Sapporo Medical Journal
Volume 57, Issue 05, May 2023

Effect of carrageenan extracting from *Kappaphycus alverazii* grown in Vietnam on gastric ulcers

Bui Huy Chich [1], Vu Ngoc Boi [2], Do Van Ninh [3], Pham Thanh Thuan [4]
Dang Xuan Cuong [5,*], Nguyen Thi My Le [5]

Department of Science and Technology – Ba Ria-Vung Tau province, Vietnam[1]
Faculty of Food Science, Nha Trang University, Vietnam[2]
Thai Binh Duong University, Vietnam[3]
Ninh Thuan Province General Hospital, Vietnam[4]
Ho Chi Minh City University of Food Industry, Vietnam[5]

Abstract— Polysaccharides of marine algae belong to anion groups such as fucoidan and alginate are known the treating ability of gastric ulcers but carrageenan has not seen the announcement yet. Therefore, the paper focused on the treating ability evaluation of gastric ulcers of carrageenan that was extracted from *Kappaphycus alverazii* grown in Vietnam. Three acid agent (hydrochloric acid, 5-sulfosalicylic acid, and acetic acid) in various concentration (1, 5, and 10%) was used for causing disease. Various concentration of carrageenan (0.5, 1.0, and 1.5%) was used for the treating of gastric ulcers. Guinea pig (*Cavia porcellus*) was observed clinical pharmacology (rate of blood sedimentation and gastric disease surgery) on day 5th and 10th after the treatment. The results showed that carrageenan has the treatment effect of gastric ulcers. A concentration of 1.5% (w/v) carrageenan is a suitable choice for the protection of the gastric mucosa of mice that drunk 10% hydrochloric acid after a 10-day treatment. In summary, carrageen extracted from *Kappaphycus alverazii* grown in Vietnam can completely be used in food and pharmaceutical products, especially in the preparation of drugs, and functional foods to support the treatment of gastric ulcers.

Keywords: Acid, carrageenan, *Cavia porcellus*, gastric ulcers, *Kappaphycus alverazii*.

1. Introduction

Carrageenan is a bioactive polysaccharide of marine resources belonging to the group of anion polysaccharides such as fucoidan and alginate. They possess sequences of α and β-D-galactopyranose residues that repeated and linked each other via the 1,3 and 1,4 linkage. Carrageenan structure is diverse, for example, kappa (κ), iota (ι), and lambda (λ) [1-3] and these structures depend on algae species, and κ – carrageenan is commonly found than other styles of carrageenan. κ – carrageenan is applied into food, [4, 5] func-tional food, [5] pharmaceutics, [6, 7] and cosmetics [8] basing on the forming characteristics of texturing, thickening, suspending, or stabilizing agents. [9, 10] Carrageenan content in marine algae *Kappaphycus alverazii* is up to 40% DW, [11] they are non-toxic, anti-heparanase, anticoagulant activities, [12] induces thrombosis, anti-cancer, anti-inflammatory, [13, 14] anti-helicobacter pylori. [15] However, the therapeutic efficacy of carrageenan from red algae *Kappaphycus alverazii* for stomach ulcers has not been found. Meanwhile, carrageenan has a large production and consumption in the world, estimated to

reach USD 1.2 billion by 2025. [16] According to the notice of WHO in 2018, peptic ulcer disease deaths in Viet Nam reached 3,613 of total deaths and ranks 81th in the world. [17] The groups of anion polysaccharides are known as the treatment agent of gastric ulcers. [18-20] Therefore, the paper focused on the therapeutic effects of carrageenan extracted from *Kappaphycus alverazii* seaweed grown in Vietnam for gastric ulcers.

2. Materials and Methods

2.1. Sample preparation

Marine algae *Kappaphycus alvarezii* (Doty) Doty grown in Nha Trang bay, Vietnam was cleaned and dried until 20±1% moisture for the extraction of carrageenan. Before the carrageenan extraction, algae was treated by using an enzyme as follows: the algae-to-aqueous (pH 5.1, 1.45% enzyme) ratio of 1/20 (w/v), the extracting temperature of 42 ^{o}C, the treatment time of 60 minutes, and then centrifugation for the collecting of residue. The treated algae were soaked aqueous at 90 ^{o}C for 80 minutes with the treated algae-to-aqueous of 1/50 (w/v). Finally, the centrifugation (10.000 rpm for 15 minutes) and the membrane filtration for the collection of the filtrate that contained carrageenan. Carrageenan was continuously purified by using 80% ethanol and dried the freeze-drying (45±2 ^{o}C, airspeed of 2 m/s) for further studies. Protein and impurities in carrageenan were removed by using 25% ethanol at 70 ^{o}C. Carrageenan in the supernatant was separated by using 80% ethanol.

