TANG [HUMANITAS MEDICINE]



**Original Article**

# Comparative investigation into the anti-ulcer activity of virgin coconut oil and coconut oil in pylorous ligated animal model

Malarvili Selvarajah[1], Zuraini Ahmad[1,2], Zainul Amiruddin Zakaria[1,2], Hoe Siong Chiong [1,3], Yoke Kin Yong [1], Kamariah Long[4], Muhammad Nazrul Hakim[1,2*]

*[1]Department of Biomedical Sciences, Universiti Putra Malaysia; [2]Halal Institute, Universiti Putra Malaysia; [3]Sports Academy, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; [4]Biotechnology Research Centre, Malaysian Agricultural Research and Development Institute, 50774 Kuala Lumpur, Malaysia*

## ABSTRACT

This current study investigated the anti-ulcer activity of 2 types of virgin coconut oil (VCO-A and VCO-B) and coconut oil (CO). Sprague-Dawley of male rats divided into 6 groups and each group consisted of ten rats. Rats were then treated with either VCO or CO and then were then anaesthetized and pyloric ligation was performed. The anaesthesia was discontinued and the animal usually recovered consciousness within less than an hour. Three hours later, the animal was then again anaesthetized and sacrificed with chloroform. Stomach removed and its content subjected to measurement of volume and pH. The results revealed VCO-B and VCO-A (100%) significantly inhibited ($p < 0.001$) the volume of gastric juice secreted by the control rats by 66.81% and 51.53%, respectively. Followed by CO 42.80%. While the inhibition of gastric juice for positive control rats which treated with ranitidine (100 mg/kg) was only 22.38%. The total acid output was reduced by the oils to 70.80%, 74.16% and 40.45% for VCO-A, VCO-B and CO respectively compared to control group. Ranitidine reduced the total acid output by 34.83%. In conclusion, prevention of gastric lesions in rats by VCO was found to increase the mucous and decrease the acid volume, total acid contents and ulcer scoring. The treatment of VCO affects the all parameters that influence the initiation and perpetuation of ulceration.

**Keywords** virgin coconut oil, coconut oil, ranitidine, ulcer, pyloric ligation, mucous secretion

## INTRODUCTION

Peptic ulcers have been considered as one of the major human illness because these diseases affect nearly 8 - 10% of the global population (Calam and Baron, 2001). Of these figure, approximately 5% endure from gastric ulcers (Bandyopadhyay et al., 2001). Various factors contribute to the pathogenesis of gastric ulcers (Rang et al., 2012) such as the *Helicobacter pylori* infections, prolong use of steroidal and non-steroidal anti-inflammatory drugs, chronic consumption of alcohol, and a stressful lifestyle. These factors trigger the imbalance between aggressive factors (i.e. acid and pepsin secretion, *Helicobacter pylori*, reactive oxygen species etc.) and mucosal defensive factors (i.e. bicarbonate secretion, mucus-bicarbonate barrier, non-enzymatic and enzymatic antioxidants, prostaglandins (PGs) release etc.) (Mota et al., 2009), which trigger gastric mucosal damages.

Despite tremendous breakthrough in the field of medicine nowadays, therapy related to peptic ulcers still becomes an important challenge to the medical officers. Being multifactorial diseases, peptic ulcers have been mostly associated with the disturbance in gastric acid secretion making the attempt to inhibit gastric acid secretion as the key therapeutic target for ulcer diseases (Jain et al., 2007; Mota et al., 2009).

One of the suitable models to study the effect of extract/compound on gastric acid secretion under experimental condition is the pyloric ligation assay in rats. Pyloric ligation is a technique used to stimulate acid secretion in rats. The method causes copious secretion of gastric acid (Shay et al., 1945, 1954). This response is probably elicited by vago-vagal reflexes (Brodie, 1966). Another study has suggested that in chronically vagotomized rats the acid response to pylorous ligation is caused by intramural reflexes activated by the gastric distension. Therefore, in overall different nervous mechanisms seem to be responsible for the acid hypersecretion following pylorous ligation in innervated and denervated animals (Hakanson et al., 1970). This technique has been widely used in the anti-ulcer studies to investigate the mechanism involve by stimulating gastric secretion and collection of gastric juices. This technique also could be used as an ulcer inducer as it could cause ulceration to the rats due to physical stress that involve.

