Open Access Maced J Med Sci **electronic publication ahead of print,**
published on November 14, 2019 as https://doi.org/10.3889/oamjms.2019.501

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences.
https://doi.org/10.3889/oamjms.2019.501
eISSN: 1857-9655
Herbal Medicine in Pharmaceutical and Clinical Sciences

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

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Citation: Lubis MR, Haryani R, Safriana S, Satria D. Ethanolic Extract of Herb Pugun Tanoh (Picria fel-terrae Lour.) Modulates TCD4+ and TCD8+ Cell Profile of Doxorubicin-Induced Immuno-Suppressed Rats. Open Access Maced J Med Sci. https://doi.org/10.3889/oamjms.2019.501

Keywords: *Picria fel-terrae* Lour.; Ethanolic Extract; Doxorubicin; Immunomodulator; $TCD4^+$; $TCD8^+$

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Received: 25-Sep-2019; **Revised:** 17-Oct-2019; **Accepted:** 18-Oct-2019; **Online first:** 14-Nov-2019

Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

AIM: To evaluate the immunomodulatory effects of ethanolic extract of herb pugun tanoh 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 Ethanolic Extract of Herb Pugun Tanoh (*Picria fel-terrae* Lour.) of dose 150 mg/kg BW orally, Group 3, receiving dose Ethanolic Extract of Herb Pugun Tanoh (*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 Ethanolic Extract of Herb Pugun Tanoh (*Picria fel-terrae* Lour.) succeeded in reducing side effect doxorubicin based on increasing the $TCD4^+$ and $TCD8^+$ blood level.

CONCLUSION: Ethanolic Extract of Herb Pugun Tanoh (*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 ethanolic 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 Ethanolic Extract of Herb Pugun Tanoh (*Picria fel-terrae* Lour.) of dose 150 mg/kg BW orally; and Group III received Ethanolic 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 Ethanolic 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 tetra-acetic 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, ethanolic 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 ethanolic 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.

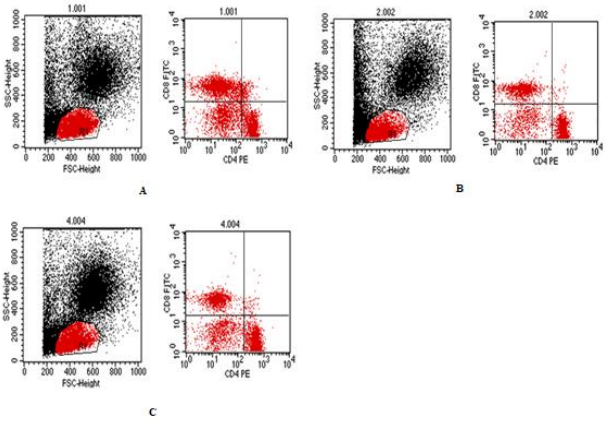

Fig. 1: Effect of Ethanolic Extract of Herb Pugun Tanoh (Picria fel-terrae Lour.) on TCD4+ and TCD8+ in rats induced by doxorubicin (DOX); A) DOX 4.6 mg/kg BW; B) Ethanolic Extract of Herb Pugun Tanoh (Picria fel-terrae Lour.) 150 mg/kg BW; C) Ethanolic Extract of Herb Pugun Tanoh (Picria fel-terrae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

References

1. van Valkenburg JL, de Padua LS, Bunyapraphatsara N, Lemmens RH. Medicinal and Poisonous Plants. Part 2. Leiden, Netherlands: Backhuys, The University of Michigan; 2001.

2. Zou JM, Wang LS, Ma XM, Shi RB, Guo YJ. Isolation and identification of a new cucurbitacin from Picria fel-terrae. Yao Xue Xue Bao. 2004; 39(11):910-2.

3. Huang YDe Bruyne TApers SMa YClaeys Mvan den Berghe DHuang Y, De Bruyne T, Apers S, Ma Y, Claeys M, Vanden Berghe D, et al. Complement-inhibiting cucurbitacin glycosides from Picria felterrae. J Nat Prod. 1998; 61(6):757-61. https://doi.org/10.1021/np9705773 PMid:9644059

4. Huang Y, de Bruyne T, Apers S, Ma Y, Claeys M, Pieters L, et al. Flavonoid glucuronides from Picria fel-terrae. Phytochemistry. 1999; 62:1701-3. https://doi.org/10.1016/S0031-9422(99)00242-3

5. Fang H, Ning DS, Liang XY. Studies on technology optimization for extracting triterpenoid saponins from Picria felterrae by multi-target grading method. Zhong Yao Cai 2009;32(12):1902-5. 25. Wang LS, Li SH, Zou JM, Guo YJ, Sun HD. Two new triterpenoids from Picria fel-terrae. J Asian Nat Prod Res. 2006; 8(6):491-4. https://doi.org/10.1080/10286020410001721131 PMid:16931422

6. AgroMedia R. Buku Pintar Tanaman Obat. Cetakan 1. Jakarta: Agromedia Pustaka, 2008.

7. Patilaya P, Husori DI. Preliminary study on the anthelmintic activity of the leaf ethanolic extract of Indonesian Curanga fel-terrae (Lour.) Merr. Int J Pharm Technol Res. 2015; 8:347-51.

8. Sitorus P, Harahap U, Pandapotan M, Barus T. Isolation of β-sitosterol from n-hexane extract of Picria fel-terrae Lour. Leave and study of its antidiabetic effect in alloxan induced diabetic mice. Int J Pharm Technol Res. 2014; 6:137-41.

