

Short Communication

THE EFFECT OF ROSELLA (*HIBISCUS SABDARIFFA* L) TREATMENT ON IL-10 AND IL-14 SECRETION ON DIMETHYLBENZ (A) ANTHRACENE (DMBA) INDUCED RAT

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ABSTRACT

Objective: The objective of this study was to determine the effect of ethanolic extract *rosella calyx* pretreatment on increasing secretion of IL-10 and IL-14 on SD rats treated with DMBA.

Methods: Rats were treated with a dose of ethanol extract of roselle with 10, 50 and 100 mg / kg BW once daily for 21 days, then treated with DMBA 15mg / rat single dose. One week after DMBA treatment lymphatic organs of mice were taken and the expression of IL-10 and IL-14 were observed by immunohistochemistry using the appropriate antibodies. Further more it was observed using a microscope at 400x magnification.

Result: The results showed that the obtained were analyzed using t-test to determine to what the effect of increased secretion of IL-10 and IL-14 between the control and treatment groups.

Conclusion: Rosella calyx extract is potential to develop as immunomodulatory agent

Keywords: Hibiscus sabdariffa, IL-10, IL14, Immunomodulator.

The calyx of *Hibiscus sabdariffa* (roselle) has been used traditionally for curing various diseases [1]. Rosella calyx extract have been reported as antioxidant [2], immunomodulator [3], antidepressant [4], antihyperlipidemia [5], antidiabetic [6], and hepatoprotective agent. The ethyl acetate fraction exhibited a significant dose-dependent immunostimulation ($p < 0.05$) higher than that observed for levamisole (positive control). The residual water-soluble fraction exhibited immunostimulatory activity at 100mg/kg body weight. The two fractions caused a significant reduction in production of tissue necrosis factor - alpha and an increase in interleukin 10 (IL-10) [7].

Interleukin-10 (IL-10) was recognized for its ability to inhibit activation and effector function of T cells, monocytes, and macrophages, is a multifunctional cytokine with diverse effects on most hemopoietic cell types. The principal routine function of IL-10 appears to be to limit and ultimately terminate inflammatory responses. In addition to these activities, IL-10 regulates growth and/or differentiation of B cells, NK cells, cytotoxic and helper T cells, mast cells, granulocytes, dendritic cells, keratinocytes, and endothelial cells. IL-10 plays a key role in differentiation and function of a newly appreciated type of T cell, the T regulatory cell, which may fig. prominently in control of immune responses and tolerance in vivo. Uniquely among hemopoietic cytokines, IL-10 has closely related homologs in several virus genomes, which testify to its crucial role in regulating immune and inflammatory responses. The objective of this research was to explore the potential therapy of rosella extract on stimulating the immune system in immunodeficiency especially caused by free radical.

Rosella calyx was found from East Java. The specimen was identified in Laboratory of Biology, Ahmad Dahlan University. Rosella was dried and grounded to powder. Extraction was carried out by 70% ethanol, followed by evaporation to get the concentrated extract.

Determination of total phenolic content was carried out by Folin Ciocalteu method with gallic acid as a standard. Absorbance was then measured by UV-Vis spectrophotometer at a 730 nm.

Sprague Dawley rats were divided into 5 groups. Group I is a base line, group II is a negative control group, treated with DMBA with dose of 15 mg/rat. Group III, IV, and V was groups treated with ethanol extract with dose 10, 50, and 100 mg/kgBW orally for 21 days, and followed by DMBA administration in corn oil with a dose of 15 mg/rat.

After treatment, the mice were sacrificed and the spleen was taken. The tissue was cleared using clearing agent (xylol) and then embedded in paraffine wax followed by sectioning. The secretion of IL-10 and IL-14 was observed by immunohistochemistry using anti IL-10 and anti IL-14. Expression of IL-10 and IL-14 was observed under light microscope.

Rosella extract contain anthocyanin one of the flavonoids group member. Flavonoids have been reported to have antioxidant effect [8, 9, 10]. The antioxidant properties of flavonoids were consider to deactivate the reactive metabolite of DMBA.

The previous research reported the high level of flavonoids and phenolics compound on *H. sabdariffa* L extract. It has been recognized that flavonoids show antioxidant activity. The mechanisms of action of flavonoids are through scavenging or chelating process [11]. Phenolic compounds are a class of antioxidant agents which act as free radical scavenger.

The total phenolic content was expressed as gallic acid equivalent. It was found that total phenolic content of rosella extract was 6.03 ± 0.21 GAE/100g. The flavonoid contents of the extracts in terms of quercetin equivalent were 2.47 ± 0.11 %. Flavonoid is also reported to have a hepatoprotective activity [12].

In this study, the animals were treated with ethanol extract for 21 days to observe the protective effect of extract treatment on DMBA administration. DMBA compounds are carcinogenic compounds that can cause immunosuppression of the immune system both humoral and cellular [13]. DMBA metabolism produce the ultimate carcinogens DMBA-3, 4-diol-1, 2-epoxide has the ability to suppress the immune system. DMBA metabolite was very active will make covalent bonds with DNA to form DNA adducts.

Interleukin is part of the immune system called cytokines that activate the immune system. IL-10 is a cytokine with pleiotropic effects in immunoregulation and inflammation. Expression of IL-10 can down regulate Th1 cytokines, MHC class II antigens and co-stimulation molecules on macrophages. IL-10 also enhances B cell survival, proliferation, and antibody production. IL-10 can block the activity of NF-kB, and is involved in the regulation of the JAK-STAT signaling pathway.

