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Anti-ulcer activity of *Hibiscus cannabinus* and *Hibiscus sabdariffa* seeds in ulcer-induced rats

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Abstract

The aim of this research is to determine the antiulcer properties and percentage protection of *Hibiscus cannabinus* and *Hibiscus sabdariffa* seed samples towards ulcer-induced *Sprague dawley* rats. Rats were divided into six groups each for each ulceration method and fed with distilled water, Omeprazole, *H. cannabinus* seed oil (HCSO), *H. cannabinus* seed extract (HCSE), *H. sabdariffa* seed oil (HSSO) and *H. sabdariffa* seed extract (HSSE), respectively via oral administration. Among the two plants tested, *H. cannabinus* showed the best protection percentage towards ethanol, non-steroidal anti-inflammatory drugs (NSAIDs) and cold restrain stress induced ulcers. *H. cannabinus* seed extract (HSSE) exhibited an exceptionally high ulcer protection of 74.98 ± 0.78% against NSAIDs induced ulcer. The gastric lesions were controlled primarily by both mucosal protection and acid inhibition. In conclusion, addition of these seeds to the daily diet may reduce free radical activity in the body and reduce the risk of developing peptic ulcer disease.

Introduction

Approximately four million people worldwide are affected by peptic ulcer disease (PUD) every year due to the imbalance between the aggressive and defensive factors (Zelickson *et al.*, 2011). Around 10 to 20% of patients' encountered complications while 10 to 40% are mortality cases (Lau *et al*., 2011). This disease is considered common around the world and therefore deserves more attention in controlling the widespread of PUD. The ingestion of non-steroidal anti-inflammatory drugs (NSAIDs) and *Helicobacter pylori (H. pylori)* infection are two major factors that increases the complications and risk of developing PUD. Synthetic drugs for instance antacids, proton pump inhibitors and histamine-2 blockers are used to treat ulceration. However, the side effects are unavoidable such as diarrhoea, headache and constipation (Srinivas *et al.*, 2013). Despite this, herbal medicine is still the mainstay of 75-80% of population and is considered a better option as they have fewer side effects, better compatibility with the human body and thus, potential for new drugs (Parekh *et al*., 2005).

Kenaf (*Hibiscus cannabinus* L.) is a warm-season annual fiber crop that has been commercially cultivated in Asia, such as China, India, Malaysia and Thailand (Liu, 2000). Many researches around the globe have been focusing on the utilization of *H. cannabinus* stems for papermaking and pulping as it was an excellent cellulose fiber source while the potential for using *H. cannabinus* seeds as a source of edible oil is overlooked. *H. cannabinus* seed is high in oil content ranging from 21.4% to 26.4% (Nyam *et al*., 2009). The oil is very similar to cotton seed oil. Edible oil is yield from seeds of this plant and is used as first-class cooking oil and margarine production. This oil is nutritionally beneficial to health because of the abundant amount of monounsaturated and polyunsaturated fatty acids available (Coetzee *et al.*, 2008). The seed oil is edible and can be kept for it has a long shelf life. Besides, the oil is a rich source of bioactive compounds filled with high antioxidative, anticancer and lipid lowering cholesterol properties (Nyam *et al*., 2009). It has been reported by Yazan *et al.* (2013) that H. cannabinus seed oil was cytotoxic towards ovarian cancer and colon cancer cell lines. Alpha-linolenic acid (omega-3 fatty acid) was also found in the seed which acts as a precursor of eicosanoids with anti-inflammatory and antithrombotic activity (Ruiz *et al*., 2002). However, a higher antioxidant yield and

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increased in antioxidant activity can be obtained from *H. cannabinus* seed extract (HCSE) (Wong *et al.*, 2014) . The findings of Lee *et al.* (2007) indicated that ethanol extract of *H. cannabinus* may produce promising anti-inflammatory properties and is capable of treating macrophage related diseases for example septic shock, arteriosclerosis and rheumatoid arthritis.

