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ScienceDirect
Procedia Environmental Sciences 23 (2015) 407 – 411
Procedia Environmental Sciences

International Conference on Tropical and Coastal Region Eco-Development 2014(ICTCRED 2014)

Evaluation of fucoidan bioactivity as anti gastric ulcers in mice

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Abstract

Fucoidan is a polysaccharide compounds containing sulfate group. Fucoidan is found in brown seaweed. In this study, we assess fucoidan activity extracted from brown seaweed Sargassum crassifolium origin from Binuangeun, Banten. Fucoidan extract was tested in mice in vivo. Observations were carried out during 16 days the control (without fucoidan) and fucoidan treatment. Fucoidan were given in various concentration of 100, 200, 300, 400 ppm. On the 14th day, aspirin was given to mice with pre-treated fucoidan 400 ppm as gastric ulcer induction. The fucoidan extracts compositions showed: water content 3.11%, uronic acid 556 ppm, 0.12 ppm sulfate and 1648 ppm total carbohydrate. Results from histopathology assay in mice tissue stomach showed that 100 ppm of fucoidan can inhibit gastric ulcers caused by 400 ppm aspirin irritation. Fucoidan was associated with an increase in the mucus layer in the gastric mucosa.

Peer-review under responsibility of scientific committee of the ICTCRED 2014

Keywords: fucoidan, induced-gastric ulcer, irritation, aspirin, bioactivity

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Peer-review under responsibility of scientific committee of the ICTCRED 2014
doi:10.1016/j.proenv.2015.01.058

1. Introduction

Fucoidan is a sulfated polysaccharide found mainly in various species of brown seaweeds. Sulfated polysaccharides extracted from seaweeds have been proven to have valuable pharmaceutical and biomedical potential activities [1], such as antioxidant, anticoagulant, antithrombic, and antiviral properties [2,3]. It was also reported that polysaccharides from the brown alga *P. pavonia* (*pavonica*) exhibit anticoagulant activity.

Research on indications of fucoidan can prevent hyperplasia in rats. Aspirin known as acetylsalicylic acid, and drugs called salicylates. Aspirin is often used to reduce pain. Aspirin is known as a class of non-steroidal drugs. Aspirin was the first-discovered member of the class of drugs known as non-steroidal anti-inflammatory drugs (NSAIDs), not all of which are salicylates, although they all have similar effects and most have some mechanism of action which involves non-selective inhibition of the enzyme cyclooxygenase [4].

Studies have indicated that fucoidan can induce apoptosis in human lymphoma cell lines and inhibit hyperplasia in rabbits. Few studies have reported the effect of fucoidan on proand anti-inflammatory cytokines. In animal models, ingestion of fucoidan has inhibitory effects on tumors, which appears to be associated with a rise in interferon-gamma (IFN- ϒ) and interleukin-12 (IL-12) and stimulation of innate immunity[5].The fucoidan is a safe substance with potential for gastric protection[6]. Therefore, the objective of this study is to investigate the effectiveness of fucoidan on the used aspirin as a cause of gastric ulcers in mice.

2. Materials and methods

2.1. Chemicals

Brown seaweed *Sargassum duplicatum* was obtained from Banten. EtOH, acetone, NaOH, and inorganic acids and salts ($CaCl_2$, NaCl) were commercial products. CMC (carboxyl methyl celulosa) was commercial products.

