ANTIULCEROGENIC EFFECTS AND POSSIBLE MECHANISM OF ACTION OF *QUASSIA AMARA* (L. SIMAROUACEAE) EXTRACT AND ITS BIOACTIVE PRINCIPLES IN RATS

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Abstract

The effects of *Quassia amara* extract (*Q. amara*) and its bioactive principles-quassin and 2-methoxycanthin-6-one on gastric ulceration were studied in albino rats. *Q. amara* (200-800 mg/kg p.o.; 5-20 mg/kg i.p) and 2-methoxycanthin-6-one (12.5, 25.0 and 50.0 mg/kg p.o; 1, 2 and 4 mg/kg i.p) but not quassin (12.5, 25.0 and 50 mg/kg p.o; 1, 2 and 4 mg/kg i.p) significantly inhibited gastric ulceration induced by indomethacin (40mg/kg). Administration of *Q. amara* (800 mg/kg p.o and 20 mg/kg i.p) and 2-methoxycanthin-6-one (12.5 mg/kg p.o; 4 mg/kg i.p) caused between 77%-85% cytoprotection against indomethacin (40 mg/kg, i.p) – induced gastric ulceration. Quassin did not cause any significant change in indomethacin-induced gastric ulceration. The inhibition of gastric ulceration produced by *Q. amara* and 2-methoxycanthin-6 one was accompanied by significant dose-dependent decreases ($P < 0.01$) in total gastric acidity. To investigate the probable mechanism of action, the individual effects of the extract and its principles alone and in combination with histamine (1 mg/kg) or cimetidine (0.12 mg/kg) on gastric acid secretion *in situ* were studied. *Q. amara* (20 mg/kg) and 2-methoxycanthin-6-one (4 mg/kg) but not quassin significantly ($P < 0.01$) inhibited the basal and histamine-induced gastric acid secretion. Inhibition of gastric acid secretion by *Q. amara* and 2-methoxycanthin-6-one was accentuated by cimetidine. The results suggest that *Q. amara* and its bioactive principle, 2-methoxycanthin-6-one possess antiulcer activity probably acting via histamine H₂ receptor. This could be a potential source of potent and effective antiulcer agents.

Keywords: *Quassia amara*; gastric ulceration; gastric acid; quassin; 2-methoxycanthin-6-one; rat

Introduction

Peptic ulcer is the most prevalent disease among the gastrointestinal diseases in most part of the world and constitutes one of the most common chronic illnesses among working-age adults. Approximately 4 million people have peptic ulcers and about 3000 people in the USA die as a result of the disease yearly (Tovey and Tunstall, 1975) listed as an area of high peptic ulcer disease prevalence with perforation being the most frequent indication for surgery. Recent studies show similar prevalence rates for duodenal ulcer and gastric ulcer in both southern and northern Nigeria (Holcombe and Okolie, 1991; Malu et al., 1994; Mustapha et al., 2007).

Consequently, many medicinal plants have been investigated pharmacologically for antiulcer activities (Njar et al, 1995; Raji et al., 2000; Raji et al., 2001; Raji et al., 2003; Raji et al., 2004; de Souza Almeida et al., 2011). Many antiulcer bioactive compounds have also been isolated from medicinal plants (Lewis and Hanson, 1991). Some of the major bioactive compounds with gastroprotection efficacy are the alkaloids, saponins, xanthenes, triterpenes and tannins (Njar et al., 1995; Ramirez and Roa, 2003; Raji et al., 2004; Baggio et al., 2005; Morikawa et al., 2006; Klein et al., 2010; Vasconcelos et al., 2010; de Souza Almeida et al., 2011). There has been increased global interest in traditional medicine and there are efforts to monitor and regulate herbal drugs and traditional medicine (Gilani and Rahman, 2005; Mehmood et al., 2010). One of the medicinal plants of interest is *Quassia amara*. *Quassia amara* belongs to the family *Simaroubaceae* and is naturally distributed in several tropical countries. The ethnobotany database lists other common names for this plant as *Amargo*, Bitterwood, *Quassia*, *Cuassia*, *Guabo*, *Hombre grande*, Jamaica bark, *Palo muneco*, *Pau amarelo*, *Pau quassia*, *Quassia de caiena*, *Quassia amarga*, *Quassia* wood, *Ruda*, *Simaruba*, *Surinam quassia*, *Surinam wood*, and *Wewe gifi*. *Quassia amara* is reputed in traditional medicine to have good stomachic, antianaemic, cytotoxic, antibiotic and antimalarial properties (Trager and Polonsky, 1981; Ajaiyeoba et al, 1999; Ajaiyeoba and Krebs, 2003; Raji 2010). In herbal medicine in the United States and Europe, *amargo* is employed as a bitter tonic for stomach, gallbladder, and other digestive problems (by increasing the flow of bile, digestive juices, and saliva); as a laxative, amebicide, and insecticide; and to expel intestinal worms.