Roselle (*Hibiscus sabdariffa* L.) is a tropical wild plant widely used in many countries as a medical herb for its antioxidant, antibacterial (Oboh and Elusiyan, 2004) and lipid-lowering properties (Faraji and Tarkhani, 1999). Jadhavet *et al.* (2009) reported that the leaves and flowers promote hair growth and aid in ulcer healing. *H. sabdariffa* seeds are used in China for vegetable oil production, sauces and as coffee substitute. *H. sabdariffa* seed oil (HSSO) is composed of mainly oleic and linoleic fatty acids (Mohamed *et al*., 2007). According to Lautenschlager (2003), linoleic acid is commonly used for cosmetic production. It moisturises the skin due to its high penetrability, aids in healing dermatoses and sunburns and may help in reducing acne lesions. It has anti-inflammatory effects, improves blood lipids and lowers blood pressure. The therapeutic effect of this extract will encourage its use in the treatment of inflammation. Lee *et al*. (2007) indicated that ethanol extract of *H. cannabinus* may produce promising anti-inflammatory properties and is capable of treating macrophage related diseases, for example septic shock, arteriosclerosis and rheumatoid arthritis.

Plants like *H. cannabinus* and *H. sabdariffa* are regaining the attention of many researches due to their many potential benefits for human health and prevention of diseases. This provides an alternative strategy and offers a bright future in the development of antiulcer drugs for the treatment of gastric ulcer. The objectives of this study was to compare the antiulcer properties of H. cannabinus seed oil (HCSO), *H. cannabinus* seed extract (HCSE), *H. sabdariffa* seed oil (HSSO) and *H. sabdariffa* seed extract (HSSE) in ulcer induced Sprague dawley rats.

Materials and Methods

Materials

Ten kilograms of dried *H. cannabinus* seeds were obtained from Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia. Ten kilograms of *H. sabdariffa* seeds were sourced from the Department of Agriculture Plantation, Rembau, Negeri Sembilan, Malaysia. Male *Sprague dawley* rats were obtained from the institutional animal house of University Kebangsaan Malaysia, Bangi, Malaysia for the antiulcer study.

H. cannabinus *(HCSE) and* H. sabdariffa *seed extracts (HSSE) preparation*

A 500 mL Scott bottle was prepared and filled with 80% ethanol to a total volume of 500 mL. A total of 50 g of grinded *H. cannabinus* seed was added to the solvent, followed by ultrasonic extraction (Ultrasonic Homogenizer Labsonic P, 400 W, Sartorius, AG) for 30 min with a 5 min pulse duration period and a 5 min pulse interval period. The extraction was repeated for 3 cycles. The *H. cannabinus* seed extract (HCSE) collected was centrifuged at 3500 rpm for 10 min. The supernatant of the kenaf seed extract was collected and filtered; the pellet was discarded. The filtered supernatant was subjected to rotary evaporation (Rotavapor R-200, Buchi, Switzerland) (Wong *et al.* 2014). The same extraction procedures were repeated for *H. sabdariffa* seed to obtain H. sabdariffa seed extract (HSSE).

H. cannabinus *seed oil (HCSO) and* H. sabdariffa *seed oil (HSSO) preparation*

The HCSO were extracted from the seeds with soxhlet extractor using hexane at 60 ºC for 3 hours. The oil was then recovered by evaporating off the solvent using rotary evaporator Model N-1 (Eyela, Tokyo Rikakikal Co., Ltd., Japan) and residual solvent was removed by flushing with 99.9% (Ng *et al*. 2014). The above procedure was repeated to obtain *H. sabdariffa* seed oil (HSSO) by replacing *H. cannabinus* seed powder with *H. sabdariffa* seed powder.

Evaluation of antiulcer activity

Experimental animals

All Sprague dawley rats weighing about 200-250 g were housed in standard cages of three to a cage. The animals were taken care under the standard laboratory conditions with controlled lighting (12 hours light and dark cycles) and at room temperature (25 ± 3ºC).