2.2. Experiments

First we extracted fucoidan from brown seaweed followed (Sinurat method). Before the extraction of fucoidan has been tested of species brown seaweed. Brown seaweed was rinsed with water and dried in open air then milled and freeze -dried. The solid powder of brown seaweed was dissolved and incubated in mixture solvent of MeOH-$CHCl_3$- H_2O with a ratio of 4: 2: 1 for 12 hours then washed with acetone and dried (F1). Moreover, this sample then was soaked with HCl 0,1N (1:10) (w / v) then mixing for 6 hours at room temperature. Planktonic filtered using 500 mesh, the filtrate collected. The filtrate was neutralized using NaOH 0.5 M and 4M $CaCl_2$ solution (1:10) while stirring mechanically for 60 minutes and heating at temperature 85 ^oC then filtered, the filtrate collected (F2). The filtrate was centrifuged and diluted in water containing 0.5 M $CaCl_2$ and 5% CPC precipitation as precipitated filtrate (F3). In addition of 3 M $CaCl_2$ and ethanol with ratio (1: 2) also water then centrifuged to the filtrate will obtain the better result of fucoidan quality. The final filtrate was rinsed with0.5 M NaCl and aquabides was obtained as extract fucoidan (F4). The quality of fucoidan determination were conducted as following methods : total carbohydrate[7] method), turbidimeter assay to quantify the sulfate content using $BaCl_2$-gelatin method and quantification, uronic acid (Scot method).

The second step fucoidan was be tested in mice *in vivo* as an anti-gastric ulcer followed Jong et al. method (2010). The methods were performed as follows: mice were used as animal model were divided into 7 groups. Group 1: control / only given CMC 0.5%. Group 2: control only CMC 0.5% was given every day during the previous 14 days, then mice were conditioned fasting for 48 hours after the mice were given aspirin (400 mg / kg body weight). Group 3: mice were given fucoidan at dose of 100 mg / kg of body weight every day, for 14 days and then fasted for 48 hours after the mice were given aspirin (400 mg) / kg body weight. Group 4: rats were given fucoidan at dose of 200 mg / kg of body weight per day during the early 14 days, then fasted for 48 hours after the mice were given aspirin (400 mg) / kg body weight. Group 5: mice were given fucoidan at dose of 300 mg / kg of body weight per day during the previous 14 days, then fasted for 48 hours after the mice were given aspirin (400 mg) / kg body weight. Group 6: rats were given fucoidan at a dose of 400 mg / kg of body weight per day during the previous 14 days, then fasted for 48 hours after the mice were given aspirin (400 mg) / kg body weight. Group 7: rats were given commercial fucoidan at a dose of 300 mg / kg of body weight per day during the previous 14 days, then fasted for 48 hours after the mice were given aspirin (400 mg) / kg body weight. Used as a coating solution of

CMC0.5%.

The observations carried out : 1. Blood samples were taken for analysis of pH by means of a centrifuge at 3000 rpm for 10 minutes. 2. Observations of gastric tissue changes under SEM/TEM, after first fixation in 10% formalin.

3. Result and Discussion

Results of species classification of the brown seaweed found that the type of brown seaweed was *Sargassum dulicatum*. The results of the analysis of the raw material brown seaweed was performed in Table 1. The yield of brown seaweed flour after soaking with methanol, $CHCl_3$ and in ratio (2: 1) respectively was containing 25% of wet seaweed. Seaweed powder was subsequently extracted using the method developed by Sinurat. Obtained yield of crude fucoidan was 5.56%.

Table 1.The quality of fucoidan composition from *Sargassum duplicatum*

No	Parameters	Concentration (ppm)
1.	Total carbohydrate (as fucosa)	1648
2.	Total sulfated	0.12
3.	Uronic acid	556
4.	Moisture content (%)	3.11

Fucoidan assay in vivo was done in BALIVET laboratory. Bioassay was conducted by using mice in various fucoidan feeding concentration of 100 mg / kg body weight to 400 mg / kg body weight in mice. The tests were grouped into 7 groups was compared with commercial fucoidan used as anti-gastric ulcer supplements currently available on the market.

Based on the in vivo test results showed that the use of fucoidan with a concentration of 100 ppm was able to prevent the occurrence of gastric ulcers. Gastric ulcer occurred with aspirin, is considered to injured gastric (not all showed). Aspirin is a drug containing strong acid that can injured gastric. The results of the pH measurements after the of mice surgery, for all treatments were given aspirin pH is 4.3. Physically seen the results of histopathology test, as shown in Figure 1.

