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Research Article
Zerumbone with Hydroxypropyl-β-Cyclodextrin Inclusion Complex as a Potential Treatment for Gastritis

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Abstract

Background and Objective: Acute and chronic gastritis both possess dyspeptic symptoms. The objective of present study was to explore the effect of zerumbone on the acute or chronic gastritis. **Materials and Methods:** The study was divided into three different groups of 220 patients each. Zerumbone with hydroxypropyl-β-cyclodextrin the inclusion complex was prepared by the freeze-drying method. All patients of vehicle group, treatment group and control group were injected with 2 mL Water For Injection (WFI), 2 mL solution containing 20 μM doses of zerumbone and 0.15 g ranitidine capsule taken orally, respectively for 20 days. All patients were analyzed by gastric endoscopic examination. The biopsies of gastric tissue were made to perform the histopathological study and urea breath tests. The one-way ANOVA/the Dunnett's multiple comparison tests were used for significant difference of scores at 95% level of confidence. **Results:** Zerumbone treatment was prevented epigastric pain, nausea, anorexia, vomiting and inflammation of gastric mucosa after 20 days of treatment ($p \leq 0.05$ for all parameters). Numbers of patients with infection of *Helicobacter pylori* were 22 and 2 ($p \leq 0.05$) for before and after zerumbone treatment. **Conclusion:** Zerumbone treatment was quite effective and safe in acute and chronic gastritis with or without *Helicobacter pylori* infection Clin.

Key words: Endoscopy, gastritis, *Helicobacter pylori*, histopathology, hydroxypropyl-β-cyclodextrin, zerumbone

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Acute gastritis causes severe and persistent abdominal pain[1]. However, the pain is for short period of time. Acute gastritis suddenly comes on and can be caused by injury, bacterial infections, viral infections, stress and ingesting irritants such as alcohol, anti-inflammatory on-steroidal drugs (NSAIDs), steroids, or aromatic foods[2]. Chronic gastritis occurs when stomach lining becomes inflamed. It can be painless or causes low and nagging stomach pain. The chronic gastritis is caused by the generation of large quantities of peroxides like hydrogen peroxide, nitric oxide and the oxide radicals. In addition, the most common type of chronic gastritis is caused by *Helicobacter pylori* (*H. pylori*) bacteria. Chronic gastritis usually gets better with treatment but may require strong follow-up[3].

Products obtained from natural sources has vital contributions in the treatment of gastritis and are considered to be safe because of having no or fewer side effects on the body[4]. Zerumbone (ZEN) is crystalline sesquiterpene that is isolated from subtropical ginger (*Zingiber zerumbet* Sm.) and is used in traditional medicine against various diseases[5]. Some of the prominent activities of ZEN includes inhibition of carcinoma cell, proliferation and quenching of various inflammatory reactions. It is reported that ZEN exhibits strong anti-oxidative, anti-*H. pylori* activities and is a potent anti-inflammatory agent. Chemically ZEN is 2, 6, 9, 9-tetramethyl-(2E, 6E, 10E)-cycloundeca-2, 6-10-trien-1-one. It is sparingly soluble in water[6]. Its treatment increases the concentration of nitric oxide synthase, cyclooxygenase-2 and CXC chemokine receptor 4 in the cells[7].

Hydroxypropyl-β-cyclodextrin (H-107) is a hydroxyalkyl β-cyclodextrin derivative. It has inclusion ability and high water solubility[8]. It is well tolerated systematically by the human body. The solubility of drugs increases linearly with increase in the concentration of it in water[6]. The improvement of solubility of ZEN will improve the bioavailability.

In the present research work, the effect of intraperitoneal injection of inclusion complex of ZEN with H-107 on the patients with acute or chronic gastritis was studied using evaluation of dyspeptic symptom, endoscopic study, histopathological examination and urea breath test.

MATERIALS AND METHODS

The 98% pure ZEN, hematoxylin-eosin (HE) and H-107 were purchased from Sigma-Aldrich, St. Louis, MO. Ranitidine capsule (150 mg) was purchased from Lanling Pharmaceutical Production, China. Sodium chloride (NaCl), Periodic Acid-Schiff (PAS) urea and agar were purchased from Merk specialties, Berlin, Germany.

