

## Adaptive Immune Response Stimulation on Nephrolithiasis Mice Model after Treatment of Tempuyung (*Sonchus arvensis* L.) Leaf Extract

Khoirin Maghfiroh<sup>1</sup>, Muhaimin Rifa'i<sup>2</sup>, Sri Widyarti<sup>2\*</sup>

<sup>1</sup>Master Program of Biology, Faculty of Mathematics and Natural Sciences, University of Brawijaya, Malang, Indonesia

<sup>2</sup>Department of Biology, Faculty of Mathematics and Natural Sciences, University of Brawijaya, Malang, Indonesia

### Abstract

Calcium crystal accumulation on kidney can cause kidney stone (nephrolithiasis). The oxalate calcium crystal which is deposited on the kidney can trigger inflammation on the epithelial that is able to induce cell death (necrosis). The necrosis is able to cause inflammation and it will affect the body's immune system. Infection agent that comes to the body will be responded by the innate immunity which will be responded later by adaptive immunity. One of herbal agents that is expected to be used to stimulate adaptive immunity response is Tempuyung (*Sonchus arvensis*) leaf extract. The aim of this study is to find out the change of relative amount of CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, and B (B220) cells on nephrolithiasis mice model after *S. arvensis* leaf extract. The mice are divided into six groups; control group, placebo, nephrolithiasis, *S. arvensis* leaf extract for 7 days, nephrolithiasis then it is continued with *S. arvensis* leaf extract for 7 days, and simultaneous (nephrolithiasis and *S. arvensis* leaf extract for 3 months). The amount of relative T lymphocyte cells is measured by using BD FACSCalibur Flowcytometer<sup>TM</sup>. The data is analyzed by using ANOVA one way ( $p < 0.05$ ) using SPSS 16.0 software for Windows. The result shows that there are changes of relative CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, and B (B220) cells on nephrolithiasis mice model after the giving of *S. arvensis* leaf extract. The treatment of *S. arvensis* leaf extract on the nephrolithiasis mice model can stimulate the homeostatic activity by suppressing the B cells. Compound of *S. arvensis* leaf extract that can inhibit of Th1 cells and the increase of Th2 cells by proliferation cells activity. The treatment of *S. arvensis* leaf extract for 7 days can suppress CD4<sup>+</sup>. The *S. arvensis* leaf extract can stimulate adaptive immune response which is caused by immunomodulatory active component.

**Keywords:** Nephrolithiasis, *Sonchus arvensis* L., leaf extract, adaptive immune response.

### INTRODUCTION

Kidney stone is formed by calcium crystallization inside urinary tract and it usually causes pain, urine stoppage, and kidney damage [1]. The crystal formation inside the kidney is normal and is not dangerous if it is not excreted by urine. Any concentration in the urine inside the kidney will maintain water and nutrition and also eliminate excretion residue. Around 1,5 L excreted blood will become urine and there is urine supersaturation alongside two types of salt: calcium phosphate (CaP) and calcium oxalate (CaOx) which form crystal sediment on the process [2]. Adhesion of calcium oxalate crystal causes inflammation through cell death (necrotic) on kidney tubules proximal. Besides, the calcium crystal is also able to induce reactive oxygen species on kidney tubules observation [3].

The change on blood vessel can be overtaken by inflammation process which stimulates

leukocyte activation. The inflammation is a response to protect and trigger tissue recovery, but it can also stimulate tissue damage. Natural immunity response is a defense form of body towards inflammation agent of epithelial barrier, phagocyte cells (neutrophils and macrophage), NK (natural killer) cells and cytokine which control and coordinate various activities of the default immunity cells. Innate immune response will trigger specific immune response activity which is played by lymphocyte T and lymphocyte B cells. T cells control antigen and is essential for adaptive immunity. T cells and B cells are produced in bone marrow while undergoing maturity while T cells have their own in thymus. When inflammation response happens, the antigen which is brought by macrophage will activate CD4<sup>+</sup> T cells. The activated CD4<sup>+</sup> T cells can activate CD8<sup>+</sup> T cells, B cells, macrophage and NK cells. CD4<sup>+</sup> T cells stimulate IL-4 secretion so that it can activate B cells and then the cells will differentiate to become plasma cells which produce antibody as a response towards specific immunity [4].