The animals were fed with meat-free rat and mouse-diet from Speciality Feeds (Glen Forrest, Western Australia) and have free access to water ad libitum throughout the duration of the experiment. Animals were allowed to acclimatise for two weeks prior to experiment to ensure animals are in a healthy state and to minimise the stress factors cause by transportation of animals. All experimental procedures were conducted after the approval of the Faculty's research ethics committee of UCSI University, Cheras, Malaysia with the approval code

Table 1. Effect of HCSO, HCSE, HSSO and HSSE on various parameters in ethanol induced, indomethacin-induced, and cold restraint stress-induced gastric ulcer in rats

	Group	Treatment	pH	Ulcer index	% Protection
Ethanol induced	I	Control (distilled water)	7.27 ± 0.51^{ab}	48.33 ± 1.53^{a}	-
	II	Omeprazole	8.29 ± 0.77^{a}	31.00 ± 2.65^{b}	35.93 ± 3.43^{c}
	III	HCSO	6.79 ± 0.23^{b}	22.00 ± 2.00^{c}	54.54 ± 2.74^{a}
	IV	HCSE	7.72 ± 0.52^{ab}	27.33 ± 2.52^{bc}	43.52 ± 3.41^{b}
	V	HSSO	6.78 ± 0.53^{b}	33.00 ± 2.65^{b}	31.78 ± 3.83^{c}
	VI	HSSE	4.12 ± 0.35^{c}	29.33 ± 1.53^{b}	39.34 ± 1.23^{bc}
Indomethacin -induced	I	Control (distilled water)	1.59 ± 0.54^{c}	33.33 ± 2.52^{a}	-
	II	Omeprazole	3.71 ± 0.05^{b}	4.67 ± 0.58^{e}	86.02 ± 1.13^{a}
	III	HCSO	3.81 ± 0.33^{b}	17.33 ± 1.16^{c}	47.95 ± 2.30^{c}
	IV	HCSE	3.33 ± 0.20^{b}	8.33 ± 0.58^{d}	74.98 ± 0.78^{b}
	V	HSSO	4.47 ± 0.21^{a}	18.67 ± 1.53^{c}	44.01 ± 1.42^{c}
	VI	HSSE	1.86 ± 0.12^{c}	23.67 ± 1.16^{b}	28.89 ± 2.67^{d}
Cold restraint stress-induced	I	Control (distilled water)	3.12 ± 0.62^{c}	35.33 ± 2.52^{a}	-
	II	Omeprazole	6.74 ± 0.22^{a}	1.67 ± 0.58^{c}	95.33 ± 1.44^{a}
	III	HCSO	3.52 ± 0.33^{c}	14.00 ± 1.00^{b}	60.38 ± 0.33^{b}
	IV	HCSE	4.83 ± 0.33^{b}	15.00 ± 1.00^{b}	57.54 ± 0.38^{c}

Values are expressed as mean ± SD (n=5). Statistical analyses were performed using ANOVA followed by Tukey test. Mean values at the same column with different superscript letters are significantly different at $p < 0.05$.

Proj-FAS-EC-13-038 and were in strict accordance to the institutional animal ethical committee guidelines for care and use of laboratory animals.

Experimental design

The animals were grouped into six groups comprising of five animals each. Food pellets were withdrawn 24 hours before the start of the experiment and while the animals had free access to water ad libitum. The compositions of experimental treatments were as shown in Table 1.

Ethanol-induced ulcer

After 24 hours of fasting, the animals were treated with treatments as shown in Table 1. One hour after the treatment, all six groups (n=5) of rats were orally administered with 5 mL/kg of ethanol each according to Robert (1979) with modifications. After one hour, the rats were euthanized with excess chloroform and their stomachs were immediately excised and opened along the greater curvature, cleared of residual matter with saline and the inner surface was examined for ulceration.

Non-steroidal anti-inflammatory drug (NSAID)-induced ulcer

The procedures were similar to that used in ethanol induced ulceration except that the control group (Group I) received indomethacin only. Lesions were induced according to the method of Nawafor *et al.* (2000) with modifications. After 24 hours of fasting, the animals were treated with treatments as shown in Table 1. One hour after the treatment, all six groups (n=5) of rats received indomethacin 100 mg/kg orally. After four hours, the rats were euthanized with excess chloroform and their stomachs were immediately excised and opened along the greater curvature, cleared of residual matter with saline and the inner surface was examined for ulceration.