2.2. Mice preparation

Guinea pig, *Cavia porcellus*, weight from 300 to 500 g were supplied by Suoi Dau experimental animal farm, Institute of Vaccines and Medical Biologicals. The animal experiments were performed at the Animal Care Division, Quality Control Department, Institute of Vaccines and Medical Biologicals (IVAC), Vietnam. The animal studies were approved by the Animal Ethical Committee of IVAC (No: 159/QĐ-VXSPYT/ Dt. 29.08.2017) and carried out following the guideline of pharmacological practices approved by the Ministry of Health Portal of Vietnam. Feeding conditions: During the quarantine period and the experimental period, rats were raised under the following conditions: a GLP-qualified, self-contained room with a controlled environment and microclimate conditions (temperature (22-25 ^{o}C), humidity (53-67%), cycle lighting (12 hours x 12 hours)). Diet: Hamster dry food is supplied by an Institute of Vaccine and Medical Biologicals food processing factory according to the registered recipe. Each feed batch is quality checked and certified to confirm its quality. Drinking water is prepared according to the formula specified with hydrochloric acid to reduce the pH to 2.2-3.0 and used immediately after preparation. Drinking water samples are monitored for quality (microbiological, chemical) twice a week. Rats were randomly distributed into cages/boxes with the amount of 5-10 animals/cage or box. Each mouse is marked on a specific position on the charcoal for identification.

2.3. Causing experimental disease

Each group of 9 rats was divided into 3 subgroups, each with 3 subgroups corresponding to 3 concentrations of 10, 5, and 1% acidity. Before drinking acid, mice were taken blood to test 2 macker of an inflammatory reaction, C reactive protein (CRP: C-Reaction Protein) and blood sedimentation rate (BSR) to determine baseline value. Each rat of 1 subgroup was given a single

dose of 1 ml of a solution of 1 of 3 types of hydrochloric acid (HCl), sulfosalicylic acid, and acetic acid at the respective concentrations by pumping through the sonde into the stomach of mice. After 24 hours, all mice were operated on to examine the stomach for macroscopic samples and sent samples as microscopic specimens to identify stomach lesions. At the same time, taking blood to test for C reactive protein and blood sedimentation rate to determine the inflammatory response that occurs due to experimental disease.

2.4. Evaluation after causing experimental disease

The degree of gastric injury was assessed based on the gross and microscopic image of the stomach after surgery and the results of the test markers of the inflammatory response (Table 1).

Table 1. Evaluating experimental pathogens of gastric ulcers

Point	Image of pathology	Macker reacts with inflammation
0	The lining of the stomach is normal and soft. The submucosa has no edema, no vasodilation or congestion.	Constant
1	The gastric mucosa is dark yellow to light pink in color, with magnifying glass showing mild congestion.	No change or slight increase
2	The gastric mucosa is pale pink to dark pink, scattered with hemorrhagic spots.	A slight increase, an insignificant difference from baseline
3	The gastric mucosa is completely congested, there are many bleeding points scattered throughout the gastric mucosa, or linked to hemorrhagic plaques 1 to several mm in diameter.	Average increase and be different from baseline.
4	The gastric mucosa completely collapsed, large plaque hemorrhage in the stomach	High increase, significantly different from background index

2.5. Treatment experiment

Probe treatment dose: 4 groups of guinea pigs (weight 350-500 g/mouse and 5 mice per group) were drunk 10% hydrochloric acid for causing experimental disease. After 1 hour of taking acid and 9 consecutive days, mice of groups II, and III were treated with 1.5% carrageenan (group II- symbol Ca), and Phospholugel (group III- symbol P) at a dose of 1 ml/day/rat. The mice of group I (symbol Cd) were not treated and followed in parallel with the above groups. Testing blood sedimentation rate at the times: before drinking acid (T0), after drinking acid 6 hours (T6h), 48 hours (T48h), 7 days (T7 days), and 21 days (T21 days).

Assessing the pharmacological effect of the product: 4 groups of guinea pigs (weight 350-500 g/mouse and 10 mice per group) were drunk 10% hydrochloric acid on the initial day (D0). After 1 hour of taking acid and for 9 consecutive days after that mice of groups II, and III were treated with

a dose of 01 ml/day/rat with the corresponding products: 1.5% carrageenan (group II), and phospholugel (group IV). Group I did not take any research products and were followed in parallel with the groups above. Surging 50% of mice on the 5th day after treatment and the remaining 50% of mice on the last day of the study (10th day) to evaluate the anatomy of the stomach. Evaluate the results based on the results of microscopic and microscopic analysis of the stomach and blood sedimentation rate test index.

