animals were housed at room temperature (27 ± 2°C; 70–80% humidity; 12 h light/darkness cycle) in the Animal Holding Unit, Faculty of Medical and Health Sciences, UPM for at least 48 h before use. Food and water were supplied ad libitum up to the beginning of the experiments. At all times, the rats were cared for in accordance with the current UPM principles and guidelines for the care of laboratory animals (reference code: UPM/FPSK/PADS/BRUUH/00221) and the UPM ethical guidelines for investigations of experimental pain in conscious animals as adopted from Zimmermann [6]. Test solutions were administered orally via gastric gavage. All experimental animals were killed by cervical dislocation according to the UPM ethical guidelines described earlier.

Antinociceptive Assays
Acetic Acid-Induced Abdominal Writhing Test. The abdominal writhing test was carried out according to the procedure described previously by Zakaria et al. [7]. Briefly, selected mice were divided into eight groups (n = 7) and pre-treated orally (p.o.) with 1% Tween 80 (control group), 100 mg/kg acetylsalicylic acid (ASA), VCOA or VCOB (10, 50 or 100%), respectively. Thirty minutes after the pre-treatment, each group was injected intraperitoneally (i.p.) with 0.6%/mg acetic acid (10 ml/kg) and the mice were placed in transparent Perspex observation boxes. After a 5-min lag period following the administration of acetic acid, the presence of contractions of abdominal muscle together with stretching of hind limbs (writhing effect) were cumulatively counted for 25 min. The number of writhing and stretching movements was used to express the percentage of analgesia using the following ratio:

\[
\% \text{analgesia} = \frac{\text{Mean of control group} - \text{Mean of test group}}{\text{Mean of control group}} \times 100
\]

Hot Plate Test. The hot plate test was performed using the method described previously by Zakaria et al. [8]. Before beginning the experiment, a group of untreated mice was subjected to a selection process by placing each on the Ugo Basile 7280 hot plate set at 53 ± 0.5°C. Only those mice with response latency times between 5–7 s were selected for this study. Briefly, selected mice were divided into eight groups (n = 7) and pre-treated orally (p.o.) with 1% Tween 80, 5 mg/kg morphine or, VCOA or VCOB (10, 50 or 100%), respectively. Thirty minutes after the pre-treatment, animals were tested. The latency time of response to discomfort, observed when the animal started licking its fore- or hind-paws or jumped when placed on the hot plate, was recorded immediately (0 min) and at 30, 60, 120, 180, 240 and 300 min after the administration of each test solution. A cut-off time of 20 s was adopted to avoid excessive tissue damage to the animals. A prolongation of latency time relative to untreated controls was taken as an indicator of antinociceptive activity.

Formalin-Induced Paw Licking Test. The method described by Zakaria et al. [9] was adopted in the present study. Rats were placed in the observation chamber to familiarize them with their surroundings for an initial 20 min of accommodation. Selected rats (n = 7) were pre-treated (p.o.) with 1% Tween 80, 100 mg/kg ASA, 5 mg/kg morphine or, VCOA or VCOB (10, 50 or 100%) 30 min prior to the intraplantar (i.pl) injection of 50 μl of 2.5% formalin into the right hind paw. The length of time following the injection of formalin the animal spent licking and biting the injected paw, which indicated pain, was recorded. Responses were recorded between 0 and 5 min, and 15 and 30 min after formalin administration – these represent the early and late phases of this test, respectively. The percentage of analgesia was calculated using the formula below:

\[
\% \text{analgesia} = \frac{\text{Mean of control group} - \text{Mean of test group}}{\text{Mean of control group}} \times 100
\]
**Anti-Inflammatory Assays**

**Carrageenan-Induced Paw Edema Test.** The procedure described by Zakaria et al. [10] was used to determine the anti-inflammatory activity of VCOs in this model of acute inflammation. Rats were divided into 5 groups (n = 7) and received (p.o.) 1% Tween 80, 100 mg/kg ASA or, VCOA or VCOB (10, 50 or 100%) following 30 min later by the administration (i.pl.) of 0.1 ml of a 1% carrageenan suspension into the right hind paw. Paw volume was measured using a plethysmometer (Model 7140, Ugo Basile, Italy). The degree of inflammation was quantified by measuring the volume displaced by the paw between the final volume (V_T) and the initial volume (V_0). The percentage of anti-inflammation was calculated using the formula:

\[
\% \text{ anti-inflammation} = \left( \frac{(V_T - V_0)}{(V_f T - V_f 0)} \right) \times 100
\]

where T = time interval.