Cold-Restraint Stress Induced Ulcer

Four groups (Group I, II, III and IV) (n=5) of rats were used. After 24 hours of fasting, Group I (control) received distilled water (10 mL/kg), Group II was given omeprazole (30 mg/kg), Groups III and IV were fed with 500 mg/kg of HCSO and HCSE (p.o), respectively. The method described by Senay and Levine (1967) was adopted with slight modifications. One hour after treatment, gastric ulceration was induced by immobilising the animals in a closed cylindrical restrainer immersed vertically up in a water bath of (18 ± 2)ºC to the xyphoid level, in the presence of intense light for three hours to induce stress ulcer. After three hours, the rats were euthanized with excess chloroform and their stomachs were immediately excised and opened along the greater curvature, cleared of residual matter with saline and the inner surface was examined for ulceration.

Measurement of ulcer index

The average length (mm) of all lesions of each

stomach were measured and classified into three levels as shown in Table 1. The ulcer index (UI) was determined as follows:

Ulcer index (UI) = 1 × (number of ulcers level I) + 2 × (number of ulcers level II) + 3 × (number of ulcers level III)

Percentage of ulcer protection was calculated as below (Navarrete *et al*. 1998):

$$\%\,\text{Protection} = \frac{(\text{Ulcer index Control} - \text{Ulcer index Test})}{\text{Ulcer index Control}} \times 100$$

Determination of pH

Gastric juice (1 mL) was diluted with 1 mL distilled water and was measured using a pH meter (Dashputre and Naikwade, 2011).

Statistical analysis

All experiments were performed in triplicate, unless stated otherwise. The results were expressed as mean ± standard deviation (SD). Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Tukey test. A p-value of <0.05 was considered statistically significant. The statistical analyses were performed using Minitab software, version 16.1.

Results and Discussion

Based on Table 1, the oral administration of HCSO, HCSE, HSSO and HSSE demonstrated gastro protective effects against gastric lesions induced by ethanol. They showed significant reduction in ulcer index, as well as increased in protection from gastric lesion. Rats fed with HCSO exhibited the highest protection percentage of 54.54 ± 2.74% while HSSO showed the lowest protection percentage of only 31.78 ± 3.83%.

All the four samples showed significant ($p < 0.05$) antiulcer effect at the dose of 500 mg/kg per oral dose. This may be due to the cytoprotective effect of the samples via antioxidant effects. Ethanol at a dose of 5 ml/kg, produced red coloration, sported ulcer and severe gastric hemorrhagic erosions in the control rat.

The development of the haemorrhagic lesions and necrotic tissue injury as observed in may be caused by stasis in gastric blood flow, in which gastric blood flow cease. Alcohol is able to penetrate the gastric mucosa rapidly, causing cell rupture and plasma membrane damage thus the intracellular membrane permeability to sodium and water was increased. The stomach appears to be larger in size and was filled with gastric contents. Besides, when an extreme amount of calcium is accumulated in the intracellular, this results in development of gastric mucosal injury which led to cell death and exfoliation in the surface epithelium (Raju *et al*., 2009). The direct damage of gastric mucosal cells that resulted in the development of free radicals and hyperoxidation of lipids would increase the gastric lesions.

From this study, ethanol is known to be very harmful to the stomach since the gastric mucosa was affected by the disruption of protective barrier and provoking prominent microvascular changes within a few minutes after application (Moleiro *et al*., 2009). Therefore, mucus secretion is vital in protecting the gastric mucosa from gastric lesions. The results clearly indicated a significant cytoprotective and gastric antiulcer activity of *H. cannabinus* and *H. sabdariffa*. Flavonoids are among the cytoprotective materials able to increase mucus, bicarbonate and prostaglandin secretion, strengthening of gastric mucosal barrier and scavenging of free radicals which are very important in preventing ulcerative and erosive lesions of gastrointestinal tract (Sachin and Archana, 2009). The results of the present study suggested that HCSO may be beneficial in the treatment of gastric lesions. The effectiveness of HCSO in preventing peptic ulcer was further investigated in non-steroidal anti-inflammatory drugs (NSAIDs) induced ulcer and cold restraint water immersion stress induced ulcer.