2.6. Data analysis

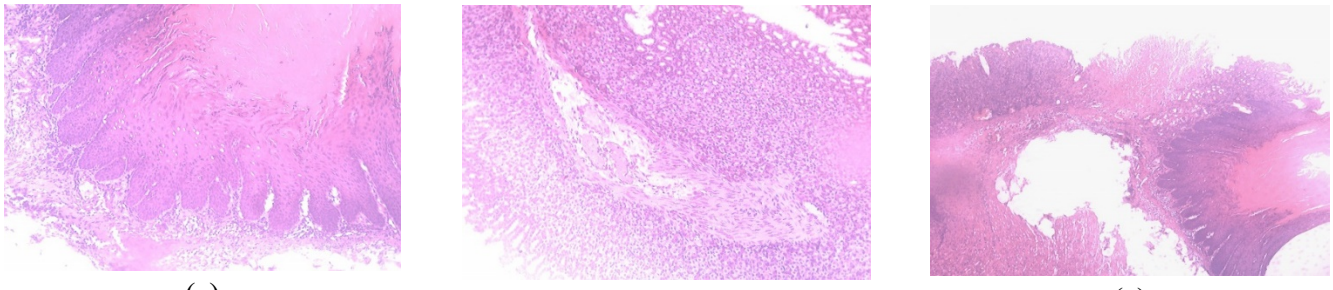
Data was exhibited under the average ± standard deviation (Mean ± SD) and analyzed ANOVA by using MS. Excel 2010. Removing unnormal values was by using the Duncan method.

3. Results and discussion

3.1. Effect of the type and the concentration of acidity on stomach damage

Anatomical stomach

General and microscopic descriptions of the stomach showed that mice taking HCl acid caused an acute inflammatory response to the stomach, including severe to mild damage to the gastric mucosa depending on the concentration. Sulfosalicylic acid causes an inflammatory reaction and erosive damage that is quite characteristic of this type of agent. Acetic acid gastric lesions are not as obvious and characteristic as with hydro-chloric acid or sulfosalicylic acid. For mice drinking 10% HCl acid, their stomach appeared severe mucosal congestion and bleeding of ¾ stomach, a micrograph of their stomach had mucous ulcers, necrosis, and inflammatory cells development (Fig. 1a). The stomach had the thin mucosa with 2 small bleeding points and the normal mucosal surface with several spots showing enlarged blood vessels corresponding to the stomach of mice that drunk 5 and 1% HCl acid, respectively. For stomach micrograph of mice that drunk 5% HCl acid showed atrophy of the mucosa and congestion (Fig. 1b). Mild congestion of the mucosa was found in the stomach micrograph of mice that drunk 1% HCl acid (Fig. 1c).

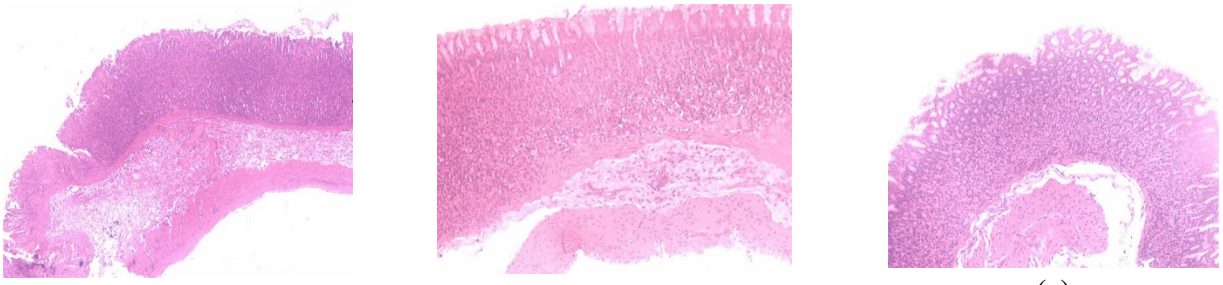


(a) (b) (c)

Figure 1 Anatomical stomach picture of mice drinking HCl acid: (a), (b), and (c) Micrograph of the mice stomach after surgery 10, 5, and 1% HCl acid, respectively

The stomach of mice that drunk 10, 5, and 1% 5-Sulfosalicylic acid appeared mucosa congestion with a few small petechiae, stomach mucosa congestion with 3 small bleeding points, and normal, respectively. It meant that 1% 5-Sulfosalicylic acid did not affect the stomach of mice. However, thin mucosa and slight atrophy appeared in the stomach of mice drinking 1% 5-Sulfosalicylic acid (Fig. 2c). The mucosa of mice that drunk 10% 5-Sulfosalicylic acid was destroyed and

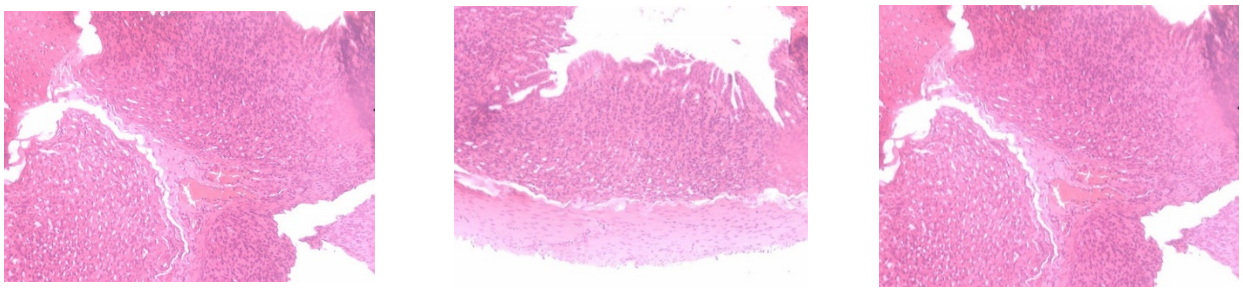
inflammation penetrates through the mucous muscle. Atrophy of the mucosa and congestion exhibited in stomach micrograph of mice that drunk 5% 5-Sulfosalicylic acid (Fig. 2b).



