Anti-ulcerogenic activity of virgin coconut oil contribute to the stomach health of humankind

Malarvili Selverajah, Zainul Amiruddin Zakaria, Kamariah Long, Zuraini Ahmad, Azhar Yaacob, Muhammad Nazrul Somchit

ABSTRACT

The aim of the present study was to determine the antulcer potential of virgin coconut oil (VCO), either extracted by wet process (VCO_A) or fermentation process (VCO_B), and to compare their effectiveness against the copra oil (CO) using the HCl/ethanol-induced gastric ulcer model. Earlier, the oils underwent chemical analysis to determine the free fatty acids composition, physicochemical properties and anti-oxidant capability. In the antulcer study, rats (n = 6) were pre-treated orally for 7 consecutive days with distilled water (vehicle), 100 mg/kg ranitidine (positive group) or the respective oils (10, 50, and 100% concentration). One hour after the last test solutions administration on Day 7th, the animals were subjected to the gastric ulcer assay. Macroscopic and microscopic analyses were performed on the collected rat’s stomachs. From the results obtained, the chemical analysis revealed i) the presence of high content of lauric acid followed by myristic acid and palmitic acid in all oils and; ii) the significant (p < 0.05) different in anisidine- and peroxide-value, percentage of free fatty acid, total phenolic content and total antioxidant activity among the oils. The animal study demonstrated that all oil possess significant (p < 0.05) antulcer activity with VCO_B being the most effective oil followed by VCO_A and CO. The macroscopic observations were supported by the microscopic findings. Interestingly, all oils were more effective than 100 mg/kg ranitidine (reference drug). In conclusion, coconut oils exert remarkable antulcer activity depending on their methods of extraction, possibly via the modulation of its antioxidant and anti-inflammatory activity.

Keywords coconut oil, virgin coconut oil, copra oil, antulcer activity, antioxidant activity, free fatty acid composition

INTRODUCTION

The utilization of natural products with therapeutic properties is as primordial as human civilization and, interminably, mineral, plant and animal products were the main sources of drugs (Ansari and Inamdar, 2010). Thus far, approximately 65 - 80% of the world’s population in developing countries depends basically on plants for their primary health care resulting from poverty and lack of access to modern medicine according to the World Health Organization (WHO). Of the 25% drugs prescribed worldwide, 121 such active compounds that are currently in use derived from plants. Moreover, of the 252 drugs regarded as and vital by the WHO, 11% are exclusively of plant origin (Gregory, 2004). Interestingly, a considerable number of these drugs are synthetic drugs acquired from natural precursors. The vast majority of these drugs cannot yet be synthesized cost-effectively and are still attained from wild or cultivated plants. It is well known that the development and utilization of herbal medicine has a long history, including in the Malay traditional culture. A vast literature of herbal medicines, related to the Chinese, Indian or Arabic traditional cultures, not only reflects the use of medicinal plant resources of people experiences, but also provides good references for the researchers. Compared with the synthesized medicine, herbal medicines have a benefit in the treatment of common diseases, chronic diseases, various incurable diseases and persistent diseases (Ansari and Inamdar, 2010).