Based on Figure 1 (A), severe injuries are seen in the gastric mucosa. Ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa. Rat pre-treated with 10 ml/kg omeprazole (30 mg/kg) had injuries of the gastric mucosa. However they are milder compared to the injuries seen in negative control rats (Figure 1 (B)). Mild injuries are seen in the gastric mucosa of rat pre-treated with HCSO (500 mg/kg) and HCSE (500 mg/kg). The oil and extract reduces the formation of gastric lesions induced by acidified ethanol (Figure 1 (D)). Moderate injuries are seen in the gastric mucosa of rat pre-treated with HSSO (500 mg/kg). The oil was able to reduce the formation of gastric lesions induced by acidified ethanol (Figure 1 (E)). Sported injuries are seen in the gastric mucosa, but they are milder compared to the injuries seen in negative control rats when pre-treated with 500 mg/kg of HSSE (Figure 1 (F)).

The gastric pH of rats that treated with omeprazole (positive control) at 30 mg/kg was 8.29 ± 0.77, slightly higher compared to the Group I (negative control). While the rats treated with HSSE showed the lowest gastric pH of only 4.12 ± 0.35 (Table 1). From Table 1, the rats that pre-treated with HCSO

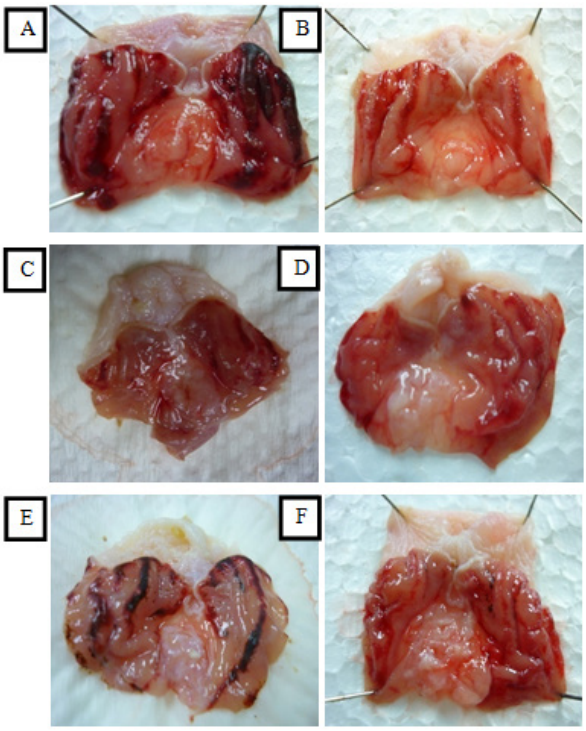

Figure 1. Gross appearance of the gastric mucosa in ethanol induced rats. (A) 10 mL/kg distilled water (negative control). (B) Omeprazole (30 mg/kg). (C) HCSO (500 mg/kg). (D). HCSE (500 mg/kg). (E). HSSO (500 mg/kg). (F) HSSE (500 mg/kg)

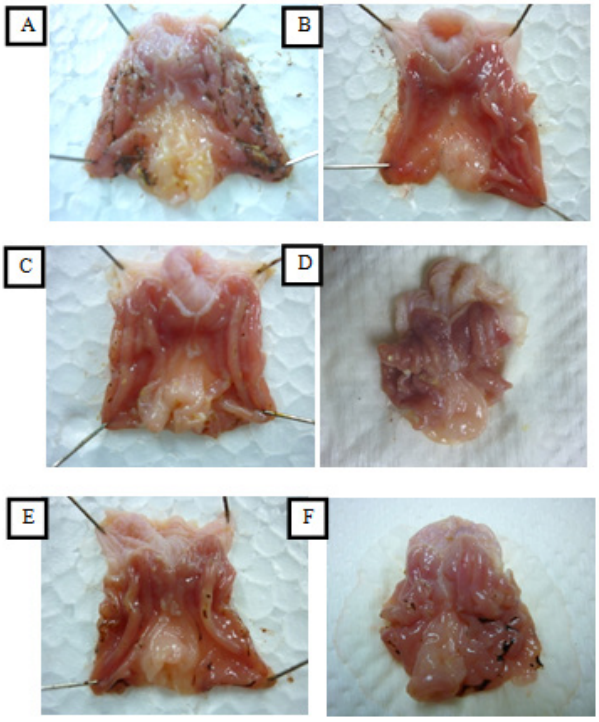

Figure 2. Gross appearance of the gastric mucosa in NSAIDs induced rats. (A) 10 mL/kg distilled water (negative control). (B) Omeprazole (30 mg/kg). (C) HCSO (500 mg/kg). (D) HCSE (500 mg/kg). (E) HSSO (500 mg/kg). (F) HSSE (500 mg/kg)