Currently available drugs that are used to treat gastric ulcers via the inhibition of gastric acid secretion include histamine $H_2$-antagonists, proton pump inhibitors, and antimuscarinics (Bighetti et al., 2005). Nevertheless, their effectiveness is often overshadowed by various side effects associated with their usage (Bandyopadhyay et al., 2002; Rang et al., 2012). In this context, the use of natural products, particularly of plant-based, has gained the interest of many researchers. Natural product uses and studies are in continuous

*Correspondence: Muhammad Nazrul Hakim
E-mail: nazrul.hakim@gmail.com
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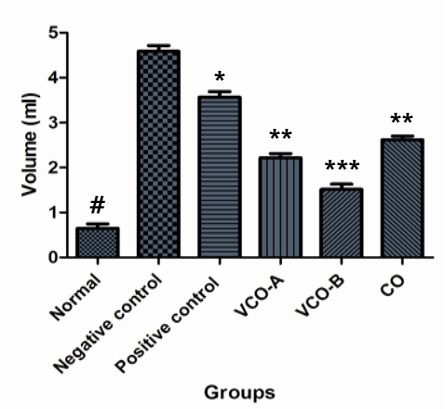


**Fig. 1.** Effects of VCO and CO on gastric ulcer induced by pylorus ligation in rats on gastric volume. $^{\#}p < 0.05$ significantly different from the control-treated group. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ - significantly different against the negative control group. Post test on selected treatment means.

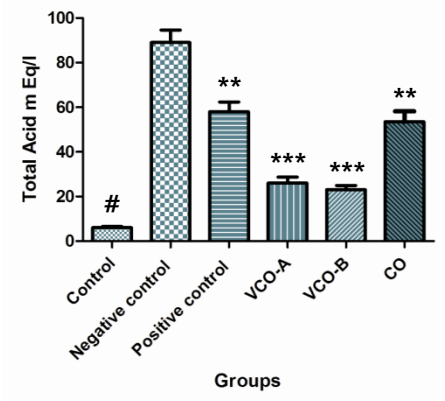


**Fig. 2.** Effects of VCO and CO on gastric ulcer induced by pylorus ligation in rats on Total Acidity. $^{\#}p < 0.05$ significantly different from the control-treated group. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ - significantly different against the negative control group. Post test on selected treatment means.

expansion all over the world and became the most attractive source of new drug for the treatment and prevention of many diseases. Diverse ranges of bioactive molecules isolated from plant natural product have been shown to produce promising results for the treatment of gastric ulcer (Borelli and Izzo, 2000). Virgin coconut oil (VCO) has been gaining a wide popularity in the research area and in the global market. Traditionally it has been widely used for the treatment of hypercholesterolemia and many other ailments. It has been taken orally by people throughout Asia who have known the beneficial effects of VCO especially in India. Little information about VCO is known on gastrointestinal functions, however based on the antioxidant properties and the high phenolic content of VCO, it should have beneficial effect on gastro intestinally.

In this study, our aim was to investigate the effect of the VCO and commercial coconut oil (CO) on gastric mucus content and acidity, an important gastrointestinal function. It is also to elucidate the possible mechanism of action employed by the agents in the oils.

## MATERIALS AND METHODS

### Preparation of VCOA and VCOB

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

Preparation of VCOA was carried out according to the methods described below (Seow and Gwee, 1997). Briefly, coconut milk emulsion was centrifuged before chilling and thawing to allow better packing of the CO globules. The temperature used were 10 and -4℃ for chilling and freezing process, respectively while the thawing process was carried out in a water bath at 40℃ until the coconut cream reached room temperature (25℃).