The main bioactive constituents of the *Quassia amara* are quassinoids which had been found to be active against chloroquine resistant *Plasmodium falciparum* (Trager and Polonsky, 1981). Quassin and the alkaloid, 2- methoxycanthin- 6- one have been isolated from the plant (Njar et al, 1993). *Quassia amara* extract and quassin have been reported to possess potent male antifertility activities in both *in vivo* and *in vitro* animal models (Njar et al., 1995; Raji and Bolarinwa 1997; Parveen et al., 2003; Faisal et al., 2006). Recently the haematological effect of *Quassia amara* extract was also reported (Raji 2010), with quassin being the bioactive principle. More recently, de Souza Almeida et al., (2011) reported the antiulcer efficacy of canthin-6-one of *Simila ferruginea* A. St-Hil in animal models. There is a dearth of information on the effect of *Quassia amara* and its active

principles on experimental gastric ulceration. In the present study, the effects of *Quassia amara* extract and its bioactive principles, quassin and 2-methoxycanthin-6-one on gastric ulceration and gastric acid secretion in rats are reported.

Materials and Methods

Plant material and extract preparation

The stem bark of *Quassia amara* (voucher No. FHI 055879) was collected at the botanical garden, University of Ibadan, Nigeria in September 2008. A voucher specimen of the plant was identified under the herbarium number FHI 055879 at the Forestry Research Institute of Nigeria (FRIN) Ibadan Nigeria. The stem bark was air-dried and pulverized to obtain 1kg of the plant material. The pulverized stem bark (1kg) was then exhaustively extracted with methanol by means of Soxhlet apparatus and the extract evaporated *in vacuo* (Njar et al., 1993). Water was added to the residue and the mixture extracted with hexane and then with methanol. The methanol extract was dried using (anhydrous magnesium sulphate (MgSO₄) and evaporated to give a residue (3.5g) called *Quassia amara* extract. The residue (3.3g) was then chromatographed on a silica gel column as previously described to yield quassin and 2-methoxycanthin-6 one (Njar et al., 1993). Fresh solutions of *Quassia amara* extract, quassin and 2-methoxycanthin-6-one were prepared in distilled water when required.

Animals

Male Wistar strain albino rats (190-200g) used for the study were obtained from the Central Animal House, College of Medicine, University of Ibadan, Nigeria. The animals kept in wire-mesh cages were acclimated to laboratory conditions (12h dark: 12h light cycles; 26 ± 1^o C) and had free access to food and water *ad libitum*. Generally the study was conducted in accordance with the recommendations from the declaration of Helsinki on guiding principles in care and use of animals. These rats were randomly assigned into the following experimental sections.

Gastric ulceration experiment

Male albino rats (190-200 g) were randomly divided into twelve groups of five animals each. Group 1 served as control and received normal saline (0.5ml) only being the vehicle for the extracts and the drugs. *Quassia amara* extract (*Q. extract*) was administered at doses of 200, 400 and 800 mg/kg body weight orally to groups 2, 3 and 4, respectively. Similarly, quassin was administered at doses of 12.5, 25.0 and 50.0 mg/kg body weight orally to groups 5, 6 and 7 while 2-methoxycanthin-6-one was also administered at the doses of 12.5, 25.0 and 50.0 mg/kg body weight orally to groups 8, 9 and 10 respectively. The doses used in this study were based on findings from previous studies (Raji and Bolarinwa 1997, Parveen et al., 2003). Propranolol (40mg/kg reference drug) and cimetidine (50mg/kg p.o) were administered to groups 11 and 12 respectively. Another subset of 12 groups of male albino rats (190-200 g) were treated with normal saline (control), *Q. amara* (5, 10, and 20 mg/kg i.p), quassin (1, 2 and 4 mg/kg i.p) and 2-methoxycanthin-6 one (1, 2 and 4 mg/kg i.p) respectively. Propranolol (40mg/kg, reference drug) and cimetidine (0.12 mg/kg i.p) were administered to groups 11 and 12 respectively. One hour after administration of propranolol, normal saline, *Q. amara*, quassin, 2-methoxycanthin-6-one or cimetidine, acute gastric mucosa lesions were induced in rats using indomethacin (40mg/kg, i.p. Merk, Sharp and Dohme). Indomethacin was dissolved in 2% sodium carbonate in water. Four hours later, the animals were killed as previously described (Raji and Bolarinwa, 1997). The stomach of each rat was removed and total gastric acidity (Lai, 1964, Raji et. al., 2004) and ulcer scores (Njar et al., 1995, Raji et al., 2004) were determined.

