Study of the mechanism of anti-ulcer effects of virgin coconut oil on gastric ulcer-induced rat model

Jie Meng[1], Taoping Chen[1], Yu Zhao[2], Sucai Lu[1], Huiling Yu[1], Ying Chang[1], Dalei Chen[1]

[1]Department of Orthopedic, Affiliated Hospital of Hebei University, Baoding, China
[2]Hebei University, Baoding, China

Submitted: 24 January 2017
Accepted: 5 June 2017

Arch Med Sci 2019; 15 (5): 1329–1335
DOI: https://doi.org/10.5114/aoms.2018.76943

Corresponding authors:
Taoping Chen MM
Department of Orthopedic
Affiliated Hospital
of Hebei University
071000 Baoding, Hebei
China
Phone: +86 312 598 1818
E-mail:
chentaoping@yahoo.com

Yu Zhao MM
Hebei University
212 Yuhua East Road Baoding
City 07100, Hebei
China
E-mail:zhaoyu162@yahoo.com

Abstract

Introduction: This study aims to evaluate the gastro-protective effects of virgin coconut oil (VCO) on different ulcer models as compared to the standard drug (omeprazole).

Material and methods: Three groups of rats (6 rats per group for each ulcer model) were pre-treated with distilled water for the negative control group, 30 mg/kg of omeprazole for the positive control group and VCO (2 ml per rat) for the treatment group. Animals were pre-treated for 7 days and ulcers were induced with cold restraint stress, piroxicam, ethanol and pylorus ligation. On day eight, animals were sacrificed and ulcer scores were determined based on macroscopic evaluation. The gastric volume, pH, total acidity and mucus content were measured in the pylorus-ligated model. The levels of antioxidants were determined from the gastric tissue homogenates.

Results: Virgin coconut oil significantly ($p < 0.001$) inhibited the ulceration caused by different inducers. The percentage of inhibition for the VCO-treated group was 78.3%, 84.7%, 72.7% and 73.1%, while for the omeprazole-treated group it was 60.8%, 61.5%, 59% and 53.8% in cold restraint stress, ethanol, piroxicam and pylorus-ligated ulcer models, respectively. Virgin coconut oil significantly ($p < 0.001$) inhibited gastric juice volume and total acidity for VCO and omeprazole treated groups as compared to the non-treated negative control group. Moreover, VCO and omeprazole caused a significant ($p < 0.001$) increase of gastric mucus content and pH. Virgin coconut oil also proved to have significantly increased glutathione (GSH) and nitrite levels, whereas the levels of SOD, GP, MDA and CAT were significantly ($p < 0.001$) reduced by VCO relative to the control group. Virgin coconut oil also significantly ($p < 0.001$) increased the level of prostaglandin in rat tissue homogenate, similar to the omeprazole treated group.

Conclusions: Virgin coconut oil shows a possible association with antioxidant properties to control the regulation of prostaglandin synthesis and protect against reactive oxygen species damage.

Key words: antioxidant, gastro-protective, peptic ulcer, treatment, virgin coconut oil.

Introduction

Peptic ulcer is a multifactorial and chronic disease [1]. To date, gastric and duodenal ulcers are dominant among the illnesses that affect a considerable number of people in the world's population [2]. Based on a systematic review, it has been pointed out that the annual incidence rate of peptic ulcer was estimated as 0.10–0.19% for physician-diagnosed cases and 0.03–0.17% for those recorded as per hospitalization date with

the prognosis closely associated with *Helicobacter pylori* infection [3].

Generally, ulceration occurs due to interruptions in the normal gastric equilibrium, which is caused by either enhanced or diminished mucosal resistance [4]. Moreover, some of the predisposing factors associated with the disease development include inadequate dietetic habits such as smoking or alcohol, duration of starvation, nature of food ingested, persistent infection with *H. pylori*, the use of acetylsalicylic acid (ASA) and non-steroidal anti-inflammatory drugs (NSAIDs), disruption of mucosal barrier due to stress, Zollinger-Ellison syndrome, and finally genetic, hereditary factors leading to higher chances of acquiring duodenal ulcers for people with a family history of the disease besides having type-O blood group [5]. Treatments for peptic ulcer that are widely used in clinical practice are muscarinic antagonists (pirenzepine), antacids (aluminium hydroxide and magnesium trisilicate), histamine-H-2 receptor antagonists (cimetidine and ranitidine), proton pump inhibitors (omeprazole and lansoprazole) and antimicrobial agents in order to eradicate *H. pylori* (amoxicillin and clarithromycin) [6].

