Antiulcer activity of methanol-chloroform extract of *Channa striatus* fillet

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Abstract: *Channa striatus* (Haruan) is Malaysian freshwater fish that is traditionally used to treat ailments related to wound and also ulcers. The aimed of the present study was to determine the mechanisms of anti-ulcer activity of chloroform: methanol extract of *C. striatus* fillet (CMCS) in rats. The antiulcer profile of CMCS, given orally in the doses of 50, 250 and 500mg/kg, was assessed using the ethanol- and indomethacin-induced gastric ulcer models. The mechanisms of antiulcer of CMCS were determined as follows; i) the antisecretory activity of CMCS was measured using the pyloric ligation rat model, and; ii) the role of nitric oxide (NO) and sulfhydryl compounds in the modulation of CMCS antiulcer activity were determined by pre-treating the rats with $_L$-NAME or NEM, respectively, followed by the pre-treatment of rats with CMCS before subjecting the animals to the ethanol-induced gastric ulcer model. From the results obtained, CMCS exerted significant ($P<0.05$) antiulcer activity in both models of gastric ulcer wherein the macroscopic and microscopic analysis of the stomach supported the antiulcer claim. With regard to its antisecretory effect, CMCS did not change the volume and pH, but reduce the total acidity only at the lower doses of the gastric juice. Moreover, CMCS demonstrated antiulcer activity was reversed by NEM, but not affected by L-NAME. In conclusion, CMCS shows antiulcer activity that is modulated via its cytoprotective, but not antisecretory effect, and in the presence of sulfhysryl compounds, but not NO.

Keywords: *Channa striatus*, chloroform: methanol extract, anti-ulcer activity, mechanisms of action, cytoprotective.

INTRODUCTION

About 8-10% of the global population suffered from peptic ulcers (Calam and Baron, 2001). Of these, approximately 5% of the patients experience from gastric ulcers (Bandyopadhyay *et al.*, 2002). Gastric ulcers arise due to the disproportion between the destructive factors (i.e. acid and pepsin secretions, refluxed bile, and reactive oxygen species (ROS)) and protective factors (i.e. bicarbonate secretion, mucus-bicarbonate barrier, prostaglandins (PGs), mucosal blood flow and non-enzymatic and enzymatic antioxidants) (Mota *et al*., 2009). These imbalances might be attributed to various factors (i.e. alcohol consumption, frequent use of steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) and drugs which stimulate gastric acid and pepsin secretions, a stressful lifestyle, *Helicobacter pylori* infections and smoking) (Rang *et al*., 2012).

Despite tremendous achievement and development in the field of antiulcer drug development, the prevention or cure of peptic ulcers remains an important challenge in the current medicine world. Generally, the secretion of gastric acid is still considered to be the major factor of this multifactor disease (Mota *et al*., 2009) and has been the main therapeutic aim for ulcer diseases (Jain *et al*., 2007). In line with this claim, the present management of gastric ulcers is generally based on the inhibition of gastric acid secretion, which is achieved using several classes of antiulcer drugs (i.e. histamine H_2-antagonists, proton pump inhibitors, and antimuscarinics) (Bighetti *et al*., 2005). Unfortunately, the successes of gastric ulcer therapy using those drugs have been overshadowed by various unwanted side effects associated with their prolonged usages (Bandyopadhyay *et al*., 2002; Rang *et al.*, 2012).

In this context, the use of natural product-based remedies to treat gastric ulcers has gained attention of many researchers as this type of remedies has been considered as safe and cheaper (Borelli and Izzo, 2000). Animal-based natural products, in particular, have been studied extensively and have become an important source of new bioactive molecules that can be potential lead in the drug development. One of the animals that have been traditionally used in Malay medicinal folklore is a freshwater fish, *Channa striatus* (family Channidae), Known as '*Haruan*' to the Malays, this snakehead fish is an indigenous fish to Malaysia. *C. striatus* is consumed throughout the Asia Pacific region and considered as a valuable source of protein (Mohsin *et al.,* 1983). Traditionally, this fish is consumed to help heal the wound and diminish post-operative pain and discomfort (Baie *et al.,* 2000; Mat Jais *et al.,* 1994; Wee *et al.,* 1982;