and HSSO had gastric pH of 6.79 ± 0.23 and 6.78 ± 0.53, respectively, which was nearer to neutral (pH 7).

Non-steroidal anti-inflammatory drugs (NSAIDs) induced ulcer

In this study, HCSO and HCSE were able to produce a significant reduction in gastric mucosal damage induced by indomethacin, indicating a possible increase of prostaglandin synthesis. The analgesic effect of NSAIDs has caused NSAID induced ulcer to be painless, thus endangering consumers. They are usually prescribed as pain killers to soothe headaches, sprains and arthritis symptoms.

The protective action of HCSE against indomethacin induced gastric lesions may be due to 5-lipoxygenase inhibitory effect of cyclooxygenase (Rainsford, 1987). Besides, it may have stimulated prostaglandin secretion or produce prostaglandins like substances to protect the stomach. Omeprazole was incorporated into the study to examine the participation of proton pump inhibitor on NSAIDs induced ulceration. The significant percentage of protection of gastric ulcer in the rats pre-treated with HCSE was compatible with omeprazole, the standard drug used in this experiment to cure ulcer. The acidity of gastric content in rats treated with HCSO, HCSE, HSSO and HSSE were higher compared to negative control group (Table 1).

The gross appearances of excised stomachs of indomethacin induced gastric lesions are shown in Figure 2. The lesions in the negative control group stomach appeared as elongated bands of thick, black coloured lesions with yellowish mucus. Mucus secretion is vital in protecting the gastric mucosa from gastric lesions. The ulcer lesions produced in HCSE fed rats were not as prominent compared to the other samples. Omeprazole, as a proton pump inhibitor has been widely used by people around the world as an acid inhibitor agent for managing gastric acid secretion related disorders and protects the stomach gastric mucosa (Li *et al*., 2004). The mechanism of omeprazole works in a way that it is able to bind very specifically to a single subunit of the gastric H+, K+-ATPase and inactivates it. This inhibits the acid secretion despite of the source of secretory stimulation. Omeprazole is very beneficial and effective for both long and short-term use in treating peptic ulcer disease (AlRashdi *et al*., 2012).

HSSO portrayed the highest gastric pH of 4.47 ± 0.21, followed by HCSO, HCSE and HSSE with gastric pH of 3.81 ± 0.33, 3.33 ± 0.20 and 1.86 ± 0.12, respectively in comparison to negative control (Group I) with the lowest pH (Table 1). The oils

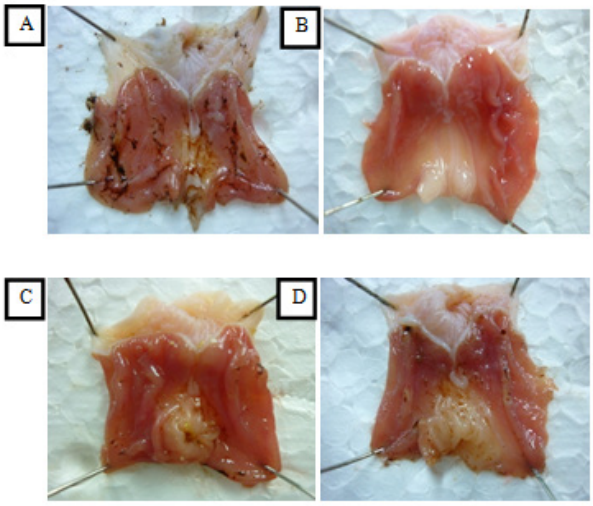

Figure 3. Macromorphological presentation of the gastric mucosa in cold restraint water immersion stress induced ulcer in rats. (A) 10 mL/kg distilled water (negative control). (B) Omeprazole (30 mg/kg). (C) HCSO (500 mg/kg). (D) HCSE (500 mg/kg)