**Cotton Pellet-Induced Granuloma Test.** The procedure described by Sulaiman et al. [11] was used to study the anti-inflammatory activity of VCOs in this model of chronic inflammation. Cotton pellets, weighing approximately 50 mg each, were autoclaved for 2 h. The autoclaved cotton pellets were implanted in intrascapular distance, or dorsum region, aseptically under the skin on the shaved backs of rats anesthetized with 1 ml/kg avertin (i.p.). The anesthetized rats were divided into 5 groups (n = 7) and received (p.o.) 1% Tween 80, 100 mg/kg ASA or, VCOA or VCOB (10, 50 or 100%). All test solutions were given once a day for a period of 7 days and the rats were killed on the 8th day by cervical dislocation according to the UPM ethical guidelines as described earlier. The wet cotton pellets, surrounded by granuloma tissue were dissected out, weighed, and then dried at 60°C and weighed again. The percentage of anti-inflammatory activity of VCOs, indicated by its ability to reduce the accumulation of granuloma tissue, was calculated using the following equation:

\[
\% \text{ anti-inflammation} = \left( \frac{T_C - T_T}{T_C} \right) \times 100
\]

where T_C = weight of granuloma tissue of the control group, and T_T = weight of granuloma tissue of the treated group.

**Statistical Analysis**

The results are expressed as mean ± SEM. Data for the abdominal constriction, formalin and cotton pellet-induced granuloma tests were analyzed using one-way analysis of variance (ANOVA) followed by Dunnet’s multiple comparison test among groups. Data for the hot plate and carrageenan-induced paw edema tests were analyzed using two-way ANOVA followed by Tukey’s post-hoc test among groups. p < 0.05 was taken as the limit of significance in all tests. All statistical analyses were carried out using the SPSS 11.0.0 for Windows (SPSS Inc., Chicago, Ill., USA) program.

**Results**

The antinociceptive profiles of VCOA and VCOB assessed using the acetic acid-induced abdominal constriction test in mice are given in table 1. Both VCOs exhibited concentration-dependent antinociception with 50% VCOB showing an antinociceptive activity that was significantly (p < 0.05) greater than that of 50% VCOA. Overall, the maximal antinociceptive activities elicited by both VCOs were significantly (p < 0.05) lower than that obtained with 100 mg/kg ASA.

The antinociceptive profiles of VCOA and VCOB assessed using the hot plate test in mice are given in table 2. Both VCOs exhibited significant (p < 0.05) antinociceptive activity with a maximal effect exhibited by the intermediate concentration. Overall, based on the results obtained, all concentrations of VCOB exhibited greater antinociceptive activity when compared to the respective concentration of VCOA. In addition, only 10 and 50% VCOA exhibited a loss of activity at the end of the study (at interval 6 h). Overall, 5 mg/kg morphine exerted an antinociceptive activity that was greater than that of either of the VCOs at all concentrations investigated.

The antinociceptive profiles of VCOA and VCOB assessed using the formalin test in rats are given in table 3. Comparison with the control group (1% Tween 80) showed that both VCOs reduced the latency of discomfort induced by formalin in both phases, indicating their antinociceptive activity. Interestingly, only 10% VCOB exhibited significant (p < 0.05) antinociceptive activity in the early phase compared to 10% VCOA (p ≥ 0.05). Overall, 5 mg/kg morphine exhibited activity that was greater than that of either of the VCOs in both phases of the formalin test, while 100 mg/kg ASA produced antinociception that was greater than that of either VCO in the late phase.

