

## Effect of *Plectranthus amboinicus* Extract on BUN and Creatinine Levels and Cellular Response Proinflammatory Factors TNF- $\alpha$ and IL-1 $\beta$ on Gout Arthritis Patients

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### Abstract

The purpose of this research was to develop anti-cytokine-based treatment using extract of *Plectranthus amboinicus* applied to gout arthritis (GA) patients. The research was quasi experimental, with a pretest-posttest randomized control group design. The samples were GA patients in the Outpatient Installation of Internal Medicine in General Hospital Haji, Surabaya. The sample was comprised of 30 respondents. The respondents were divided into a treatment group and a control group. The treatment group was asked to take medicine from the hospital, coupled with *P. amboinicus* extract capsules, for 7 days, during which time patients' joint inflammation was observed. The control group was provided with only medication from the hospital, and their joint inflammation was likewise observed. Blood samples were taken before and after treatment, to measure the levels of blood urea nitrogen (BUN) and creatinine, as well as the concentrations of TNF- $\alpha$  and IL-1 $\beta$ . There was a decrease in BUN and creatinine levels in the control group, but it was not significant, decreasing by 3% and 8%, respectively. The treatment group also showed elevated levels of BUN and creatinine, which also was not significant at 3% and 7%, respectively. There was a decrease in the concentration of TNF- $\alpha$  in the control group by 9% and 22%. The concentration of IL-1 $\beta$  in the control group increased by 18%, whereas, in the treatment group, it decreased by 3%; however, the decreases in both groups were not significant.

### Abstrak

**Pengaruh Ekstrak Daun Jintan (*Plectranthus amboinicus*) terhadap Kadar BUN dan Kreatinin serta Respon Seluler Faktor Proinflamasi TNF- $\alpha$  dan IL-1 $\beta$  Pasien Gout Arthritis.** Pengobatan gout arthritis (GA) saat ini dikembangkan berbasis anti sitokin yaitu blokade kemokin, penghambatan pelepasan IL-1 $\beta$  dan TNF- $\alpha$ . Tujuan penelitian ini adalah mengembangkan pengobatan berbasis anti sitokin dengan menggunakan ekstrak daun jintan yang diaplikasikan pada penderita GA. Penelitian ini adalah penelitian eksperimental semu dengan desain penelitian *randomized pretest-posttest control group design*. Pengambilan sampel pasien GA di Instalasi Rawat Jalan Poli Penyakit Dalam RSU Haji Surabaya yang memenuhi kriteria inklusi dan eksklusi. Jumlah sampel sebanyak 30 responden terbagi menjadi kelompok perlakuan dan kelompok kontrol masing-masing 15 responden. Pada kelompok perlakuan diminta untuk minum obat dari Rumah Sakit ditambah dengan kapsul ekstrak daun jintan selama 7 hari, dengan dilakukan pengamatan peradangan sendi. Pada kelompok kontrol minum obat dari Rumah Sakit dan dilakukan pengamatan peradangan sendi. Sampel darah diambil sebelum dan sesudah perlakuan untuk mengukur kadar BUN, kreatinin, konsentrasi TNF- $\alpha$  dan IL-1 $\beta$ . Terdapat penurunan kadar BUN dan kreatinin pada kelompok kontrol namun tidak signifikan yaitu menurun sebesar 3% dan 8%. Sementara pada kelompok perlakuan terdapat peningkatan kadar BUN dan Kreatinin namun juga tidak signifikan sebesar 3% dan 7%. Terdapat penurunan konsentrasi TNF- $\alpha$  pada kelompok kontrol sebesar 9% dan kelompok perlakuan sebesar 22%, keduanya tidak signifikan. Sementara konsentrasi IL-1 $\beta$  terjadi peningkatan pada kelompok kontrol sebesar 18%, sementara pada kelompok perlakuan terjadi penurunan sebesar 3%, keduanya tidak signifikan.

