



T cells regulation modulated by *Sambucus javanica* extracts in DMBA-exposed mice

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ABSTRACT

Introduction: *Sambucus* is a genus of the Adoxaceae family and has been widely used as a food and medicinal source. In this study, we aimed to evaluate the effects of *S. javanica* extracts toward T cells regulation in the 7,12-dimethylbenzene (a) anthracene (DMBA)-exposed mice.

Methods: Thirty mice were used in this experiment which divided into six treatment groups and five times repetition. Three-month-old BALB/c mice were administrated with 2.8 mg.kg⁻¹ BW of DMBA as carcinogen agent for ten times within four weeks, then continuously treated with 400 and 800 mg.kg⁻¹ BW of *S. javanica* berries leaves extracts daily for two weeks. Treatment groups in this study were vehicle as negative control, DMBA 2.8 mg.kg⁻¹ BW as positive control; BD1 group, DMBA 2.8 mg.kg⁻¹ + berries extracts 400 mg.kg⁻¹ BW; BD2 group, DMBA 2.8 mg.kg⁻¹ + berries extracts 800 mg.kg⁻¹ BW; LD1 group, DMBA 2.8 mg.kg⁻¹ + leaves extract 400 mg.kg⁻¹ BW; and LD2 group, DMBA 2.8 mg.kg⁻¹ + leaves extract 800 mg.kg⁻¹ BW. The relative number of CD4⁺CD25⁺CD62L⁺ cells and CD4⁺CD62L⁺ cells were measured and analyzed by using flow cytometer.

Results: DMBA induction decreased the relative number of CD4⁺CD25⁺CD62L⁺ cells while the leaves extract of *S. javanica* significantly increased their expression. Furthermore, DMBA promoted the relative number of CD4⁺CD62L⁺ cells expression while leaves and berries extracts of *S. javanica* sharply suppressed its expression.

Conclusion: The results suggest that, *S. javanica* extracts may exert their medicinal properties by modulating T cells regulation in DMBA-induced mice.

Implication for health policy/practice/research/medical education:

Berries and leaves extracts of *Sambucus javanica* modulated the T cells regulation by increasing the relative number of CD4⁺CD25⁺CD62L⁺ regulatory T cells and decreasing the relative CD4⁺CD62L⁺ cells after exposed by DMBA. These results suggest that *Sambucus javanica* has therapeutical potential against the unfavorable effects of DMBA by modulating the T cells regulation.

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Introduction

DMBA (7,12-dimethylbenzene (a) anthracene) is a part of polycyclic aromatic hydrocarbons family known as a carcinogenic pollutant abundantly distributed in the environment (1,2). As a carcinogenic agent, DMBA is profoundly utilized as a tumor initiator in experimental animals (3-5). Chemicals exposed like DMBA positively affect the biological system into the adverse condition. A study conducted by Paliwal et al stated that numerous adverse effects of DMBA's toxicity might affect on a broad range of biological systems such as excretion, immune, and transportation systems. Therefore, the strategy to

reduce the unwanted impacts of DMBA's toxicity is highly needed (6).

Recently, traditional medicines have been reached their popularity in primary health care. Affordable, less harmful side effects and cost-effectiveness might be the more significant reasons for utilizing traditional medication (7). For a long time, several ethnic groups have already used *Sambucus* as a food and medicinal source (8). *Sambucus* is a group of herbs widely known for its therapeutic potencies as an anti-inflammatory, anti-oxidant, anti-diabetes remedy, and hematopoiesis promoting agent (9-12). In the present study, we systematically evaluated the

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effects of leaves and berries extracts of *S. javanica* toward T cells regulation in DMBA-exposed mice.

Materials and Methods

Materials Preparation

Plant materials were obtained from Materia Medica Batu, The Ministry of Health Indonesia. Ethanolic extraction was performed for both leaves and berries of *S. javanica*. In this study, the treatment doses of each extract were 400 and 800 mg.kg⁻¹ BW. The *S. javanica* extracts were stored at -20°C refrigerator for further purposes.