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Mat Jais *et al.,* 1997; Zakaria *et al.,* 2004). Scientifically, *C. striatus* has been demonstrated to exert various pharmacological activities (Somchit *et al.,* 2004; Zakaria *et al.,* 2005) and reported to contain high amount of arachidonic acid (Mat Jais *et al.,* 1994; Zuraini *et al.,* 2006), which is a precursor for the production of prostaglandin required later for the synthesis of mucus that is important as a barrier against those aggressive factors (Beil *et al*., 1995)**.** Despite various reports on *C. straqtus* pharmacological activities, no study has been performed to determine its antiulcer potential. Taking into account that *C,. striatus* contained high arachidonic acid, a precursor for prostaglandins synthesis that is required in gastric mucus production, and the lack of antiulcer study on this fish, the present study was carried out to establish the antiulcer potential of chloroform: methanol extract of *C. striatus* (CMCS) using various rats models.

MATERIALS AND METHODS

Preparation of fresh Channa striatus fillet

Throughout the study, 6-month-old *C. striatus* fish weighing 250-400 g were used. Fish were cultured in Pasir Puteh, Kelantan, and were transferred by road (approximately 485km) to Universiti Putra Malaysia (UPM), Malaysia and acclimatized for at least 3 days prior to experiments. The preparation of fresh *Channa striatus* fillets was as described by Mat Jais *et al.* (1997). Briefly, precleaned live fish were weighed and killed in ice. The fish fillets were obtained by carefully cutting the fish lengthwise along the backbone to gain the maximum amount of flesh without any backbone. The ethical clearance for the handling and sacrificing of *C. striatus* was obtained from The Animal Care and Use Committee (ACUC), The Faculty of Medicine and Health Sciences, UPM [Approval Number: (UPM/IACUC/AUP-R054/2014)] together with that of the rats.

Preparation methanol: Chloroform extract of Channa striatus

The CMCS were prepared by soaking the fresh fillet in chloroform: methanol (2:1 (v: v)) system overnight (Zakaria *et al.*, 2007). The supernatant obtained was collected by filtration using Whatman No. 1 filter papers and then left for 30 min to settle down into two layers. The lower layer (CMCS) was collected and evaporated to remove any solvent residue present. The upper layer (aqueous extract) was stored at 4°C for future use. The CMCS obtained was considered as the stock solution with 100% concentration strength and used throughout the study.

Experimental animals

Male Sprague Dawley rats (180-250g; 8-10 weeks old) were used in the present study and were, at all times, cared and handled in accordance with the ACUC guidelines (UPM/FPSK/PADS/BR-UUH/00451) for the care of laboratory animals (Zakaria *et al*., 2014) and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann *et al*., 1983). Details on the care and handling of animals prior to the experiments can be found in report by Mamat *et al*. (2013).

Acute toxicity studies

The acute toxicity of CMCS was evaluatedusinga single dose oral administration of 5000 mg/kg assay (Mohamed *et al*., 2011). Twenty male rats (n=10) were fasted for 24 h before the administration of CMCS. A group that received 10% DMSO (as vehicle) represented the control group. The toxicity signs and symptoms or any abnormalities were observed at 0, 30, 60, 120, 180 and 240 min after CMCS administration. The observation was continued once a day for the next 14 days. The number of rats that survived was recorded at the end of the study period.

Antiulcerogenic activity

Ethanol- and indomethacin-induced gastric ulcer assay

The detail procedures for ethanol- and indomethacin-induced gastric ulcer assay have been described by Wan Zainuldin *et al.* (2015). The test solutions used were 10% DMSO (10 ml/kg; as negative control), ranitidine (100 mg/kg; as positive control) and CMCS (50 mg/kg, 250 mg/kg, and 500 mg/kg). Macroscopic and microscopic analyses on the collected stomachs were carried out as previously explained (Wan Zainuldin *et al*., 2015).

Pylorus ligation-induced gastric ulcer assay

The pylorus ligation-induced ulcer model was carried out according to the detail procedures as described by Balan *et al*. (2014). The test solutions used were 10% DMSO (10ml/kg), 100mg/kg ranitidine or CMCS (50, 250 and 500mg/kg). Following the surgical procedures, the gastric juice content's volume, pH and total acidity, and gastric wall mucus content were determined. The gastric lesion was also examined as described earlier.