9. Lestari P, Hadisahputra S, Ilyas S, Satria D. Combinational effects of n-hexane extract of poguntano leaves (Picria fel-terrae Lour.) with doxorubicin on MCF-7 breast cancer cells. J Chem Pharm Res. 2015; 7:353-5.

10. Satria D, Furqan M, Hadisahputra S, Rosidah. Combinational effects of ethylacetate extract of Picria fel-terrae Lour and doxorubicin on T47D breast cancer cells. Int J Pharm Pharm Sci. 2015; 7:73-6.

11. Furqan M, Hadisahputra S, Rosidah. Effects of inhibition cell cycle and apoptosis of poguntano leaves ethylacetate extract (Picria fel-terrae Lour.) on breast cancer cells. Int J Pharm Technol Res. 2014; 6:1096-9.

12. Dalimunthe A, Harahap U, Rosidah G, Pandapotan NM. Evaluation of diuretic activity of Picria fel-terrae Lour leaves extracts. Asian J Pharm Clin Res. 2015; 8:204-5.

13. Sihotang Y, Silalahi J, Hadisahputra S, Anjelisa P, Satria D. Cardioprotective effect of ethylacetate extract of poguntano (Picria fel-terrae Lour.) against doxorubicin-induced cardiotoxicity in rats. Int J Pharm Clin Res. 2016; 8:466-70.

14. Harahap U, Husori DI, Marianne, Yuliasmi S, Patilaya P, Laila L, et al. Inhibitory effect of ethanolic extract of Curanga fel-terrae (pugun tano) leaves on acetylcholine muscarinic-3 receptors induced on isolated guinea pig tracheal. Asian J Pharm Clin Res. 2017; 10:95-8. https://doi.org/10.22159/ajpcr.2017.v10i1.14163

15. Harahap U, Marianne, Yuliasmi S, Husori D I, Patilaya P, Laila L. Hepatoprotective activity from ethanol of Pugun Tano's leave (Curanga fel-terrae (Lour.) Merr.). Asian J Pharm Clin Res. 2017; 10(11):78-82. https://doi.org/10.22159/ajpcr.2017.v10i11.19127

16. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nature reviews immunology. 2008; 8(1):59-73. https://doi.org/10.1038/nri2216 PMid:18097448

17. Lu X, Ding ZC, Cao Y, Liu C, Habtetsion T, Yu M, Lemos H, Salman H, Xu H, Mellor AL, Zhou G. Alkylating agent melphalan augments the efficacy of adoptive immunotherapy using tumor-specific CD4+ T cells. The Journal of Immunology. 2015; 194(4):2011-21. https://doi.org/10.4049/jimmunol.1401894 PMid:25560408 PMCid:PMC4324150

18. Tan ML, Choong PF, Dass CR. Review: doxorubicin delivery systems based on chitosan for cancer therapy. J Pharm Pharmacol. 2009; 61(2):131-42. https://doi.org/10.1211/jpp.61.02.0001 PMid:19178759

19. Nugroho AE, Hermawan A, Nastiti K, Suven S, Elisa P, Hadibarata T, et al. Immunomodulatory Effects of Hexane Insoluble Fraction of Ficus septica Burm. F. in Doxorubicin-treated Rats. APJCP. 2012; 13(11):5785-90. https://doi.org/10.7314/APJCP.2012.13.11.5785 PMid:23317257

20. Zhang XY, Guang LW, Jie WY, Tang GM. Amelioration of doxorubisin-induced myocardial oxidative stress and immunosuppression by grape seed proanthocyanidins in tumour-bearing mice. J Pharm Pharmacol. 2005; 57:1043-1051. https://doi.org/10.1211/0022357056523 PMid:16102261

21. Rukhsana S, Fabio DD, Tseng M, Cai J, Noel T, Chelvarajan RL, Pierce WD, Cini C, Bondada S, Clair DK, Butterfield BD. Doxorubicin-induced thymus senescence. J Proteome Res. 2010; 9:6232-6241. https://doi.org/10.1021/pr100465m PMid:20945937

22. Sumardi TH, Sasmito E. Ant plant (Myrmecodia tuberosa) hypocotyl extract modulates TCD4+ and TCD8+ cell profile of doxorubicin-induced immune-suppressed sprague dawley rats in vivo. Scientia Pharmaceutica. 2013; 81(4):1057-1069. https://doi.org/10.3797/scipharm.1302-03 PMid:24482773 PMCid:PMC3867240

23. Silalahi J, Rosidah R, Yuandani Y, Satria D. Virgin coconut oil modulates TCD4+ and TCD8+ cell profile of doxorubicin-induced immune-suppressed rats. Asian Journal of Pharmaceutical and Clinical Research. 2018:37-8. https://doi.org/10.22159/ajpcr.2018.v11s1.25562

24. Uspenskaya YA, Mikhutkina SV, Taksanova EI, et al. Induction of apoptosis in bone marrow cells is mediated via purinergic receptors. Buletinof Experimental Biology and Med. 2004; 8:116-9. https://doi.org/10.1023/B:BEBM.0000048365.80201.fc PMid:15662449

25. Harahap U, Husori DI, Marianne N, YuliasmiS, PatilayaP, LailaL, et al. Inhibitory effect of ethanolic extract of Curanga fel-terrae (Pugun Tano) leaves on acetylcholine muscarinic-3 receptors induced on isolated guinea pig tracheal. Asian J Pharm Clin Res. 2017; 10(1):95-8. https://doi.org/10.22159/ajpcr.2017.v10i1.14163