ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antihypertensive Activity of Ethanol Extract Of Macroalgae *Gracilaria Verrucosa* (Hudson) Papenfuss. In Rats Using Non-Invasive Blood Pressure Method.

Ahmad Muhtadi[1]*, Byantari Prameswari Nyoman[1] and Moelyono Moektiwardoyo[2]

[1]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran,J atinangor 45363, Sumedang, Indonesia
[2]Department of Biological of Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran,J atinangor 45363, Sumedang, Indonesia

ABSTRACT

Hypertension is a worldwide problems of epidemic proportions, affecting 15-20% of adults. This study was conducted to antihypertensive activity of ethanol extract of macroalgae *Gracilaria verrucosa* (Hudson) Papenfuss. were investigated on rats induced epinephrine dose of 1.2 µg / kgBW given intraperitoneally and using three doses (125, 250 and 500 mg/kg BW) ethanol extract also captopril dose (2.25 mg/kgBW) as positive control drug given orally with a non-invasive method. The result showed that ethanol extract of macroalgae *Gracilaria verrucosa* (Hudson) Papenfuss. possessed antihypertensive properties. The extract can reduce blood pressure with inhibitory percent of 14.56 for systolic blood pressure and 15.12 for diastolic blood pressureat a dose of 125mg / kgBW in the 45thminute after oral administration.

Keywords: *Gracilaria verrucosa*, antihypertensive, rats, ethanol extract.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Hypertension or high blood pressure is a condition where the increase of blood pressure is above the normal, blood pressure is more than 140/90 mmHg [1]. Prevalence of hypertension is globally increase, and at 2025 predicted 29% of adults throughout the world would have hypertension [2]. In Indonesia hypertension is a medical issue with also a high prevalence 25,8% and become one of cause of death [3].

Macroalgae known as seaweed is a Thallophyta division, one of majorly found in Indonesia is *Gracilaria sp*. which commonly consumed since ages in Asia's continent, whereas in Western country it is used as source of phycocolloids, thickening and gelling agent in food industry. In addition macroalgae is also used as antihypertensive [4], by obstruct ACE (angiotensin Converting Enzyme) work as vasodilator [5]. Study of ACE inhibitory activity using macroalgae is not spreadly done except *Gracilaria verrucosa* [6]. Thus *Gracilaria verrucosa* (Hudson) Papenfuss. have more potential to be further researched as antihypertensive using a different method such as non-invasive blood pressure method, which is not only more practical but also easier because the blood pressure measurement was conduct without any invasive or surgery, using VPR (Volume Pressure Recording) on rat's tail [7].

MATERIALS AND METHODS

Chemicals

Chemicals used in this experiment Ethanol 70% (*Merck*), Ephinephrine Injection 1 ml (*Phapros*), Aqua Pro Injectio, Captoprile tablets 12.5 mg (*Indofarma*), and PGA 2%

Collection of material

Fresh Macroalgae *Gracilaria verrucosa* (Hudson) Papenfuss. harvested from polyculture pond of Milkfish and Seaweed at Domas Village, Serang, Banten, Indonesia. Then its identity being verified with plant determination at The Indonesian Science Institute, Oceanographic Research Center, Ancol, Jakarta, Indonesia.

Preparation solvent extraction

Fresh macroalgae is rinsed and dried by solar evaporation method so dried simplicial were obtained. Then dried simplicial of *Gracilariaverrucosa* being extracted with maceration method using ethanol 70% for 3x24 hours, the macerate is being compact with rotary evaporator at 40-45^{o}C so a consistent viscous macerate is obtained, then the percent yield of extract is calculated. Viscous Extract is dissolved in PGA 2% before administrated to mice.

Experimental animals

White male Wistar Rats (*Rattus norvegicus*) weighing 150 – 400 g were obtained from School of Biological Sciences and Technology, Bandung Institute of Technology. The animals were housed in a controlled environment (25$\pm$4 ^{0}C, 50-60% humidity and 12-hour light dark cycle). The animals were fed nutrition with pellets and water *ad libitum*for one week (adaption), and were then randomly assigned to 6 groups (4 rats each). The experiment was approved by the Health Research Ethics Committee of the Faculty of Medicine, UniversitasPadjadjaran No: 400/UN6.C1.3.2/KEPK/PN/2016

Instruments:

Digital blood pressure monitor *CODA^{TM} Non-Invasive Blood Pressure Kent Scientific Corporation*.