Total gastric acidity and ulcer score

The stomach was opened along the greater curvature and gastric contents were drained into a centrifuge tube and centrifuged for 10min. The supernatant was then titrated with NaOH (0.01 M) to an end-point using phenolphthalein as an indicator. The total gastric acidity was expressed as $\mu\text{Eq./100g}$ of the rat. Assessment of the degree of ulceration was carried out by examining the inner surface of the stomach with a dissecting binocular microscope. The gastric lesions formed were scored and the mean ulcer index and percentage inhibition of ulceration were calculated as earlier described (Njar et al., 1995, Raji et al., 2004), thus:

$$\text{Ulcer index} = \frac{\text{Mean degree of ulceration} \times \% \text{ of group of ulceration}}{100}$$

$$\% \text{ Inhibition of ulceration} = \frac{\text{Ulcer index in control} - \text{ulcer index in test}}{\text{Ulcer index in control}} \times 100$$

Gastric acid secretion experiment

Q. amara (20 mg/kg), quassin (4 mg/kg) and 2-methoxycanthin-6 one (4 mg/kg) were used in this experimental section because these doses produced the maximum effects in the ulcer experiment and they did not cause death of any rat when administered intravenously (i.v.). Consequently, the individual effects of *Q. amara*, quassin and 2-methoxycanthin-6 one on basal and histamine-induced gastric acid secretion in albino rats (190-200 g) fasted for 24 h were studied as described by Ghosh and Schild (1958) and employed by others (Raji et al., 2004). Briefly adult male rats (190-200 g) were anaesthetized with i.p. injection

of 0.6ml/100 g 25% ethyl carbamate. The femoral vein (route of drug administration), oesophagus and pyloro-duodenal junction were calculated. The stomach was perfused with normal saline (37⁰ C) (via oesophageal cannula) and gastric effluent was collected (via pyloro-duodenal cannula) at a constant rate of 1 ml/min. The effluent was titrated against M/400 NaOH solution for basal (normal saline) and histamine-induced gastric acid secretion as previously described (Njar et al., 1995, Raji et al., 2004). The individual effects of *Q. amara* (20 mg/kg) alone, quassin (4 mg/kg) alone and 2-methoxycanthin-6 one (4 mg/kg) alone and in combination with histamine (1mg/kg) or cimetidine (0.12 mg/kg) on gastric acid secretion were also studied. The results are expressed as mEq/l.

Statistical analysis

Statistical analysis was performed using Student's t-test and ANOVA. The significance of difference was accepted at $P < 0.05$. Data are presented as mean $\pm$ S.E.M.

Results

Experimental gastric lesions in rats

The results shown in Tables 1 and 2 indicate that *Q. amara* and 2-methoxycanthin-6-one produced a dose-dependent gastro-protective action on indomethacin-induced gastric ulceration in rats. The cytoprotection produced by propranolol (40 mg/kg) was lower than that caused by administration orally or intraperitoneally of either *Q. amara* (200-800 mg/kg, p.o., 5-20 mg/kg i.p) or 2-methoxycanthin-6 one (12.5-50 mg/kg p.o., 1-4 mg/kg i.p) in male adult rats. The ulcer index in the control rats was significantly higher ($P < 0.01$) than each of the *Q. amara*, 2-methoxycanthin-6 one and the standard drug tested groups. *Q. amara* and 2-methoxycanthin-6 one also produced a dose-dependent reduction in total gastric acidity (Tables 1 and 2). This reduction in gastric acidity was significant ($P < 0.01$) when each group was compared with the control group. The highest doses (p.o or i.p) of the *Q. amara* and 2-methoxycanthin-6-one produced the highest percentage inhibition of gastric ulceration (Tables 1 and 2). However, quassin administered orally or intraperitoneally at these doses produced no significant effect on indomethacin-induced gastric ulceration and total gastric acidity in rats (Tables 1 and 2)

