Rosella (*Hibiscus sabdariffa*, l) extract increase the macrophage phagocytic activity in dmba (7,12-dimetilbenz(α)antrasen) induced sprague dawley rat

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Abstract The immune system can be modulated with immunogen, including immunogen from plants. *Over* exposure of free radicals were reported to suppress the immune functions. Previous study reported the potency of *H.sabdariffa* (rosella) extract to increase the phagocytic activity in vitro. The objective of this study was to explore the effect of rosella extract in increasing macrophage phagocytic in DMBA induced rat in *vivo*.

Rosella calyx were extracted with maceration method uing 70% ethanol. The animals were induced with DMBA 15mg/kgBW single dose. After DMBA induction, at the same day, animals were treated with rosella extract with dose 10, 50 and 100mg/kgBW for 33 days. After treatment the animals were sacrified and macrophage were isolated from peritoneal fluid.

The results showed that phagocytic activity of macrophages showed significant differences between the groups with the DMBA and EE dose 10, 50 and 100 mg/kgBW. Nitric oxide (NO) secretion levels, secretion of Reactive Oxygen Intermediate (ROI) and expression of IL-12 levels were also found to incease. The total flavonoid content was obtained 2,465% and total polyphenol content was obtained 6,03 g GAE/100 g extract.

Introduction

Macrophages are the first line of defense and constitute important participants in the bi-directional interaction between innate and specific immunity. Macrophages are in a quiescent form and are activated when given a stimulus. This cells are activated by a widely variety of stimulus such as bacterial components, cytokines and chemicals [1].

Hibiscus sabdariffa, L extract was reported to stimulate immunity in vitro[2] and in vivo[3]. The DMBA used as PAH model which have been widely reported as immunotoxicity [4]

Materials and Methods

1) *H. sabdariffa* calyx were found from East Java, Indonesia.

2) *H. Sabdariffa* calyx were extacted with 70% ethanol by maceration and followed by evaporation to get the concentated extract.

3) The SD female rats were divided into 5 groups i.e; baseline, DMBA 15 mg/rat single dose and treatment group was given extract with dose 10, 50 and 100 mg/kgBW. In the treatment groups, the animals were induced by DMBA 15mg/rat single dose on the first day and the sameday were treated with extract. The duration treatment of extract was 33 days.

4) Phagocytic activity test was observed using latex beads, NO secretion was determined by Griess assay, ROI secretion was observed by NBT assay method, and the expression of IL-12 was conducted using immunocytochemistry using anti-IL-12 antibody.

5) The total flavonoid content were detected using spectrophotometric method with AlCl3[5] and total phenolic content were determined using Folin-Ciocalteu method [6]

Results and discussion

Polycyclic aromatic hydrocarbons (PAHs) produced by incomplete combustion of organic fuels are present universally as environmental pollutants. High levels of PAHs are present in cigarette smoke, vehicle exhaust, and charcoal-grilled foods. Many of the members of the PAH family are toxicants, and can induce carcinogenic and immunotoxic effects [7]. As a model PAH agent, DMBA is widely used as immunotoxicity in various species, tissues and cells types [7,8]. Treatment with rosella extract were expected to enhance the immune response. The effect of *H.sabdariffa* L extract on macrophage phagocytic activity were perfomed at Table 1.

Table 1. The effect of H.sabdariffa extract on macophage phagocytic activity in DMBA induced SD rats

Group	Phagocytic activity ± SD	Phagocytic capacity ± SD	Phagocytosis index ± SD
Normal	83.56±5.75*	287.6±86.15	2.160±0.116*
DMBA	59.49±22.69	178.2±42.06	2.153± 0.356
Treatment 1 (10 mg/kg BW)	66.38±15.49*	318±129.7*	2.427± 0.214
Treatment 2 (50 mg/kg BB)	79.578±3.73*	343.2±102.5*	1.970±0.194
Treatment 3 (100 mg/kg BB)	82.98± 4.505*	311.8±85.08*	1.950±0.371

*significantly different with DMBA ($p<0.05$)

The result showed that DMBA treatment were suppress the macrophage phagocytic activity and treatment with *H.sabdariffa* extract could enhance the phagocytic activity. The activation of macrophage were followed by the secretion of reactive oxygen intermediate (ROI) and nitrite oxide (NO) which is playing an important physiological role as a defence molecule in the immune system. The secretion of ROI, NO and IL-12 after H.sabdariffa teatment in DMBA induced rat were shown in Table 2.

Table 2. The secretion of ROI, NO and IL-12 after H.sabdariffa teatment in DMBA induced rat

Group	NO secretion ± SD	ROI secretion ± SD	IL-12 secretion ± SD
Normal	0.191±0.04	116±14.05*	41.8±26.16
DMBA	0.17± 0.036	56.4± 8.76	23.6±7.43
Treatment 1 (10 mg/kg BW)	0.335±0.105*	129.6± 12.38*	35.6± 20.18
Treatment 2 (50 mg/kg BB)	0.389± 0.146*	123.8± 15.38*	56±13.76*
Treatment 3 (100 mg/kg BB)	0.361± 0.074*	125.4 ± 14.8*	62.2±25.14*

*significantly different with DMBA ($p<0,05$)

Macrophages are widely distributed in different tissues and play an essential role in the development of the specific and nonspecific immune response. These cells are activated by a variety of stimuli such as bacterial components, cytokines and chemicals. Some herbal extract were also found to activate the macrophage. Once activated, macrophages produce and release numerous secretory products which have biological activity [6]. These include several cytokines such as IL-12, and reactive nitrogen intermediates. Activated macrophages generally produce large quantities of nitric oxide (NO) which is biologically active at nanomolar concentrations, playing an important physiological role as a defence molecule in the immune system [9].

Induction with DMBA suppressed the macrophage phagocytic activity leading in reducing the NO, ROI and IL-12 secretion. DMBA and the immunotoxic properties suppress the inflammatory reaction leading in decreasing the phagocytic activity of macrophages [4]. After treatment with H.sabdariffa extract the NO, ROI and IL-12 secretion increase significantly. .

The quantitatif analysis of total flavonoid showed 2.465% and total phenolic content was 6.03%. Flavonoid and poliphenolic were widely known as immunostimulating agent.

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