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Effect of *Physalis minima* L. on Interferon-γ and Interleukin-1β Levels in 7,12-Dimethylbenz[a]anthracene Administered Mice

Physalis minima'nın 7,12 Dimetilbenzantrasen Uygulanmış Farelerde Interferon-γ ve İnterlökin 1β Seviyelerine Olan Etkisi

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Abstract

Introduction: 7.12-Dimethylbenz[a]anthracene (DMBA) is a carcinogenic substance that can have immunosuppressive effects. *Physalis minima* L. is one of the herbal medicinal substances that was assumed to modulate immunosuppressive effects in DMBA-induced mice toward interferon γ (IFN-γ) and interleukin-1β (IL-1β).

Materials and Methods: Female BALB/c mice were given DMBA. DMBA-induced mice were administered with *Physalis* extract, once a day for two weeks. The level of IFN-γ and IL-1β were measured using a flow cytometer.

Results: *Physalis minima* L. extract was able to increase ($p<0.05$) the level of IFN-γ in DMBA-given groups. Meanwhile, *Physalis minima* L. extract could increase the level of IL-1β in group C+D1 ($p<0,05$).

Conclusions: *Physalis minima* L. extract could increase the level of IFN-γ and IL-1β in DMBA Induced Mice

Keywords: 7.12-Dimethylbenz[a]anthracene, IFN-γ, IL-1β, *Physalis minima* L.

Öz

Giriş: Karsinojenik etkileri olan 7,12-Dimetilbenzantrasen (DMBA) aynı zamanda bağışıklık sistemini de baskılar. Physalis minima'nın bu baskılanmayı DMBA verilmiş farelerde interferon γ (IFN-γ) ve interlökin 1γ (IFN-1β) yolu ile düzenleyebileceği düşünülmektedir.

Gereçler ve Yöntemler: Dişi BALB/c farelere DMBA verildi. Bu DMBA uygulanmış deneklere ayrıca iki hafta boyunca her gün bir kez Physalis ekstresinden verildi. Bu deneklerin IFN-γ ve IFN-1β seviyeleri akan hücre ölçer kullanılarak ölçüldü.

Bulgular: Physalis minima L ekstresi, DMBA verilmiş farelerde IFN-γ ve IFN-1β seviyelerini artırdı ($p<0,05$).

Sonuçlar: Phsalis minima ekstresi, DMBA uygulanmış farelerde IFN-γ ve IFN-1β seviyelerini artırmaktadır.

Anahtar Sözcükler: 7-12 Dimetilbenzantrasen, IFN-γ, IL-1β, *Physalis minima* L.

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Introduction

7.12-Dimethylbenz[a]anthracene (DMBA) is a carcinogenic substance that was often used to induce cancer in mice or rats. DMBA consists of polycyclic aromatic hydrocarbons (PAHs) which has various structures and toxicity effects.[1] The specific characteristic of PAHs is consisting of more than one fused aromatic rings.[2] In the previous study, DMBA had been used to induce mammary cancer in Wistar rats.[3] It was also used to induce skin tumor formation in C57BL/6 mice.[4] In addition, DMBA was reported to be able to create an immunosuppressive microenvironment.[5] The immunosuppressive microenvironment is frequently addressed to immunotoxicity where the immune system is suppressed.[6]

Continuity of the immunosuppressive microenvironment may enhance the progression of cancer.[7] An immunosuppressive environment suppresses the level of Interferon-γ (IFN-γ) and Interleukin-1β (IL-1β).[8] IFN-γ and IL-1β can support anti-tumor immunity.[9] IFN-γ is a pro-inflammatory cytokine, predominantly produced by T-cell

lymphocytes.[10] IFN-γ has a fundamental role in cell-mediated immunity that might enhance anti-tumor effects by boosting cytotoxic T-cell lymphocytes.[11] On the other hand, IFN-γ can enhance the expression of Major Histocompatibility Complex (MHC), which indirectly induce cytotoxic T-cell lymphocytes to recognize tumor cells.[12] Similar to IFN-γ, IL-1β is a member of IL-1 family which is a group of pro-inflammatory cytokines.[13] Specifically, IL-1β promotes the activation of Th1 and Th17, which possess anti-tumorigenic effect.[14]

Physalis minima L. is a member of solanaceae family which is often used as food and a herbal medicine.[15] *Physalis minima* L. contains a lot of secondary metabolites such as physalins, withaphysalins, and phytosterols.[16] The study shows that the pharmacological effect of *Physalis minima* L. is a potential source of immunomodulatory and anti-cancer activity.[17] The immunomodulatory effects of *Physalis minima* L. is expected to modulate cytokines which has anti-tumorigenic effects.[17]

The aim of this study is to investigate the effect of *Physalis minima* L. on IFN-γ and IL-1β level on DMBA administered mice.