The Ethics Committee for Human Experiments of the Affiliated Hospital of Nanjing University of Traditional Chinese Medicine, Nanjing, China approved the experimental protocol under the reference number of Chi CTR-IPR-15005760 and the Ethical Guidelines for biomedical research on human participants in accordance with Chinese law was followed[9].

Inclusion criteria: Outdoor and indoor patients of the hospital of Traditional Chinese Medicine, Nanjing, China of both sexes, aged 18 years and older with dyspeptic symptoms were included in the study after signing the informed consent form. The three different groups were made randomly as per Table 1.

Exclusion criteria: Patients difficulty to understand the informed consent form, refusal to sign the informed consent form, impossibility to return for the study follow-up, age younger than 18 years and with gastric surgery were excluded from the study.

Preparation of the ZEN solution for injection: The ZEN was stored at -20°C in LN2 Chamber (Southern Scientific Lab, Instruments, Chennai, India) before preparation[10]. The ZEN

Table 1: Selection of subjects for study

		Total No. of patients		Males		Females			Acute gastritis patients		Chronic gastritis patients	
Groups	Drugs	No.	%	No.	%	No.	%	Age (years)	No.	%	No.	%
Vehicle group	Water for injection	220	100	108	49	112	51	30.5±2.11	209	95	11	5
Treatment group	Zerumbone	220	100	101	46	119	54	32.0±2.12	198	90	22	10
Control group	Ranitidine	220	100	103	4	117	53	34.0±2.13	187	85	33	15

All female patients were non-pregnant. Results of age presented as a Mean±Standard Deviation, Data for bifurcation patients were represented as No. (%), None of the patients had a waist-to-hip ratio more than 0.81 and body mass index more than 28 kg m^{-2}

and H-107 (1:1 M ratio) was dissolved in 20 mL WFI (Ranbaxy (I) Pvt. Ltd). The mixture was agitated in a shaker bath (Innova 4230, Brunswick Scientific, NJ, USA) for 3 days at 25±2°C temperature and filtered through 0.45 µm membrane. The filtrate was frozen at -80°C and freeze-dried (vacuum freeze dryer, SP Scientific, New York, US) for a day at-60°C[6]. The 400 mg freeze dried powder and 50 µg of NaCl were added into WFI and make the volume up to 2 mL[11]. The solution for injection was sterilized by ethylene oxide sterilizer (Raphael, Murcia, Spain)[12].

All patients of vehicle group, treatment group and control group were injected with 2 mL WFI, 2 mL solution containing 20 µM doses of ZEN and 0.15 g ranitidine capsule taken orally respectively in the morning and evening daily for 20 days. Neither any antisecretory drug nor antibiotics were given to patients in the 2 weeks before and during the test[13].

Evaluation of dyspeptic symptoms: The dyspeptic symptoms of all patients as epigastric pain, nausea, anorexia and vomiting were evaluated before and after treatment of 20 days by the way of scoring as 0: Absent, 1: Normal, 2: Mild, 3: Moderate to severe, 4: Severe[14].

Endoscopic examination: Upper gastrointestinal endoscopy was performed for all patients before and after 20 days of the treatment using a disposable medical injection endoscope (Changzhou, Jiuhong Medical Instruments Co., Ltd, Jiangsu, China). The inflammation of gastric mucosa was graded as 0: Normal stomach mucosa, 1: Mild gastritis, 2: Moderate to severe gastritis, 3: Severe gastritis and 4: Erosive gastritis[15].

Histopathological study: For each of the patient, tissue samples from corpus and antrum were collected by disposable biopsy forceps (Jiangsu Medical Technology Co., Ltd., Changzhou, China). From the gastric biopsies, 4 mm tissue sample was taken, fixed in 10% formalin and stained with HE and PAS. The pathologist blind for the study did the histopathological examination using the light microscope (Opto-Edu Beijing, Co., Ltd., Beijing, China). The classification and grading of gastritis were performed according to 0: Normal, 1: Mild gastritis, 2: Moderate to severe gastritis, 3: Intense gastritis[16].

Urea breath test: The presence of *H. pylori* is a biomarker of chronic gastritis[17]. From the slides of gastric biopsies of tissues, one piece of tissue was put into 2% w/w urea agar with a needle and incubated at 25°C in BOD (Yorko, USA) and results were recorded after 4 h from inoculation[18].