B cells also have signal receptors which is B220 (CD45) [5]. B220 is a B cell marker that comes

\* Correspondence author:

Sri Widyarti

Email :swid@ub.ac.id

Address : Dept. of Biology, Faculty of Mathematics and Natural Sciences, University of Brawijaya, Jl. Veteran, Malang, 65145

from glycoprotein on cells surface of lymphoid. The B220 marker is only found in B cells and bone marrow subset which covers B cells precursor in the progress and B cells differentiation [6].

Immune response modulation is an immunomodulator activity through response suppression which is known as immunosuppressant and immune response increase is known as immunostimulant [7]. The work of immunostimulant includes augmentation of anti-inflammation immunity by the cells form of the body immune system which covers lymphocyte subset, macrophage, dendritic and natural killer (NK) cells. The next mechanism occurs through competent cells induction which is involved in adaptive immunity [8]. Immunosuppressant activity through cells suppression which produce cytokine pro inflammation so that it can prevent T cells activation [9]. Wahyudi shown that *S.arvensis* as herbal medicine is the basic material which is proven to be able to destroy kidney stone [10]. Up to now the mechanism of *S.arvensis* in modulating immune response is still unknown; that is why scientific study about the plant's immunomodulator activity is needed.

## MATERIALS AND METHODS

### Ethical Clearance

All experimental procedures were performed with the approval of the ethic committee of research of the Brawijaya University, ethical clearance no.127-KEP-UB.

### Mice

Specific pathogen free 2-months-old male BALB/c mice were obtained from the Galaxy Science, Wringinangung Jombang Jember, Indonesia. Mice were acclimated for 7 days before treatment. Mice were maintained in animal chamber pathogen free.

### Induction of Nephrolithiasis

Nephrolithiasis model mice were made by administrated with 6 mg/100g BW/day of porang tuber (*Amorphopallus muelleri*) powder orally for 3 months [11].

### Treatment of Tempuyung (*Sonchus arvensis*) Leaf Extract

Giving 3.3 mg/g BW/ day of *S.arvensis* leaf extract orally for 7 days. As a placebo, hydrochlorothiazide administrated orally with 0.00142 mg/g BW / day for 6 days [12].

## Lymphocytes Cells Isolations

Experimental animals were killed by neck dislocation, sectioned using standard method. The spleen was removed and washed three times with sterile PBS, transferred another petri dish which containing PBS. The spleen crushed by syringe base and then suspension are filtered with BD cells strainer and transfered into propylene tube. Then added PBS until 10 ml and centrifuged by 2500 rpm for 5 minutes in 4°C. Pellet resuspended in 1 ml PBS. Cells suspension is taken 200 µl and transferred into micro tube and added with 500 µl PBS, and then it is centrifugated by 2500 rpm for 5 minutes in 4°C. The pellet is incubated with antibodies : washperm 1 : 200 [13].

## Antibody Staining and Flow Cytometry Analyze

Antibody are FITC – conjugated anti-mouse CD4, PE – conjugated anti-mouse CD8 and PE-conjugated anti-B220. Analytical flow cytometry were performed by using FACS Calibur flow cytometer (BD Bioscience). Preparative cells sorting were performed by using FACS Vantage cells sorter (BD Bioscience).

## Statistical Analysis

The data is analyzed by using one way ANOVA (Analysis of Variance) ( $p < 0,05$ ) with SPSS (Statistical Product and Service Solution) 16.0 software, followed by Tukey HSD.

## RESULT AND DISCUSSION

### CD4<sup>+</sup> and CD8<sup>+</sup> T cells Profiles

The nephrolithiasis treatment can increase significantly CD4<sup>+</sup> T cells profile by 20.29±0.89%. Calcium crystal can stimulate secretion of pro inflammation cytokine through NLRP3 (NOD like receptor). Activation of NLRP3 can causes recruitment to the inflammasome triggers caspase-1 for cleaves pro inflammatory cytokine into their active and secreted forms. The pro inflammatory cytokine secretion is stimulated by CD4<sup>+</sup> T cells activation [14,15,16]. CD4<sup>+</sup> T cells profile is T helper (Th) cells which has matured because of activation of antigen exposure from the outside. Macrophage brings antigen and it is brought to be represented through MHC II. The process is adaptive immunity response as a response towards antigen exposure [7].