*Keywords: BUN, creatinine, Gout arthritis, IL-1 $\beta$ , Plectranthus amboinicus extract, TNF- $\alpha$*

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## Introduction

On the occurrence of gout arthritis (GA), acute symptoms are easily observed in the form of disruptive pain in the joint, which requires prompt treatment to control pain and inflammation. GA symptoms are caused by a complex inflammatory reactions involving the proinflammatory factors TNF- $\alpha$  and interleukin-1 $\beta$ .<sup>1,2</sup> The emergence of GA is acutely stimulated by trauma, surgery, and the consumption of alcohol and certain drugs that alter serum urate levels. High levels of urate are distributed to various tissues, and deposition of urea crystals in the joints can cause recurrent acute inflammation. In appropriate treatment can lead to chronic inflammation, can cause urea crystals to form tophi deposition (tophi gout), and cause structural damage in joints. In cases of continued chronic polyarthritis, other tissues will be affected, such as kidney (nephropathy and uric acid nephrolithiasis), juxta-articular, cardiac, and subcutaneous tissue.<sup>2</sup>

The deposition of urea crystals into the renal tubules will interfere with the function of kidney filtration, and it can be detected by increasing levels of blood urea nitrogen (BUN) and creatinine concentrations. Therefore, it can be used as initial parameters for analyzing the condition of the kidney filtration function. Increased BUN and creatinine may occur simultaneously or, sometimes, creatinine levels will increase while the BUN level rises, when kidney damage has reached 60%.<sup>3</sup>

*Plectranthus amboinicus* belongs to the family Lamiaceae, once identified as *Coleus amboinicus*, and, in Indonesia, as *daun bangun bangun*. This plant has been used traditionally as food, and as herbal remedies, especially to cure various diseases. The chemical composition of *P. amboinicus* in the form of ethanol extract consists of flavonoids, saponins, polyphenols, essential oils, and Antraquinon.<sup>4</sup>

Existing treatments for GA are symptomatic, blocking only the formation of leukotrienes and prostaglandins in the inflammatory process, without eliminating the major cause.<sup>5</sup> However, long-term use of these anti-inflammatory drugs potentially damages kidney and liver function.<sup>6</sup>

The current treatment of GA was developed based on the anti-cytokine chemokine blockade,<sup>7</sup> inhibition of IL-1 $\beta$ ,<sup>8</sup> and TNF- $\alpha$  inhibition.<sup>9-11</sup> Anti-cytokine-based treatment is more effective against the main causes GA than symptomatic treatment is. Several studies has shown that use of *P. amboinicus* leaf extract has inhibitory effects on anti-cytokine release in induced inflammation in mice.<sup>12</sup> However, research on applications of *P. amboinicus* leaf extract for the development of anti-cytokine-based treatment for patients with GA has not been done. This study aims to

analyze the effect of *P. amboinicus* leaf extract in patients with GA, by measuring the levels of BUN, creatinine, TNF- $\alpha$  and IL-1 $\beta$ , before and after treatment in the treatment group and the control group.

## Methods

A quasi-experimental research method was used in the study, to provide treatment for patients with GA. The study design was a randomized pretest-posttest control group design with single blind administration.

**Sample criteria.** The subjects of this study were patients with GA in the Outpatient Installation of Internal Medicine in the General Hospital (RSU) Haji, Surabaya, who met the inclusion criteria, being diagnosed with GA (established in accordance with specific clinical symptoms) and agreeing to follow the research by completing the informed consent, and the exclusion criteria, no history of gastritis, duodenal ulcer, hypersensitivity to the drugs indomethacin and *P. amboinicus*, and not a pregnant or lactating woman. The exclusion criteria were used to restrict the study group and reduce the risk of treatment interventions. These data were derived from the results of the interview and physical examination by the doctor; however, participants did not require detailed examination. Indomethacin served as the drug of choice of GA patients.

**Clinical research.** The sample consisted of 30 respondents. The respondents were grouped into one of two groups: 15 in the treatment group and 15 in the control group. Blood samples of respondents were taken to measure levels of BUN, creatinine, TNF- $\alpha$ , and IL-1 $\beta$  as the initial data before the treatment. Those in the treatment group were asked to take standard medication from the hospital, coupled with *P. amboinicus* leaf extract capsules for 7 days, 1 capsule daily, and they underwent observation of the inflammation in the joints. Those in the control group were provided only the standard medication from hospital and the inflammation of their joints was observed. After 7 days, in each group, blood sample were collected to test the levels of BUN, creatinine, TNF- $\alpha$ , and IL-1 $\beta$ . Then the results were analyzed to determine differences in the levels of BUN, creatinine, TNF- $\alpha$ , and IL-1 $\beta$ , before and after treatment, between the treatment group and the control group.

**Ethical clearance.** This study was approved by the Ethical Committee of General Hospital Haji, Surabaya, No. 073/18/KOM.ETIK/2013. Blood samples were collected by experienced medical personnel (nurses of General Hospital Haji, Surabaya).