One of the diseases wherein herbal medicines have been extensively used as alternative therapy is peptic ulcer. Peptic ulcer is a chronic and appalling disease. Today, gastric and duodenal ulcers are dominant among the illnesses that affect considerable number of people in world’s population. A current estimate shows that prevalence rate of peptic ulcer is approximately 1 in 54 or 1.84% in 5 million people in USA (WHO). The pathophysiological processes involved in the peptic ulcer formation leading to the disturbances in the gastric equilibrium between the aggressive and protective factors have been discuss in detail elsewhere (Arrieta et al., 2003; Falcao et al., 2008; Victor et al., 2007). Due to the serious treat associated with the peptic ulcer formation, medical research to find cure for peptic ulcer has been progressing worldwide. In line with this progress, therapy for peptic ulcer disease has also advanced from vagotomy to the development of several classes of synthetic antulcer agents (i.e. anti-cholinergic drugs,
histamine H-2 receptor antagonists, antacids and, later, proton pump inhibitors) (Bighetti et al., 2005; Wallace and Granger, 1996). Nevertheless, their effectiveness is often overshadowed by various side effects associated with their usage (Bandypadhyay et al., 2002; Rang et al., 2012). In most cases, incidence of relapses and adverse reactions is seen following the synthetic antiulcer therapy (Awaad et al., 2013). Thus, a widespread search has been initiated to identify new anti-ulcer remedies from natural sources to substitute currently used drugs of uncertain efficiency and safety. Medicinal plants, herbs, spices, vegetables and crude drug substances are believed to be an impending source to manage various diseases including gastric ulcer. In the scientific literature, a large number of medicinal plants and their secondary metabolites with antiulcer potential have been reported (Awaad et al., 2013; Borelli and Izzo, 2000).

One of the plant-based natural products that have been studied extensively in recent years is virgin coconut oil (VCO). Coconut, and its copra-extracted oil, in general, has served man as important foods for centuries. On the other hand, VCO has gained a wide popularity in the global market in comparison to the copra oil (CO) due to the mode of extraction used to prepare them wherein the extraction of VCO is believed to retain more biologically active components such as vitamin E, polyphenols and other fatty acids (Nevin and Rajamohan, 2004). Moreover, VCO has been taken orally by people throughout Asia, especially in India, due to high medicinal values especially due to its high antioxidant properties and phenolic content. Scientifically, VCO has been reported to exert various pharmacological activities such as anti-arthritis and antioxidant (Vysakh et al., 2014), anti-thrombogenicity (Voorn et al., 2015), antihyperlipidemia (Nevin and Rajamohan, 2008) cardioprotective (Babu et al., 2014), antimicrobial (Shilling et al., 2013), anti-osteoporosis (Hayatullina et al., 2012), hepatoprotective (Zakaria et al., 2011a) and, antiinocceptive and anti-inflammatory (Zakaria et al., 2011b). Interestingly, recent clinical studies demonstrated that VCO possesses at least the antihypercholesterolemia (Cardoso et al., 2015) and anti-Alzheimer (Hu Yang et al., 2015) activities. The ability of VCO to exert antioxidant and anti-inflammatory activities, in particular, has led to the belief that the extract might also possess antiulcer potential. This assumption was raised because of the link between the three activities (Nagulsamy et al., 2015). Recently, we have reported on the ability of VCO and CO to reduce the volume of gastric juice, total acid output and ulcer scoring while increasing the gastric wall mucus secretion when measured using the pyloric ligation model (Selvarajah et al., 2015). This study indirectly demonstrated the effectiveness of VCO over CO to affect several parameters that influence the initiation and perpetuation of ulcer formation. Therefore, in an attempt to further contribute to the search for plant-based natural antiulcer agent, the ability of VCO and CO to demonstrate antiulcer activity was investigated using the HCl/ethanol induced gastric lesions in rats.

MATERIALS AND METHODS

Preparation of VCOA, VCOB and CO

Preparation of VCOA was performed according to the method described by Seow and Gwee (1997) while preparation of VCOB was carried out according to the methods described by Che Man et al. (1997). In addition, CO was prepared according to the method described by Nevin and Rajamohan (2004). The detail methods used to prepare both types of VCO and CO are given below. Two types of VCO, labeled as VCOA and VCOB, were used in the present study and were donated by Dr. Kamariah Long from the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Malaysia. They were differentiated by the way they were prepared and the detail procedures were as described below.

Preparation of VCOA

Briefly, coconut milk emulsion was centrifuged before chilling and thawing to allow better packing of the coconut oil globules. The temperature used were 10 and +4°C for chilling and freezing process, respectively while the thawing process was carried out in a water bath at 40°C until the coconut cream reached room temperature (25°C).