CD4<sup>+</sup> T cells activity increase after *S.arvensis* leaf extract treatment and nephrolithiasis induction is not different significant. But, there is CD4<sup>+</sup> T cells profile decrease for 18.77±0.85%. The research in line also argued that flavonoid

can suppress CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells activation through activation suppression of nuclear factor kappa beta (Nf-Kβ) [17]. CD4<sup>+</sup> T cells activity increase after *S.arvensis* leaf extract treatment and nephrolithiasis induction is not different significant. But, there is CD4<sup>+</sup> T cells profile decrease for 18.77±0.85%. The research in line also argued that flavonoid can suppress CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells activation through activation suppression of nuclear factor kappa beta (Nf-Kβ) [17]. The treatment of *S.arvensis* leaf extract after nephrolithiasis induction is not different significantly from placebo group with average 7.52±0.65%. The 7 days treatment of *S.arvensis* leaf extract is different significantly in decreasing CD4<sup>+</sup> T cells profile for 7.58±0.14%.

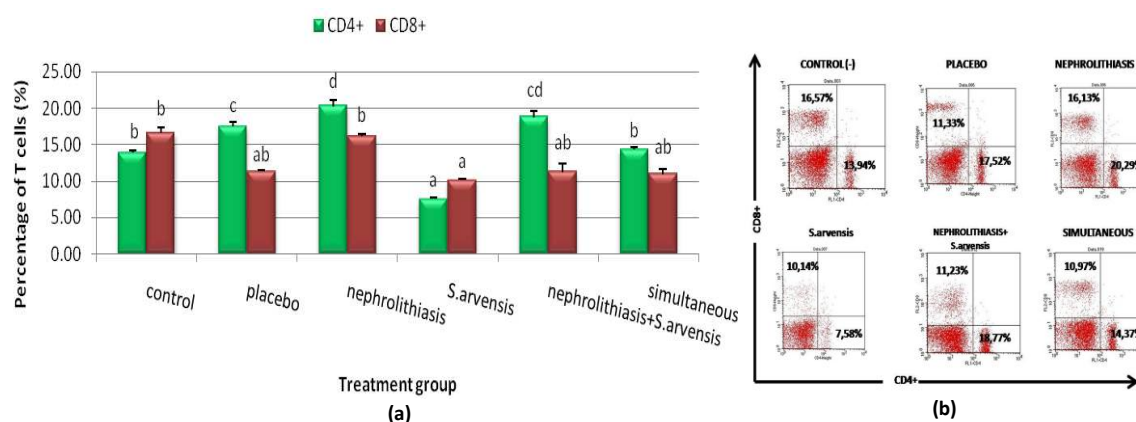
Flavonoid is able to obstruct Nf-kβ and MAPK (mitogen activated protein kinase) activation. Nf-kβ transcription factor obstruction through ERK1/2 (extracellular signal-regulated kinase) regulation decrease, JNK (Jun N-terminal kinase), and protein p38 signaling pathways [18]. MAPK obstruction is related to the activation of macrophage activity which synthesizes cytokine pro inflammation. Besides, it is effectively able to decrease MCP-1 (monocyte chemoattractant protein) and ICAM-1 (intracellular adhesion molecule) regulation which have important role to activate lymphocyte [7,19]. Treatment of *S.arvensis* leaf extract simultaneously on the average 14.37±0.27% of CD4<sup>+</sup> T cells. This were not significant difference.

The activated CD4<sup>+</sup> T cells can stimulate cytokine IL-2 secretion to activate CD8<sup>+</sup> T cells. The treatment of *S.arvensis* leaf extract for 7 days and it significantly can decrease the CD8<sup>+</sup> T cells profile for 10.14±0.17% (Fig.1). Activity of