Methods

Animal Study and DMBA Administration

In this study, we used female BALB/c mice obtained from LPPT Gadjah Mada University, Yogyakarta. Mice were subcutaneously given with 15 mg/kg BW of DMBA at a dose of 15 mg/kg BW in the mammary gland. The DMBA administration was carried out for 6 weeks, once a week. The mice were divided into 5 groups, consist of N group: healthy mice; K: 15 mg/kg BW DMBA; C+D group: 15 mg/kg BW DMBA + 25 mg/kg BW *Physalis minima* L. extract; C+D2 group: 15 mg/kg BW DMBA + 50 mg/kg BW *Physalis minima* L. extract; C+D3 group: 15 mg/kg BW DMBA + 100 mg/kg BW *Physalis minima* L. extract.

Preparation of *Physalis minima* L. Extract

Physalis minima L. was obtained from UPT Materia Medica, Batu City, East Java. *Physalis minima* L. was extracted with methanol (w/v 100 mg/mL). The extraction process was done with maceration. *Physalis minima* L. extract was administered daily for 2 weeks.

Antibody Staining and Flow Cytometry

Spleens of the mice were removed, homogenized and centrifuged at 2.500 rpm at 10°C for 5 minutes. The obtained cell suspension was marked with anti-CD4-fluorescein isothiocyanate (FITC), then incubated at 4°C for 20 minutes. The suspension was added 50 µl cytofix (BioLegend, San Diego, CA) was added then it was washed with 500 µl washperm (Biolegend, San Diego, CA). The level of cytokines was stained with anti-IFN-γ, and anti-IL-1β (Biolegend, San Diego, CA) antibodies. To optimize the bonding, antibody-stained cells were incubated at 4°C for 20 minutes, then 400 µl phospahe buffer saline (PBS) was added. The antibody-stained cells were analyzed using a flow cytometer (BD FACS Calibur, USA).

Data Analysis

Data were analyzed with BD CellQuest ProTM software (USA). Statistical analysis was done by one-way ANOVA with significance threshold of 0,05 HSD Tukey's test was also used.

Results

IFN-γ Levels

IFN-γ levels were shown in Figure 1. The level of IFN-γ level in the Group C was decreased ($p<0.05$ compared to the Group N. It was decreased from 11.72% to 5.26%. Administration of *Physalis minima* L. extract increased ($p<0.05$) the level of IFN-γ in the C+D2 group and C+D3 group becoming 8.23% and 9.23%. Meanwhile, the C+D1 group had no statistically significantly different level of IFN-γ ($p<0.05$) compared to the C group.

IL-1β Levels

The IL-1β levels were shown in Figure 2. IL-1β was decreased in groups C compared to group N ($p<0.05$). *Physalis minima* L. extract was able to increase IL-1β in Group C+D1 ($p<0.05$). Yet, *Physalis minima* L. extract could not increase IL-1β level in the Group C+D2 and Group C+D3 ($p<0.05$).

Discussion

The decrease in IFN-γ and IL-1β in DMBA given mice is interpreted as immunosuppresive impact of DMBA. A number of studies showed that DMBA can suppress the

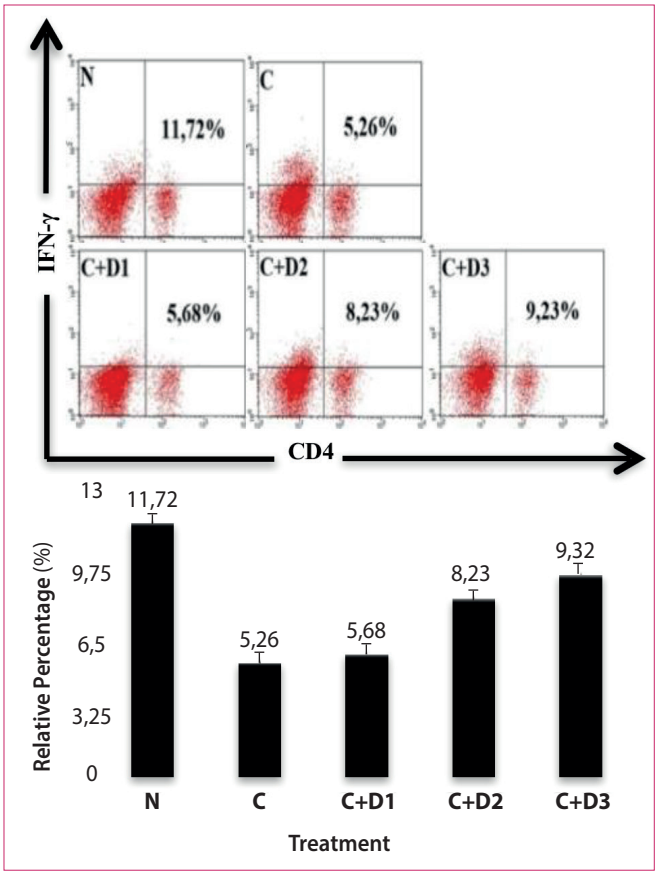

Figure 1. a, b. Administration of *Physalis minima* L. extract was able to increase the level of IFN-γ, in the C+D2 group and C+D3 group: The level of IFN-γ after was administered with *Physalis minima* L. extract for 2 weeks **(a)**. Data were mean ± SD (n=5) each group (p<0.05) **(b)** (N, healthy mice; C, DMBA-induced mice; C+D1, DMBA-induced mice + 25 mg/kg BW *Physalis minima* L. extract; C+D2, DMBA-induced mice + 50 mg/kg BW *Physalis minima* L. extract; C+D3, DMBA-induced mice + 100 mg/kg BW *Physalis minima* L. extract).