Preparation of VCOB

Pure culture of Lactobacillus plantarum 1041 IAM was used to extract coconut oil. Grated coconut meat and water at 30°C were mixed at a ratio of 1:1 and allowed to settle for 2 to 6 h. The coconut milk emulsion was then separated by adjusting the pH of the coconut milk emulsion between pH 3 and 5.6.

Preparation of CO

Endosperm of mature coconut meat was dried under sunlight for five days to remove the moisture content. The dried copra was then pressed in mill to obtain coconut milk, which was later subjected to the high heating using cooking pan. The resulting oil was filtered through the Whatmann filter papers to remove residues and was later used for the present study.

Drugs, reagents and solvents

The chemicals used in this study are of analytical grades and had been prepared immediately before use. The following drugs were used: ranitidine (Sigma Aldrich, MO, USA), sodium methoxide, hexane, standard fatty acids (FAME), hydrochloric acid and ethanol. They were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals

The experiments were performed on male Sprague Dawley rats (180 – 200 g; 8 - 10 weeks old) obtained from the Animal Unit, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM), Malaysia. The animals were kept in polypropylene cages with wood shaving in the Animal Holding Unit (UPM) set at the standard conditions (25 ± 3°C; 70-80% humidity; 12 h light/darkness cycle). The animals were fed with standard pellet and allowed free access to water but subjected to fasting prior to all assays. Standard drug and samples were administered orally (p.o.) by gavage with distilled water (10 ml/kg) as the vehicle. The use of animals in this study was approved by the International Animal Care and Used Committee (IACUC) of UPM (Approval No: UPM/FPSK/PADS/BR-UUH/00221).

GC–MS analysis of VCOA, VCOB and CO

Preparation of Fatty Acid Methyl Esters (FAMES) Prior to the GC-MS analysis, all oils were subjected to the methyl-esterification process using the BF3-MeOH method after alkaline hydrolysis. Approximately 20 mg of the respective oils were added with 2 ml of 0.5 mol/l NaOH-methanol solutions, and the combination was heated for 7 min at 1000°C. Following cooling, 3 ml of 14% BF3-MeOH reagent was added, and the vessel was closed and heated for 5 min at 1000°C. Subsequent to cooling, 7 ml of saturated NaCl solution and 2 ml of hexane were added, followed by a careful shaking. The resultant hexane layer (2 ml) was used as a sample solution.
Table 1. Fatty acid composition of virgin coconut oil (VCOA), fermented virgin coconut oil (VCOB), and coconut oil (CO).

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>VCOA</th>
<th>VCOB</th>
<th>CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4-0 (Butyric)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C6-0 (Caproic)</td>
<td>0.64</td>
<td>0.79</td>
<td>0.52</td>
</tr>
<tr>
<td>C8-0 (Caprylic)</td>
<td>6.92</td>
<td>6.21</td>
<td>8.63</td>
</tr>
<tr>
<td>C10-0 (Capric)</td>
<td>5.23</td>
<td>5.38</td>
<td>5.84</td>
</tr>
<tr>
<td>C12-0 (Lauric)</td>
<td>49.62</td>
<td>45.72</td>
<td>45.56</td>
</tr>
<tr>
<td>C14-0 (Myristic)</td>
<td>19.36</td>
<td>19.79</td>
<td>18.40</td>
</tr>
<tr>
<td>C16-1 (Myristoleic)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C16-0 (Palmitic)</td>
<td>8.37</td>
<td>11.20</td>
<td>8.93</td>
</tr>
<tr>
<td>C16-1 (Palmitoleic)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C18-0 (Stearic)</td>
<td>3.27</td>
<td>3.52</td>
<td>3.07</td>
</tr>
<tr>
<td>C18-1 (Oleic)</td>
<td>5.30</td>
<td>6.68</td>
<td>5.88</td>
</tr>
<tr>
<td>C18-2 (Linoleic)</td>
<td>0.77</td>
<td>0.94</td>
<td>1.98</td>
</tr>
<tr>
<td>C20-0 (Arachidic)</td>
<td>0.08</td>
<td>0.09</td>
<td>0.07</td>
</tr>
<tr>
<td>Others</td>
<td>0.44</td>
<td>0.45</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Means within each row with different superscript are significantly different at *p < 0.05. The relative standard deviation was less than 6% for all samples.