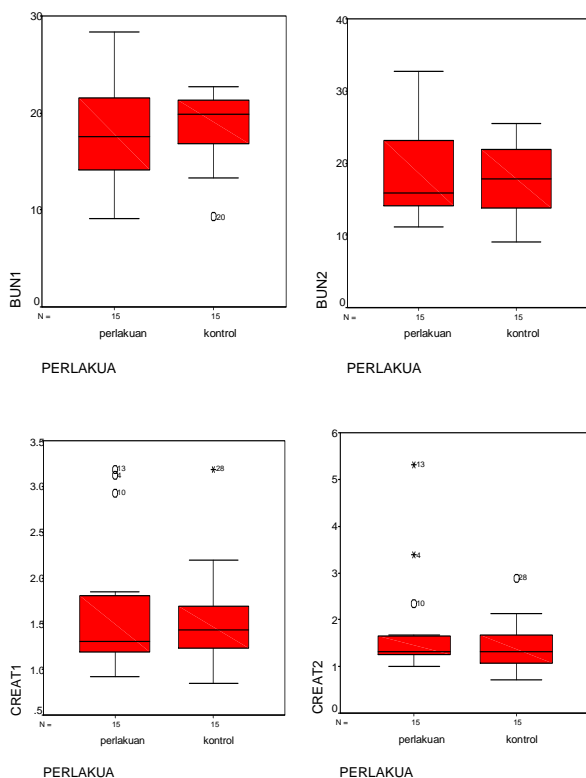
**Data analysis techniques.** Blood samples were tested to determine the concentration of BUN, creatinine, TNF- $\alpha$ , and IL-1 $\beta$ . Measurements of TNF- $\alpha$  and IL-1 $\beta$

concentrations were obtained by using the Elisa technique. Paired sample t-tests (paired t-test) were employed to determine the significance level of the differences in BUN, creatinine, TNF- $\alpha$ , and IL-1 $\beta$  concentrations before and after the treatment.

## Results and Discussion

**Clinical Research.** Preparation of *P. amboinicus* leaf extracts was done in Integrated Research and Testing Laboratory (Laboratorium Penelitian dan Pengujian Terpadu/LPPT Universitas Gadjah Mada/UGM). It was prepared into capsules using pharmaceutical technology at a dose corresponding to a daily intake of fresh *P. amboinicus* leaves in humans is 210 g/70 kg body weight. Leaf extract capsulation was carried out in the laboratory of the pharmacology and clinical pharmacy UGM. Levels of BUN and creatinine measurements were performed in the Clinical Laboratory “Klinika” Surabaya. Analysis of the cellular response of TNF- $\alpha$  and IL-1 $\beta$  were conducted in the laboratories of the Institute for Tropical Diseases (ITD) Universitas Airlangga.

**BUN and creatinine levels.** The following Figure 1 shows the concentrations of BUN and creatinine in both group before and after treatment.



**Figure 1. Boxplot of BUN and Creatinine Concentrations of Both Groups of Patients Before and After Treatment**

The results of the measurement of BUN and creatinine (mg/dl) levels in both group before and after treatment are shown in Table 1.

Normal levels of BUN in adults are between 14 and 23 mg/dL, whereas normal creatinine levels are between 0.7 and 1.3 mg/dL.<sup>13</sup> In the control group, there were decreases in creatinine levels by 8%, and, in BUN levels, by 3% before and after treatment. In the treatment group, BUN and creatinine concentrations increased by 3% and 7%, respectively, before and after treatment. The results of the statistical analysis in the control group showed a decrease in the levels of BUN and creatinine, but these were not significant ( $p > 0.05$ ). Meanwhile, there was an increase in BUN and creatinine levels in the treatment group, but, likewise, the increase was not significant ( $p > 0.05$ ).

BUN and creatinine concentration are blood chemistry parameters used to analyze the normality of kidney filtration. The mean concentrations of BUN and creatinine from blood samples taken from the entire group of patients remained at the normal level. This result shows that the patients in this study did not have the impaired renal function associated with GA. Increasing BUN and creatinine may be indicative of a disturbance in the renal filtration both in tubules and glomeruli. Increments of BUN and creatinine may occur simultaneously. In addition, the creatinine and the BUN levels will rise when kidney damage has reached 60%.<sup>3</sup>