Table 1: Experimental gastric lesions in rats following individual oral administration (p.o) of *Quassia amara* extract (*Q. amara*) extract, quassin and 2-methoxycanthin-6 one in male rats

Treatment groups (n = 5)	Mean ulcer score (Mean $\pm$ S.E.M.)	Ulcer index (Mean $\pm$ S.E.M.)	Inhibition of Ulceration (%)	Total gastric acidity (μ Eq./100 g)
1. Control, distilled water (0.5ml)	20.1 $\pm$ 1.6	20.1 $\pm$ 1.6	-	11.45 $\pm$ 0.47
2. <i>Q. amara</i> extract (200 mg/kg)	9.3 $\pm$ 1.2*	9.3 $\pm$ 1.2*	53.73	7.92 $\pm$ 0.40*
3. <i>Q. amara</i> extract (400 mg/kg)	7.2 $\pm$ 0.8*	7.2 $\pm$ 0.8*	64.18	7.45 $\pm$ 0.35*
4. <i>Q. amara</i> extract (800 mg/kg)	4.0 $\pm$ 0.8*	4.0 $\pm$ 0.8*	80.10	6.36 $\pm$ 0.21*
5. Quassin (12.5 mg/kg)	19.2 $\pm$ 2.2	19.2 $\pm$ 2.2	4.48	10.92 $\pm$ 0.40
6. Quassin (25 mg/kg)	19.3 $\pm$ 1.8	19.3 $\pm$ 1.8	3.98	11.25 $\pm$ 0.35
7. Quassin (50 mg/kg)	19.1 $\pm$ 2.0	19.1 $\pm$ 2.0	4.98	11.36 $\pm$ 0.21
8. 2-Cant (12.5 mg/kg)	7.3 $\pm$ 1.4*	7.3 $\pm$ 1.4*	63.68	7.92 $\pm$ 0.40*
9. 2-Cant (25.0 mg/kg)	5.6 $\pm$ 1.1*	5.6 $\pm$ 1.1*	72.14	7.11 $\pm$ 0.35*
10. 2-Cant (50.0 mg/kg)	3.2 $\pm$ 1.0*	3.2 $\pm$ 1.0*	84.08	6.08 $\pm$ 0.21*
11. Propranolol (40 mg/kg)	8.9 $\pm$ 1.3*	8.9 $\pm$ 1.3*	55.72	7.44 $\pm$ 0.32*
12. Cimetidine (50 mg/kg)	4.9 $\pm$ 0.8*	4.9 $\pm$ 0.8*	75.62	6.23 $\pm$ 0.32*

*Significantly different from the control ($P < 0.01$)

Gastric acid secretion and *Quassia amara* extract

In order to investigate the possible mechanism of ulcer inhibition, the effects of *Q. amara* (20 mg/kg i.v.) on basal, histamine – and cimetidine-induced gastric acid secretion in rats were studied. The results shown in Fig. 1 indicate that *Q. amara* significantly ($P < 0.01$) inhibited basal and histamine-induced gastric acid secretion. Cimetidine significantly ($P < 0.05$) augmented *Q. amara* inhibition of gastric acid secretion. In the combined cimetidine and *Q. amara* experiment, the inhibition of gastric acid secretion was higher than that produced by cimetidine alone (Fig. 1).