(a) (b) (c)

Figure 2 Anatomical stomach picture of mice drinking 5-Sulfosalicylic acid: (a), (b), and (c) Micrograph of the mice stomach after surgery 10, 5, and 1% 5-Sulfosalicylic acid, respectively

Various concentrations (10, 5, and 1%) of acetic acid did not affect the stomach of mice, exhibited in Figures 3a, 3 c, and 3e, respectively. The mucous membrane is slightly atrophy and congested still exhibited in the stomach of mice drinking 10% acetic acid. The mucosa is slightly atrophy and fibrosis in the stomach of mice drinking 5% acetic acid (Fig. 3b). For mice using 1% acetic acid, their stomach had mild atrophy of the mucosa (Fig. 3c).



(a) (b) (c)

Figure 3 Anatomical stomach picture of mice drinking acetic acid: (a), (b), and (c) Micrograph of the mice stomach after surgery 10, 5, and 1% acetic acid, respectively

Stomach damage

Stomach damage decreased in the order to hydrochloric acid, sulfosalicylic acid, and acetic acid. C-reactive protein was negative for all acids with various concentrations. The vulnerability in the stomach of mice increased according to the concentration of acid (Table 2).

Table 2. Stomach damage of mice after impacting by using various acids

Acid	Acid concentration	The vulnerability *(Point)			Blood sedimentation rate (VS) after 1 hour and 2 hours (mm)			C-reactive protein (CRP)		
		Mice 1	Mice 2	Mice 3	Mice 1	Mice 2	Mice 3	Mice 1	Mice 2	Mice 3
Hydrochloric acid	10%	3	3	2	20/45	12/27	5/16	(-)	(-)	(-)
	5%	2	1	2	5/12	2/2	2/6	(-)	(-)	(-)

	1%	0	1	1	1/2	2/2	3/7	(-)	(-)	(-)
Sulfosalicylic acid	10%	2	2	2	5/17	2/6	2/2	(-)	(-)	(-)
	5%	2	1	1	5/12	2/6	3/6	(-)	(-)	(-)
	1%	0	0	1	1/2	2/2	3/7	(-)	(-)	(-)
Acetic acid	10%	1	2	2	1/2	2/2	3/7	(-)	(-)	(-)
	5%	1	1	1	2/2	1/2	2/5	(-)	(-)	(-)
	1%	0	0	1	1/1	1/2	1/2	(-)	(-)	(-)

Note: (-) negative, *(point): Score 0: The lining of the stomach is normal and soft. The submucosa is not edematous, vasodilating, or congestive. Point 1: The gastric mucosa is dark yellow to light pink in color, with magnifying glass showing mild congestion. Point 2: The gastric mucosa is pale pink to dark pink, scattered with hemorrhagic spots. Point 3: The gastric mucosa is completely congested, there are many bleeding points scattered throughout the gastric mucosa, or linked to a hemorrhagic plaque 1 to several mm in diameter. Point 4: The gastric mucosa completely collapses, bleeding large plaques in the stomach.

3.2. Therapeutic effect

Table 3. Blood sedimentation rate of mouse

Group	Mice	Blood sedimentation rate (mm)									
		T0		T6h		T48h		T7 days		T21 days	
		1h	2h	1h	2h	1h	2h	1h	2h	1h	2h
Non-treatment (Cd)	1	1	1	1	1	3	6	3	6	2	2
	2	1	1	5	16	23	50	5	13	4	11
	3	1	1	1	1	3	6	2	2	1	1
	4	2	2	2	2	4	13	3	6	3	6
	5	1	1	2	2	4	11	4	11	4	7
Phospholugel (P)	1	1	1	2	2	2	2	1	1	1	1
	2	1	1	5	16	4	11	4	11	3	6
	3	2	2	2	2	2	2	2	2	2	2
	4	2	2	3	6	3	6	4	11	2	2
	5	1	1	2	2	1	1	1	1	2	2
Carrageenan (Ca)	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	1	1	1
	3	2	2	5	16	4	11	3	6	1	1
	4	1	1	4	11	4	13	4	11	3	6
	5	2	2	1	1	2	2	2	2	1	1

Notet: T0: Blood sedimentation rate of mice before drinking acid (baseline value)

Rate of blood sedimentation

Table 3 showed that BSR of all mice reached 1-2 mm after 1 hour and 2 hours. After 6 hours of taking acid (T6h), BSR of all mice accounted for 20–80%, depending on the group with the increase after the first hour is 3-5 mm. After the second hour reached 5-16 mm, non-difference occurred. Increased BSR response occurred on T48h, there was a significant difference in higher