Experimental design :

Rats is grouped into 6 group, each group have 4 rats as subject and given treatment as below:

Group I, group II and group III fed ethanol extract of macroalgae *Gracilaria verrucosa* (Hudson) Papenfuss.dose 125mg/kgBW, 250mg/KgBW and 500mg/kgBW [8] with PGA 2%; group IV captopril dose 2.25mg/kg bw with PGA 2% (positive control); group V ephinephrine 1.2 µg/kg bw in aqua pro injectio [9] (negative control) and group VI PGA 2% (normal control).

Blood Pressure measurement is proceed by digital blood pressure monitor *CODA^{TM} Non-Invasive Blood Pressure Kent Scientific Corporation* with steps as below:

a. Every groups is given treatment perorally, except epinephrine given intraperitonially.
b. Normal blood pressure measurement:
   Then normal blood pressured of rats being monitored and noted as Blood Pressure (BP) I. Normal BP is at initial time (0^{th} minute).
c. Blood pressure measurement after induction:
   Rats induced by epinephrine dose 1,2µ/kgbw (Runadi, 2010) intraperitoneal in 30 minutes after given PGA 2% for every groups except normal group. Blood pressure is being monitored every 15 minutes after induction (15^{th}, 30^{th}, 45^{th}, 60^{th} minutes after induce of epinephrine), noted as BP II.
d. Blood pressure measurement after treatment:
   Blood pressure of rats will be back to normal in 60 minutes after induction, then every groups is given teratment: group I, II, III given ethanol extract of macroalgae *Gracilaria verrucosa* (Hudson) Papenfuss. in variated dosage, group IV given captopril as positif control, group VI givenPGA 2% as normal control, and than induced again with epinephrine 30 minutes after administration. Blood pressure is being monitored every 15 minutes after induction (15^{th}, 30^{th}, 45^{th}, 60^{th} minutes after induce of epinephrine), noted as final blood pressure (BP III).
e. Calculation of antihypertensive activity:
   Antihypertensive activity can be calculated from the difference between BP II and BP III, with the percentage of blood pressure decrease, with the formula s below:

$$\% \text{ of Inhibition} = \left(\frac{BP\ II - BP\ III}{BP\ II}\right) \times 100\%$$

Explanation:
BP II = Blood pressure after induced by epinephrine (mmHg).
BP III = Blood pressure after given treatment/extract (mmHg).

Statistical analysis

Data analyzed using ANOVA and Tukey's HSD.

RESULTS AND DISCUSSION

The result of antihypertensive activity of ethanol extract ofmacroalgae *Gracilaria verrucosa* (Hudson) Papenfuss. in rats induced epinephrine, is obtained the blood pressure data of systolic and diastolic in every time observed which is 15^{th}, 30^{th}, 45^{th}, and 60^{th}minutes after treatment, converted into average percent of inhibition activity showed at Table 1 and Figure 1 (Systolic) also Table 2 and Figure 2 (Diastolic) as below:

Table 1. AveragePercent of Inhibition Systolic Blood Pressure

Treatment	Time (minutes)			
	15^{th}	30^{th}	45^{th}	60^{th}
Group I	14.24± 14.56	8.06± 3.71	14.55± 9.30*	-4.82± 14.45
Group II	2.72± 10.45	15.07± 23.35	8.42± 10.37	10.88± 17.89
Group III	-9.18± 24.65	4.78± 13.53	6.27± 8.43	-6.49± 3.28
Group IV	2.29± 13.02	8.42± 10.39	14.21± 17.38	10.46± 16.17
Group V	6.78± 11.71	1.71±13. 89	2.51± 14.44	8.23± 17.59
Group VI	-7.16± 22.72	-11.26± 15.56	-3.84± 9.59	9.78± 14.50

