

Ethanollic Extract of Herb Pugun Tanah (*Picria fel-terrae* Lour.) Modulates TCD4+ and TCD8+ Cell Profile of Doxorubicin-Induced Immuno-Suppressed Rats

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Abstract

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AIM: To evaluate the immunomodulatory effects of ethanollic extract of herb pugun tanah on TCD4 and TCD8 cells in Doxorubicin-induced rats.

METHODS: Fifteen male Sprague Dawley rats were divided into five groups consisting of six rats each as follows: Group 1, DOX-treated rats (4.67 mg/kg BW body weight on day 1 and 4) and were administered normal saline 0.9% orally once daily for 7 consecutive days, Group 2, receiving Ethanollic Extract of Herb Pugun Tanah (*Picria fel-terrae* Lour.) of dose 150 mg/kg BW orally, Group 3, receiving dose Ethanollic Extract of Herb Pugun Tanah (*Picria fel-terrae* Lour.) 300 mg/kg BW orally. The rats of group 2-3 were intramuscularly administered with doxorubicin at a dose of 4.67 mg/kg BW at the days 1-4 to suppress immune functions.

RESULTS: Treatment of 300 mg/kg BW of Ethanollic Extract of Herb Pugun Tanah (*Picria fel-terrae* Lour.) succeeded in reducing side effect doxorubicin based on increasing the TCD4⁺ and TCD8⁺ blood level.

CONCLUSION: Ethanollic Extract of Herb Pugun Tanah (*Picria fel-terrae* Lour.) could increase the level of TCD4⁺ and TCD8⁺ in rats which induced by doxorubicin.

Introduction

The utilisation of traditional medicines in various the area is inherited from generation to generation based on experience/empirical, then develop through scientific proof through pre-clinical and clinical trials. Puguntano (*Picria fel-terrae* Lour.) is one of the medical plants of the family Scrophulariaceae that grows in the Asian region like China, India, Indonesia, Philippines, Malaysia, and Myanmar. In Indonesia, this plant Spread in Sumatra, Java, Kalimantan, and Maluku [1], [2]. The medicinal plant is potential to be developed into a safe and easy-to-use herbal medicine preparation. Reportedly showed that this plant contained glycoside, flavonoid, saponin, terpenoid, curangin, and bitter compound [2], [3], [4], [5], [6], [7], [8]. The pharmacological activity of

this plant also has been studied such as anthelmintic, antidiabetic, anti-breast cancer, diuretic effect, cardioprotective effect, and antimuscarinic receptor [2], [9], [14].

Chemotherapy is a major treatment modality for many types of cancer. Even though chemotherapeutic agents are chosen for their cytotoxicity toward cancerous cells, many widely used anticancer drugs have been found to exert immunomodulatory effects [15], [16]. There are several approaches used for treating cancer including chemotherapy. One of the most popular chemotherapeutics is doxorubicin [17], [18], [19]. Unfortunately, its damaging effects not only occur on cancer cells, but also to normal ones [18], [19]. Doxorubicin can affect the immune system by decreasing interleukin-2 (IL-2) and production of interferon- γ (INF- γ), natural killer cells, lymphocyte

cells, the CD4⁺ / CD8⁺ ratio, as well as damaging the thymus organ [20], [21], [22].

In this study, the effect of ethanol extract of pugun tano (*Picria fel-terrae* Lour.) modulates tcd4⁺ and tcd8⁺ cell profile of doxorubicin-induced immune-suppressed rats.

Material and Methods

Materials

The chemicals used in this study were an ethanollic extract of herb *Picria fel-terrae* Lour. And Doxorubicin (Kalbe Farma, Indonesia).

Animal

Wistar rats (weighing 150-200 g) were housed and maintained under the standard conditions a 12-h light/dark cycle, 25°C ± 2°C were fed with standard rat chow and water ad libitum. The experimental protocol was conducted by the Guideline for Care and Use of Animals Laboratory [23].

Experimental animals

Fifteen normal rats were divided randomly into three groups of five rats in each group and used in the experiments. Group I, DOX-treated rats (4.67 mg/kg body weight on day 1 and 4) and were administered normal saline 0.9% orally once daily for 7 consecutive days, Group II, served Ethanollic Extract of Herb Pugun Tanoh (*Picria fel-terrae* Lour.) of dose 150 mg/kg BW orally; and Group III received Ethanollic Extract of Herb Pugun Tanoh (*Picria fel-terrae* Lour.) of dose 300 mg/kg BW orally. The rats in Group II and III were administered Ethanollic Extract of Herb Pugun Tanoh (*Picria fel-terrae* Lour.) once daily for 7 consecutive days and DOX doses of 4,67 mg/kg BW on day 1 and day 4 [23].