Experimental Treatments

Thirty free pathogen BALB/c mice were purchased from Animal Laboratory, Gadjah Mada University. Experimental mice then maintained in the animal facility, Department of Biology, Brawijaya University, Indonesia. In this study, the mice were administrated by 2.8 mg.kg⁻¹ BW DMBA (Tokyo Chemical Industry Co. Ltd.) for ten times in a month, then followed by 400 and 800 mg.kg⁻¹ BW of *S. javanica* extracts treatment every day for two weeks. Treatment groups in this study were vehicle as negative control, DMBA 2.8 mg.kg⁻¹ BW as positive control; BD1 group, DMBA 2.8 mg.kg⁻¹ + berries extract 400 mg.kg⁻¹ BW; BD2 group, DMBA 2.8 mg.kg⁻¹ + berries extract 800 mg.kg⁻¹ BW; LD1 group, DMBA 2.8 mg.kg⁻¹ + leaves extract 400 mg.kg⁻¹ BW; and LD2 group, DMBA 2.8 mg.kg⁻¹ + leaves extract 800 mg.kg⁻¹ BW. All contents of this study were confirmed by the Research Ethics Committee, Brawijaya University.

Flow cytometry analysis

The flow cytometry procedure in this study was similar to our previous research (13,14). Splenocytes from each treatment group were stained by several antibodies including mouse anti-CD4, mouse anti-CD25, mouse anti-CD62L antibody (Biolegend). FACS Calibur™ flow cytometer (BD-Biosciences, San Jose, CA) and BD CellQuest Pro™ software were used in this study.

Statistical analysis

Data analysis was done by student t-test with a significance level (α) of 0.05. The data used in this study was based on a calculation of the relative number of CD4⁺CD25⁺CD62L⁺ regulatory T cells and CD4⁺CD62L⁺ naive T cells.

Results

Regulatory T cells population after *S. javanica* treatment

As stated in the above explanation, BALB/c mice were treated by *S. javanica* extracts after exposed by DMBA. In the present study, we evaluated the regulatory T cells and naive T cells population by flow cytometer (Figure 1). According to the results, DMBA induction significantly suppressed the relative number of CD4⁺CD25⁺CD62L⁺, regulatory T cells. Interestingly, the relative number of

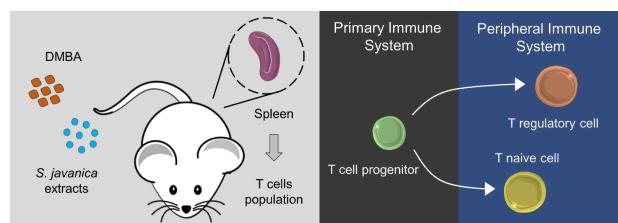


Figure 1. Schematic diagram about experimental treatments in this study. DMBA was occupied as carcinogenic agent, however *S. javanica* extracts could ameliorate the adverse effects of DMBA by modulating the T cells population.

regulatory T cells was promoted with the leaves extract with a dose of 800 mg.kg⁻¹ BW (Figure 2).

Naive T cells population after *S. javanica* treatment

To greater extends, we have evaluated and analyzed the relative number of CD4⁺CD62L⁺ naive T cells. In this study, we found that the relative number of naive T cells was contradicted with regulatory T cells. The results demonstrated the increasing number of CD4⁺CD62L⁺ naive T cells after DMBA induction, however it was sharply decreased after treated by leaves and berries extracts of *S. javanica* (Figure 2).

Discussion

T cells have critical roles in the immune system as fine defender against microorganism infections and other foreign invaders. T cells were originated from bone marrow as the progenitor. During its development, T cells underwent the selection and maturation in the thymus before it becomes functional T cells (15,16). After leaving the thymus, another subset of T cell population migrates into the secondary lymphoid organs via blood stream and lymph. Specifically, the migrated cells which carry particular markers such as CD62L and CCR7 are called naive T cells (17). Another subset of T cells with a specific function in self-tolerance respond and immunosuppressive properties is known as regulatory T cells (18,19). More details, numerous studies have suggested that regulatory T cells are divided into two types, natural and inducible regulatory T cells which are originated from naive T cells in the periphery immune system (20). Unlike naive T cell, regulatory T cells are identified by the appearance of FOXP3 in CD4⁺CD25⁺ T cell subset (19,21).

Regarding the above results, we found the crucial role of *S. javanica* in T cells regulation of DMBA-exposed mice. It has been clearly demonstrated that the leaves extract of *S. javanica* contribute to modulating regulatory T cells population. On the other hand, the relative number of naive T cells is down-regulated toward leaves and berries extracts of *S. javanica* (Figure 2). These findings suggested that T cells regulation were altered by bioactive compounds of *S. javanica* after DMBA administration.

