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Anti-Inflammatory Activity of Marine Sponge *Aaptos sp.* to the Plasma Interleukin-1β Level in Wistar Male Rats

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Abstract

Inflammation is the response of the body to injury and infection characterized by swelling, heat, pain, and redness. This study aimed to investigate the anti-inflammatory effect of *Aaptos sp.* ethanolic extract to plasma interleukin (IL)-1β level of Wistar male rats. *Aaptos sp.* was macerated with 96% ethanol for 3 x 24 hours. Inflammation was induced with administration of 1% carrageenan intraplantarly. Animals were divided into 5 treatment groups, *i.e.,* positive control (diclofenac sodium 3598 ppm); *Aaptos sp* extract 50 ppm; *Aaptos sp* extract 100 ppm *Aaptos sp* extract 200 ppm; and negative control (0.5% Na CMC). After 1 hour, blood was collected and assayed using enzyme-linked immunosorbent assay (ELISA) kit. The results showed that plasma IL-1β levels of animals were decreased by *Aaptos sp* ethanolic extract. The administration of 50 ppm of extract showed no significant difference ($p>0.05$) in IL-1β level in first and second hour measurement, but indicated a statistically significant decrease after three hour ($p<0.05$). The administration of 100 ppm of extract showed no significant difference ($p>0.05$) in every hour. Significant reduction was observed in the administration of 200 ppm of extract, but the elevation of IL-1β levels was also observed at third hour measurement. In conclusion, ethanolic extract of *Aaptos sp.* had anti-inflammatory activity and its effective dose was 50 ppm.

Keywords: anti-inflammatory; ethanolic extract; *Aaptos sp*.

Introduction

Inflammation is the reaction of living tissue towards infection and injury, characterized by tumor, calor, dolor, and rubor. It comprises local and systemic response. Nevertheless, the consumption of anti-inflammatory drugs in the long-term might trigger potential side effect, such as increased risk of peptic ulcer, constipation, kidney problem, etc.[1,2]

Terrestrial and marine biodiversity of Indo nesia are abundant. These natural resources can be utilized in many aspects, including as traditional medicine. Around 31.4% of Indonesian people utilize traditional medicine to self medicate themselves.[3] Marine sponge such as *Aaptos sp*. is empirically used to treat several diseases. Based on the previous study, *Aaptos sp*. has been reported to have antibacterial, anti-cancer, and anti-depressant activities.[3] Aaptamine, an alkaloid from *Aaptos sp*., is predicted to be responsible for anti-inflammatory activity because of its ability to

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reduce cytokines such as IL-1β as a regulator for inflammatory response.[4,5]

There is limited information regarding anti-inflammatory activity of *Aaptos sp*. ethanolic extract with regards to its activity towards IL-1β level in plasma. Thus, this study aimed to investigate the anti-inflammatory effect of *Aaptos sp*. ethanolic extract to plasma IL-1β level of Wistar male rats.

Methods

The conduct of this study was approved by Ethics Comitee of Halu Oleo Univeristy (No.2685/UN29.20/PPM/2018).

Sample collection and extraction

Aaptos sp. was collected from Bintang Samudra Marine Edu-Park, Soropia Sub District, Konawe, Southeast Sulawesi, Indonesia. *Aaptos sp*. was obtained by scuba diving and collected by hand at reef slope (70º), 10 m above sea level. *Aaptos sp*. was then sorted, washed and cleaned from impurities. Thereafter, the sponge sample was put in storage for further process. *Aaptos sp*. was put in a clear jar and macerated with 96% ethanol (1:2) for 3 days. The extract was stirred everyday. The crude extract was obtained by filtrating the solvent using filter paper (Whatmman®). The crude extract was then concentrated using vacuum rotary evaporator at 50 ^{0}C and waterbath at 37 ^{0}C.

Anti-inflammatory assay

Experimental animals used in this study were Wistar male rats (200-250 g). Animals were obtained from animal farm in Surabaya, Indonesia. Wistar male rats were acclimatized for 1 month. Wistar male rats were put in standard environmental (22±2 ^{0}C) with free access to pellet chow and water *ad libitum*.

Wistar male rats (n=20) were divided into 5 groups (n=4). At the day of the experiment, 1% carrageenan was injected intraplantarly to induce inflammation. Subsequently, each group was given treatment orally; positive control (diclofenac sodium 3598 ppm); *Aaptos sp* extract 50 ppm; *Aaptos sp* extract 100 ppm; *Aaptos sp* extract 200 ppm; and negative control (0.5% Na CMC). Animals were left for 1 hour and blood was collected via tail vein at the first and second hours and cardiac puncture at the third hour post injection. Blood was collected and centrifugated (Thermo Scientific™) at 1000 rpm for 15 minutes. It was examined using ELISA Kit (Elabscience®) to measure the plasma IL-1β levels of animals. Data obtained from ELISA assay was then analyzed using SPSS with Saphiro-Wilk, continued by One Way ANOVA followed by post-hoc LSD test.