Preparation of VCOB was performed according to the methods described below (Che Man et al., 1997) was prepared according to the procedure described by Seow and Gwee (1997) with several modifications. Pure culture of *Lactobacillus plantarum* 1041 IAM was used to extract coconut oil. Grated coconut meat and water at 30℃ 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.

### Drugs and chemicals

Ranitidine, Alcian blue, Phenolphtalein, Sodium hydroxide, Magnesium Chloride, sucrose, Ketamil, ethanol, and sodium citrate were purchased from Sigma Aldrich (St Louis, MO, USA).

### Animals

Sprague- Dawley of male rats weighing between 150 - 180 g was obtained from Universiti Putra Malaysia. The animals were housed in separate large cages in group of 5 rats per cage in environmentally controlled room (25 ± 2℃, 12 h light/dark cycles) with free access to standard laboratory chow. Tap water was supplied ad libitum. The animals were deprived of feed but not water 18 h before the experiment. Animal studies were reviewed and approved by Institutional Committee in accordance with National guidelines before the study.

### Experimental design

Rats were divided into 6 groups and each group consisted of ten rats with two cages for each group.

Group I: Control group. Animals in this group not treated and not pylorous ligated.

Group II: Negative control. Animals pre-treated with distilled water 10 ml/kg.

Group III: Positive control. This group pre-treated with 100 mg/kg ranitidine and pyloric ligated.

Group IV: 100% VCO-A pre-treated (10 ml/kg) and pylorous ligated.

Group V: 100% VCO-B pre-treated (10 ml/kg) and pylorous ligated.

Group VI: 100% CO pre-treated (10 ml/kg) and pylorous ligated.

### Pylorous ligation

The method of Shay et al. (1945) was used. The animals were fasted 48 h starting in early morning, to ensure complete emptying of the stomach and water was permitted ad libitum.

The oils and control rats were administered orally to the test, respectively, 1 h before the experiment. Then, the animals were anaesthezied using 1 ml of Ketamil (2-fold) injected intraperitoneally. Under anaesthesia, the abdomen of the rats was shaved and a midline incision was made extending 2 cm

**Table 1**. Effect of VCO and CO on gastric ulcer induced by pylorus ligation in rats

| Design of treatment | Dose | Volume (ml) | Acidity (Total acid) (mEq/l) | Gastric mucus content (µg/g) | Ulcer Score |
|---|---|---|---|---|---|
| **Control** | - | 0.65 ± 0.10 | 6.04 ± 0.59 | 13.26 ± 0.36 | 0.00 ± 0.00 |
| **Negative control** | 10 ml/kg | 4.58 ± 0.13$^{\#}$ | 89.02 ± 5.59$^{\#}$ | 4.42 ± 0.25$^{\#}$ | 4.33 ± 0.33$^{\#}$ |
| **Positive control** | 100 mg/kg | 3.57 ± 0.12$^{*}$ | 58.03 ± 4.35$^{**}$ | 5.32 ± 0.38$^{*}$ | 3.33 ± 0.21$^{*}$ |
| **VCO type-A** | 10 ml/kg | 2.22 ± 0.10$^{*}$ | 26.03 ± 2.67$^{***}$ | 8.34 ± 0.32$^{*}$ | 2.67 ± 0.33$^{*}$ |
| **VCO type-B** | 10 ml/kg | 1.52 ± 0.112$^{**}$ | 23.01 ± 1.93$^{***}$ | 10.19 ± 0.63$^{**}$ | 1.33 ± 0.42$^{*}$ |
| **CO** | 10 ml/kg | 2.62 ± 0.09$^{*}$ | 53.51 ± 4.71$^{**}$ | 5.45 ± 0.50$^{*}$ | 3.00 ± 0.37$^{*}$ |