Determination of TCD4⁺ and TCD8⁺ profiles by flow cytometry

Blood samples were collected from treated rats on day 8 under the anaesthetised condition and kept in a vacutainer containing ethylenediamine tetraacetic acid. Sample preparation was performed by mixing 5 µL of whole blood, and 10 µL was rat antibody and then was vortexed gently and allowed to stand in a dark room for 15 min. For dilution, the lysing reagent was added, then allowed to stand in a dark room for 15 min. After immunolabeling, cells were analysed on a FACS Calibur flow cytometer (Becton Dickenson, Mountain View, Ca, USA) [23].

Results

In this study, ethanollic extract of herb *Picria fel-terrae* Lour. was evaluated the effect on TCD4⁺ and TCD8⁺ profile using FACS Flow cytometer and the results presented in Figure 1. Doxorubicin dose 4.67 mg/kg BW at the day 1 and 4 succeeded to suppressed TCD4⁺ and TCD8⁺ by 33.41% ± 0.48 and 43.05% ± 0.2. Treatment of 7 consecutive days ethanollic extract of herb *Picria fel-terrae* Lour. Concomitantly with Dox of dose 150 mg/kg BW could the decrease of TCD4⁺ and TCD8⁺ by 57% ± 0.63 and 26.62% ± 0.47 and 300 mg/kg BW by 60.70% ± 0.59 and 22.42% ± 1.30 could the decrease of TCD4⁺ and TCD8⁺ due to Dox administration.

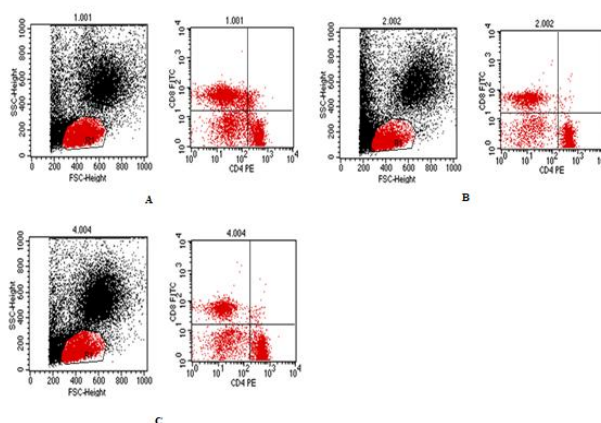


Fig. 1: Effect of Ethanollic Extract of Herb Pugun Tanoh (*Picria fel-terrae* Lour.) on TCD4⁺ and TCD8⁺ in rats induced by doxorubicin (DOX); A) DOX 4.6 mg/kg BW; B) Ethanollic Extract of Herb Pugun Tanoh (*Picria fel-terrae* Lour.) 150 mg/kg BW; C) Ethanollic Extract of Herb Pugun Tanoh (*Picria fel-terrae* Lour.) 300 mg/kg BW

Discussion

Doxorubicin may affect the function of the immune system of mice induced cancer by lowering the production of gamma interferon and interleukins significantly, so the cytotoxic cells are causing a decrease in Natural killer (NK), the proliferation of lymphocytes, and the ratio of TCD4⁺ / TCD8⁺. Doxorubicin suppressed immune system on doxorubicin treated-rats, shown by inhibition of lymphocyte proliferation, suppression of phagocytosis activity and capacity of macrophages, suppression of CD8⁺ cytotoxic T cells and downregulation of IL-10. Macrophages play a role in the nonspecific immune response by phagocytosis, while T cells and B cells are responsible for a specific immune response. Doxorubicin caused DNA damage to bone marrow cells [24].

Preliminary phytochemical screening of ethanollic extract of *Picria fel-terrae* leaves indicated

that the presences of flavonoids, saponins, triterpenes, and steroids [25]. Some flavonoids showed anticancer and immunomodulatory activity. Previous in vivo anticancer combinatorial study of doxorubicin showed that the combination of doxorubicin and proanthocyanidin strongly improved the anti-tumour effect of doxorubicin and immune responses, and eliminated myocardial oxidative stress on rats induced by doxorubicin [19], [20].

In conclusion, ethanolic Extract of Herb Pugun Tanoh (*Picria fel-terrae* Lour.) could increase the level of TCD4⁺ and TCD8⁺ in rats which suppressed by doxorubicin.

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