Statistical analysis: The data obtained were analyzed using SPSS13.0 for Windows (IBM Corp., Armonk, NY, USA). The data obtained for the dyspeptic symptom, endoscopic examinations and histopathological study were statistically analyzed using the one-way ANOVA followed by Dunnett's multiple comparison tests (considering $q>2.273$ significant)[19]. Chi-square test was used for assessment of the statistical difference of *H. pylori* infection between the groups and within the group[20]. The difference was considered significant statistically at the 95% level of confidence.

RESULTS

The 21 patients excluded from endoscopic examinations study (eight, seven and six patients from vehicle group, treatment group and control group respectively) because they were refused to do anendoscopy. Remaining 639 patients were included in endoscopy examination study. Forty three patients excluded from the histopathological study (18, 12 and 13 patients from vehicle group, treatment group and control group, respectively) because they were refused to do abiopsy. Remaining 617 patients were included in histopathological examination study.

The results showed that there was significant difference of scores of dyspeptic symptoms (Table 2), gastric mucosa (Table 3), histopathological examination (Table 4) and *H. pylori* infection (Table 5) for after treatment between vehicle group and treatment group and also between vehicle group and control group ($p\leq0.05$ for all).

There were no significant differences in scores of endoscopy and histopathology between before treatment (Fig. 1a, 2a) and after treatment (Fig. 1b, 2b) of vehicle group ($p\geq0.05$ for all). There were significant differences ($p\leq0.05$ for one-way ANOVA and $q>2.273$ for Dunnett's multiple comparison tests) for endoscopy and histopathology scores between prior application of drug (Fig. 1c, 2c) and after application of drug (Fig. 1d, 2d) for treatment group and control groups. All patients of treatment (Fig. 1e) and control (Fig. 2e) groups had gastritis at beginning of the study.

There was no significant differences for endoscopy and histopathology scores for after treatment between treatment group (Fig .1f) and of the control group (Fig. 2f) ($p\geq0.05$ for all).

Table 2: Effect of treatment on dyspeptic symptoms

	Vehicle group				Treatment group				Control group			
	Before treatment		After treatment		Before treatment		After treatment		Before treatment		After treatment	
Dyspeptic symptoms	No	%	No	%	No	%	No	%	No	%	No	%
Epigastric pain												
0	22	10	0	0	0	0	132	60	0	0	154	70
1	55	25	57	26	82	37	13	6	78	35	33	15
2	62	28	66	30	58	26	35	16	65	30	22	10
3	50	23	64	29	48	22	25	11	56	25	11	5
4	31	14	33	15	32	15	15	7	21	10	0	0
Nausea												
0	33	15	0	0	0	0	169	77	0	0	204	93
1	66	30	71	32	73	33	22	10	69	31	11	5
2	55	25	65	30	81	37	15	7	58	26	5	2
3	44	20	50	23	39	17	11	5	55	25	0	0
4	22	10	34	15	27	13	3	1	38	18	0	0
Anorexia												
0	18	8	3	1	3	1	172	79	0	0	183	83
1	49	22	55	25	78	35	25	11	97	44	35	16
2	42	19	45	20	55	25	11	5	85	39	2	1
3	61	28	64	29	44	20	7	3	23	10	0	0
4	50	23	53	25	40	19	5	2	15	7	0	0
Vomiting												
0	80	36	41	19	12	5	187	85	0	0	198	90
1	60	27	69	31	79	36	22	10	103	47	22	10
2	40	18	51	23	68	31	11	5	85	39	0	0
3	35	17	48	22	32	15	0	0	23	10	0	0
4	5	2	11	5	29	13	0	0	9	4	0	0

Data were represented as numbers (%) for all parameters. 0: Absent, 1: Normal, 2: Mild, 3: Moderate to severe, 4: Severe, n = 220 for all groups

Table 3:Endoscopic examinations

	Vehicle group (n = 212)				Treatment group (n = 213)				Control group (n = 214)			
	Before treatment		After treatment		Before treatment		After treatment		Before treatment		After treatment	
Inflammation of gastric mucosa score	No	%	No	%	No	%	No	%	No	%	No	%
0	0	0	0	0	0	0	153	72	0	0	177	83
1	65	31	58	27	61	29	25	12	73	34	19	9
2	55	26	59	28	59	28	19	9	59	28	11	5
3	51	24	55	26	48	23	11	5	43	20	7	3
4	41	19	40	19	45	20	5	2	39	18	0	0