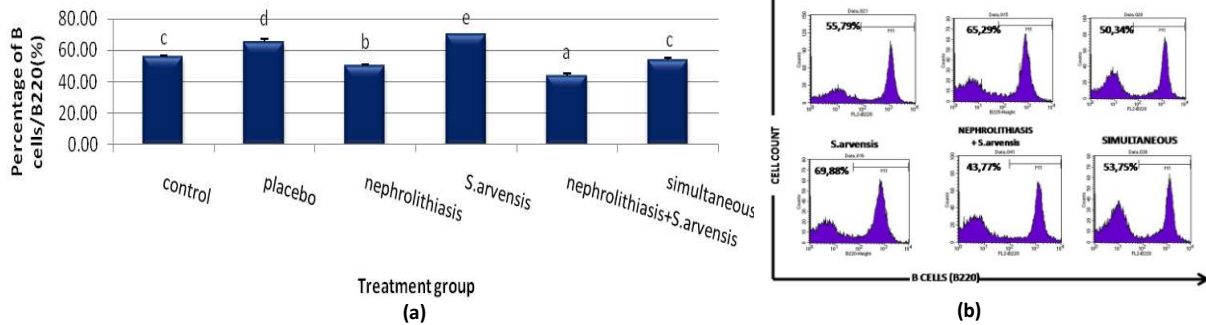
compound flavonoid in the plants extract can suppress CD8<sup>+</sup> T cells activity. Nephrolithiasis group with average 16.13±0.37% of CD8<sup>+</sup> T cells is not significantly different from control gorup. The treatment of the *S.arvensis* leaf extract after nephrolithiasis induction 11.23±1.16% is not significantly different from nephrolithiasis group and control group but there is a decrease of CD8<sup>+</sup> T cells profile. The treatment of the extract simultaneously on average 10.97±0.74% is not significantly different in affecting CD8<sup>+</sup> T cells profile change. Quercetin activity from flavonoid can suppress CD8<sup>+</sup> T cells activity through TCF 1 (T cells Factor) or β catenin obstruction via P13K/AKT/ERK pathway [20]. TCF-1 has a role in expanding selection positive in Thymus [21]. CD8<sup>+</sup> T cells proliferation is stimulated by IL-2 secretion. Flavonoid can decrease IL-2 secretion so that it also can decrease CD8<sup>+</sup> T cells activity [22].

### B Cells Profile

B cells profile indicates the amount of B cells which has matured to produce antibody. Based on the data in Figure 2, it is shown that nephrolithiasis significantly can decrease B cells profile by 50.34±0.66%. However, it is not significantly different from placebo group. The treatment of *S.arvensis* leaf extract after nephrolithiasis induction significantly can decrease B cells profile by 43.77±1.50%. B cells profile significantly increases 69.88±0.58% in the 7 day treatment. B cells profile increase significantly in the 7 days treatment for the *S.arvensis* leaf extract seems to be able to increase the Th2 proliferation and prevent Th1 cells activation through TGFβ stimulation.



**Figure 1.** CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells in BALB/c mice spleen. (a) Relative numbers of each CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells subpopulation derived from BALB/c mice determined by FACS analysis. Data are mean ± SD values of three mice in each group. (b) Spleen cells were obtained from 13-wk-old BALB/c mice, stained with indicated fluorescence-conjugated antibodies, and analyzed by flow cytometry. Percentages of CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells are shown in each panel.



**Figure 2.** B cells in BALB/c mice spleen. (a) Relative numbers of each B cells subpopulation derived from BALB/c mice determined by FACS analysis. Data are mean  $\pm$  SD values of three mice in each group. (b) Spleen cells were obtained from 13-wk-old BALB/c mice, stained with indicated fluorescence-conjugated antibodies, and analyzed by flow cytometry. Percentages of B cells are shown in each panel.

Flavonoid in the *S.arvensis* leaf extract suggest can stimulate TGF- $\beta$ . The treatment of *S.arvensis* leaf extract after nephrolithiasis is significantly decrease B cells profile. It is estimate that *S.arvensis* leaf extract can modulate immune response through increasing Th1 and Th2. Th1 and Th2 become the down-regulator and up-regulator each other to keep the homeostatic [23]. Treatments of nephrolithiasis induction can suppression significantly B cells. It is suspect that proliferation after CaOx accumulation in nephrolithiasis and suppression of Th2 proliferate can cause suppression B cells production.

Nephrolithiasis followed by *S.arvensis* leaf extract treatment can suppression significantly B cells profile. It is suspect that *S.arvensis* leaf extract can modulate immunity response through increase homeostatic of Th1 and Th2[24]. Flavonoid is suspected can stimulate of proliferation Th-2 to suppress of Th-1. The Th-2 cytokine is role plays stimulatory B cells for antibody secretion[8].

## CONCLUSION

*S.arvensis* leaf extract can stimulate adaptive immunity response by suppressing CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells activation. Besides, the suppression of B cells can induce the homeostatic activity. The *S.arvensis* leaf extract can increase immunomodulator activity in nephrolithiasis.

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