Ethanol- induced gastric lesion N^G-ω-nitro-L-arginine methyl ester ($_L$-NAME)-pretreated rats

The experiment was carried out according to Lima *et al*. (2006) with slight modification. The rats were divided six into each group and fasted for 24h. The rats were pretreated with N^G-omega-nitro-L-arginine methyl ester ($_L$-NAME) or normal saline (i.p.). After 30 min, the rats received an oral dose of vehicle (10mL/kg), cabbenoxolone (100mg/kg) or CMCS (500mg/kg). For the L-arginine pre-treated groups, the rats received an intraperitoneal administration (i.p.) of L-arginine (70 mg/kg) after 30min of $_L$-NAME or normal saline (i.p.) administration. After 1 hr, all groups of rats were treated with 1mL of absolute ethanol to induce gastric ulceration and 1 hr after the administration of the ulcerogenic agent, the rats were euthanized by cervical dislocation. The stomachs were then excised and gastric damage was determined.

Table 1: Antiulcer effect of CMCS against the ethanol- induced gastric ulcer in rats

Ulcer model	Pretreatment	Dose (mg/kg)	Ulcer area (mm^2)	Protection (%)
Ethanol	Vehicle	-	20.50±3.24	-
	Ranitidine	100	5.00±1.21***	75.61
	CMCS	50	20.17±2.85	1.610
		250	11.33±1.50*	44.73
		500	6.00±1.07***	70.73

Table 2: Histopathological evaluation of CMCS against the ethanol- and indomethacin-induced gastric ulcer in rats

Model	Pre-treatment	Dose (mg/kg)	Architecture of the mucosa	Haemorrhage	Inflammatory exudates	Oedema
Ethanol	10% DMSO	-	++	+++	+	++
	Ranitidine	100	+	++	-	+
	CMCS	50	++	++	-	++
		250	+	++	-	+
		500	+	+	-	-
Indomethacin	10% DMSO	-	++	++	+	++
	Ranitidine	100	+	+	-	+
	CMCS	50	++	++	+	+
		250	+	+	-	-
		500	-	+	-	-

The severity of various features of ethanol-induced gastric ulcer were evaluated according to the following scoring scheme: - = normal; + = mild effect; ++ = moderate effect; +++ = severe effect

Table 3: Antiulcer effect of CMCS against the indomethacin- induced gastric ulcer in rats

Ulcer model	Pretreatment	Dose (mg/kg)	Ulcer area (mm^2)	Protection (%)
Indomethacin	Control	-	10.83±2.762	-
	Ranitidine	100	0.33±0.333***	96.95
	CMCS	50	5.50±0.764*	49.22
		250	3.17±1.01**	70.76
		500	1.83±0.601***	83.07

Table 4: Effect of CMCS on gastric juice parameters following the pyloric ligation-induced gastric ulcer in rats

Pre-treatment	Dose (mg/kg)	Volume of gastric juice (ml)	pH	Total acidity
10% DMSO	-	5.83±1.01	1.36±0.04	1527.00±232.20
Ranitidine	100	1.55±0.26***	4.18±0.81***	620.00±154.10**
	50	7.89±1.87	1.62±0.05	820.00±136.90*
CMCS	250	7.27±2.27	1.75±0.24	713.00±184.00**
	500	5.57±1.18	1.39±0.05	1247.00±86.67

Table 5: Anti-secretory effect of CMCS on the gastric wall mucus secretion following the pylorus ligation-induced assay in rats

Pre-treatment	Dose (mg/kg)	Gastric wall mucus (Alcian blue µg/g wet tissue)
10% DMSO	-	1.30±0.08
Ranitidine	100	3.31±0.20***
	50	2.41±0.49*
CMCS	250	2.64±0.0.22*
	500	3.33±0.38***

Data are present as mean ± S.E.M. Thirty rats (n=6 in each group) were used in this study. Statistical analysis was done by one-way ANOVA followed by Dunnet's multiple comparison tests. *P<0.05 and ***P<0.001 as compared to vehicle-treated group.