**Table 1.** Antinociceptive activity of MARDI-produced VCOs assessed by the acetic acid-induced writhing test in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>No. of writhing responses ± SEM</th>
<th>Percentage of analgesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>groups (n = 7)</td>
<td>strength, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1% Tween 80</td>
<td>–</td>
<td>47.6 ± 2.4</td>
<td>–</td>
</tr>
<tr>
<td>100 mg/kg ASA</td>
<td>10</td>
<td>12.2 ± 1.1*</td>
<td>74.4</td>
</tr>
<tr>
<td>VCOA</td>
<td>50</td>
<td>36.4 ± 1.7*</td>
<td>23.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>30.1 ± 2.2*</td>
<td>36.8</td>
</tr>
<tr>
<td>VCOB</td>
<td>10</td>
<td>18.3 ± 1.7*</td>
<td>61.6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>31.3 ± 1.6*</td>
<td>34.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>22.5 ± 0.8*</td>
<td>52.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.9 ± 2.1*</td>
<td>56.1</td>
</tr>
</tbody>
</table>

ASA = Acetylsalicylic acid; VCOA = virgin coconut oil A; VCOB = virgin coconut oil B. The writhing response was expressed as mean ± SEM.

* p ≤ 0.05 compared with the 1% Tween 80-treated group.

# p < 0.05 compared with the 50% VCOB-treated group.

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Table 2. Antinociceptive activity of MARDI-produced VCOs assessed by the 50°C hot plate test in mice

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Concentration strength, %</th>
<th>Latency of discomfort (s)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>1% Tween 80</td>
<td>–</td>
<td>7.4 ± 0.2</td>
<td>7.9 ± 0.4</td>
</tr>
<tr>
<td>5 mg/kg morphine</td>
<td>–</td>
<td>7.8 ± 0.6</td>
<td>15.9 ± 0.8*</td>
</tr>
<tr>
<td>VCOA</td>
<td>10</td>
<td>7.4 ± 0.5</td>
<td>8.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>7.7 ± 0.3</td>
<td>7.8 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7.3 ± 0.6</td>
<td>9.2 ± 0.1*</td>
</tr>
<tr>
<td>VCOB</td>
<td>10</td>
<td>7.2 ± 0.5</td>
<td>8.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>7.3 ± 0.6</td>
<td>10.5 ± 0.8*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7.4 ± 0.1</td>
<td>10.2 ± 0.5*</td>
</tr>
</tbody>
</table>

VCOA = Virgin coconut oil A; VCOB = virgin coconut oil B. The latency for licking of the hind paws, shaking or jumping off from the surface was expressed as mean ± SEM.

* p ≤ 0.05 compared with the 1% Tween 80-treated group. # p ≤ 0.05 compared with the 10% VCOA-treated group at the respective time interval. $ p ≤ 0.05 compared with the 50% VCOA-treated group at the respective time interval. ^ p ≤ 0.05 compared with the 100% VCOA-treated group at the respective time interval.

The present study has indicated the potential of MARDI-produced VCOs as antinociceptive and anti-inflammatory agents, and validated the traditional use of C. nucifera in the treatment of pain- and inflammation-related ailments such as fever and headache. The VCOs were found to exhibit antinociceptive activity when assessed using the abdominal constriction and hot plate tests, thus indicating their effectiveness in blocking peripheral and centrally mediated nociception induced by chemical and thermal stimuli [12, 13]. Furthermore, because both VCOs inhibited the hot plate test, this suggested their ability to block a noninflammatory, acute nociceptive input. The peripheral and central effects of VCOs on nociceptive processes were further confirmed by the effectiveness of VCOs in attenuating the formalin test [14]. The antinociceptive effects of the VCOs seen in both phases of the formalin test indicated their ability to directly block the formalin-induced nociceptor stimulation seen with the early phase and to block the subsequent antalgia seen with the late phase.

**Discussion**

The present study has indicated the potential of MARDI-produced VCOs as antinociceptive and anti-inflammatory agents, and validated the traditional use of C. nucifera in the treatment of pain- and inflammation-related ailments such as fever and headache. The VCOs were found to exhibit antinociceptive activity when assessed using the abdominal constriction and hot plate tests, thus indicating their effectiveness in blocking peripheral and centrally mediated nociception induced by chemical and thermal stimuli [12, 13]. Furthermore, because both VCOs inhibited the hot plate test, this suggested their ability to block a noninflammatory, acute nociceptive input. The peripheral and central effects of VCOs on nociceptive processes were further confirmed by the effectiveness of VCOs in attenuating the formalin test [14]. The antinociceptive effects of the VCOs seen in both phases of the formalin test indicated their ability to directly block the formalin-induced nociceptor stimulation seen with the early phase and to block the subsequent antalgia seen with the late phase.
release of inflammatory mediators. The anti-inflammatory activity of VCOs was demonstrated with the carrageenan-induced rat paw edema test, but not the cotton pellet granuloma test. The former, usually used as a basic test to study the anti-inflammatory activity of compounds/bioactive agents, is considered to be an acute model of inflammation [9, 15]. The ability of both VCOs to reduce paw edema therefore indicates their ability to reduce acute inflammation. However, both VCOs were inactive in the chronic model of inflammation.