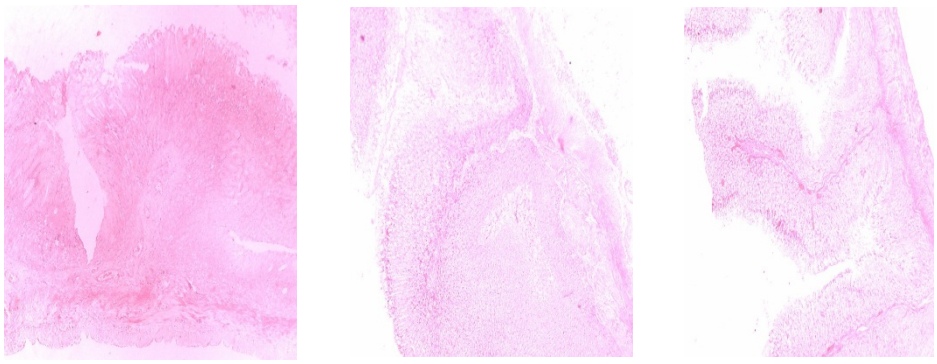
BSR increase between the non-treatment group and the treatment groups. BSR of all the without treatment mice (the positive control group) was higher, compared to the treatment groups (the lower ratios of reactive). BSR value decreased on T7 days, compared to T48h but still higher than the baseline value. However, the level and frequency differed between groups with and without treatment. BSR values on T21 days fell to normal or close to normal in all groups with or without therapy. However, the non-treatment group with 3/5 rats still had BSR level higher than the baseline value. Whereas in the treatment groups this rate was only 1/5.

The blood sedimentation rate test results showed that the blood sedimentation rate value changed (increased) from the 6th hour after taking acid, and peaked at 48 hours then decreased gradually but still higher than the baseline value, occurred in a certain proportion in both the with and without treatment. This also proves that the inflammatory response of the stomach caused by hydrochloric acid, whether treated or not, can recover over time, but the recovery process will be faster when treated with carrageenan in the current study that was similar to phosphalugel.

Gastric surgery

Anatomy of 10 mice on the 5th day after treatment, the macroscopic and microscopic of the stomach showed macroscopically pale pink gastric mucosa, no signs of congestion or hemorrhage, no signs of edema or thin cells for the group of mice with therapy (Carrageenan and phosphalugel). The gastric mucosa surface, in general, returned to a nearly normal level. Microscopic showed that the mucosa is still slightly atrophy, the ability to regenerate from mild to moderate. The group of mice that did not treat macroscopic therapies no longer showed signs of bleeding or edema, but still showed congestion or slight vasodilation, while mice still showed a thin cell mucosal surface. Microencapsulation of the majority found inflammation, atrophy, and fibrosis of the mucosa.

Anatomy of 10 mice on the 10th day after treatment, the examination of macroscopic and microscopic of the stomach showed that the surface of the gastric mucosa is normal, about 2-3 mm thick. Microcosmic showed that the mucosa can regenerate from medium to good for a group of mice with therapy (Carrageenan and phosphalugel). Generally, there are no signs of congestion or edema, the mucosal surface is about 1-2 mm thick for the group of rats without treatment. Microscopic also phenomenon atrophy of the mucosa and fibrosis (Fig. 4a).



(a) (b) (d)

Figure 4 Microimage of the gastric mucosa of mice after therapy 10 days: (a) No treatment; (b) carrageenan; (c) phospholugel

Mucosa was slightly atrophy and fibrosis increased less in the stomach of mice drinking sterile

distilled water (Fig. 4a). In the stomach of mice that drunk carrageenan appeared the mucous membrane regenerates quite well (Fig. 4b). The mucous membrane regenerates quite well for the mice that used phospholugel (Fig. 4c). Thus, carrageenan regenerated the stomach lining damaged by 10% HCl acid in terms of BSR testing and gastric disease anatomy. From the anatomical results, it is possible to preliminary assess the mucosal protective effects of carrageenan equivalent to phospholugel after 10 consecutive doses.