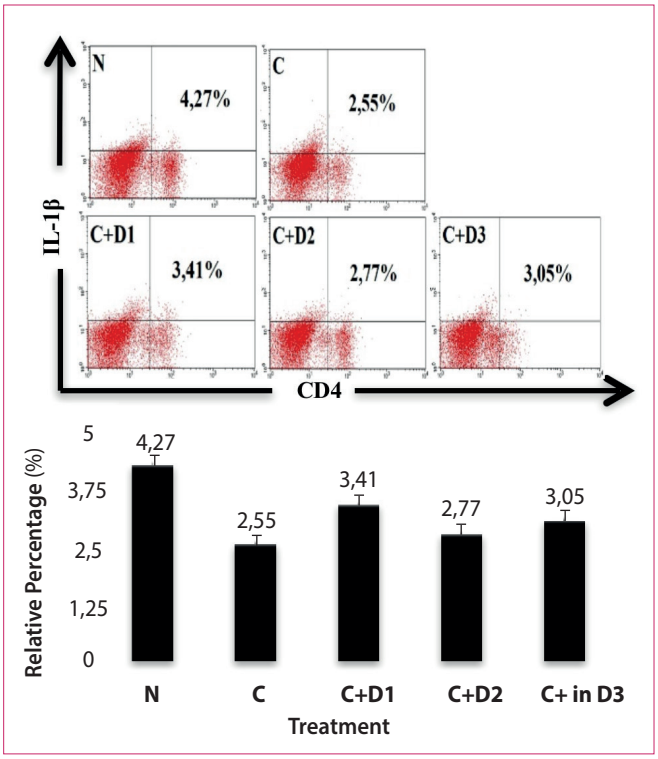

Figure 2. a, b. Administration of *Physalis minima* L. extract can increase the level of IFN-γ, in the C+D2 and C+D3 groups: The level of IFN-γ after was administered with *Physalis minima* L. extract for 2 weeks **(a)**. Data were mean ± SD (n=5) each group (p<0.05) **(b)** (N, healthy mice; C, DMBA-induced mice; C+D1, DMBA-induced mice + 25 mg/kg BW *Physalis minima* L. extract; C+D2, DMBA-induced mice + 50 mg/kg BW *Physalis minima* L. extract; C+D3, DMBA-induced mice + 100 mg/kg BW *Physalis minima* L. extract).

immune system.[18] It works by reducing the immune and stromal cells inside spleen and bone marrow via apoptosis.[19] A recent study showed that there was a close link between immunosuppression and cancer development.[20] Suppression of immune cells may lead to suppress cell-mediated immune responses to eliminate cancer cells.[21] Therefore, immunosuppressive effects of DMBA can increase the susceptibility of cancer risk.[22]

IFN-γ and IL-1β were reported to be associated with tumor development, although several studies showed different results.[23] IFN-γ has a role as central mediator of cell-mediated response.[11] Studies showed that IFN-γ can enhance anti-tumor by increasing motility and killing ability of cytotoxic T-cells.[11] IFN-γ is also involved in enhancing expression of major histocompatibility complex (MHC), both MHC class-I and MHC class-II.[24, 25] The up-regulation of MHC class-I is highly important for host response against tumor cells.[26] Its response assists in cancer cells recognition of cytotoxic T-cells.[27] Meanwhile, up-regulation of MHC class-II on antigen presenting cells (APCs) is more crucial for $CD4^+$ T-cell function by boosting its peptide-specific activation.[28] IL-1β specifically induces the activation of Th-1 cells.[29] The previous study showed that IL-1β can eliminate tumor cells through exogenous injection method.[30] It exerted its effect by increasing Th-1 mediated response against tumor cells.[22]

The up-regulation of IFN-γ and IL-1β levels after being treated proves that *Physalis minima* L. has immunomodulatory effects. Even though, *Physalis minima* L. only could show its effect at a certain dose.

Immunomodulatory effects of *Physalis minima* L. may be originated from its phytochemical contents.[31]

Conclusion

In summary, *Physalis minima* L. increased the levels of IFN-γ and IL-1β in DMBA-administered mice. at a certain concentration. Thus, the best concentration is yet to be determined.

Ethics Committee Approval: The study has been approved by Brawijaya's University Ethics Commission (No. 1013-KEP-UB. Date: 12.09.2018)

Contribution of authors: We state that the research was carried out by Hary Isnanto, Fatiha Kamilah, Kurnilia Dewi Astuti, Citra Sefrilla Ramadhani, and Muhaimin Rifa'I. Hary Isnanto executed the study conception and drafting of manuscript. Fatiha Kamilah, Kurnilia Dewi Astuti, Citra and Sefrilla Ramadhani performed statistical analysis. Muhaimin Rifa'i performed the critical revision of the manuscript.

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Conflict of interest: There was no conflict of interest linked with this research.

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