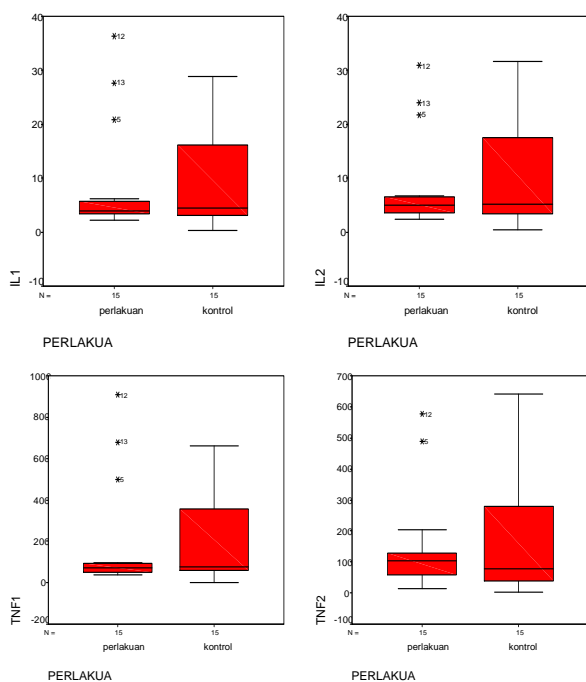
Following the administration of *P. amboinicus* leaf extract and standard drug in the treatment group and only standard drug administration in the control group, no changes in BUN and creatinine levels were shown. The results might have occurred because the GA patients in the study had relatively normal kidney function. In the case of continued GA, deposition of urea crystals will enter into the kidney tissue, filling the tubular lumen, and, thereby, interfering with the function of filtration and the absorption of body fluids.<sup>2,14,15,16</sup>

**Concentrations of IL-1 $\beta$  and TNF- $\alpha$ .** The results of the concentration of IL-1 $\beta$  and TNF- $\alpha$  in all groups of GA patients before and after treatment is shown in Figure 2.

The mean concentration of IL-1 $\beta$  and TNF- $\alpha$  in the treatment group and the control group of patients before

**Table 1. Mean Concentrations of BUN and Creatinine in Both Groups of GA Patients Before and After Treatment in the RSU Haji, Surabaya, in 2013**

Time	Control Group		Treatment Group	
	BUN	Creatinine	BUN	Creatinine
Before	18.58	1.54	18.08	1.66
After	17.97	1.42	18.71	1.78
p	0.667	0.158	0.678	0.505



**Figure 2. Boxplot of IL-1 $\beta$  and TNF- $\alpha$  Concentrations in the Whole Group of GA Patients Before and After Treatment**

and after treatment in the RSU, Haji, Surabaya, is described in Table 2.

In the control group, there were increased levels of IL-1 $\beta$  by 18%, but these were not significant ( $p > 0.05$ ), whereas in the treatment group, it decreased 3% before and after treatment, which was also not significant. Table 2 also shows a decrease in the concentration of TNF- $\alpha$  in the control group by 9%, although it was not significant ( $p > 0.05$ ), whereas there was a decrease of TNF- $\alpha$  by 22% in the treatment group.

The analysis of IL-1 $\beta$  secretion and TNF- $\alpha$  in the control group showed increased concentration levels of IL-1 $\beta$  and decreased TNF- $\alpha$  concentrations levels before and after treatment. These results indicate that the administration of the non-steroidal anti-inflammatory drug (NSAID) indomethacin may reduce the occurrence of inflammation in patients with GA. Moreover, partially marked changes in TNF- $\alpha$  level were lower after treatment, but it was not followed by a decrease in the levels of IL-1 $\beta$ . Target non-steroidal anti-inflammatory drugs are blocking the secretion of prostaglandin (PGF2- $\alpha$ ) and leukotrienes in the inflammatory process<sup>6</sup>; therefore, the secretions of good TNF- $\alpha$  cytokine and IL-1 $\beta$  are not dependent on the administration of NSAIDs, but they do inhibit migration neutrophil.<sup>17</sup> Meanwhile, the treatment group showed a decline in the level of secretion of IL-1 $\beta$  by 3% and decreased levels of TNF- $\alpha$  secretion by 22% after

**Table 2. The Mean Concentration of IL-1 $\beta$  in Both the Treatment and Control Group of GA Patients Before and After Treatment in the RSU, Haji, Surabaya, in 2013**

Time	Control Group		Treatment Group	
	IL-1 $\beta$	TNF- $\alpha$	IL-1 $\beta$	TNF- $\alpha$
Before	9.231	517.125	8.84	478.5
After	10.982	470.625	8.603	372.166
p	0.914	0.627	0.497	0.832

treatment with *P. amboinicus* leaf extract capsules and standard medication. The decline in the level of IL-1 $\beta$  and TNF- $\alpha$  secretion showed that the GA inflammation inhibition mechanism was different from that of the model of inhibition by NSAIDs. It can be assumed that the leaf extract of *P. amboinicus* contributes in reducing the level of cytokines IL-1 $\beta$  and TNF- $\alpha$  that is not shown in the control group of patients with NSAIDs.