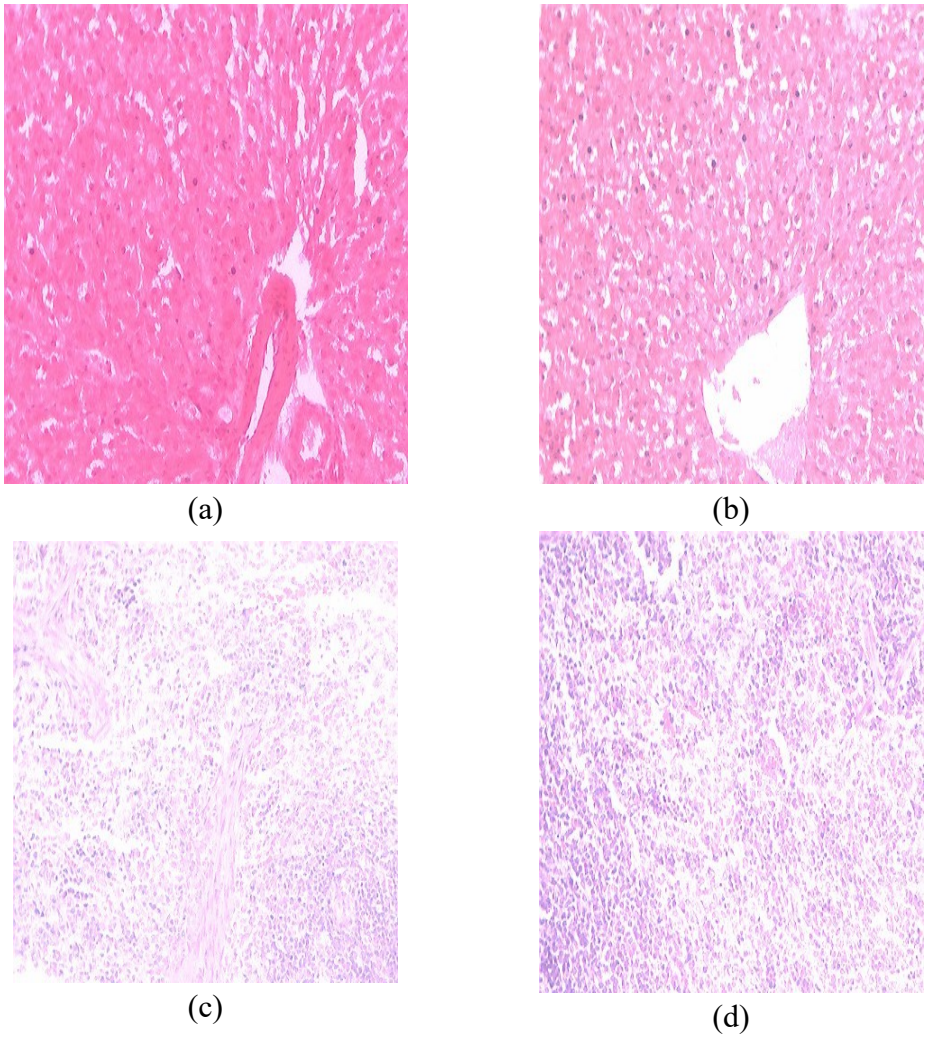
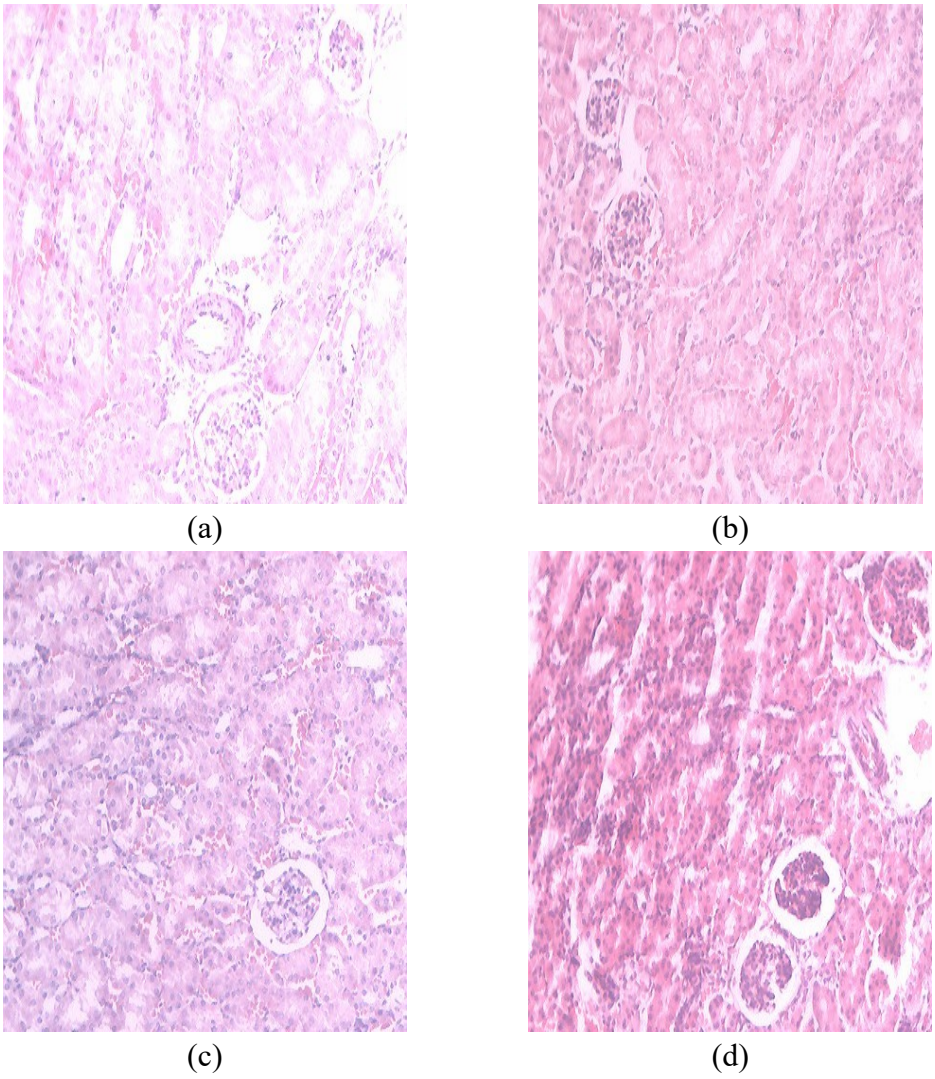