Table 2. Chemical composition and anti-oxidant capability of virgin coconut oil (VCOA), fermented virgin coconut oil (VCOB), and coconut oil (CO).

<table>
<thead>
<tr>
<th>Component</th>
<th>VCOA</th>
<th>VCOB</th>
<th>CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anisidine Value</td>
<td>0.27</td>
<td>1.58</td>
<td>0.63</td>
</tr>
<tr>
<td>Peroxide Value</td>
<td>0.21</td>
<td>0.62</td>
<td>0.10</td>
</tr>
<tr>
<td>Iodine Value</td>
<td>5.8</td>
<td>7.7</td>
<td>7.0</td>
</tr>
<tr>
<td>Saponification value</td>
<td>256.08</td>
<td>249.12</td>
<td>259.64</td>
</tr>
<tr>
<td>Free Fatty Acid (%)</td>
<td>0.094</td>
<td>0.981</td>
<td>0.103</td>
</tr>
<tr>
<td>Total phenolic content</td>
<td>12.77</td>
<td>23.47</td>
<td>11.39</td>
</tr>
<tr>
<td>Antioxidant activity (%)</td>
<td>75.67</td>
<td>82.42</td>
<td>69.35</td>
</tr>
</tbody>
</table>

Means within each row with different superscript are significantly different at *p < 0.05. The relative standard deviation was less than 7% for all samples.

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VCOA Group 6 - 100% VCOA, Group 7 - 10% VCOA, Group 8 - 50% VCOA, Group 9 - 100% VCOA, Group 10 - 10% CO, Group 11 - 50% CO and Group 12 - 100% CO. Rats were pretreated with distilled water, ranitidine or various concentrations of the respective oils intra-gastrically for 7 days. On Day 7th, one hour after the last test solutions administration, HCl/ ethanol was administered orally to induce gastric ulcer. Sixty minutes later, the animals were sacrificed with an overdose of diethyl ether. Their stomachs were removed, opened along the greater curvature, and rinsed with physiological saline, then blotted dry for macroscopic evaluation. The rat stomachs were then stretched on balsa boards. Digital pictures were taken using camera (Olympus 7.0 megapixel) for morphometrical analysis.

Macroscopic and histopathological analysis

The lesion size in mm was determined by measuring each lesion along its greatest length/ diameter using a transparent grid. The severity score was assigned according to Minano et al. (1987). Score 0, no pathological changes; Score 1, mucosal oedema and petechial haemorrhages; Score 2, 1-5 small ulcers (1 - 2mm); Score 3, more than 5 small ulcers or 1 medium ulcer (3 - 4mm); Score 4, more than 4 medium ulcers or 1 large ulcer (more than 4 mm) and Score 5, perforated ulcers. The sum of the total activity score in each group divided by the number of rats in the group was expressed as mean ulcer index. The percentage of lesion area in relation to total stomach area was analyzed from the digital pictures taken using transparent grid paper (mm2) (Sergio et al., 2007). Tissues were immediately immersed in the fixative 10% formalin solution for histopathological studies. The sections of the stomach, stained with hematoxylin and eosin, were assessed for histopathological changes such as congestion, edema, hemorrhage and necrosis (Shah and Khan, 1977).

Statistical analysis

Results are expressed as mean ± SEM. Statistical significance determined by one-way analysis of variance (ANOVA) followed by Turkey’s post-hoc test with the minimum level of significance set at *p < 0.05.

RESULTS
The fatty acid composition of VCO is tabulated in Table 1. Lauric acid (C12:0) was the highest fatty acid concentration in all oils wherein VCO A had significantly higher lauric acid compared to VCO B and CO. Myristic acid (C14:0), capric acid (C10:0), stearic acid (C18:0) and oleic acid (C18:1) were all statistically similar in all oils. Interestingly, caprylic acid (C8:0) of CO was the highest.