Table 6: Effect of $_{L}$-NAME or NEM on the antiulcer activity of CMCS assessed using the ethanol-induced gastric ulcer model

Pre-treatment	Treatment	Dose (mg/kg)	Ulcer Index
Normal Saline	10% DMSO	-	20.67±1.02
	Carbenoxolone	100	3.17±0.40[a]
	CMCS	500	7.33±1.12[a]
	L- arginine	70	20.33±1.04[a]
$_{L}$-NAME	10% DMSO	-	59.00±9.92[a]
	Carbenoxolone	100	8.00±0.68[bc]
	CMCS	500	7.00±1.39
	L-arginine	70	53.67±4.93[d]
NEM	10% DMSO	-	44.83±4.45[a]
	Carbenoxolone	100 mg/kg	18.50±2.26[ef]
	CMCS	500 mg/kg	20.17±1.68[gh]

Data are present as mean ±S.E.M. Thirty rats (n=6 in each group) were used in this study. Statistical analysis was done by one-way ANOVA followed by Dunnet's multiple comparison tests..
[a]Data differed significantly (P<0.05) against the (Normal saline + 10% DMSO)-treated group.
[b]Data differed significantly (P<0.05) against the ($_{L}$-NAME + 10% DMSO)-treated group.
[c]Data differed significantly (P<0.05) against the (Normal saline + Carbenoxolone)-treated group.
[d]Data differed significantly (P<0.05) against the (Normal saline + $_{L}$-arginine)-treated group.
[e]Data differed significantly (P<0.05) against the (NEM + 10% DMSO)-treated group.
[f]Data differed significantly (P<0.05) against the (Normal saline + Carbenoxolone)-treated group.
[g]Data differed significantly (P<0.05) against the (NEM + 10% DMSO)-treated group.
[h]Data differed significantly (P<0.05) against the (Normal saline + Carbenoxolone)-treated group.

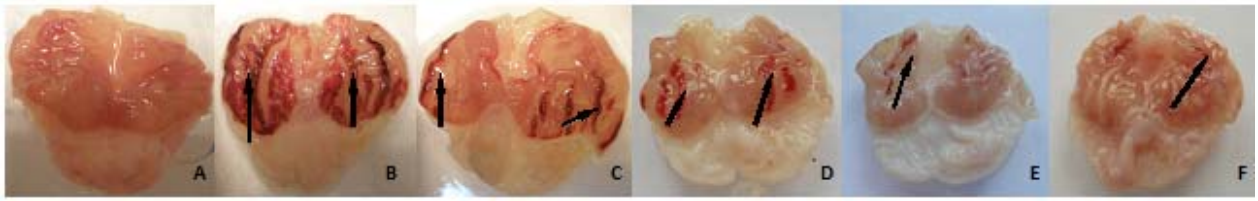

Fig. 1(a): Macroscopic evaluation of antiulcer activity of MCECS against ethanol-induced gastric ulcer in rats. Stomach of rat: (A) normal untreated (B) pre-treated with 10% DMSO (negative control); (C) pre- treated with 100 mg/kg ranitidine (positive control); (D) pre-treated with 50 mg/kg MCECS; (E) pre-treated with 250 mg/kg MCECS; (F) pretreated with 500 mg/kg MCECS. The arrow showed the hemorrhage.

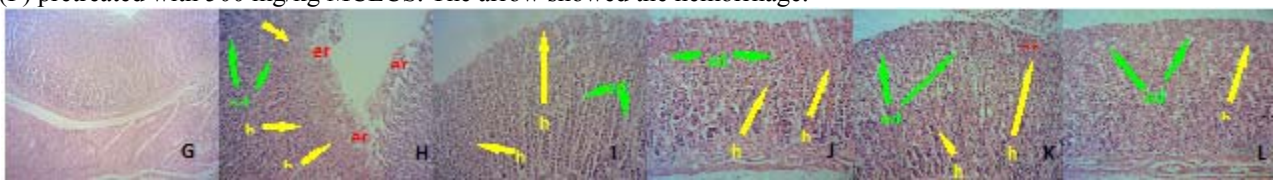

Fig. 1(b): Microscopic evaluation (at x10 magnification) of tissue of rats' stomach pre-treated with MCECS against ethanol-induced gastric ulcer in rats. Tissue of the stomach of rat: (G) normal untreated shows normal mucosal architecture; (H) pre-treated with 10% DMSO showing severe erosion (er) and hemorrhage (h;), and the presence of edema (ed;); (I) pre- treated with 100 mg/kg ranitidine shows mild erosion and hemorrhage with the presence of edema; (J) pre-treated with 50 mg/kg MCECS shows moderate erosion on the mucosa layer with the presence of moderate hemorrhage and edema; (K) pre-treated with 250 mg/kg MCECS shows mild erosion and hemorrhage on the mucosa layer with the presence of edema; (L) pre-treated with 500 mg/kg MCECS treated animal shows almost normal mucosa with slight hemorrhage and the presence of mild edema. H&E staining; x10 magnification.