Figure 5 Micro-image of guinea pig liver and spleen in the toxicity study: (a) Liver of mice using carrageenan; (b) Liver of mice using phospholugel; (c) Spleen of mice using carrageenan; (d) Spleen of mice using phospholugel



(a) (b)

(c) (d)

Figure 6 Micro-image of guinea pig kidney in the toxicity study: (a) Right kidney – Carragenan; (b) Right kidney – phospholugel; (c) Left kidney – carrageenan; (d) Left kidney – phospholugel

The difference in micro-image of liver, kidney, and spleen of guinea pig between the group drinking phospholugel and carrageenan did not occur (Fig. 5 and 6). Carrageenan is widely applied in the pharmaceutical field in the role of drug delivery and medicine-based. Carrageenan possesses bioactive because they contain sulfate groups. Specific evidence for the regeneration of mouse gastric membranes with carrageenan had not been found. [21] Carrageenan belongs to an anion polysaccharide group of marine algae, similar to fucoidan, although their structure is different. Some studies showed fucoidan is effective in treating the stomach because it possesses sulfate groups. [22-24] The sulfate group in the structure of alginate is identified as one of the factors determining the ability to support stomach treatment. [18] Therefore, the ability to support peptic ulcer treatment may also depend on the molecular weight of carrageenan. This is also demonstrated in fucoidan and alginate. The biological activity of fucoidan in gastric protection depends on the molecular weight of fucoidan, low molecular mass fucoidan has higher activity than high molecular mass fucoidan, [19, 20] and this is no exception for alginate. [25, 26] In summary, the carrageen extracted from the *Kappaphycus alverazii* seaweed grown in Vietnam are non-toxic and potentially ulcerative. The safety of carrageenan does not depend on the species of seaweed, the

geographic location, and growing season of the seaweed species, this is shown in comparison with the previously published carrageenan toxicity. [27]

4. Conclusion

Carrageenan extracted from *Kappaphycus alverazii* based on the treatment of the algae surface by using enzyme and extracting by using aqueous was effective in the treatment of gastric ulcers that damaged by acid. 1.5% (w/v) carrageenan had a protective effect and regenerated the gastric mucosa of mice that acutely damaged by 10% hydrochloric acid after a 10-day treatment. Carrageenan was prepared according to the method in the current study was friendly to the environment and they exhibited a good potency for applicating into functional food and pharmacy such as support and treatment of stomach ulcers or minimize the impact of foods or medicines on the stomach.

5. Conflict of interest

No conflict of interest to declare.

6. Financial disclosure

The study did not receive any specific grant from funding agencies in the public, commercial, or not-forprofit sectors.

7. References

[1] Álvarez R., Araya H., Navarro-Lisboa R., Lopez d.D.C. Evaluation of polyphenol content and antioxidant capacity of fruits and vegetables using a modified enzymatic extraction. Food Technol Biotechnol 2016;54:462-467.

[2] Necas J., Bartosikova L. Carrageenan: a review. Vet Med-Czech 2013;58:187-205.

[3] Sankalia M., Mashru R., Sankalia J., Sutariya V. Stability improvement of alpha-amylase entrapped in kappa-carrageenan beads: physicochemical characterization and optimization using composite index. Int J Pharm 2006;312:1-14.

[4] Sarah H., Mariel B., Ross C., Kevin P., Angie T. Chapter 10 - The use of carrageenan in food. In Carrageenans: Sources and extraction methods, molecular structure, bioactive properties and health effects, 1st ed., Leonel P., Eds.: Nova Science Publishers, Inc: USA, 2016; pp. 229 -243.

[5] Błażej B., Grażyna G., Alexander S. Carrageenan as a functional additive in the production of cheese and cheese-like products. Acta Sci Pol Technol Aliment 2018;17:107–116.

[6] Li L., Ni R., Shao Y. Mao, S. Carrageenan and its applications in drug delivery. Carbohydr Polym 2014;103:1-11.

[7] Abida K.K., Ain U.S., Shamyla N., Fahad A., Rehana R., Sadullah M., Bushra N., Furqan I., Samina A., Fahad P., Ghulam M. Carrageenan based bionanocomposites as drug delivery tool with special emphasis on the influence of ferromagnetic nanoparticles. Oxid Med Cell Longev 2017;2017:1-13.