Data were represented as numbers (%) for all parameters. 0: Normal stomach mucosa, 1: Mild gastritis, 2: Moderate to severe gastritis, 3: Severe gastritis and 4: Erosive gastritis

Table 4: Histopathological examination study of stomach tissues

	Vehicle group (n = 202)				Treatment group (n = 208)				Control group (n = 207)			
	Before treatment		After treatment		Before trseatment		After treatment		Before treatment		After treatment	
Histopathological examination score	No	%	No	%	No	%	No	%	No	%	No	%
0	0	0	0	0	0	0	108	52	0	0	149	72
1	74	37	71	35	79	38	34	16	81	39	24	12
2	60	30	62	31	62	30	25	12	62	30	15	7
3	45	22	46	23	38	18	23	11	37	18	14	7
4	23	11	23	11	29	14	18	9	27	13	5	2

Data were represented as numbers (%) for all parameters. 0: Normal; 1: Mild gastritis; 2: Moderate to severe gastritis, 3: Intense gastritis

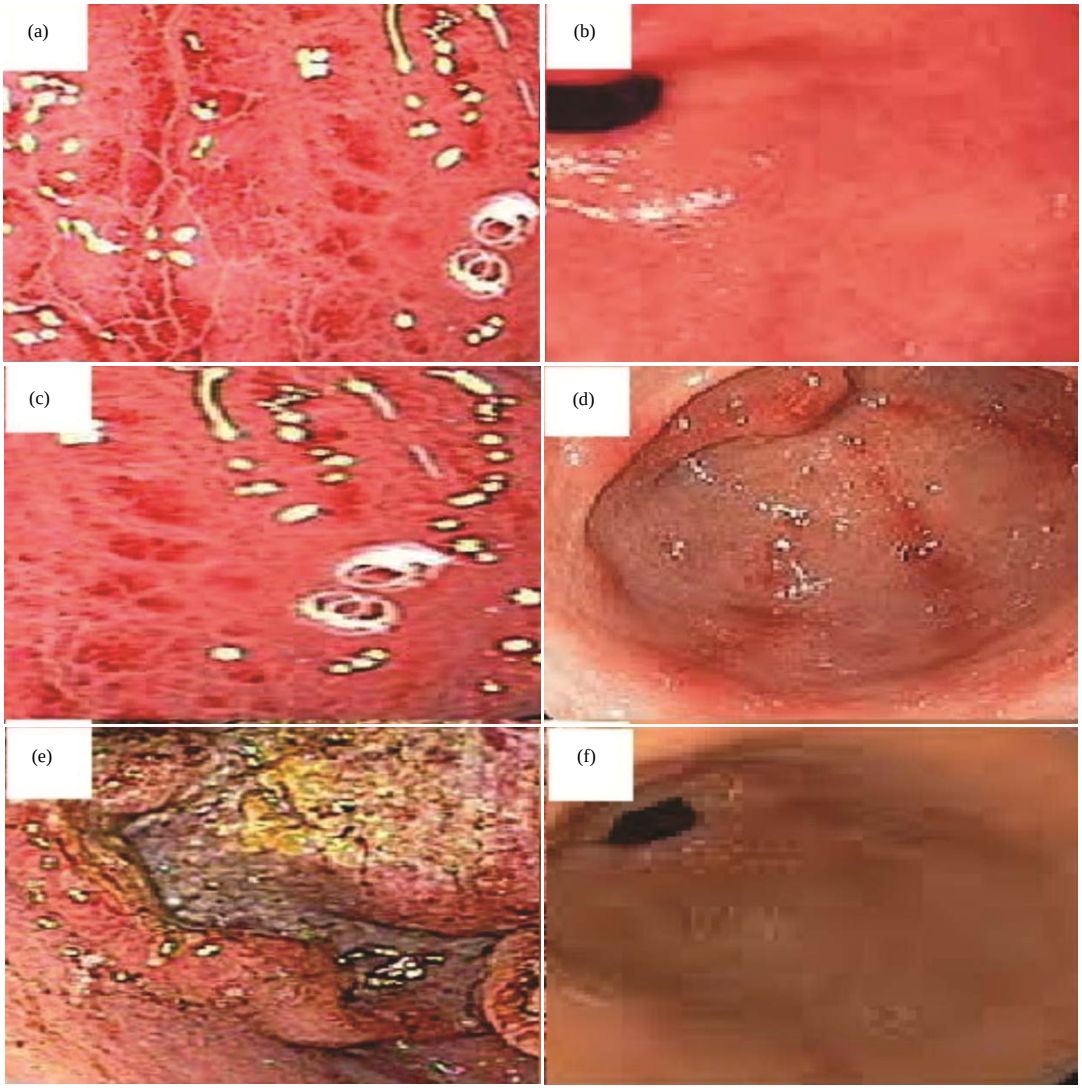

Fig. 1(a-f): Endoscopic view of stomach tissue before treatment and after treatment

Table 5: Results of urea breath test

	Vehicle group (n = 202)				Treatment group (n = 208)				Control group (n = 207)			
	Before treatment		After treatment		Before treatment		After treatment		Before treatment		After treatment	
H. pylori	No	%	No	%	No	%	No	%	No	%	No	%
Positive	11	5	12	6	22	11	2	1	33	16	2	1
Negative	189	94	188	93	184	88	204	98	171	83	202	98
Unpracticed results	2	1	2	1	2	1	2	1	3	1	3	1

Data were represented as numbers (%) for all parameters

DISCUSSION

The ZEN has anti-inflammatory[21], antisecretory, gastroprotective, antioxidant and anti-*H. pylori* activities[22]. However, it is sparingly solublein water[7]. It is soluble in dimethyl sulfoxide (DMSO) but DMSO has systematic toxicity for ahuman. Cyclodextrins are used to form inclusion complexes[23]. The segment of H-107 has a hollow cone which forms ahigh water soluble complex with ZEN[6]. In the study ZEN-H-107 inclusion complex was prepared to make the intraperitoneal injection of ZEN.

In acute or chronic gastritis, patients are suffering from damage of gastric and the esophageal mucosa and development of ulcer which are characterized by dyspeptic