Ethanol- induced gastric lesion in NEM- pretreated rats

The method was carried out according to Lima *et al.* (2006) with slight modification. The rats were divided into six rats per group and fasted for 24h. The rats were pretreated with N-ethylmaleimide (NEM) (10 mg/kg) or saline (i.p). After 30 min, the rats received an oral dose of vehicle (10mL/kg), cabbenoxolone (100 mg/kg) or CMCS (500 mg/kg). After 1 hr, all groups of rats were treated with 1mL of absolute ethanol to induce gastric ulceration and 1 hr after the administration of the ulcerogenic agent, the rats were euthanized by cervical dislocation. The stomachs were then excised and gastric damage was determined.

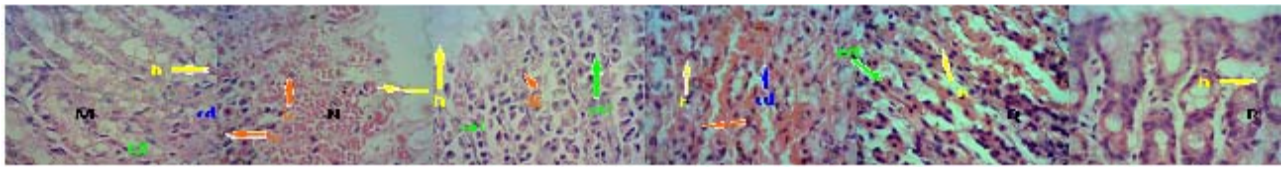

Fig. 1(c): Microscopic evaluation (at x40 magnification) of tissue of rats' stomach pre-treated with MCECS against ethanol-induced gastric ulcer in rats. Tissue of the stomach of rat: (M) normal untreated shows normal mucosal architecture; (N) pre-treated with 10% DMSO shows the presence of severe erosion and hemorrhage that are accompanied by edema, leukocytes infiltration (lc) and cellular debris (cd); (O) pre-treated with 100 mg/kg ranitidine shows mild hemorrhage accompanied by edema formation and leukocytes infiltration; (P) Pretreated with 50 mg/kg MCECS treated animal shows the mucosa with severe hemorrhage formation with the presence of edema, leukocytes infiltration and celluar debris; (Q) pre-treated with 250 mg/kg MCECS shows moderate hemorrhage accompanied by the presence of edema and mild cellular debris, and; (R) pre-treated with 500 mg/kg MCECS shows almost normal mucosa with very mild hemorrhage (h). H&E staining; x40 magnification.

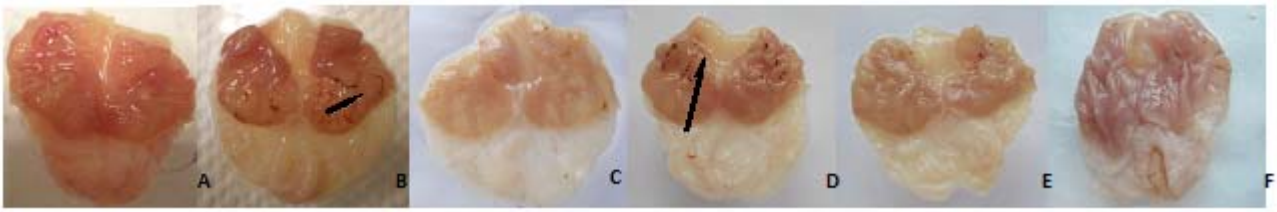

Fig. 2(a): Histological evaluation of antiulcer activity of MCECS against indomethacin-induced gastric ulcer in rats. (A) Stomach of normal rat; (B) Stomach of an ulcer control rats (10% DMSO+ IND); (C) Stomach of rat pre- treated with 100 mg/kg ranitidine; (D) Stomach of rat treated with 50 mg/kg MCECS; (E) Stomach of rat treated with 250 mg/kg MCECS; (F) Stomach of rat treated with 500 mg/kg MCECS. The black arrow showed the hemorrhage (h).