$^{\#}p < 0.05$ significantly different from the control-treated group.
$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ - significantly different against the negative control group.

downwards from the xyphoid. The junction between the pylorus and the duodenum was picked gently using a curved probe without disturbing the stomach. Pyloric ligation was made using silk thread, care being taken to avoid damage of blood vessels or traction on the stomach. Then the abdominal wall was then closed by interrupted sutures. The abdominal wound was cleaned thoroughly with physiological saline, dried and covered with a solution of flexible collodion. The anaesthesia was discontinued and the animal usually recovered consciousness within less than an hour. Three hours later, the animal was then again anaesthetized and sacrificed with chloroform. Stomach removed and its content subjected to measurement of volume and pH.

**Measurement of gastric acidity**

Gastric content obtained from each pylorus ligated rat was centrifuged to obtain a clear solution. A total of 1 ml of gastric content was analyzed for Hydrogen ion concentration by titration with 0.01 N NaOH. Gastric acidity was expressed in m Eq/l.

**Determination of gastric wall mucus content**

Adherent gastric mucus was determined as described by Corne et al. (1994). The stomach was removed, opened along the lesser curvature and rinsed in cold saline. The glandular part of the stomach was excised, weighed and immersed for 2 h in 10 ml of 0.1% w/v Alcian blue in 0.16 M sucrose solution buffered with sodium acetate (pH 5.8). The excess dye was removed by two rinses in 0.25 M of sucrose (15 min each). The mucus-bound dye was extracted by immersing the gastric tissue in 0.5 M $MgCl_2$ solution, which was intermittently shaken for 1 min at 30 min intervals during a 2 h period. The blue extract was shaken vigorously with an equal volume of diethyl ether. The emulsion was then centrifuged at 5000 g for 10 min and the optical density of the aqueous phase was measured spectrophotometrically at 580 nm. The amount of mucus adherent to the gastric mucosal was estimated from a standard curve established using concentrations from 0 to 20.

**Ulcer scoring**

The severity score 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 - 2 mm); Score 3, more than 5 small ulcers or 1 medium ulcer (3 - 4 mm); Score 4, more than 2 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 ($mm^2$) (Sergio et al., 2007).

**Statistical Analysis**

The results were expressed as mean ± standard error of mean (SEM) and analyzed for statistical significance by One way ANOVA followed by Turkey multiple post test $p$ values < 0.05 were considered significant. The results analyzed using GraphPad Prism Version 5.

## RESULTS AND DISCUSSION

The three different types of oils evoked gastric juice secretion in rats. The gastric juice induced by each different types of oil showed significantly lower total acidity, high in mucus content (µg/g) and lower in ulcer scoring (Table 1). Table 2 shows the percentage of inhibition for volume of gastric juice, total acid output and ulcer scoring.

VCO-B and VCO-A (100%) significantly inhibited ($p < 0.001$) the volume of gastric juice secreted by the control rats by 66.81% and 51.53%, respectively. Followed by CO 42.80%. While the inhibition of gastric juice for positive control rats which treated with ranitidine (100 mg/kg) was 22.38%. The total volume of gastric juice secreted by each groups of rats shown in the figure 1.

The total acid output was reduced by the oils to 70.80%, 74.16% and 40.45% for VCO-A, VCO-B and CO respectively compared to control group. Ranitidine reduced the total acid output by 34.83% which is lower than the treatment groups. Figure 2 shows the total acid output in m Eq/l for all the experimental groups.