*) = P< 0.05 Vs Group V with Tukey's HSD Test; n=4

ISSN: 0975-8585

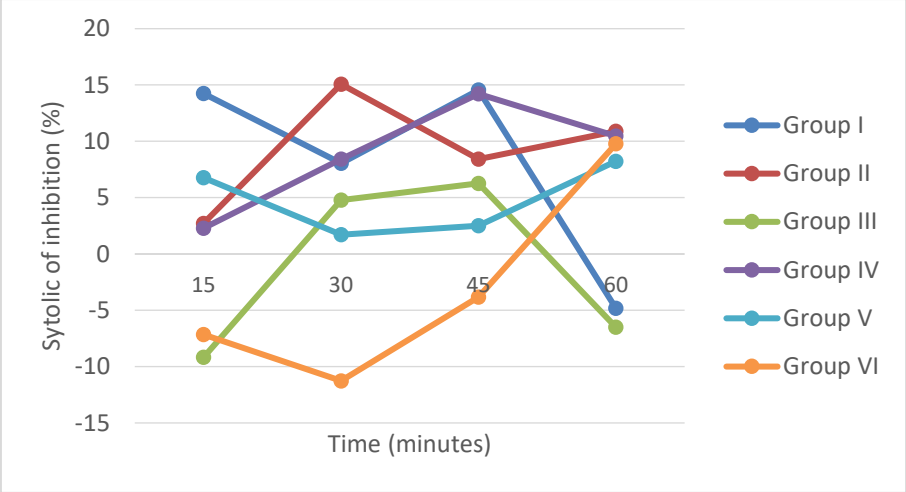

Figure 1. Average Percent of Inhibition Systolic Blood Pressure

The average of the percent of inhibition on systolic blood pressure showed, that group I (extract dose 125mg/kgBW) indicated have greater on systolic blood pressure activity with the other gruops, followed group IV (captroprildose 2.25mg/kgBW as positive control), group II (extract dose 250 mg/kgBW) and group III (extract dose 500 mg/kgBW) against group V (ephinephrine 1.2 µg/kgBW as negative control) onTukey's HSD Test indicated on Table 1 and Figure 1. Percent of inhibition of systolic blood pressure optimum occurs in observation at the 45th minute, then followed at minute 60, 30, and 15 after administration, in observation with Tukey's HSD Test.

Table 2. Average Percent of Inhibition Diastolic Blood Pressure

Treatment	Time (minutes)			
	15th	30th	45th	60th
Group I	10.56± 5.73	11.24± 12.14	15.12± 8.94 *	-3.71±6.31
Group II	10.91± 14.60	11.55± 18.57	10.16± 11.20	17.74± 20.90
Group III	-15.48± 36.63	7.45± 14.65	14.28± 11.68	-7.48± 3.68
Group IV	-7.17± 20.78	8.54± 11.68	14.04± 18.87	13.04± 20.50
Group V	-7.78± 19.81	3.88± 11.31	3.71± 23.61	9.22± 29.50
Group VI	-5.38± 20.99	-15.39± 22.20	-1.32± 14.32	11.67± 19.01

*) = P< 0.05 Vs Group V with Tukey's HSD Test; n=4

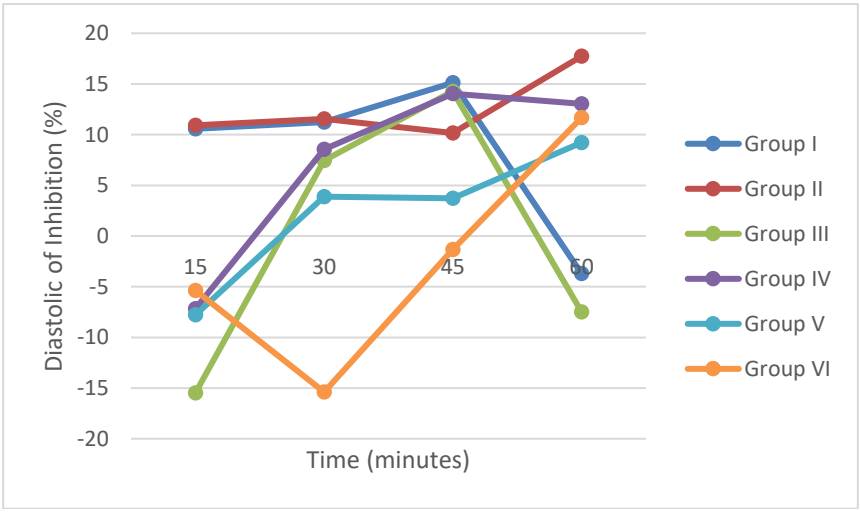

Figure 2. Average Percent of Inhibition Diastolic Blood Pressure

The percent of inhibition on diastolic blood pressure showed, thatgroup I (extract dose 125mg/kgBW) indicated have greater on diastolic blood pressure activity with the other gruops, followed Group III (extract dose 500 mg/kgBW), group IV (captoprildose 2.25mg/kgBW as positive control), group II (extract dose 250 mg/kgBW) and against group V (ephinephrine 1.2 µg/kgBW as negative control) on Tukey's HSD Test indicated on Table 2 and Figure 2. Percent of inhibition of diastolic blood pressure optimum occurs in observation at the 45th minute, then followed at minute 60, 30, and 15 after administration, in observation with Tukey's HSD Test.