The current aims of peptic ulcer treatment are relief of pain, healing and preventing the recurrence of ulcer. The pharmacology of each category of drugs is different. However, the common mechanism or rule that applies to all categories is that they all in general act to inhibit gastric acid secretion by blocking the gastric H^+K^+-ATPase (proton pump inhibitors) [7], blocking H-2 receptors (H-2 receptor antagonist) or via stimulation of mucus and bicarbonate secretion to alter the mucosal blood flow [8].

Nevertheless, there is no complete recovery for peptic ulcer using the current pharmacological approaches. Apart from that, many adverse effects have been addressed in relation to continuous use of the drugs such as arrhythmia, hypomagnesemia, hypersensitivity, impotence and gynecomastia [9]. In addition, some of these medications are costly [10] and have high risk of causing gastric cancer and later stage disease [11]. Thus, this enigma has encouraged the researchers to identify a new therapeutically safe and effective treatment to completely eradicate the issue. From this perspective, plants are gaining importance as they are the major contributors of bioactive molecules having anti-ulcerogenic properties [12]. Among medicinal plants, oils derived from plant species are also gaining wide popularity for their therapeutic properties.

Recently, several studies have reported on the effectiveness of virgin coconut oil (VCO) in treating and preventing peptic ulcer disease significantly as compared to commercial coconut oil, also known as copra oil (CO) [13]. The major differences between VCO and CO are due to the extraction method, which leads to differences in their benefits. Virgin coconut oil is classified as oil extracted from fresh-dry mature kernel of coconut (*Cocos nucifera* L.) using enzymatic and/or fermentation technique without involving direct heating, whereas copra oil is produced via the direct heating process [14]. As the production process does not involve heat, VCO does not go through any transformation and thus retains the beneficial components such as vitamins, antioxidants, the fatty acid component lauric acid and other medium chain fatty acids (MCFA) which help in digestion [15]. Moreover, VCO is an edible food which can be included in our diet and taken directly without prescription from a physician, without causing any side effects.

Although studies have reported on the effects of VCO on peptic ulcer [13], there has been no study yet to elucidate the mechanism involved in inhibiting ulceration. The present study aims to reveal the possible mechanism involved in the action of VCO in ulcerative models.

Material and methods

Preparation of virgin coconut oil

Low temperature technique was applied in the preparation of VCO. The solid endosperm of matured fresh coconut was crushed and made into a viscous slurry [16]. It was later mechanically squeezed through a cheese cloth to collect the coconut milk. The milk was kept in the refrigerator and frozen for 48 h, then heated at 500°C using a thermostat oven. After the heating process, the collected oil was filtered using a cheese cloth. The total yield was approximately 40–50%.

Drugs and chemicals

All chemicals and solvents were of analytical grade and included omeprazole (Sigma Aldrich, St. Louis, MD, USA), ethanol (Merck, UK), phenolphthalein (Merck, UK), alcian blue (Merck, UK), magnesium chloride (Merck, UK), sucrose (Merck, UK) and sodium citrate (Merck, UK).

Animal preparation

A total of 72 Wistar rats (40 days old), weighing 200–250 g, were obtained from our animal feeding center. All studies carried out were approved by the animal care and ethical committee following the employed protocols (ACUC No: HDFY-LL-2016-19). The animals were kept at room temperature (25–28°C) for a minimum of seven days for acclimatization to room conditions prior to the experimental procedure. The animals were housed in groups of six animals per standard cage for each experimental group in standard environ-

mental conditions (25 ±3°C), with 12 h dark/12 h light cycles, and food and water ad libitum [17].

Anti-ulcer and cytoprotective studies

The experimental rats were divided into three groups consisting of 6 rats per group. Rats of each group were orally pre-treated with distilled water (10 mg/kg) for the negative control group, omeprazole (30 mg/kg) [13, 18] for the positive control group and VCO (2 ml per rat) [19] for the treatment group for seven days and fasted for 24 h into the eighth day before the ulcer induction procedure. The grouping was the same for each ulcer induction model.