Fig. 3 shows the amount of gastric mucus produced by rats in the experimental design. The results were tabulated in Table

**Table 2**. Percentage of inhibition for the effects of VCO and CO on pylorous ligated rats

| Design of treatment | Dose | Volume (%) | Acidity (%) | Ulcer score (%) |
|---|---|---|---|---|
| **Control** | - | - | - | - |
| **Negative control** | 10 ml/kg | - | - | - |
| **Positive control** | 100 mg/kg | 22.28$^{*}$ | 34.83$^{*}$ | 23.08$^{*}$ |
| **VCO-type-A** | 10 ml/kg | 51.53$^{***}$ | 70.80$^{***}$ | 38.45$^{*}$ |
| **VCO-type-B** | 10 ml/kg | 66.81$^{***}$ | 74.16$^{***}$ | 69.28$^{***}$ |
| **CO** | 10 ml/kg | 42.80$^{**}$ | 40.45$^{**}$ | 30.76$^{*}$ |

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ - significantly different against the negative control group.

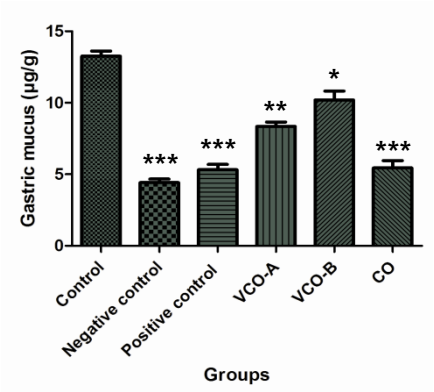


**Fig. 3.** Effects of VCO and CO on gastric ulcer induced by pylorus ligation in rats on gastric mucus. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ - significantly different against the negative control group. Post test on selected treatment means.

1 above. The highest gastric mucus content was reported to have in normal group of rats. The amount of gastric mucus produced was 13.256 µg/g of rat stomach. The pylorous ligated and negative control group produced significantly ($p < 0.001$) lesser gastric mucus 4.415 µg/g. The oils VCO-A, VCO-B and CO produced gastric mucus content of 8.338 µg/g, 10.190 µg/g and 5.447 µg/g respectively compared to the control groups. Ranitidine, positive control treated group possessed significantly ($p < 0.001$) the lowest mucus content of 4.415 µg/g.

Table 1 shows the ulcer score obtained for the experimental rats according to their groups. Table 2 shows the percentage of ulcer scoring inhibition. Oral administration of three different types oils respectively decreases the ulcer scoring in pylorous ligated rats. Figure 4 shows the effects of oils on ulcer score for pylorous ligated rats. Negative control group have ulcer scoring of 4.333. The percentage of ulcer inhibition for all the treatment oils were 38.45%, 69.28% and 30.76% for VCO-A, VCO-B and CO respectively compared to the negative control group. The percentage of inhibition for positive control was 23.08.

In most cases the etiology of ulcer is unknown, it is generally accepted that ulcer results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism (Piper and Stiel, 1986). To regain the balance equilibrium, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production.

Evidently pylorus ligation is a technique to stimulate gastric acid secretion in the rat by entirely different mechanism which is not well elucidated. However, previous study reported that pylorus occlusions will increase the acid output and the volume of the gastric content progressively for 6 - 8 hours after pyloric ligation procedure (Hakanson et al., 1980). Another study by Ridley (1973) reported that pylorus ligation induced a true stimulation of the acid output rather than causing accumulation of basal gastric secretion in the stomach. Another study reported that, pylorus ligation is an ulcer inducer which will lead to auto digestion of the gastric mucosal barrier due to excess production and accumulation of hydrochloric acid in the stomach (Moumita et al., 2010).

Earlier studies have indicated that the vagus is important for the acid response also to pylorus ligation (Harkins, 1947; Shay et al., 1949). The suppression of acid secretion after vagotomy was found to be transient and almost fully reversible. Acid secretion stimulated when the stomach was continuously drained via a gastric fistula and distension of the stomach (Hakanson et al., 1980). Besides that, receptors in the pyloric part of the stomach wall are activated by the ligature itself (Brodie, 1966).