Fig. 2(b): Respective histopathological section: (G) Stomach of normal rat; (H) Stomach of control animal showing severe effect on mucosa with hemorrhagic erosion (h), (I) Stomach of 100 mg/kg ranitidine-treated animal show moderate effect on mucosa with mild hemorrhage (h); (J) Stomach of 50 mg/kg MCECS treated animal showing mild effect on mucosa with hemorrhagic erosion (h); (K) Stomach of 250 mg/kg MCECS treated- animal show mild effect of hemorrhage (h); (L) Stomach of 500 mg/kg MCECS treated animal showing almost normal mucosa with mild effect of hemorrhage (h). H&E stain: x10 magnification.

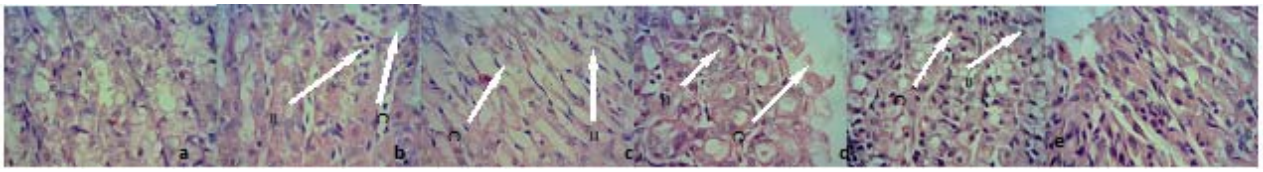

Fig. 2(c): Respective histopathological section: (a) Stomach of normal rat; (b) Stomach of control animal showing severe effect on mucosa with hemorrhagic erosion (h), oedema (o), moderate infiltrate leucocytes (IL); (c) Stomach of 100 mg/kg ranitidine-treated animal show moderate effect on mucosa with mild infiltrate leucocyte (IL) and oedema (o); (d) Stomach of 50 mg/kg MCECS treated animal showing oedema (o), moderate infiltrate leucocytes (IL); (e) Stomach of 250 mg/kg MCECS treated- animal show mild infiltrate leucocytes (IL) and oedema (o); (f) Stomach of 500 mg/kg MCECS treated animal showing almost normal mucosa. H&E stain: x40 magnification;

STATISTICAL ANALYSIS

The results were expressed as mean ± S.E.M. and analyzed using One-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison tests. Results were considered significant when $p \leq 0.05$.

RESULTS

Percentage yield of CMCS and acute toxicity study in rats

The fillet of *C. striatus* (1050 g) were soaked in methanol: chloroform solvent system (1:2 (w: v) ratio) and yielded

approximately 5.15g (≈0.49%) of crude CMCS. The extraction processes were carried out for another two times to ensure enough CMCS yielded for the whole experiment. A single dose oral administration of CMCS, at 5000 mg/kg, did not show any symptoms or sign of toxicity in treated animals after observation for 14 days, which indicated that the toxic dose of CMCS might be higher than 5000 mg/kg.

Antiulcer activity of CMCS against the ethanol-induced gastric ulcer in rats

Macroscopic analysis of the stomachs from the negative control group (vehicle-treated) showed complete and severe ulceration (fig. 1B) when compared to the normal untreated stomach (fig. 1A). Pretreatment with CMCS, at the doses of 50, 250 and 500 mg/kg, exhibited significant ($P<0.05$) reduction of gastric ulcer formation with the percentage of protection ranging between 2-71%, when compared to the control group (fig. 1D-1F) while 100 mg/kg ranitidine attenuate the ulcer formation by approximately 76% protection (fig. 1C; table 1).These dose-dependent observations were supported by the significant ($P<0.05$) decrease in the ulcer area formation from approximately 21 mm^2 as shown by the negative control group, to 6.0 mm^2, as shown by the 500 mg/g CMCS-treated group. Interestingly, the antiulcer strength of 500 mg/kg CMCS was similar to the one produce by 100 mg/kg ranitidine indicated by their similar percentage of protection. The histopathological evaluation of the stomach section is summarized in table 2.