and extracts showed protection against gastric lesions produced by indomethacin. Omeprazole also accelerates ulcer healing by reducing the acid secretion thus pH of gastric juice increases. NSAID such as indomethacin have the ability to cause gastro-duodenal ulcer by reducing prostaglandin synthesis, which is important for increasing blood flow, secretion of mucus and bicarbonate and in inhibiting the secretion of hydrochloric acid (Konturek *et al*., 2005). Thus, the gastric juice pH for indomethacin induced ulcer method was much lower compared to ethanol induced ulcer method.

According to Figure 2 (A), rat pre-treated with 10 mL/kg distilled water (negative control) had severe injuries in the gastric mucosa. Indomethacin produced elongated bands of thick, black coloured lesions with yellowish mucus. There are some red dots seen on the gastric mucosa in rat pre-treated with omeprazole (30 mg/kg) however, they were much milder compared to the injuries seen in negative control rats (Figure 3.2 (B)). Dotted erosions are seen in rat fed with HCSO (500 mg/kg) (Figure 3.2 (C)). Rat pre-treated with HCSE (500 mg/kg) showed mild dotted lesions (Figure 3.2 (D)). Moderate injuries are seen in rat pre-treated with HSSO (500 mg/kg) (Figure 3.2 (E)). For the rats pre-treated with 500 mg/kg HSSE, erosion injuries on the gastric mucosa are milder compared to the injuries seen in negative control rats (Figure 3.2 (F)).

Cold restraint water immersion stress induced ulcer

The cytoprotective activity of HCSO and HCSE was examined on cold restrained water immersion stress rats. This is because HCSO and HCSE portrayed the best protection percentage in ethanol induced ulceration and NSAID induced ulceration, respectively. Cold restrained water immersion stress provides both emotional stress as well as physiological stress to the animals. It is one of the best methods for inducing stress-induced gastric lesions in rats. Some of the factors known to play a role in inducing gastric lesions are by auto-digestion of gastric mucosal barrier, accumulation of hydrochloric acid and generation of oxygen derived free radicals. Stress-related mucosal bleeding has known to be the cause of morbidity and mortality in critically ill patients (Metz 2004). Therefore, necessary prevention steps are important to avoid stress-related ulceration. Arakawa *et al.* (1997) stated that stress causes increases in plasma norepinephrine and epinephrine levels, whereas stress-induced syndromes are reduced by inhibitors of adrenaline receptors or sympathetic nerve excitation.

Cold restrained water immersion stress produced visible gastric lesions in all rats after three hours as anticipated. In the cold restraint stress induced gastric ulcer rats, a significant reduction (14.00 ± 1.00 and 15.00 ± 1.00) in ulcer index was observed in rats treated with 500 mg/kg of HCSO and HCSE in comparison with negative control group (Group I) (Table 1). The average ulcer index in negative control group (Group I) was very high (35.33 ± 2.52). The ulcers produced are most likely mediated by production of histamine followed by acid secretion and reduction in mucus production. The blood flow reduces drastically in cold stress immobilisation leading to local hypoxia and ischemia (Hase and Moss, 1973). Lesions observed were clearly visible and were spread throughout the stomach area. The percentages of protection against ulcer were 60.38 ± 0.33, 57.54 ± 0.38 and 95.33 ± 1.44 for groups treated with HCSO, HCSE and omeprazole, respectively. Thus, omeprazole had more antiulcer effect in cold restrained water immersion stress induced rats followed by HCSO and HCSE.

According to Table 1, animal treated with omeprazole portrayed the highest gastric pH of 6.74 ± 0.22, followed by HCSE and HCSO with gastric pH of 4.83 ± 0.33, 3.52 ± 0.33, respectively in comparison to negative control (Group I) with the lowest pH (Table 1). There is a correlation between pH and % protection as shown in Table 1. As gastric pH increases, % protection increases. The blood flow may decline when stress is present and this would enhance the production of certain substance present in the stomach such as acid, bile and pepsin. Thus, the gastric pH dropped due to the over production of gastric acid and this would cause gastro-duodenal

ulceration. Rats fed with omeprazole (Group II) portrayed a significant ($p < 0.05$) increase in gastric pH of 6.74 ± 0.22 and increase in protection percentage of 95.33 ± 1.44 compared to negative control group.