Table 2: Experimental gastric lesions in rats following individual intraperitoneal (i.p) administration of *Quassia amara* extract (*Q. amara*), quassin and 2-methoxycanthin-6 one in male rats

Treatment group (n = 5)	Mean ulcer score (Mean $\pm$ S.E.M.)	Ulcer index	Inhibition of Ulceration (%)	Total gastric acidity (μ Eq./100 g)
1. Control, distilled water (0.5ml)	19.4 $\pm$ 1.4	19.4 $\pm$ 1.4	-	12.10 $\pm$ 0.34
2. <i>Q. amara</i> extract (5 mg/kg)	8.2 $\pm$ 0.7*	8.2 $\pm$ 0.7*	57.73	7.46 $\pm$ 0.18
3. <i>Q. amara</i> (10 mg/kg)	7.3 $\pm$ 0.3*	7.3 $\pm$ 0.3*	62.37	6.08 $\pm$ 0.22*
4. <i>Q. amara</i> (20 mg/kg)	4.5 $\pm$ 0.8*	4.5 $\pm$ 0.8*	76.80	3.90 $\pm$ 0.19*
5. Quassin (1 mg/kg)	19.2 $\pm$ 0.7	19.2 $\pm$ 0.7	1.03	11.46 $\pm$ 0.18
6. Quassin (2 mg/kg)	19.0 $\pm$ 0.3	19.0 $\pm$ 0.3	2.06	11.18 $\pm$ 0.21
7. Quassin (4 mg/kg)	18.7 $\pm$ 0.0	18.7 $\pm$ 0.0	3.61	10.90 $\pm$ 0.19
8. 2-Cant (1 mg/kg)	7.0 $\pm$ 0.7*	7.0 $\pm$ 0.7*	63.92	6.46 $\pm$ 0.18*
9. 2-Cant (2 mg/kg)	4.2 $\pm$ 0.3*	4.2 $\pm$ 0.3*	78.35	6.08 $\pm$ 0.22*
10. 2-Cant (4 mg/kg)	3.0 $\pm$ 0.0*	3.0 $\pm$ 0.0*	84.53	2.90 $\pm$ 0.19*
11. Propranolol (40 mg/kg)	9.2 $\pm$ 1.0*	9.2 $\pm$ 1.0*	52.57	7.00 $\pm$ 0.21*
12. Cimetidine (0.12 mg/kg)	4.7 $\pm$ 0.7*	4.7 $\pm$ 0.7*	75.77	5.10 $\pm$ 0.20*

*Significantly different from the control ($P < 0.01$).

Gastric acid secretion and quassin

As shown in Fig. 2, quassin administered alone or in combination with histamine or cimetidine produced no significant effect on gastric acid secretion in rats. There was no significant change in acid secretion when quassin plus cimetidine and cimetidine alone were compared or when quassin plus histamine and histamine alone were compared. However, histamine caused significant increase ($P < 0.01$) while cimetidine caused significant decrease ($P < 0.01$) in gastric acid secretion.

Figure 2: Effects of Quassin on gastric acid secretion in rats. H, histamine; Qsin, quassin alone; QsinH, quassin plus histamine; C, cimetidine alone; QsinC, quassin plus cimetidine; CH, cimetidine plus histamine. Point of injection of (a) normal saline and (b) drug/quassin

Gastric acid secretion and 2-methoxycanthin-6-one

The results shown in Fig. 3 indicate that 2-methoxycanthin-6-one significantly ($P < 0.01$) inhibited basal and histamine-induced gastric acid secretion. 2-methoxycanthin-6-one significantly ($P < 0.01$) accentuated cimetidine inhibition of gastric acid secretion. In the combined cimetidine and 2-methoxycanthin-6-one experiment, the inhibition of gastric acid secretion was higher (point for point) than that produced by cimetidine alone. The doses of 2-methoxycanthin-6-one that produced significant inhibition of gastric acid secretion either administered alone or in combination with histamine or cimetidine are much lower than those that produced similar but lower effects in *Q. amara* treated rats.

Figure 3: Effects of 2-methoxycanthine-6-one (2-Cant) on gastric acid secretion in rats. H, histamine; 2-CantH, 2-Cant plus histamine; C, cimetidine; 2-CantC, 2-Cant plus cimetidine; CH, cimetidine plus histamine. Point of injection of (a) normal saline and (b) drug/2-Cant