Groups	Gastric organ	Remarks
A	Normal	Mice surgery in stomach tissue first day (before treatment)
B	Mild ulcerative gastritis	Mices were given 0.5% carboxy methyl cellulose (100,200,300mg /kg body weight, p.o.) for 14 days and fasted for 48 h and post-orally gavaged with aspirin (400 mg/kg body weight
C	Fucoidan-pretreated ulcerated	Mices were given fucoidan (100,200,300mg /kg body weight, p.o.) for 14 days and fasted for 48 h and post-orally gavaged with aspirin (400 mg/kg body weight, suspended in 0.5% carboxy methyl cellulose)

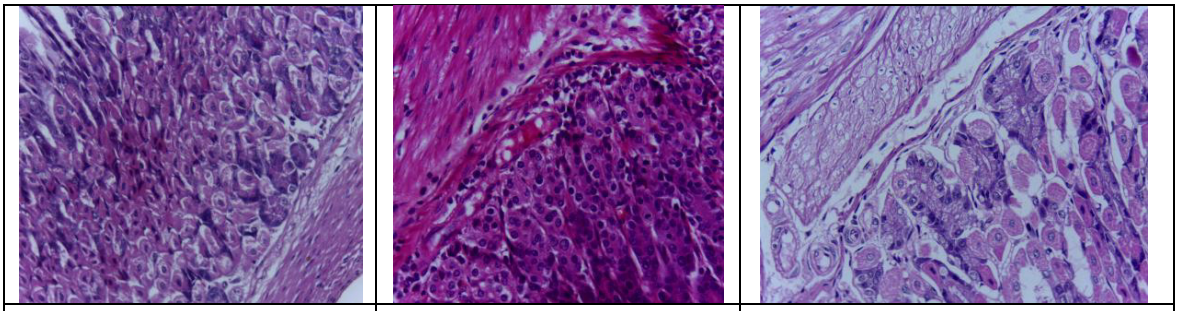

Figure 1. Test hispathology in stomach tissue of normal an ulcer experimental mice.

Based on figure results of hispatology performance that given aspirin without fucoidan (Figure B) caused blood clots this show that ulcer gastric in stomach tissue. Different performance on (Figure A without aspirin and C given aspirin and fucoidan) not see many blood clots. This show that fucoidan can be prevent ulcer gastric with make be layer in mucosa because fucoidan make so it does not injure the lining of the stomach tissue. The mucosa is always covered by a layer of thick mucus that is secreted by tall columnar epithelial cells. Gastric mucus is a glycoprotein that serves two purposes: the lubrication of food masses in order to facilitate movement within the stomach and the formation of a protective layer over the lining epithelium of the stomach cavity. This protective layer is a defence mechanism the stomach has against being digested by its own protein-lysine enzymes, and it is facilitated by the secretion of bicarbonate into the surface layer from the underlying mucosa. The acidity, or hydrogen ion concentration, of the mucous layer measures netral at the area immediately adjacent to the epithelium and becomes more acidic at the luminal level. When the gastric mucus is removed from the surface epithelium, small pits, called foveolae gastricae, may be observed with a magnifying glass.

The toxic dose of aspirin did not fully dissolve in normal saline or water. Hence it was preferred as using a vehicle and the vehicle was also given for the control animals. Carboxy methyl cellulose is generally being used as the vehicle because of its high viscosity, non-toxicity, biodegradability and safe [8].

These findings support the hypothesis that fucoidan effectively attenuates the inflammatory cytokines release mediated gastric mucosal damage on give aspirin. As ulcer healing is a complex process involving various factors, this study is at too primitive stage to conclude its exact action. Mucosal damage can be easily produced by the generation of reactive metabolite. An improvement in mucus production guides the healing process by protecting the ulcer crater against the endogenous aggressors, like stomach secretions and oxidants as well as against exogenous damaging agents, such as NSAIDs. The ulcer prevention or healing by fucoidan was associated with an increase in the mucus layer in the gastric mucosa [9].

4. Conclusion

The yield of was 5.56% crude fucoidan from seaweed powder dried. Based on organ histopathology test of mice obtained using 100 ppm fucoidan can inhibit gastric ulcer irritation after given aspirin 400 mg/kg body weight. Fucoidan was associated with an increase in the mucus layer in the gastric mucosa.

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