ABSTRAK

MEKANISME ANTI ULSER LAMBUNG EKSTRAK KITIN DAN SERBUK CANGKANG RAJUNGAN (*Portunus pelagicus* Linn.) MELALUI PENGHAMBATAN NF-κB p65 PADA LAMBUNG TIKUS YANG DIINDUKSI ETANOL SERTA TOKSISITAS AKUT EKSTRAK KITIN

Oleh
RENNY AMELIA
NPM: 260130190041

Penyakit ulser lambung atau *peptic ulcer desease* (PUD) adalah lesi pada lapisan mukosa lambung yang disebabkan oleh kerja pepsin, asam lambung, dan faktor lainnya. Terdapat peningkatan prevalensi PUD sebesar 25,82% dari tahun 1990 hingga tahun 2019. Eradikasi dalam mengatasi PUD telah banyak dilakukan dengan menggunakan obat sintesik. Namun obat sintesik dapat menyebabkan efek samping apabila dikonsumsi dalam jangka waktu yang panjang atau diberi dalam dosis besar.

Kitin dapat diperoleh dari berbagai macam sumber, produksinya diperoleh dari golongan krustasea. Dalam beberapa penelitian, kitin mampu berefek sebagai penyembuh luka dan pencegah ulser lambung. Pada umumnya kitin yang diuji merupakan kitin hasil ekstraksi dari pelarut konvensional seperti HCl dan NaOH. Pada penelitan ini, dilakukan ekstraksi kitin dari cangkang rajungan menggunakan pelarut ramah lingkungan, *natural deep eutectic solvents* (NADES), yaitu campuran kolin klorida dan asam malat dengan perbandingan molaritas (1:1) memiliki rendemen sebesar 35,43%. Ekstraksi dilakukan dua kali untuk mengurangi kandungan abu di dalam ekstrak.

Pengujian jenis isomer ekstrak kitin telah dilakukan dengan *fourier transform infrared* (FTIR). Beberapa pita yang terbentuk dari ekstrak kitin adalah adanya puncak di bilangan gelombang 1100 cm^{-1} (C-O alifatik); 1380 cm^{-1} (C-N *stretch*); 1600 cm^{-1} (C=O *stretch*); 2890-2968 cm^{-1} (C-H alkil); and 3400-3550 cm^{-1} (OH alkohol). Pita yang terdapat pada bilangan gelombang 2900 cm^{-1} biasanya digunakan sebagai referensi pita dalam menganalisis kitin. Berdasarkan hasil FTIR terdapat adanya puncak tak terbagi gugus amida I di bilangan gelombang 1620 cm^{-1} yang menunjukkan jenis isomer kitin yang didapat adalah β-kitin.

Uji proksimat yang dilakukan meliputi kadar air, kadar lipid, kadar abu, kadar protein, kadar karbohidrat. Metode pengujian kadar abu, kadar air kadar protein, kadar lipid berdasarkan SNI 01-2891:1992. Penentuan kadar karbohidrat dengan metode *by difference*. Kadar air dan abu dari ekstrak kitin secara berturut turut

1,93% dan 30,61% dengan syarat kadar air dan abu menurut SNI secara berturut-turut adalah 12% dan 5%. Dapat disimpulkan kadar abu dari ekstrak kitin tidak memenuhi persyaratan SNI. Sedangkan kadar logam diuji dengan *Inductively Coupled Plasma – Optical Emission Spectrometry* (ICP-OES). Kadar logam ekstrak kitin yang disyaratkan SNI meliputi As dan Pb. Kadar As dan Pb ekstrak kitin keduanya adalah < 0,0001 mg/kg dan syarat SNI maksimal keduanya adalah 5 mg/kg. Sehingga dapat disimpulkan ekstrak kitin memenuhi syarat logam.

Aktivitas anti ulser lambung telah dilakukan dengan metode *in vivo* dimana induksi ulser diberikan setelah pemberian sampel. Mekanisme penghambatan protein NF-κB p65 diujikan menggunakan metode *western blot*. Dosis ekstrak kitin yang digunakan pada uji anti ulser lambung adalah 150, 300, dan 600 mg/kgBB sedangkan dosis serbuk cangkang rajungan yang digunakan adalah 500 dan 1000 mg/kgBB. Dari hasil pengujian, semua kelompok perlakuan mampu menurunkan indeks ulser lambung dibandingkan kelompok kontrol negatif. Kelompok ekstrak kitin 600 mg/kgBB merupakan kelompok yang memiliki indeks ulser lambung (1,08±0,77%) paling mendekati kelompok normal (0,06±0,06%) dibanding kelompok perlakuan lain. Hasil uji mikroskopik organ lambung menunjukkaan kelompok ekstrak kitin 150 mg/kgBB adalah kelompok yang paling mendekati kelompok normal baik dilihat dari parameter kualitatif maupun kuantitatif. Berdasarkan pengujian ekspresi protein NF-κB p65, terlihat bahwa kelompok ekstrak kitin 600 mg/kgBB memiliki kemampuan menghambat ekspresi NFκB p65 paling kuat dengan nilai rasio relatif NF-κB p65 (0,188±0,114) lebih baik dibanding kelompok normal (0,366±0,208).