Gastric ulcer induction

Cold restraint stress-induced gastric ulceration

On day 8, the rats were paralyzed by strapping the fore and hind limbs on a flat wooden plank, and transferred into the refrigerator (4–6°C). After two hours, the rats were sacrificed by cervical dislocation. The stomach was incised along the greater curvature and the lumen was rinsed with normal saline. The ulcer scoring was carried out based on the method described by Minano *et al*. [20]. Then, the fundic part of the stomach was homogenized (5%) and centrifuged. The acquired supernatant was used to study activity using an antioxidant enzyme assay. The percentage of inhibition was calculated according to the following formula [21]: % of inhibition = (UI for control (negative) – UI for treatment group)/UI for control (negative).

Ethanol-induced gastric ulceration

The experimental rats of each group were pre-treated as explained previously for 7 days and fasted for 24 h into the eighth day. On day 8, ulceration was induced in the animals by oral administration of absolute ethanol (1 ml/200 g) [22, 23]. After 1 h of ulcer induction, the animals were sacrificed by cervical dislocation. The stomach was dissected out for the macroscopic examination of ulcers. The scoring for ulcer was determined using a method described in a previous study.

Piroxicam-induced ulcer model

Another set of rats were pre-treated for 7 days as described earlier. The animals were then fasted for 24 h into the eighth day. On day 8, piroxicam (30 mg/kg) was administered to the rats [4]. After 6 h of piroxicam induction, the animals were sacrificed by cervical dislocation. The rats were dissected and their stomachs were cut open along the greater curvature. The gastric lumen was rinsed with normal saline and examined. The ulcer scoring was carried out using a method described in a previous study.

Pylorus-ligated rats

Animals were pre-treated for a period of seven days as described earlier and the rats were fasted for 24 h into the eighth day to ensure complete emptying of the stomach and water was permitted *ad libitum*. On day 8, the animals were anaesthetized by intraperitoneally injection of pentobarbitone sodium (35 mg/kg) [24, 25]. After anesthetic, the abdomen was opened and pylorus ligation was carried out without causing any damage to its blood supply. Then, the stomach was replaced carefully and the abdominal wall was closed in two layers with interrupted sutures followed with a moist swab of normal saline. After 4 h, each stomach was dissected out and cut open along the greater curvature [26, 27]. The ulcer index (UI) was determined using the scoring scaling of Minano *et al*. [20].

Measurement of gastric juice volume, total acidity, pH and gastric mucus content

After dissection and removal of the stomach, gastric juice was collected. Than it was centrifuged for 5 min at 3000 × g, while the supernatant was separated and used to analyze for pH, gastric juice volume, and total acidity. Total acid was estimated by titrating against 0.01 N sodium hydroxide using Topfer's reagent as an indicator to obtain the total acidity, expressed as mEq/l [28]. Gastric mucus content was determined using the method described by Corne *et al*. [29] with modification. The stomach was removed, opened along the lesser curvature, and rinsed with cold saline.

Analysis

Antioxidant enzyme assay

The levels of superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GP), catalase (CAT), malondialdehyde (MDA) and nitrite were determined using a commercially available ELISA kit (Cayman, USA) [30].

Determination of prostaglandin E_2 synthesis

The level of prostaglandin was determined using the prostaglandin E_2 (PGE_2) Monoclonal Enzyme Immunoassay (ELISA) kit (R&D Systems, USA) [31].

Statistical analysis

All the grouped data were presented as mean ± standard error of the mean (SEM). Statistical significance was determined by one-way analysis of vari-

ance (ANOVA) followed by Tukey's post hoc test with the minimum level of significance at $p < 0.05$.

Results

Effects of virgin coconut oil on cold restraint stress-induced ulcer

Table I shows that VCO pre-treatment among the ulcerative rats significantly ($p < 0.001$) reduced the ulcer score and index as compared to the negative control group. Similarly, the positive control drug treated group also had a significantly ($p < 0.001$) lower ulcer score compared to the negative control group. The percentage of inhibition was 78.3%, similar to the positive control drug, which exhibited 60.8% inhibition.

Table I. Anti-ulcer effects of virgin coconut oil (VCO) on cold-stress-induced rats

Treatment	Ulcer index	Percentage of inhibition (%)
Negative control	3.83 ±0.17	–
Positive control (omeprazole – 30 mg/kg)	1.50 ±0.22	60.8
VCO (10 ml/kg)	0.83 ±0.31	78.3

Values are expressed as mean ± SEM; n = 6.