Results and Discussion

The results showed plasma IL-1β levels of animals were decreased by administration of *Aaptos sp* ethanolic extract. Extract 50 ppm showed greater effect in decreasing plasma IL-1β level of Wistar male rats compared to the dose of 100 ppm and 200 ppm (Figure 1). The administration of 50 ppm of extract showed no significant difference ($p>0.05$) in IL-1β level in first and second hour measurement compared to negative control group, but indicated a statistically significant decrease after three hour ($p<0.05$). The administration of 100 ppm of extract showed no significant difference with negative control group ($p>0.05$) in every hour. Significant reduction was observed in the administration of 200 ppm of extract, but the elevation of IL-1β levels was also observed at third hour measurement. (Figure 1). Nevertheless, compared to positive control group, the anti-inflammatory activity of *Aaptos sp* was slightly lower. Effect of *Aaptos sp.* extract 50 and 100 ppm had a significant difference ($p<0.05$) compared to psotive control group on 1st hour measurement (Table 1).

Table 1. Anti-Inflammatory Activity of *Aaptos sp*

Group	P values		
	1st Hour*	2nd Hour**	3rd Hour*
C+ vs C-	0.021	0.000	0.564
C+ vs A50	0.021	0.292	0.021
C+ vs A100	0.021	0.000	0.149
C+ vs A200	0.386	0.473	0.564
C- vs A50	0.149	0.000	0.021
C- vs A100	0.021	0.000	0.564
C- vs A200	1.000	0.000	0.386
A50 vs A100	0.021	0.000	0.021
A50 vs A200	1.000	0.088	0.021
A100 vs A200	0.021	0.000	0.248

*=Mann-Whitney Test; **= LSD test; $p<0.05$ = significant difference; $p>0.05$ = no significant difference

Il-1β is one of the pro-inflammatory cytokines that is primarily associated with innate immunity. It regulates the innate inflammation response. It also involved in cancer progression and affecting the growth and invasion of almost all types of tumor cells.[6]

One of the substances in *Aaptos sp.* that is responsible for its anti-infammatory properties is Aaptmine. The possible mechanism of Aaptmine as anti-inflammatory agent toward IL-1β are: (1) aaptamine incorporates into IL-1β DNA chain with low affinity thus interrupts the protein arrangement; (2) at low-dose, it inhibits proteasome in degradation of IL-1β forming protein and at high-dose it induces pro-apoptosis; or (3) aaptamine inhibits IL-1β signal which initiates metabolism of arachidonic acid (AA) in lipooxigenase (LOX) pathway.[7-10]

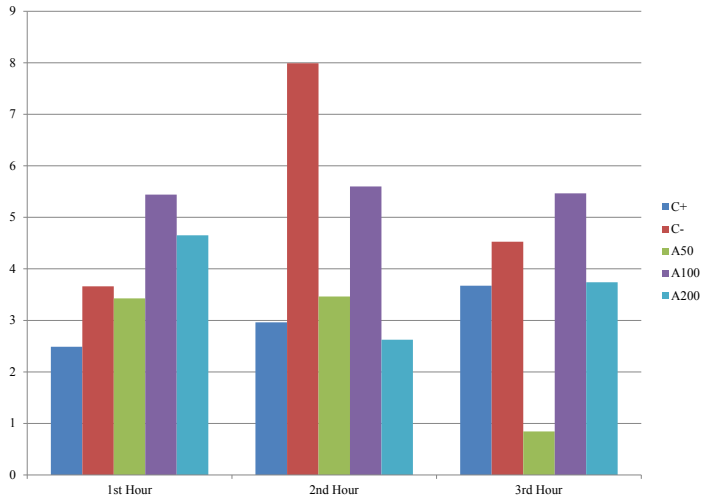

Figure 1. Mean Decrease of IL-1β in EachTreatment Group over Three Hours

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We found that the anti-inflammatory activity of *Aaptos sp.* depends on onset and concentration. At the dose of 50 ppm, the effect was observed during second and third hour measurement post injection. It even decreased IL-1β level significantly ($p<0.05$) after three hours. The delayed effect was occurred since ethanolic extract might takes longer time to distribute into the inflammation site.[8,9] The dose of 100 ppm showed no significant difference ($p>0.05$) between the first, second, and third hour measurement. It was possibly due to equilibrium process between inhibition ability and toxicity.[10,11] The dose of 200 ppm showed that optimal effect occurred at the second hour of measurement. However, it increased the IL-1β on third hour measurement. It was possibly due to the toxicity effect of a higher dose *Aaptos sp*. ethanolic extract. [12,13]

Conclusion

Ethanolic extract of *Aaptos sp.* had anti-inflammatory activity and its effective dose was 50 ppm. Further study is needed for investigating the effect of ethanolic extract of *Aaptos sp.* by using different cytokines parameter.

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Conflict of Interests

None declared

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