Toksisitas akut diujikan pada ekstrak kitin dengan prosedur yang telah disesuaikan pedoman BPOM. Dosis ekstrak kitin yang digunakan pada uji toksisitas meliputi 500, 1000, 2000, 4000 dan 6000 mg/kgBB. Hasil uji toksisitas akut menunjukkan persentase relatif berat organ (lambung, jantung, hati, ginjal, dan paru-paru) semua kelompok secara statistik tidak berbeda signifikan dibandingkan dengan kelompok kontrol negatif. Jumlah sel normal pada dosis ekstrak kitin 6000 mg/kgBB pada semua organ mengalami penurunan yang signifikan dibanding kelompok kontrol, hal ini diikuti peningkatan jumlah sel yang mengalami nekrosis

Berdasarkan data yang dihasilkan pada penelitian ini, dapat disimpulkan bahwa ekstrak kitin dapat dijadikan sebagai alternatif dalam pengobatan ulser lambung. Penelitian lebih lanjut dibutuhkan guna memaksimalkan kemampuan kitin sebagai anti ulser lambung.

Kata kunci: kitin, mikroskopis, ulser lambung, NF-κB p65, toksisitas akut.

ABSTRACT

Mechanism of Anti Gastric Ulcer of Chitin Extract and Blue Swimming Crab Shell Powder (Portunus Pelagicus Linn.) Through Inhibition of NF-κB p65 in Ethanol Induced Rats and Acute Toxicity of Chitin Extract

By:

Renny Amelia
NPM: 260130190041

Peptic ulcer disease (PUD) is a lesion of the gastric mucosal layer caused by the action of pepsin, gastric acid, and other factors. There has been an increase in the prevalence of PUD by 25.82% from 1990 to 2019. Eradication in overcoming PUD has been widely carried out using synthetic drugs. However, synthetic drugs can cause side effects if taken for a long time or given in large doses.

Chitin can be obtained from various sources, where the main source of its production is obtained from the crustacean group. In several studies, chitin can affect a wound healer and prevent gastric ulcers. In general, the chitin tested is extracted from conventional solvents such as HCl and NaOH. In this study, chitin was extracted from crab shells using environmentally friendly solvents, natural deep eutectic solvents (NADES), a mixture of choline chloride and malic acid with a molarity ratio (1:1) having a yield of 35.43%. The extraction was carried out twice to reduce the ash content in the extract.

Testing of isomeric types of chitin extract has been carried out using Fourier transform infrared (FTIR). Some of the bands formed from the chitin extract were peaks at wave number 1100 cm^{-1} (aliphatic C-O); 1380 cm^{-1} (C-N stretch); 1600 cm^{-1} (C=O stretch); 2890-2968 cm^{-1} (C-H alkyl); and 3400-3550 cm^{-1} (OH alcohol). The band contained in the wave number 2900 cm^{-1} is usually used as a reference band in analyzing chitin. Based on the FTIR results, it can be seen that there is an undivided peak of the amide I group at wave number 1620 cm^{-1}, which indicates that the type of chitin isomer obtained is β-chitin.

The proximate test carried out included water content, lipid content, ash content, protein content, and carbohydrate content. The testing method for ash, water, protein, and lipid content is based on SNI 01-2891:1992. Determination of carbohydrate content by the process by difference. The water and ash content of the chitin extract was 1.93% and 30.61%, respectively, provided that the water and ash content according to SNI were 12% and 5%, respectively. It can be concluded that the ash content of the chitin extract does not meet the requirements of SNI. Meanwhile, the metal content was tested by Inductively Coupled Plasma – Optical

Emission Spectrometry (ICP-OES). The metal content of chitin extract required by SNI includes As and Pb. As and Pb levels of chitin extract were both < 0.0001 mg/kg and the maximum SNI requirement for both was 5 mg/kg. So it can be concluded that the chitin extract meets the metal requirements.

Acute toxicity was tested on chitin extract where the procedure was according to BPOM guidelines. The doses of chitin extract used in the toxicity test included 500, 1000, 2000, 4000 and 6000 mg/kgBW. The acute toxicity test results showed that the relative percentage of organ weight (stomach, heart, liver, kidney, and lungs) of all groups was not statistically significantly different from the negative control group. The number of normal cells at a dose of 6000 mg/kgBW of chitin extract in all organs showed a significant decrease compared to the control group, an increase followed this in the number of cells undergoing necrosis.

The anti-ulcer activity of the stomach has been carried out by an in vivo method in which ulcer induction is given after administration of the sample. Mechanism of inhibition of NF-κB p65 protein by western blot method. The doses of chitin extract used in the anti-gastric ulcer test were 150, 300, and 600 mg/kgBW, while the crab shell powder doses used were 500 and 1000 mg/kgBW. The test showed that all treatment groups were able to reduce gastric ulcer index compared to the negative control group. The chitin extract group of 600 mg/kgBW was the group that had the gastric ulcer index (1.08±0.77%) closest to the normal group (0.06±0.06%) compared to the other treatment groups. The microscopic examination of the gastric organs showed that the 150 mg/kgBW chitin extract group was the closest to the normal group in terms of qualitative and quantitative parameters. Based on the protein expression test of NF-B p65, it was seen that the chitin extract group of 600 mg/kgBW had the strongest ability to inhibit the expression of NF-B p65 with the relative ratio value of NF-B p65 (0.188±0.114) better than the normal group (0.366±0.208).

Based on the resulting data, it can be concluded that chitin extract can be used as an alternative in the treatment of gastric ulcers. Further research is needed to maximize the ability of chitin as an anti-gastric ulcer.

Keywords: Chitin, microscopic, NF-κB p65, gastric ulcer, acute toxicity.