Antiulcer activity of CMCS against the indomethacin-induced gastric ulcer in rats

Macroscopically, indomethacin, at the dose of 100 mg/kg, induced severe gastric lesion as seen in the negative control group (fig. 2B) in comparison to the normal untreated group (fig. 2A). Pretreatment with 50, 250 and 500mg/kg of CMCS demonstrated significant ($P<0.05$) attenuation in the area of ulceration (fig. 2D-2F), which also occurred in a dose-dependent manner with the percentage of protection ranging between 49-83% in comparison to the negative control group. However, only pretreatment with the 250 and 500 mg/kg CMCS caused significant ($P<0.05$) reduction of gastric lesion area with the total ulcer area of 3.2 and 1.8 mm^2, respectively, in comparison to the negative control group (10.8 mm^2) (table 3). Assessment using the indomethacin-induced model revealed that ranitidine, at 100 mg/kg, exerted the highest percentage of protection (approximately 97%). The histopathological evaluation of the stomach section is summarized in table 2.

Histopathological studies of ulcer-induced stomach

CMCS effect on the ethanol-induced gastric ulcer in rats

Microscopic observation of ethanol-induced stomach tissue pretreated with 10% DMSO (vehicle) showed severe lesion and damaged to the gastric mucosa. Severed hemorrhagic erosion, oedema, necrosis and leucocytes infiltration in the sub mucosal layer were clearly seen (fig. 1I) I comparison to the normal untreated tissue (fig. 1H). Interestingly, the stomach tissue of ethanol-induced rats pretreated with 250 or 500 mg/kg of CMCS, or 100 mg/kg ranitidine demonstrated mild lesions, with mild to moderate effect of hemorrhage and oedema to almost normal mucosa architecture (fig. 1J-1M).

CMCS effect on the indomethacin-induced gastric ulcer in rats

Microscopic examination of indomethacin- induced stomach tissue pretreated with 10% DMSO (negative control group) showed the presence of mild erosion accompanied by the moderate necrosis and severe oedema (fig. 2H). In contrast, the stomach tissue of rats pretreated with 100 mg/kg ranitidine, or 250 or 500 mg/kg CMCS demonstrated normal mucosal architecture while the group pretreated with 50 mg/kg CMCS exerted mild oedema (fig. 2I-2L).

Mechanisms of antiulcer activity of CMCS

Antisecretory activity of CMCS assessed by the pylorus ligation assay in rats

The biochemical parameters of gastric juice collected following the investigation on the effect of CMCS towards pylorus ligation test are shown in table 4. The CMCS did not caused significant ($P<0.05$) change to the volume and pH of the gastric juice, but significantly ($P<0.05$) reduced the total acidity in a dose-independent manner. Interestingly, the gastric wall mucus content was significantly reduced at all doses tested (table 5). On the other hand, 100mg/kg ranitidine significantly ($P<0.05$) reduced the volume and total acidity of the gastric juice while increasing the pH of the juice and secretion of the gastric wall mucus.

Effect of $_L$-NAME and NEM on antiulcer activity of CMCS assessed using the ethanol- induced gastric lesion rats

Oral pretreatment with normal saline followed by the oral administration of 100mg/kg carbenoxolone, 70 mg/kg L-arginine and 500mg/kg CMCS significantly ($P<0.05$) reduced the ulcer formation when compared to the 10% DMSO-treated group (negative control) (table 6). Pretreatment with $_L$-NAME significantly ($P<0.05$) altered the cytoprotection induced by carbenoxolone but not CMCS when compared against the 10% DMSO-treated group (table 6). On the other hand, pretreatment with NEM caused a significant ($P<0.05$) increase in the gastric ulcer formation in group treated with 10% DMSO (negative control) (table 6). NEM also significantly ($P<0.05$) reversed the antiulcer potential of 100 mg/kg carbenoxolone and 500 mg/kg CMCS.