Chemical analysis and anti-oxidant activity of VCO is demonstrated in Table 2. All three oils possessed an Anisidine value, a measure used to indicate the level of oxidation/decomposition of the oil, of less than 2 indicating that they were of good quality. In addition, all oils possessed a peroxide value, a measure that indicates the freshness of the oils, that was much lower than the Codex maximum value of 15 mequiv oxygen/kg oil. Furthermore, all oils showed insignificant saponification value, a measure of the average molecular weight of all fatty acid in the oil, when compared to the other oils suggesting that those oils have almost similar number of short chain fatty acids. Moreover, VCO B was demonstrated to have a very high free fatty acid value, a measure of the free fatty acids formed by hydrolytic process, in comparison to VCO A and CO. However, all data obtained were within the Codex standard. Additional analysis also revealed that VCO A had the highest phenolic content and antioxidant activity in comparison to VCO A and CO (Table 2).

In the acute toxicity studies, the animals treated with the oils (5 ml /rat (p.o.)) once did not show any clinical signs of toxicity at any times of observation and this observation was further supported by the macroscopic examination of the important organs (i.e. liver and kidney) (data not shown). In the sub-acute toxicity studies, treatment with the oils (5 ml/rat (p.o.) for 14 days also did not cause mortality to the rats. No conspicuous signs of toxicity were observed during the experimental period. The body weight increased normally and there was no ulcer formation in the stomach of rats (data not shown).

The gastroprotective effects of VCO A, VCO B and CO at 10%, 50% and 100% concentrations, against the HCl/ethanol induced gastric damage was macroscopically examined and show in Fig. 1 (A-L). Oral administration of HCl/ethanol induced remarkable haemearias in the stomachs of vehicle-pretreated rats while in the groups pre-treated with VCO A, VCO B, CO or ranitidine, the haemearias reduced significantly in comparison to the vehicle-pretreated group (Table 3). There was no ulcer lesion detected after in oral administration of the distilled water-administered (normal) group. The recorded ulcer index shows that all doses of the three oils and had ranitidine had significant gastroprotective effect against the gastric damages caused by HCl/ethanol with the effectiveness of the oils followed the sequence of VCO A > VCO B > CO > ranitidine (a H2-receptor blocker) (Table 3). The recorded ulcer index was paralleled to the recorded ulcer length (Table 4).

Microscopic analysis of the stomach’s tissue pre-treated with vehicle followed by the administration of HCl/ethanol demonstrated the presence of necrosis, erosion, congestion and hemorrhage of the stomach wall. Pretreatment with both VCOs offered significant protection against all such damages to the gastric mucosa (Fig. 2A-L).

**DISCUSSION**

Coconut oil is widely used in food industry possibly because of its high content of medium chain fatty acids (MCFA) and ability to exert good digestibility. Depending on the types of extraction, either through dry or wet processing, different types of coconut oil can be obtained. The later mode of processing involves the use of dried copra which is the most commonly used form of extraction and has been traditionally used to obtain CO. The dried copra is pressed (i.e. wedge-, screw- or hydraulic-press) to obtain coconut oil, which was then subjected to the refining, bleaching, and deodorizing (RBD) processes, during which heating process at high temperature (204 - 245°C) is applied (O’Brien, 2004). A few years ago, a trend towards producing coconut oil by wet processing, which involves the extraction of cream from the fresh coconut milk, has captured the public attention. This process is more enviable as the oil, named VCO, is not exposed to chemical or high heat treatment as seen during the dry processing (Villarino et al., 2007). Unlike CO, which is generally used for cooking purposes, VCO is usually promoted as functional oil.