Table II. Anti-ulcer effects of virgin coconut oil (VCO) on ethanol-induced rats

Treatment	Ulcer index	Percentage of inhibition (%)
Negative control	4.33 ±0.21	–
Positive control (omeprazole – 30 mg/kg)	1.67 ±0.21	61.5
VCO (10 ml/kg)	0.67 ±0.21	84.7

Values are expressed as mean ± SEM; n = 6.

Table III. Anti-ulcer effects of virgin coconut oil (VCO) on piroxicam-induced rats

Treatment	Ulcer index	Percentage of inhibition (%)
Negative control	3.67 ±0.21	–
Positive control (omeprazole – 30 mg/kg)	1.50 ±0.22	59
VCO (10 ml/kg)	1.00 ±0.26	72.7

Values are expressed as mean ± SEM; n = 6.

Effects of virgin coconut oil on ethanol-induced ulcer

As shown in Table II, VCO-treated rats and omeprazole-treated rats had a significantly ($p < 0.001$) lower UI compared to the negative control group. The percentage of inhibition was 84.7%. Comparatively, the percentage of inhibition for the omeprazole-treated group was 61.5%.

Effects of virgin coconut oil on piroxicam-induced ulcer

As reported above, similar trends were observed for the piroxicam-induced ulcer model. The highest UI (3.67) was reported in the negative control group. Virgin coconut oil CO significantly ($p < 0.001$) reduced the UI to 1.0, having 72.7% inhibition, whereas the omeprazole-treated group had a percentage of inhibition of 59% (Table III).

Effects of virgin coconut oil on pylorus ligation-induced ulcer model

In the pylorus experimental model, VCO evoked gastric acid secretion in rats. The gastric juice induced by VCO showed significant reduction of total acidity and ulcer scoring. In addition, VCO exhibited a significant ($p < 0.001$) increase in mucus content (Table IV). Virgin coconut oil significantly ($p < 0.001$) reduced the volume of gastric juice from 4.1 ml in the negative control to 1.98 ml, compared to 2.20 ml in the omeprazole-treated group. On the other hand, the total acid, for the negative control reported as 92.00 mEq/l, was found to be significantly ($p < 0.001$) lower in both treatment groups, 39.67 mEq/l in the omeprazole-treated group and 30.50 mEq/l in the VCO-treated group. The gastric mucus content of pylorus ligation negative control rats was 4.03 µg/g, whereas VCO showed a significant ($p < 0.001$) increase in gastric mucus content of 11.09 µg/g followed by the positive control group (8.70 µg/g). The pH for the negative control group was found to be 2.77, while it

Table IV. Anti-ulcer effects of virgin coconut oil (VCO) on pylorus ligated model

Treatment	Gastric juice volume [ml]	Acidity (total acid) [mEq/l]	Gastric mucus content [mg/g]	pH	Ulcer index [UI]	% of inhibition for UI
Negative control	4.1 ±0.27	92.00 ±2.08	4.03 ±0.18	2.77 ±0.08	4.33 ±0.17	–
Positive control (omeprazole – 30 mg/kg)	2.20 ±0.31	39.67 ±1.38	8.70 ±0.20	5.18 ±0.12	2.00 ±0.26	53.8
VCO (10 mg/kg)	1.98 ±0.24	30.50 ±1.48	11.09 ±0.35	6.52 ±0.11	1.17 ±0.31	73.1

Values are expressed as mean ± SEM; n = 6.

Table V. Anti-ulcer effects of virgin coconut oil (VCO) on antioxidant assays

Treatment	GP [U/mg protein]	SOD [U/mg protein]	MDA [U/mg protein]	CAT [U/mg protein]	GSH [U/mg protein]	Nitrite [nmol/g]
Negative control	0.97 ±0.12	4.15 ±0.30	0.70 ±0.04	11.53 ±0.35	4.62 ±0.17	78.60 ±2.53
Positive control (omeprazole – 30 mg/kg)	0.62 ±0.07*	1.85 ±0.1***	0.32 ±0.02***	7.00 ±0.12***	7.13 ±0.18***	141 ±4.21***
VCO (10 mg/kg)	0.20 ±0.06	1.02 ±0.13	0.17 ±0.02	4.66 ±0.16	10.27 ±0.24	171.2 ±4.47