The study on knockout mice suggest this cytokine function as important immuno regulator in the intestinal tract. Patients with Crohn's disease

react positively to treatment with recombinant bacteria that produce interleukin-10, shows the importance of IL-10 to prevent a hyperactive immune response in the human body. A study on mice has shown that IL-10 is also produced by mast cells, counteracting the effects of inflammation at the site of an allergic reaction.

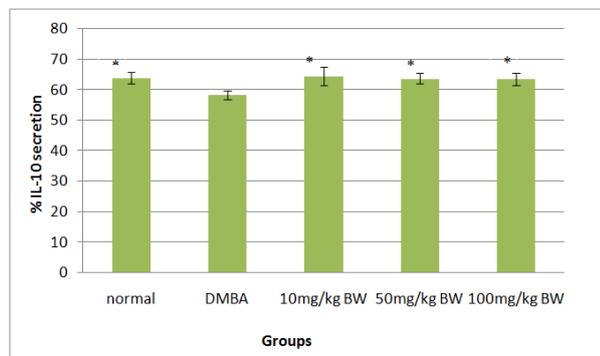


Fig. 1: Expression of IL-10 in spleen DMBA-induced SD rats and treated with ethanol extract of Roselle, * indicates significant differences with the negative control group (DMBA)

DMBA treatment lowered the expression of IL-10, compared with other groups. Roselle extract treatment in all groups compared with DMBA group increased the expression of IL-10 significantly. But an increase in the dose does not increase the secretion of IL-10 significantly.

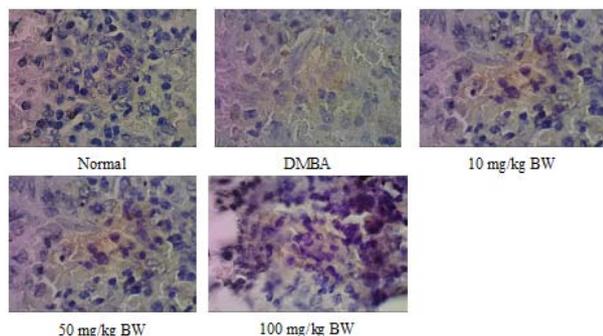


Fig. 2: The immunohistochemistry of IL-10-induced on Sprague Dawley rats spleen treated with DMBA and ethanol extract of roselle

The study found that IL-10 increase on rosella extract treated rats, but the increasing of doses cannot increase the IL-10 significantly. Fakeye also found the increasing of IL-10 after subchronic treatment with rosella extract [7]. The interplay of the reduction in concentration of TNF- α and increase in IL-10 has been linked with stress, inflammation and tumors. IL-10 has been known to down regulate factors present in inflammation.

Expression of IL-10 occurred in many cells of adaptive immune system such as CD8+T cells, B cells, and cells of the innate immune system such as dendritic cells, macrophage, mast cells and natural killer (NK) cells [14]. Upregulation of IL-10 increased activation and differentiation of CD8+cytotoxic cells [15]. Expression of IL-10 in blood is equal to its expression on spleen, the higher IL-10 expression on spleen, the higher IL-10 expression in blood circulation.

Interleukin-14 is a cytokine that controls the growth and proliferation of normal and cancerous B cells. This molecule is also called taxilin. IL-14 induces the proliferation of B-cells, and inhibits the secretion of antibodies. Interleukin-14 is produced mainly by T cells and certain malignant B cells. Expression of IL-14 was detected

using immunohistochemical methods. Expression of IL-14-induced spleen SD rats treated with DMBA and ethanol extract of roselle can be seen in fig. 3 and fig. 4.

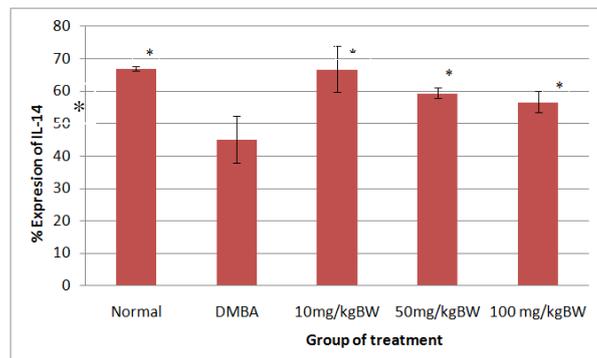


Fig. 3: Expression of IL-14 on spleen of DMBA induced SD rats treated with ethanol extract of Roselle, * indicates significant differences with the negative control group (DMBA)

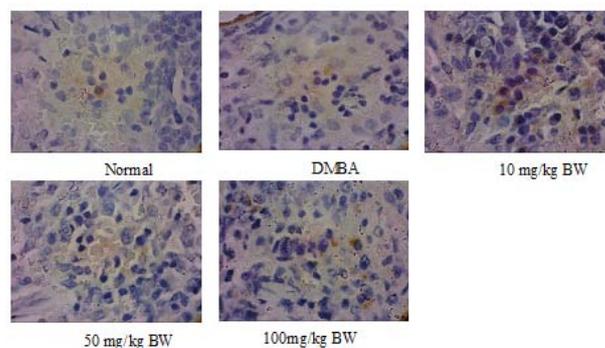


Fig. 4: Immunohistochemistry of IL-14 of spleen of DMBA induced rats treated with roselle extract

The results showed that the rosella extract treatment increases the secretion of IL-14 compared to the negative control group. However, increasing the dose does not cause any increase or decrease in a significant expression. It shows a small dose treatment (10 mg / kg BW) is most effective in increasing the secretion of IL-14. The immunohistochemistry observation can be seen in fig. 4

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CONFLICT OF INTEREST

The author declares there is no conflict of interests

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