Interestingly, various scientific studies have demonstrated various pharmacological potentials of VCO. Of these, the antioxidant and anti-inflammatory activities of VCO have triggered the present study as oxidation and inflammation have been widely known to contribute to the development of ulcer. In the present study, the VCOs exerted a more effective antulcer activity when compared to CO with VCO A being the most effective antulcer agent. The ability of all oils to exert antulcer activity could be associated with their FFAs content as some of the FFAs have been reported to possess pharmacological effects such as antioxidant (i.e. palmitic acid, myristic acid) and anti-inflammatory (i.e. linoleic acid, oleic acid, lauric acid) activities (Henry et al., 2002). Interestingly,
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Fig. 1. Photomicrographs showing mucosal surface of rat stomach after oral administration of HCl/ethanol. A) Normal control, B) negative control (HCl/ethanol induced), C) Rat treated with Ranitidine at dose of 100 mg/kg, D) Rat treated with VCOA 10%, E) Stomach from rat treated with VCOA 50%, F) Rat treated with VCOA 100%, G) Rat treated with VCOA 10%, H) Stomach of rat treated with VCOB 50%, I) Rat treated with VCOB 100%, J) Stomach of rat treated with CO 10%, K) Stomach of rat treated with CO 50%, L) Rats treated with CO 100%. Arrows indicate hemorrhage sites. Hemorrhage sites were observed extensively in comparison with (I).

The fatty acid compositions of VCOs and CO were comparable to the Codex standards and previous studies (Henry et al., 2002). On the other hand, the difference in antiulcer intensity between the oils could be associated with the way they were processed. VCO, which was extracted directly from coconut milk under controlled temperature either via a wet (VCO A) or fermentation (VCO B) processes, tends to retain most of its beneficial components in comparison to CO, which was produced from sunlight-exposed, dried coconut meat that was exposed to chemical and heat during the RBD processes. The changes in components within the oils can be postulated based on the differences in the value of certain parameters such as the anisidine and peroxide values, percentage of FFA, TPC value and total antioxidant activity.

There are several factors that may induce ulcer such as: stress, chronic use of anti-inflammatory drugs and continuous alcohol ingestion (Barocelli et al., 1997). The candidate for an effective drug against peptic ulcer should act either by reducing the aggressive factors on gastroduodenal mucosa or by increasing mucosal resistance against them (Sergio et al., 2007). More recently, HCl/ethanol has been used to induce gastric lesions in rats as a model. The exact mechanism of pathogenesis by HCl/ethanol is still unclear (Matsumoto et al., 1993), but it has been demonstrated that active oxygen species might be involved in the formation of gastric mucosal lesions (Itoh and Guth, 1985; Smith et al., 1987; Yoshikawa et al., 1990). On the other hand, it has been found that oxygen-derived free radicals are involved in the mechanism of acute and chronic ulceration in the gastric mucosa (Pihan et al., 1987). Furthermore, disturbances in gastric secretion, damage to the gastric mucosa, alterations in permeability, gastric mucus depletion and free radical production are observed after the administration of ethanol (Salim, 1990). These data suggest that antioxidant compounds could be active in this experimental model, producing antulcerogenic effects. Indeed, VCO possessed this activity hence may be one of the potential candidates for gastroprotection. Moreover, those oils might also work via several other mechanisms of gastroprotection such as by increasing gastric hexosamine level and enhancing the strength of the gastric barrier either physically or by blocking the H⁺,K⁺-ATPase pump, stimulation of membrane stabilization by interference with Ca⁺⁺ influx and inhibition of biological membranes (Akhtar et al., 1992; Akhtar and Ahmed, 1995; Cholbi et al., 1991; Koch and Löffler, 1985). Further study is, therefore, required to confirm on the possible mechanisms of gastroprotection used by the VCOs to demonstrate the antiulcer activity.

CONCLUSION

VCOs, either produced via wet or fermentation processes, exert better antiulcer activity in comparison to CO that was produced using the dry processes possibly due to the former ability to preserve its beneficial components from the action of chemicals and heat. The antiulcer activity of the oils could be associated with their antioxidant and anti-inflammatory activities and the free fatty acids composition.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.
REFERENCES