[8] Abdul B.A.A., Mohaddeseh A, Pegah K., Mahvash P. Chapter 10 - Pharmaceutical, cosmeceutical, and traditional applications of marine carbohydrates. Adv Food Nutr Res

2014;73:197-220.

[9] Roos G., Cheshire A., Nayar S., Clarke S.M., Zhang W. Harnessing marine macroalgae for industrial purposes in an australian context: emerging research and opportunities: Emerging research and opportunities, 1st ed.: IGI Global: Hershey PA, USA, 2018; pp. 34.

[10] Nussinovitch A. Hydrocolloid Applications: Gum technology in the food and other industries, 1st ed., Springer Science+Business Media Dordrecht: Doyle Graphics, Tullamore, Ireland, 1997; pp. 40-58.

[11] Vanessa W., Sabrina M.D.C., Paulo J.O., Leila H., Pedro L.M.B. Optimization of the extraction of carrageenan from Kap-paphycus alvarezii using response surface methodology. Ciênc Tecnol Aliment Campinas 2012;32:812-818.

[12] Hugo G., Rémi C., Caroline C.-P., Maheva M., Nicolas B., Jean-Marie P., Thierry M., Ingrid F.-A. λ-carrageenan oligosaccharides of distinct anti-heparanase and anticoagulant activities inhibit MDA-MB-231 breast cancer cell migration. Mar Drugs 2019;17:140-155.

[13] Zhiwei L., Tianheng G., Ying Y., Fanxin M., Fengping Z., Qichen J., Xian S. Anti-cancer activity of porphyran and carra-geenan from red seaweeds. Molecules 2019;24:4286-4299.

[14] Ning M., Xi-Wang L., Ya-Jun Y., Jian-Yong L., Isam M., Guang-Rong L., Ji-Yu Z. Preventive effect of aspirin eugenol ester on thrombosis in κ-carrageenan-induced rat tail thrombosis model. PLoS One 2015; 10: e0133125.

[15] Cristian G.-Z., Marisela G.-Á., Gustavo D.-B., Carlos T.S., Carlos G.-C., Apolinaria G.-C. Increased anti-helicobacter pylori effect of the probiotic Lactobacillus fermentum UCO-979C strain encapsulated in carrageenan evaluated in gastric simulations under fasting conditions. Food Res Int 2019;121:812-816.

[16] https://www.marketdataforecast.com/market-reports/carrageenan-market

[17] https://www.worldlifeexpectancy.com/viet-nam-peptic-ulcer-disease

[18] Leiman D.A., Riff B.P., Morgan S., Metz D.C., Falk G.W., French B., Umscheid C.A., Lewis J.D. Alginate therapy is effective treatment for gastroesophageal reflux disease symptoms: a systematic review and meta-analysis. Dis Esophagus 2016;00:00–00. DOI: 10.1111/dote.12535.

[19] Bo Li, Fei L., Xinjun W., Ruixiang Z. Fucoidan: structure and bioactivity. Molecules 2008;13:1671–1695.

[20] Yu W., Maochen X., Qi C., Aiguo J., Hao L., Shuliang S. Biological activities of fucoidan and the factors mediating its therapeutic effects: a review of recent studies. Mar Drugs 2019;17:183-201.

[21] Edisson-Mauricio P.-Q., Roberto R.-C., María-Dolores V. Review Carrageenan: Drug Delivery Systems and Other Biomedical Applications. Mar Drugs 2020;18:2-39.

[22] Ellya S., dan R.P. Evaluation of fucoidan bioactivity as anti gastric ulcers in mice. Procedia Environ Sci 2015;23:407 – 411.

[23] Geert v.W., Marcin B., Karolina O., Willem J.v.W., Andrea R., Johanna M.A.P. Fucoidan structure and activity in relation to anti-cancer mechanisms. Mar Drugs 2019;17:32-61.

[24] Hitoshi K., Yasunari M., Takayuki K., Katsunori T., Tsuyoshi N., Makoto K., Hideyuki M. Effects of fucoidan from mozuku on human stomach cell lines. Food Sci Technol Res 2006;12:218-222.

[25] Hampson F.C., Farndale A., Strugala, V., Sykes J., Jolliffe I.G., Dettmar P.W. Alginate

rafts and their characterisation. Int J Pharm 2005;137–147.

[26] Marta S., Agata P., Emilia S., Patrycja C., Katarzyna W. Alginate: Current use and future perspectives in pharmaceutical and biomedical applications. Int J Polym Sci 2016;2016:1-17. http://dx.doi.org/10.1155/2016/7697031

[27] James M.M., Jamin A.W.Sr., William R.B., Myra L.W. Clarifying the confusion between poligeenan, degraded carrageenan, and carrageenan: A review of the chemistry, nomenclature, and in vivo toxicology by the oral route. Crit Rev Food Sci Nutr 2019;59:3054-3073.