DMBA can induce oxidative damage during metabolic

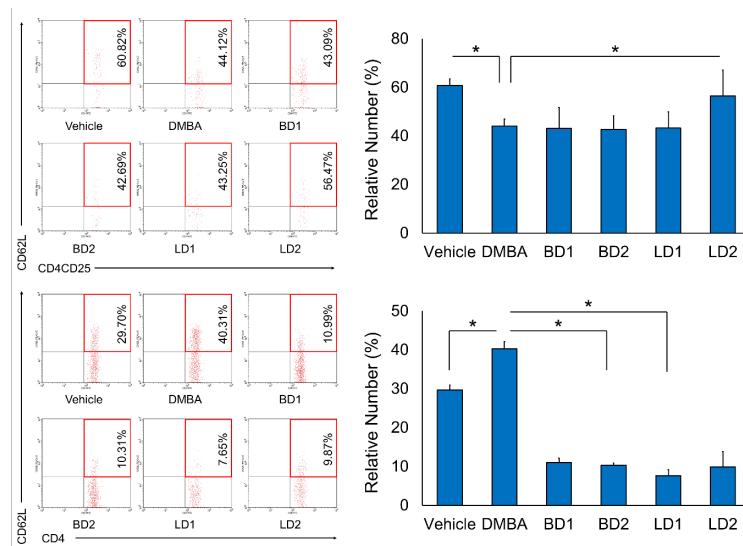


Figure 2. Effect of the leaves extract of *S. javanica* on the number of CD4⁺CD25⁺CD62L⁺ regulatory T cells after down regulation by DMBA treatment. The number of CD4⁺CD62L⁺ naïve T cells decreased by *S. javanica* extracts. The bars are calculation of the relative number of CD4⁺CD25⁺CD62L⁺ and CD4⁺CD62L⁺ cells expression, respectively. The results in this study were represented as the mean \pm SD. *P< 0.05. Treatment groups in this study were vehicle, DMBA 2.8 mg.kg⁻¹ BW;BD1, DMBA 2.8 mg.kg⁻¹ + berries extract 400 mg.kg⁻¹ BW;BD2, DMBA 2.8 mg.kg⁻¹ + berries extract 800 mg.kg⁻¹ BW;LD1, DMBA 2.8 mg.kg⁻¹ + leaves extract 400 mg.kg⁻¹ BW;and LD2, DMBA 2.8 mg.kg⁻¹ + leaves extract 800 mg.kg⁻¹ BW.

activation by promoting the production of reactive oxygen species such as superoxide anion, hydrogen peroxidase, and hydroxyl radical (22). A study conducted by Manoharan and Selvan revealed that geraniol could exert the protective effect against tumor formation in DMBA exposed mice by modulating the activities of free radical scavenging (23). Similarly, the extract of *Syzygium cumini* seed and *Aloe vera* also showed the ameliorative effect toward adverse alteration of DMBA in Swiss albino mice (24,25). The successive methanol extract of *Ficus benghalensis* has been known to have immunostimulant activity in lymphocyte proliferation such as T cells and B cells (26). Another *in vivo* experiment in tumor-bearing mice proved the methanol extract of *Nitraria retusa* leaves significantly promoted splenocyte proliferation and boosted the activity of natural killer cells and cytotoxic T lymphocytes (27). Similarly, *in vitro* and *in vivo* experiments revealed that *Mesembryanthemum crystallinum* extract was able to enhance human B and T cell lines proliferation (28).

Conclusion

In this study, DMBA induction suppressed the relative number of CD4⁺CD25⁺CD62L⁺ regulatory T cells while the leaves extract of *S. javanica* significantly promoted their expression. Oppositely, in the case of naive T cells, DMBA enhanced the relative number of CD4⁺CD62L⁺ cells expression while leaves and berries extracts of *S. javanica* precipitously decreased its expression.

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Authors' contributions

WEP performed the experiment, analysis, and wrote the manuscript. ARM and ATKR performed the experiment. MR designed the experiment and revised the manuscript. All authors approved the final version of this manuscript for publication.

Conflict of interests

No conflict of interest.

Ethical considerations

All aspects of ethical issues have been observed by the authors. These experimental studies were approved by the ethical committee of Brawijaya University, Malang, Indonesia with ethical clearance no. 312-KEP-UB and were conducted adhering to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1978).

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