*Values are expressed as mean ± SEM; n = 6. ***p < 0.001 VCO vs. negative control. ***p < 0.001 omeprazole vs. negative control. ***p < 0.001 VCO vs. omeprazole.*

was 5.18 for the positive control group and 6.52 for the VCO-treated group. The UI for the negative control group was 4.33, whereas VCO inhibited it significantly ($p < 0.001$) to 1.17 and the positive control to 2.00. The percentage of inhibition noted for the positive control group versus the pylorus-ligated group was 53.8%, whereas for VCO it was 73.1%.

Effects of virgin coconut oil on gastric antioxidant enzyme assay

Virgin coconut oil at the dose of 10 ml/kg significantly ($p < 0.001$) increased GSH and nitrite levels as compared to the negative control group followed by the positive control (treated group). The levels of SOD, GP, MDA and CAT were significantly ($p < 0.001$) reduced by VCO relative to the control group (Table V).

Effects of virgin coconut oil on mucosal prostaglandin E_2 content

The level of PGE_2 content in the negative control was 50.50 pg/mg tissue, whereas the level of PGE_2 in VCO was significantly ($p < 0.001$) increased to 158.3 pg/mg tissue and 110.70 pg/mg tissue in the positive control group, respectively (Table VI).

Table VI. Anti-ulcer effect of virgin coconut oil (VCO) on prostaglandin E_2 (PGE_2) synthesis

Treatment	PGE_2 [pg/mg tissue]
Negative control	50.50 ±2.93
Positive control (omeprazole – 30 mg/kg)	110.70 ±5.77
VCO (10 mg/kg)	158.3 ±5.27

Values are expressed as mean ± SEM; n = 6.

Discussion

Different factors are involved in the pathogenesis of peptic ulcer in human beings such as chronic use of NSAIDs, stress, *H. pylori* infection, alcohol consumption, smoking and inappropriate dietary lifestyle. Although various kinds of medication are available to treat peptic ulcer disease such as H-2 receptor antagonist, proton pump inhibitors (PPIs), antacids and anti-muscarinics [32], most of them cause side effects to patients, yet do not providing a complete recovery [33]. Earlier studies revealed the effectiveness of VCO in treating ulcer in animal models [13, 19]. In the last decade, the exploitation of VCO for their fatty acid and vitamin E composition followed by antioxidant properties and their effects on various ailments have been reported by researchers [15, 16]. There has been growing interest and attention on VCO and their potential effects on humans include antimicrobial, anti-viral, anti-diabetic, hypocholesterolemic [16] and anti-ulcer effect. To the best of our knowledge, no detailed study has been conducted on different ulcer models that elucidated the possible mechanism. Therefore, the present study was designed to further evaluate the antiulcer activity of VCO compared to a standard drug (omeprazole). For this purpose, the effects of VCO were evaluated using different ulcer models induced by cold restraint stress, ethanol, piroxicam (NSAIDs), ethanol and pylorus ligation. Moreover, the effects of VCO on the gastric secretions from a pylorus-ligated model, level of PGE_2 secretion from a piroxicam model and antioxidant assays from an ethanol-induced model were also studied.

Omeprazole is an extensively used anti-ulcer drug belonging to proton pump inhibitors (PPIs), to treat peptic ulcer disease caused by stress, non-steroidal anti-inflammatory drugs and by *H. pylori* infections [34]. Generally, PPIs are known to act by inhibiting acid secretion and causing irreversible interaction with the H^+K^+- ATPase proton pump. However, another study revealed that omeprazole acts as a potent antioxidant to scavenge the oxygen free radical and prevents oxidative damage by increasing lipid peroxidation and protein oxidation, in an experiment conducted using three different ulcer models, stress, indomethacin-induced and pylorus ligation-induced models [35]. Thus, in the present investigation omeprazole was chosen as the standard drug for the positive control treated group.