DISCUSSION

The present study reported the antiulcer potential of CMCS against the ethanol- and indomethacin-induced

gastric ulcer models. The activity occurs in a dose-dependent manner and supported the traditional use of *C. striatus* for the healing of wound. The ability of CMCS to attenuate both models of gastric ulcer suggested that the extract possess an ability to prevent ulcer formation via different mechanisms of action. Ethanol exerted a direct toxic effect on the epithelium and leading to the formation of necrotic lesion due to a reduction in the mucus production and bicarbonate secretion (Massignani *et al*., 2009). Based on report Bagchi *et al*. (1998), ethanol increased superoxide anion and hydroxyl radical production and lipid peroxidation in the gastric mucosa and together with other reactive metabolites react with the most of the cell component. Marotta *et al.* (1999) reported ethanol reduces the gastric blood flow, solubilization the component of mucus in stomach and oxidative stress. Ethanol also increases the xantine oxidase activity and levels of malodialdehyde with reducing the level of gluthatione (Massignani *et al*., 2009).

NSAIDs, like indomethacin, induces ulcer formation during the course of anti-inflammatory therapy by inhibiting the action of cyclo-oxygenase (COX-1) and (COX-2) activities leading to inhibition of prostaglandins synthase action and direct cytotoxic effect on the epithelium (Bjarnason *et al*., 2007; Moleiro *et al*., 2009; Rainsford *et al*., 1987). Secretion of bicarbonate and mucus are stimulated by prostaglandins for maintaining the blood flow of the mucosa and regulate the mucosal cell renewal. The indomethacin or ibuprofen is traditional NSAIDs non-selective inhibitors of both COX-1 and COX-2 cause damage in the stomach with a marked decrease in gastric mucosal PGE2 content (Delaney *et al*., 2007). This activity occurs through inhibition of COX-1 that caused the gastric environment to be susceptible to tropical attack by endogeneous and exogeneous factors (Vane *et al*., 1995). Furthermore, this lead to inhibition of platelet production by thromboxane, which further worsens the gastrointestinal bleeding (Lanas *et al*., 2007). The present findings also could suggest that CMCS could be administered together with NSAIDs with the aim of minimizing the latter's gastric ulcer effect.

Pylorus ligation model enables the determination of possible changes in parameters relative to the gastric content (de Barros *et al*., 2008) and indirectly allows researchers to determine the status of mucosal defense barrier against the unpleasant effect of acid-pepsin and quantity of gastric mucus secretion (Rachchh *et al*., 2008). In this assay, the ligation of the pyloric end of the stomach causes leads to accumulation of gastric acid in the stomach. Hence, agents that reduce the gastric acid and/or enhance mucus secretion are useful in preventing the ulcer formation (Khare *et al*., 2008). From the present findings, CMCS did not cause changes in volume and pH of the gastric juice although there was decrease in total acidity at the low dosages of CMCS used suggesting its lack of anti-secretory effect.

Nitrite oxide (NO) plays a major role in mucosal defense by modulating the mucosal circulation in the gastrointestinal system via its potent vasodilator effect (Laine *et al*., 2008; Berg *et al*., 2004). Administration of $_{L}$-NAME, a NO synthesis inhibitor, alone certainly is harmful to the gastric mucosa and caused delayed and enhanced healing (Moura Rocha *et al*., 2010) as seen in the present study. However, L-NAME, which able to reverse carbenoxolone action, was unable to affect CMCS-produced antiulcer activity.

An endogenous non-protein sulfhydryl (NP-SH) compounds have been related in the mucosal protection against ethanol-induced gastric injury (Szabo *et al*., 1990). The NP-SH compounds are thought to be involved in the scavenging of oxygen-derived free radicals and controlling the production and nature of mucus (Allen *et al*., 1984; Salim *et al*., 1993). The development of gastric mucosa damage is related to the decrease in the concentration of mucosal NP-SHs because the SH-groups bind with the free radicals derived from the noxious agents metabolism (Andreo *et al*., 2006). In the present study, CMCS activity was found to decrease when NP-SH compounds were reduce by the administration of NEM. This indicates a strong participation of the endogenous SHs in the gastroprotective effect of this extract.

CONCLUSION

CMCS demonstrates remarkable antiulcer activity against the ethanol- and indomethacin-induced gastric ulcer models via its cytoprotective effect and involves, partly, the presence of sulfhydryl compounds, but not NO. The results fortify the etnopharmacological importance of *C. striatus* as a wound and ulcer healing agent.

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