Discussion

The results of this study showed that *Quassia amara* extract and its bioactive component 2-methoxycanthin-6-one possess antiulcer properties. Quassin did not seem to have antiulcer activity at the doses employed in the rat model used in this study. According to Tsukimi et al., (2007), the pathophysiology of the gastric ulcer has not been fully elucidated. However, it is well established that an imbalance between aggressive (acid and pepsin secretion) and cytoprotective factors of the gastric mucous membrane (mucus and bicarbonate secretion) leads to gastric ulceration (Raji et al., 2001; Ramakrishnan and Salinas, 2007). Endogenous factors such as prostaglandin E2 (PGE2), somatostatin, nitric oxide (NO) and sulfhydryl compounds (Tsukimi et al., 2001) are involved in the pathophysiology of the gastroprotection. Environmental factors such as alcoholic beverages and non-steroidal anti-inflammatory drugs (NSAIDs) use, *Helicobacter pylori* and genetic factors are involved in gastric ulcerogenesis (Bech et al., 2000; Konturek et al., 2005; Mustapha et al., 2007). Although NSAIDs are useful in the treatment of inflammatory diseases, its aggressive properties on the gastrointestinal tract constitute a major setback in its therapeutic use (Ávila et al., 1996). Inhibition of PG synthesis is the principal ulcerogenic mechanism of the NSAIDs (Guth, 1992). NSAIDs also cause generation of free radicals leading to lipid peroxidation, leukocyte infiltration and apoptosis induction (Bech et al., 2000).

Q. amara and its bioactive constituent 2-methoxycanthin-6-one produced remarkable reduction in ulcer indices induced by indomethacin. The mechanism by which *Q. amara* and 2-methoxycanthin-6-one produced antiulcer protective effect might be through inhibition of gastric acid secretion by the parietal cell. This is demonstrated by the dose-dependent reduction in total gastric acidity produced by *Q. amara* and 2-methoxycanthin-6-one in this study. Moreover, the extract also significantly reduced basal and histamine-induced gastric acid secretion, with cimetidine accentuating the extract reduction in gastric acid secretion. These findings may suggest that the extract inhibits H₂ receptor, causing blockade of histamine release—a potent gastric acid secretagogue. The stimulatory action of histamine is mediated by the H₂ receptor as previously demonstrated in other pharmacological studies (Berglinde, 1977; Chew et al., 1980; Dial et al., 1981; Bottcher et al., 1989). Since in the presence of H₂ receptor antagonist, cholinergic stimulation of acid secretion is weak and often transient (Berglinde et al., 1980), it is possible that *Q. amara* contains a histamine antagonist acting via H₂ receptor. That the 2-methoxycanthin-6-one is the likely histamine antagonist in *Q. amara* was demonstrated by the inhibition of gastric acid secretion by this compound in this study. Endogenous histamine is known to cause gastric ulceration by actions, which may involve alterations in microcirculation (Black et al., 1972). Therefore, the gastric cytoprotection of *Quassia amara* extract could probably be due to its acid reduction effect through an antihistaminergic mechanism.

Several canthinones have been reported from *Simaroubaceae* plants such as *Quassia amara*, *Simaba multiflora*, *Simaba polyphylla* and *Eurycoma longiflora* (Njar et al., 1995; Falcao et al., 2008). Several biological activities are associated with plants alkaloids among which are antiulcer, antitumor, diuretic, antihypertensive, and anti-inflammatory (Falcao et al., 2008).

The phytochemical analysis of *Quassia amara* extract had earlier been reported (Njar et al., 1993). Recently de Souza Almeida et al., (2011) reported the antiulcer activity of canthin-6-one isolated from *Simaba ferruginea* A. St-Hil, a plant that belongs to the *Simaroubaceae* family as *Quassia amara*. While the present study focused on canthin-6-one from *Quassia amara*, gastric ulceration and gastric acid secretion, that of de Souza Almeida et al., (2011) focused on canthin-6-one from *Simaba ferruginea* A. St-Hil and the protective factors in gastric mucosa. The functional integrity of gastric mucosa depends on equilibrium between the aggressive and the protective factors. Since the success of the pharmacological treatment of gastric ulcer is dependent not only upon inhibition of the acid secretion, but also on the increase in the protective factors in the gastric mucosa (Dajani and Klamut, 2000), further studies would be needed to fully elucidate the mechanisms of antiulcer action of *Q. amara* and 2-methoxycanthin-6-one. The present study therefore showed that 2-methoxycanthin-6-one is one of the antiulcer principles in *Quassia amara* extract whose more detailed pharmacological actions could be explored for potential development of potent and effective antiulcer agents. Efforts are in progress to investigate other possible mechanisms of antiulcer effects of *Quassia amara* extract and its 2-methoxycanthin-6-one.

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