To speculate on the possible mechanisms involved in the ability of both VCOs to attenuate acute, but not chronic, inflammation, it is worth mentioning the different types of mediators that are found in both phases of inflammation. During acute inflammation, signaling molecules and chemoattractants (e.g. bradykinin, histamine, serotonin, nitric oxide, eicosanoids, etc.) are released at the site of an infection or injury by various types of cells (such as mast cells, platelets, nerve endings, endothelial cells, and other resident cells) [16]. Chronic inflammation, on the other hand, is mediated primarily by macrophages that are involved in perpetuating the pro-inflammatory response via the release of various chemical mediators (i.e. IL-1, IL-6 and tumor necrosis factor [TNF]-α). Based on the difference between the types of inflammatory mediators released during acute and chronic inflammations, and the ability of both VCOs to inhibit it only the former, it is speculated that VCOA and VCOB act against signaling molecules and chemoattractants that are short-lived and released at the site of an infection or injury rather than those released during chronic inflammation. Another mechanism might involve the attenuation of ROS activity. This might involve antioxidant activity of both VCOs associated with the presence of polyphenolic compounds [unpubl. data]. Other than that, it can also be speculated that the VCOs possess a short duration of action and are rapidly metabolized and eliminated from the body. This could explain their failure to affect the chronic inflammatory processes seen with cotton pellet-induced granuloma test. However, further studies are required before a final conclusion could be reached on this matter.

VCO has been reported to contain polyphenols [2, 3] and polyphenols have been reported to possess various biological actions, including antinociceptive and anti-inflammatory activities [17, 18] and to affect signal transduction, activation of pro-inflammatory transcription factors and gene expression [19]. In a recently submitted study, we have demonstrated the antiulcerogenic and antioxidant activities of VCOA and VCOB [unpubl. data] and have shown that these activities are due, at least in part, to their polyphenol content. The total antioxidant activity and total phenolic content of VCOA and VCOB were 75.7 and 82.4%, and 12.8 and 23.5 mg GAE/100 g oil, respectively. Thus, the presence of polyphenolic compounds may contribute to the observed antinociceptive and anti-inflammatory activities of VCOA and VCOB.

In another study, the antihypercholesterolemia activity and fatty acid composition of VCOA and VCOB was explored [20]. The main fatty acids found in VCOA in-
included lauric (50%), myristic (18%), caprylic (9%), palmitic (7%), capric (7%), oleic (5%) and stearic (3%) acids, while those of VCOB include lauric (47%), myristic (19%), palmitic (9%), caprylic (8%), oleic (6%), capric (6%) and stearic (3%) acids. Fatty acids such as oleic and stearic acids have been shown to attenuate the activity of polymorphonuclear leukocytes leading to the suppression of inflammatory processes [21]. It is suggested that the proportion of fatty acids integrated into membrane phospholipids affect membrane fluidity, which in turn can influence cell function. This is due to the fact that membrane fluidity is affected by the chain length and saturation of phospholipid fatty acids [21]. The presence of unsaturated (i.e. palmitic and stearic acids) and monounsaturated (i.e. oleic acid) fatty acids in both VCOs undoubtedly influences membrane fluidity and will affect many cell functions, including PMN activation. It has been claimed that compounds with anti-inflammatory activity might also exhibit antinociceptive activity [9]. It is therefore plausible to suggest that the pharmacological activities observed in the present study could be associated with the presence of polyphenols and fatty acids in both VCOs.

Conclusion

The MARDI-produced VCOs possess antinociceptive and anti-inflammatory activities with the former mediated via peripheral and central nociceptive mechanisms while the later being effective only against the acute model of inflammation, and thus requiring further in-depth investigation.

Acknowledgements

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