Generally, the results showed that VCO type-A and type-B decreased significantly the formation of ulcers followed by CO in pylorus ligated rats. VCO at the dose levels of 100% (10 ml/kg) significantly reduced the volume of gastric juice, total acid output, ulcer index and increased the mucus content. As the defense potential of mucus perimeter of gastric mucosa depends upon a delicate balance between the process affecting the synthesis and secretion of its mucin constituents. Mucus is a viscous colloid containing antiseptic enzyme such as lysozyme and immunoglobulins that serves to protect GI tract. Thus, the antiulcer effect caused by VCO could be due to the antiulcer agents which involve in maintaining a delicate balance of factors controlling the synthesis, secretion and breakdown of its proteins, glycoproteins and lipid components, so as to strengthen the mucosal integrity (Brown, 1978).

It has been widely researched and reported that VCO contains highly Vitamin E. A study has been reported by Susan et al. (1999), Vitamin E has got a protective effect of on gastric ulcerogensis. Besides that, Tariq (1998) had demonstrated the protective role of Vitamin E against drug induced gastric ulcers in rats and suggested an increase in PG synthesis as a possible mechanism for the anti-ulcer activity of Vitamin E.

PGs are form of an important component of gastric mucosal defense. PGs that involve in protecting gastric mucosa derived from Cyclooxygenase enzyme (COX-1 enzyme). The three main roles of PGs in causing gastric mucosal defense are in maintaining gastric blood flow during exposure to noxious substances such as nitric oxide and hydrogen sulfide, in secretion of bicarbonate and mucus by the surface epithelial cells and as a superficial injury through the process of epithelial restitution. Thus, further investigations required to estimate the levels of PGs to confirm the association of Vitamin E in VCO and the activity of PGs in GI tract whether VCO protects gastric mucosa by PG mediated mechanism.

Vitamin E has been extensively shown for its antioxidant property. Besides that, VCO has been reported has been reported to contain high contain of total phenolic content (Marina et al., 2009) which also lead to high antioxidant activity. Reactive oxygen metabolites have been implicated in the pathogenesis of peptic ulcer (Desai et al., 1997). And it is well known 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

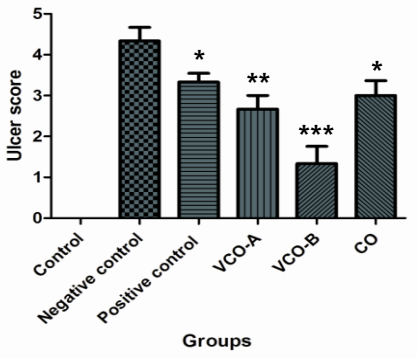


**Fig. 4.** Effects of VCO and CO on ulcer scoring for pylorus ligated rats. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ - significantly different against the negative control group. Post test on selected treatment means.

model, producing antiulcerogenic effects. This effect is known as cytoprotection.

The present study indicated highest antiulcer activity possessed by VCO-B followed by VCO-A. VCO-B is the oil produced through fermentation technique while VCO-A produced through wet process. As the fermentation technique uses lesser heating temperature compared to other techniques, it able to retain the beneficial constituents in it such as Vitamin E and phenols which could lead to high antioxidant activity. On the other hand, CO produced the lowest effect compared to the VCO as it produced from copra and a very high heating used as it is going through the RBD process.

Gastric secretion is also blocked by histamine $H_2$-receptor blockers (Schultz, 1979). In the group of rats received Ranitidine at dose of 100 mg/kg, the mean acid output, volume of gastric juice were significantly reduced probably by blocking the histamine $H_2$-receptors and allowing the muscarinic-receptors to predominate despite the stability in gastric juice volume.

## CONCLUSION

The prevention of gastric lesions in rats was found to increase the mucous and decrease the acid volume, total acid contents and ulcer scoring. The treatment of oils affects the parameters that influence the initiation and perpetuation of ulceration. Present study also proved the involvement of PG mediated pathway and cytoprotection effect of VCO and CO in the anti-ulcer mechanism for the ulcer formed in pylorus ligated rats.

## CONFLICT OF INTERESTS

The authors have no conflicting financial interests.

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