Stress-induced ulcer may be caused by histamine secretion by the parietal cells. In addition, presence of reactive oxygen species (ROS) due to stress also may provoke damage to he stomach leading to ulceration. Apart from that, acid and pepsin-associated factors also play a role in

the pathogenesis of stress-induced ulcer. Increase in generation of ROS during stress will lead to oxidative damage and cause injury to the mucosal layer. Stress-induced ulcer also leads to a decrease in mucus production. In the current study, VCO significantly ($p < 0.05$) inhibited stress-induced ulcer compared to omeprazole. This finding indicates that VCO may enhance mucus secretion and also play a role in suppressing formation of ROS. As VCO has been reported to contain high antioxidant properties followed by flavonoid and other fatty acid components, VCO might contribute to the opposing effect [8]. From the present investigation, the increase in the levels of GSH and nitrite corresponding to the reduction in MDA, CAT, SOD and GP shown by VCO suggests that there is a strong correlation of its antiulcer activity with the free radical scavenging activity, similar to omeprazole [35].

Several previous studies have reported on the pathogenesis of ethanol in ulcer induction. It has been proven that exposure to ethanol leads to gastric lesions and mucosal damage [36]. Similarly, another study mentioned that the occurrence of cellular damage caused by ethanol exposure is dose dependent. The higher the dose of ethanol, the greater is the damage to the mucosal layer [37]. Moreover, the involvement of oxygen-derived free radicals has been associated with ethanol-induced ulceration. Ethanol-induced ulcers are also strongly related to the generation of leukotriene C4 and mast cell secretory products, which also alter the mucosal permeability, reduce gastric mucus and cause severe damage to the gastric mucosal layer [38]. Therefore, the above information suggests that an agent with high antioxidant properties such as VCO can act as a protectant for the mucosal layer by protecting against necrotizing agents such as ethanol. Based on the present results, VCO significantly ($p < 0.001$) inhibited UI with 84% inhibition as compared to the untreated group. This indicates that VCO displays a defensive characteristic against ethanol. As similar trend was observed in cold restraint stress-induced ulcer. The increase in the levels of GSH and nitrite corresponding to the reduction in MDA, CAT, SOD and GP shown by VCO may also be associated with its defensive action in the ethanol-induced model.

Piroxicam is a known anti-inflammatory drug widely prescribed for patients but it produces side effects and induces ulcer. NSAIDs act via inhibition of cyclooxygenase (COX)-1 and COX-2 enzymes, which leads to accumulation of intracellular arachidonic acid that inhibits PG synthesis [7]. Alteration in the PG level promotes acid secretion in the mucosa which disturbs the gastric equilibrium, increases the neutrophil infiltration, induces TNF-α expression and disrupts the balance between nitric oxide (NO) and other free radical expression [39]. As PG plays an important role in preventing mucosal injury and shows a protective role via enhancing bicarbonate and mucus production, it is important to prevent the suppression of PG. Virgin coconut oil shows a significant ($p < 0.001$) reduction of UI in the piroxicam-induced ulcer model. These results suggest the possibilities of PG and mucus involvement in the anti-ulcer activity of VCO. This has also been proven in our present investigations as the level of PGE_2 in the VCO-treated group was significantly ($p < 0.001$) increased three-fold as compared to the control group. In addition, it can be also supported by the significant ($p < 0.001$) increase of mucus production noted in VCO-treated pylorus-ligated rats, which is associated with preventing NSAIDs-induced ulcer.

Pylorus ligation is a procedure used in enhancing the secretion of gastric acid and breakdown of the gastric mucosal barrier [40]. In this present investigation, a significant ($p < 0.001$) decrease in gastric juice secretion, decrease in total acidity and significant ($p < 0.001$) increase in mucus content and gastric pH were observed in the VCO-treated group compared to the control group. This indicates that VCO possesses a protective role in inhibiting ulceration. Again, the enhanced production of PG as well as up-regulation and down-regulation of antioxidants observed might also be responsible for their anti-ulcerogenic effect.

A previous study reported the presence of phenols, flavonoids, and vitamin E in VCO, which highlights its high antioxidant properties [9]. Furthermore, presence of some fatty acid components such as lauric acid might also contribute to the effective role of VCO in preventing ulceration.

In conclusion, virgin coconut oil shows potential gastro-protective activity among different kinds of ulcer models. As pathogenesis of peptic ulcer disease is associated with various factors, VCO can be considered as a potential therapy to be used for treating and preventing this ailment.

Conflict of interest

The authors declare no conflict of interest.