Based on evaltuated on above it is showed that a change of blood pressure occurs significantly, at the systolic blood pressure data which shows the highest decrease of blood pressure is dose group of ethanol extractdose 125 mg/kgBW (14.55 %) followed by captoprildose 2.25mg/kgBW (14.21%) as positive control ,ethanol extract dose 250 mg/kgBW (8.42%), and ethanol extract dose of 500 mg/kgBW(6.27%) compared to epinephrinedose 1.2 µg/kgBW as the negative control. Percent of inhibition of systolic blood pressure optimum occurs in observation at the 45th minute, then followed at minute 60, 30, and 15 after administration. Similar antihypertensive activity occurs in the diastolic blood pressure, a great decreased blood pressure also occurred in dose group of ethanol extract dose 125 mg/kgBW (15.12 %) followed by group of ethanol extract dose 500 mg/kgBW (14.28 %) captoprildose 2.25mg/kgBW (14.04%) as positive control, and ethanol extract dose 250 mg/kgBW (10.16%) and against ephinephrine 1.2 µg/kgBW as negative control, while optimum percent of inhibition for diastolic blood pressure occur sequentially in the 45th minute observation, 60, 30, and 15 minute after administration. The result showed that ethano lextract of macroalgae *Gracilaria verrucosa* (Hudson) Papenfuss. possessed antihypertensive properties. The extract can reduce blood pressure with inhibitory percent of 14.55 for systolic blood pressure and 15.12 for diastolic blood pressure at at a dose of 125 mg/kgBW in the 45th minute after oral adminitration. The crude extract of Gracilaria sp also was invstigated in the other method on anaesthetsized rats using two doses (250 and 500 mg/kg given intraperitoneally [8] and the results showed that the crude extract posessed antihypertensive properties.

CONCLUSIONS

This study was conducted to antihypertensive activity of macroalgae *Gracilaria verrucosa* (Hudson) Papenfuss. were investigated on rats induced epinephrine dose of 1.2 µg / kgBW given intraperitoneally and using three doses (125, 250 and 500 mg/kgBW) ethanol extract also captopril dose (2.25 mg/kgBW) as positive control drug given orally with a non-invasive method. The result showed that ethanol extract of macroalgae *Gracilaria verrucosa* (Hudson) Papenfuss. possessed antihypertensive properties. The extract can reduce blood pressure with inhibitory percent of 14.55 for systolic blood pressure and 15.12 for diastolic blood pressure at at a dose of 125 mg/kgBW in the 45thminute after oral administration.

REFERENCES

[1] The Indonesian Heart Association (IHA).PedomanTatalaksana Hipertensi pada Penyakit Kardiovaskular. Edisi I. Jakarta. 2015;Indonesian Heart Associaton.

[2] Sari, A., N. Y. Lestari, D. A. Perwitasari. Validasi St European Quality of Life – 5 Dimensions (EQ-5D) for Indonesia Hypertension Patient in Puskesmas Kotagede II Yogyakarta. Pharmaçiana.2015; 5(2): 131-138. Yogyakarta: Pharmacy Faculty of Ahmad Dahlan University.

[3] Ministry of Health Republic of Indonesia(MH RI), RisetKesehatanDasar.2013; Jakarta: Research and Development Agency for Health, Ministry of Health Republic of Indonesia.

[4] Sahat, H. J. Rumput Laut Indonesia. Warta Ekspor September 2013; 3. Jakarta. 2013; Ministry of Trade Republic of Indonesia.

[5] Cornish, M. L., D. J. Garbary. Antioxidants from macroalgae: potential applications in human health and nutrition. J Algae .2010;25(4) : 155 - 171. Canada: James S. Cragie Research Centre and Departement of Biology St. Francis Xavier University.

[6] Cha, S.H., K. W. Lee, and Y. J. Jeon. Screening of Extracts from Red Algae in Jeju for Potentials Marine Angiotensin - I Converting Enzyme (ACE) Inhibitory Activity. Algae. 2006; 21(3): 343 – 348. South Korea: Faculty of Applied Marine Science, Cheju National University, Jeju 690-756.

[7] Kent Scientific Corporation. CODATM Multi – Channel, Computerized, NonInvasive Blood Pressure System for Mice and Rats. 2011; Torrington, Connecticut.

[8] Ratnasooriya, W. D., Premakumara G. A. S., and Tillekeratne, L. M. V. Hypotensive Acfivity of Crude Extracf of Marine Red Algae, Gracilaria sp. in Rats. Vidyodaya J.1991; 3(2): 35 – 39. Sri Lanka: Department of Zoology and Department of Chemistry, University of Colombo, Colombo 3.

[9] Runadi, Dudi. Telaah Fitokimia dan Uji Antihipertensi Ekstrak Air Daun Salam [Syzgiumpolyanthum (Wight) Walp, Myrtaceae]. [Tesis]; 2010. Bandung: Program Studi Farmasi Institut Teknologi Bandung.