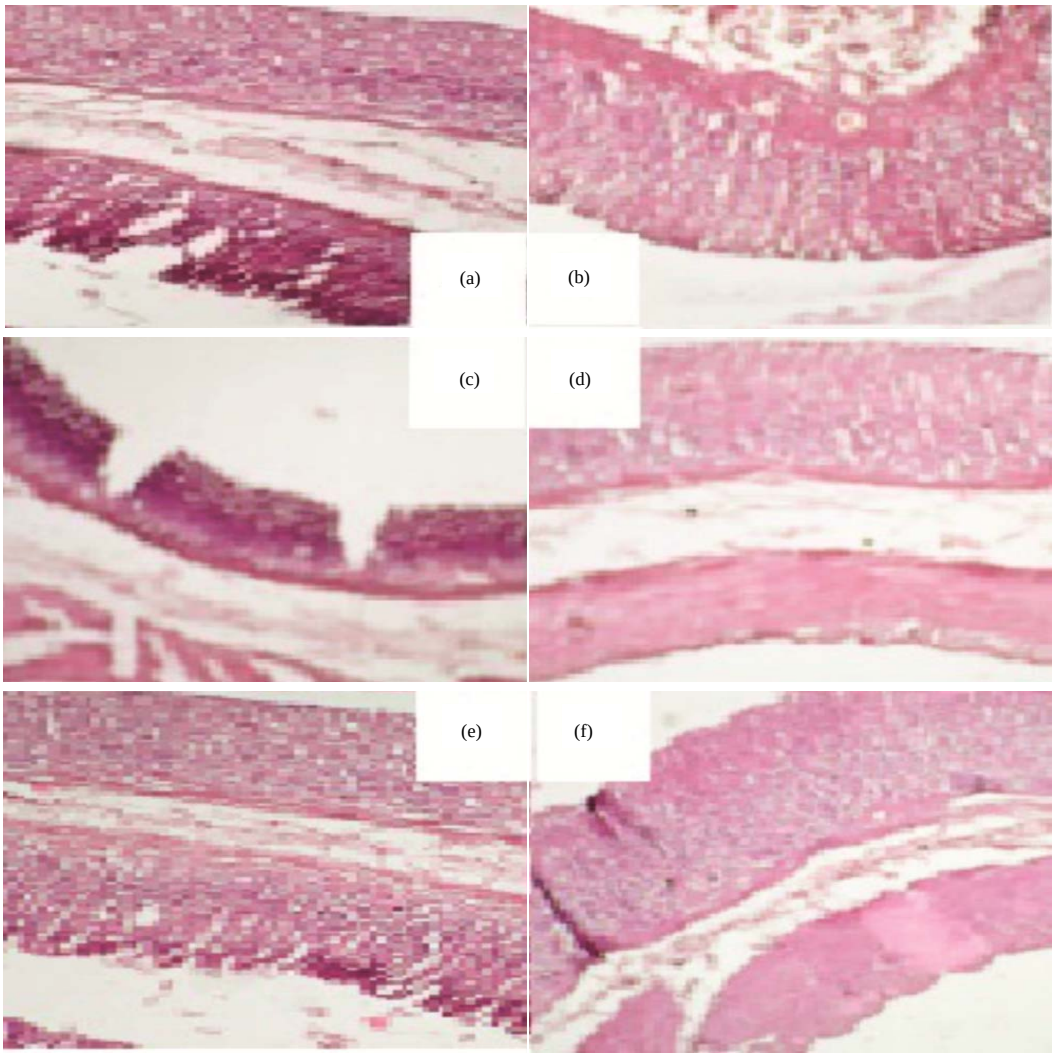

Fig. 2(a-f): Histopathological of stomach tissues (45×view) before treatment and after treatment, (a) Photograph of patient belongs to vehicle group before treatment, (b) Photograph of patient belongs to vehicle group after treatment, (c) Photograph of patient belongs to treatment group before treatment, (d) Photograph of patient belongs to treatment group after treatment, (e) Photograph of patient belongs to control group before treatment and (f) Photograph of patient belongs to control group after treatment

symptom[24]. Gastritis and dyspeptic symptom are developed due to the disturbances with the mucosal protective factors and the aggressive factors[25]. Results from the present study demonstrated that zerumbone treatment for 20 days inhibits the dyspeptic symptom. The probable reason behind these is the antisecretory activity of ZEN[22].

Endoscopy and biopsy of the stomach in acute or chronic gastritis is a major tool to diagnose inflammation of gastric mucosa[20]. Endoscopy and histopathology of biopsies samples revealed that there was complete removal of inflammation of gastric mucosa after 20 days treatment of ZEN. The probable reason behind anti-inflammatory effect of gastric mucosa by ZEN is the preservation of gastric mucosal content of prostaglandins-2[19].