References

1. Arrieta J, Benitez J, Flores E, Castillo C, Navarrete A. Purification of gastroprotective triterpenoids from the stem bark of Amphipterygium adstringens: role of prostaglandins, sulfhydryls, nitric oxide and capsaicin-sensitive neurons. Planta Medica 2003; 69: 905-9.
2. Mustafa M, Menon J, Muiandy RK, Fredie R, Sein MM, Fariz A. Risk factors, diagnosis, and management of peptic ulcer disease. J Dental Med Sci 2015; 14: 40-6.
3. Sung J, Kuipers E, El-serag H. Systematic review: the global incidence and prevalence of peptic ulcer disease. Aliment Pharm Ther 2009; 29: 938-46.

4. Barbastefano V, Cola M, Luiz-Ferreira A, et al. Vernonia polyanthes as a new source of antiulcer drugs. Fitoterapia 2007; 78: 545-51.
5. Ode OJ, Asuzu OV. Investigation of cassia singuena leaf extract for antiulcer effects using ethanol-induced gastric ulcer model in rats. Int J Plant Animal Environ Sci 2011; 1: 1-7.
6. Sabiu S, Garuba T, Sunmonu T, et al. Indomethacin-induced gastric ulceration in rats: protective roles of Spondias mombin and Ficus exasperata. Toxicol Rep 2015; 2: 261-7.
7. Shin JM, Sachs G. Pharmacology of proton pump inhibitors. Curr Gastroenterol Rep 2008; 10: 528-34.
8. Cohen M. Role of endogenous prostaglandins in gastric secretion and mucosal defense. Clin Invest Med 1987; 10: 226-31.
9. Oliveira FD, Andrade LN, Vieira de Sousa EB, Pergentino de Sousa D. Anti-ulcer activity of essential oil constituents. Molecules 2014; 19: 5718-49.
10. Klein LC Jr, Gandolfi RB, Santin JR, Lemos M, Cechinel-Filho V, de Andrade SF. Antiulcergenic activity of extract, fractions, and some compounds obtained from Polygala cyparissias St. Hillaire & Moquin (Poligalaceae). Naunym Schmiedebergs Arch Pharmacol 2010; 381: 121-6.
11. Sheen E, Triadafilopoulos G. Adverse effects of long-term proton pump-inhibitor therapy. Dig Dis Sci 2011; 56: 931-50.
12. Zayachkivska OS, Konturek SJ, Drozdowicz D, Brzozowski T, Gzhegotsky MR. Influence of plant-originated gastroprotective and antiulcer substances on gastric mucosal repair. Fiziol Zh 2004; 50: 118-27.
13. Selverajah M, Zakaria ZA, Long K, Ahmad Z, Yaacob A, Somchit MN. Anti-ulcerogenic activity of virgin coconut oil contribute to the stomach health of humankind. Tang 2016; 6: 12-8.
14. Handayani R, Sulistyo J, Rahayu RD. Extraction of coconut oil (Cocos nucifera L.) through fermentation system. Biodiversitas 2009; 10: 151-7.
15. Mansor TS, Che Man YB, Shuhaimi M, Abdul Afiq MJ, Ku Nurul FK. Physicochemical properties of virgin coconut oil extracted from different processing methods. Int Food Res J 2012; 19: 837-45.
16. Nevin K, Rajamohan T. Influence of virgin coconut oil on blood coagulation factors, lipid levels and LDL oxidation in cholesterol fed Sprague-Dawley rats. e-SPEN 2008; 3: e1-8.
17. Abd El Motteleb DM, Hasan MM. Gastroprotective effect of simvastatin against experimetnally induced gastric ulcers in rats: role of ATP-sensitive K^+ channels. J Am Sci 2011; 7: 760-9.
18. Almasaudi SB, El-Shitany NA, Abbas AT, et al. Antioxidant, anti-inflammatory, and antiulcer potential of manuka honey against gastric ulcer in rats. Oxid Med Cell Longev 2016; 2016: 3643824.
19. Nneli RO, Woyike OA. Antiulcerogenic effects of coconut (Cocos nucifera) extract in rats. Phytother Res 2008; 22: 970-2.
20. Miñano FJ, Serrano J, Pascual J, Sancibrián M. Effects of GABA on gastric acid secretion and ulcer formation in rats. Life Sci 1987; 41: 1651-8.