Results showed that rats pre-treated with HCSO and HCSE before stressing had significantly reduced in ulcer index compared to rats pre-treated with distilled water (negative control, Group I). It was observed that the protection of gastric mucosa was more prominent in rats pre-treated with 500 mg/kg HCSO as demonstrated in Table 1. The plant oil and extracts are used to stimulate the mucosal defence mechanism of the stomach by increasing the amount of mucus production to protect and facilitate in repairing of damaged epithelial cells (Goel and Sairam, 2002). HCSO significantly acts as a first line defence against cold restraint stress-induced gastric ulcers by showing cytoprotective property. There may be some antioxidant properties present in HCSO that provides some protection towards the rats induced with cold restrained water immersion stress.

Rats fed with omeprazole (Group II) showed a significantly ($p < 0.05$) lower ulcer index (1.67 ± 0.58) compared to negative control group. As according to Brzozowaski *et al.* (2000), proton pump inhibitors and histamine H_2-receptor antagonists are known for speeding up the healing process of gastric lesions and in inhibiting mucosal injury. Therefore, gastric ulcer was significantly inhibited showing a protection percentage of 95.33 ± 1.44. The stomach surface remained pink, showing there was neither inflammation nor bleeding (Figure 3). The pH of gastric contents in HCSO was significantly lower compared with HCSE (pH 3.52 ± 0.33 vs. pH 4.83 ± 0.33). Omeprazole showed the highest gastric pH of 6.74 ± 0.22. The auto digestion of gastric mucosal barrier caused by the overproduction and build-up of gastric acid is known to be the most important reason for the formation of gastric lesions caused by stress (Sairam *et al*., 2003). This decreases the gastric mucus in reducing the mucosal damage of stomach. Apart from that, the gastric acid secretion plays an important role in the progression from an erosive mucus layer to a gastric lesion.

The protective activity against cold restraint stress may be due to the antioxidant activity present in HCSO, which helps in strengthening the rats' physiological competence to reduce stress induced ulcers. The results obtained indicate that the HCSO had the ability to maintain the cell membrane integrity, to protect the gastric mucosa against oxidative damage and to strengthen the mucosal barrier, which acts as the first line defense against ulcer agents. The diagrams in Figure 3 show the excised stomach of respective groups and the severity of ulcer on the lining.

Figure 3.3 shows that the (A) Rat pretreated with 10 mL/kg distilled water (negative control) suffered from severe injuries in the gastric mucosa. Cold restrained water immersion stress produced brownish-black coloured lesions with mucus. Not much gastric ulcer was formed in rat pre-treated with 10 ml/kg omeprazole (30 mg/kg) however, there were a few small dots on the surface of the stomach [Figure 3.3 (B)]. Dotted erosions are seen in the gastric mucosa of rat pre-treated with HCSO (500 mg/kg) and HCSE (Figure 3.3 (C) and 4.4 (D)). HCSO and HCSE reduced the formation of gastric lesions induced by cold restrained water immersion stress.

Conclusion

Both plant (*H. cannabinus* and *H. sabdariffa*) seed oils and extracts reduced the ulcer index and increased percentage of protection against ulceration, suggesting their role in protecting against gastric ulcer. This suggests the potential of *H. cannabinus* and *H. sabdariffa* in strengthening the stomach defense against inflammation and further progress to gastric ulcer. All samples showed protective activity against ethanol, NSAIDs and cold restrain induced ulcer activities. However, H. cannabinus samples produced better protection against ulcer compared to *H. sabdariffa* samples. HCSO showed the highest protection against ethanol and cold restrained stress ulcer, whereas HCSE exhibited the highest protection against NSAID ulcers. Hence, this result has revealed that *H. cannabinus* seed is a superior potential of an antiulcer agent.

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