The association between *H. pylori* infection and chronic gastritis is strong[18]. It is Gram negative pathogen[22]. The study showed that treatment with WFI for 20 days increased the *H. pylori* colonization density in the antrum. However, treatment with ZEN for 20 days decreased the *H. pylori* colonization density in the antrum, which was significantly higher ($p \leq 0.05$) at the beginning of the treatment. The probable reason for anti-*H. pylori* activity of ZEN is its remarkable MIC value equal to 250 μg mL^{-1} for *H. pylori*[22].

CONCLUSION AND LIMITATIONS

The clinical study of inclusion complex of zerumbone with hydroxypropyl-β-cyclodextrin/H-107 showed that 20 μM twice a day intraperitoneal zerumbone injection treatment for 20 days was quite effective in acute and chronic gastritis with or without *Helicobacter pylori* infection. The study results were validated by evaluation of dyspeptic symptoms, endoscopic study, histopathological examinations and urea breath test.

Different *H. pylori* strains have a significant role in gastritis. However, the study was failed to diagnose differently *H. pylori* strains present in biopsies samples.

SIGNIFICANCE STATEMENTS

This study discovered the significance effect of zerumbone treatment in acute and chronic gastritis with or without *Helicobacter pylori* infection that can be beneficial for peptic ulcer diseases. This study will help the researcher to uncover the critical areas of zerumbone in gastritis. Thus a new theory on acute and chronic gastritis management may arrive with the natural product zerumbone.

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