21. Okabe S, Takeuchi K, Urushidani T, Takagi K. Effects of cimetidine, a histamine H2-receptor antagonist, on various experimental gastric and duodenal ulcers. Am J Dig Dis 1977; 22: 677-84.
22. Al-Jaber N. The relationship between antioxidant and antiulcer activities in saudi honey samples harvested from various regions in different seasons. Food Nutr Sci 2013; 4: 131-8.
23. Rozza AL, Moraes TD, Kushima H, Nunes DS, Hiruma-Lima CA, Pellizzon CH. Involvement of gluthathione, sulfhydryl compounds, nitric oxide, vasoactive intestinal peptide, and heat-shock protein-70 in the gastroprotective mechanism of Croton cajucara Benth. (Euphorbiaceae) essential oil. J Med Food 2011; 14: 1011-7.
24. Shay H, Komarov SA, Fell SS. A single method for the uniform production of gastric ulceration in the rat. Gastroenterology 1945; 5: 43-5.
25. Verma VK, Singh N, Saxena P, Singh R. Anti-ulcer and anti-oxidant activity of Moringa Oleifera (Lam) leaves against aspirin and ethanol induced gastric ulcer in rats. Int Res J Pharm 2012; 2: 46-57.
26. Shay H, Sun DC, Gruenstein M. A quantitative method of masuring a spontaneous gastric secretion in the rat. Gastroenterology 1954; 26: 906.
27. Bhave AL, Bhatt JD, Hemavathy KG. Antiulcer effect of amlodipine and ist interaction with H2 blocker and proton pump inhibitor in pylorus ligated rats. Indian J Pharmacol 2006; 38: 403-7.
28. Kumar V, Bhat ZA, Kumar D, Khan NA, Chashoo IA, Ara I. Gastroprotective effect of leaf extracts of Basella alba against experimental gastric ulcers in rats. Br J Pharmacognosy 2012; 22: 657-62.
29. Corne SJ, Morissey SM, Woods RJ. A method for the quantitative estimation of barrier mucus. J Physiol 1974; 242: 116-7.
30. Akindele AJ, Aigbe FR, Olawe JA, Oduntan BO, Adeyemi O. Gastroprotective effects of DAS-77 (a Phytomedicine) in ulcer models in rats. Trop J Pharm Res 2012; 11: 783-91.
31. Lima ZP, Calvo TR, Silva EF, et al. Brazilian medicinal plant acts on prostaglandin level and Helicobacter pylori. J Med Food 2008; 11: 701-8.
32. Wallace JL, Granger D. The cellular and molecular basis of gastric mucosal defense. FASEB J 1996; 10: 731-40.
33. Rates SM. Plants as source of drugs. Toxicon 2001; 39: 603-13.
34. Fellenius I, Berglindh T, Sachs G, et al. Substituted benzimidazoles inhbit gastric acid secretion by blocking (H^+K^+) ATPase. Nature 1981; 290: 159-61.
35. Biswas K, Bandyopadhyay U, Chattopadhyay I, Varadaraj A, Ali E, Benerjit RK. A novel antioxidant and antiapoptotic role of omeprazole to blockgastric ulcer through scavenging of hydroxyl radical. J Biol Chem 2003; 278: 10993-1001.
36. Hamauzu Y, Forest F, Hiramatsu K, Sugimoto M. Effect of pear (Pyrus communis L.) procyanidins on gastric lesions induced by HCl/ethanol in rats. Food Chem 2007; 100: 255-63.
37. Mutoh H, Hiraishi H, Ota S, Ivey KJ, Terano A, Sugimoto T. Role of oxygen radicals in ethanol-induced damage to cultured gastric mucosal cells. Am J Physiol 1990; 258: G603-9.
38. Salim A. Removing oxygen-derived free radicals stimulates healing of ethanol-induced erosive gastritis in the rat. Digestion 1990; 47: 24-8.
39. Hayllar J, Bjarnason I. NSAIDs, Cox-2 inhibitors, and the gut. Lancet 1995; 346: 521-2.
40. Sairam K, Rao CV, Babu MD, Kumar KV, Agrawal V, Goel RK. Antiulcerogenic effect of methanolic extract of Emblica officinalis: an experimental study. J Ethnopharmacol 2002; 82: 1-9.