The GA disease process begins when an increase in the level of monosodium urate crystals (MSU), which are further distributed into tissues and joints, causing acute inflammation mediated by many factors and proinflammatory cytokine. In acute GA, inflammatory processes involving cell activation endothelium (intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)) induced by TNF- $\alpha$  cytokine and IL-1 $\beta$ . In addition, acute GA inflammation occurs due to vasodilatation, increased blood flow, increased permeability of plasma proteins, and secretion of leukocytes into the tissues.<sup>2,18</sup>

In both groups, all patients after treatment for 7 days, either with NSAIDs (control) and with leaf extract (treatment) showed improvement of the inflammation of GA, as stated by patients at the beginning of the examination. The *P. amboinicus* leaf extract group was not shown to be significantly statistically different ( $p > 0.05$ ) in reducing the levels of TNF- $\alpha$  and IL-1 $\beta$ , but there was a decrease in the percentage TNF- $\alpha$  and IL-1 $\beta$  concentration of post-treatment compared with the control group (treated with NSAIDs) that did not decrease IL-1 $\beta$ . The inflammatory process in patients with GA in both the treatment and control groups clinically did not have the same degree of severity, as it can be seen from the variations in symptoms of GA, both the level of pain and the swelling of the joints. Conditions related to nutrition, diet, and the severity of the inflammation of GA may affect the healing process and the secretion of proinflammatory factors that follow the occurrence of GA in patients.

In the case of GA, the inflammation process that occurs is mediated by complex secretion of proinflammatory factors and cytokine. The classical inflammatory process in GA occurs in multiple pathways including phagolysosomes, inactivation of neutrophils, complement

activation, bradykinin and kallikrein, and activation of eicosanoids, free radicals and TNF- $\alpha$  sitokine and IL-1 $\beta$ <sup>2,18</sup> and induction of Toll-like receptor (TLR) -2 ligands that activate IL-1 $\beta$  inflammasome.<sup>19-22</sup> With a variety of pathways and the activation of proinflammatory factors in GA, cytokine secretion of IL-1 $\beta$  has a vital role in mediating the inflammation of complex inflammatory inflammasome.<sup>23</sup> Therefore, appropriate treatment of inflammation in GA is targeted at the inhibition of IL-1 $\beta$  secretion.<sup>8,24,25</sup>

Anti-cytokine-based treatment promises more effective therapies than the symptomatic treatment to eliminate the main causes of GA. The group treated with leaf extract capsules combined with standard medication showed decreased levels of TNF- $\alpha$  secretion by 22%, and IL-1 $\beta$  secretion decline by 3% post-treatment. The control group treated with standard medication (NSAIDs) increased IL-1 $\beta$  by 18%. The results show that the potential of *P. amboinicus* leaf extract in reducing inflammation in patients with GA has different mechanisms compared to NSAIDs (control group). Recently, the treatment of GA and other arthritis diseases developed based on anti-cytokine-based treatment by blockade of chemokine,<sup>7</sup> inhibition of IL-1 $\beta$  release<sup>8</sup> and release of TNF- $\alpha$  inhibition.<sup>9-11</sup> However, to obtain high potency of the active compound *P. amboinicus* leaf extract requires a process of repetition ranging from isolation, processing, testing and pharmacological responses in experimental animals and patients with GA.

## Conclusions

This study found that the levels of BUN and creatinine in the control group decreased, while there was an increase in the treatment group although not significant, but the BUN and creatinine levels of the two groups are remain within normal range. The concentration of TNF- $\alpha$  in the control and treatment groups both decreased, but not significantly, while the concentration of IL-1 $\beta$  in the control group increased, but decreased in the treatment group, although not significant. Therefore, for patients with GA, *P. amboinicus* leaf extract capsules can be used as an additional medication to treat the disease, as evidenced during this study there was no significant complaints and patient felt more comfortable to drink the leaf extract capsule.

**Recommendations:** Further studies could be developed by administering varying concentrations with a larger sample.

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