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RESEARCH ARTICLE

EFFECT OF RED ONION POWDER ON INFLAMMATORY BIOMARKERS IN A GOUTY ARTHRITIS MOUSE MODEL

(EFEK BUBUK BAWANG BOMBAI MERAH TERHADAP BIOMARKER INFLAMASI MENCIT MODEL ARTHRITIS GOUT)

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ABSTRACT

Gouty arthritis is an inflammatory joint disorder caused by the accumulation of monosodium urate (MSU) crystals. It is characterized by elevated levels of several inflammatory biomarkers, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP). Acute inflammation can be reduced by natural substances containing quercetin, which has anti-inflammatory properties, such as red onions (Allium cepa L.). This study aimed to determine the effect of red onion powder on inflammatory biomarkers in mice induced with MSU crystals. This study was conducted at the Food and Nutrition Study Center (PSPG) Laboratory, Gadjah Mada University (UGM), Yogyakarta, from July to August 2024. This true experimental study used a post-test control group design. Twenty-seven male Balb/C mice were divided into three groups: (1). control, (2). AG: mice induced with MSU crystals, and (3). ROP: mice induced with MSU crystals and treated with red onion powder. Gouty arthritis induction in the AG & ROP groups was performed using an intra-articular injection of MSU crystals at 1 mg/20 g BW, confirmed by the presence of plantar edema. The ROP group received red onion powder at a dose of 27 mg/20 g BW/day for seven consecutive days. IL-6, TNF-α, and CRP levels were measured using ELISA one day after treatment. Data were analyzed using one-way ANOVA with a significance level of 0.05. The mean of IL-6 (95.33±0.99 pg/mL), TNF-α (8.09±0.51 pg/mL), and CRP (0.93±0.01 ng/mL) levels in the ROP group were lower than those in the AG group (139.65±1.01 pg/mL; 16.07±0.27 pg/mL; 2.41±0.02 ng/mL) ($p < 0.05$). Administration of red onion powder affected inflammatory biomarkers in mice induced with MSU crystals.

Keywords: gouty arthritis, inflammatory biomarkers, red onion powder

ABSTRAK

Arthritis gout merupakan penyakit metabolik peradangan sendi akibat penumpukan kristal monosodium urat (MSU) yang ditandai dengan peningkatan berbagai biomarker inflamasi

seperti interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), dan C-reactive protein (CRP). Inflamasi akut bisa diminimalisir dengan pemberian bahan alam yang mengandung quercetin salah satunya bawang bombai merah (Allium cepa L.) yang mempunyai properti antiinflamasi. Tujuan penelitian ini untuk mengetahui pengaruh bubuk bawang bombai merah terhadap biomarker inflamasi pada mencit yang diinduksi kristal MSU. Penelitian ini dilakukan di Laboratorium Pusat Studi Pangan dan Gizi (PSPG) Universitas Gadjah Mada (UGM) Yogyakarta pada bulan Juli-Agustus 2024. Rancangan penelitian true experimental ini adalah post-test control group design. Subjek uji yang digunakan 27 ekor mencit jantan galur Balb/C dibagi menjadi 3 kelompok: (1). kontrol, (2). AG: mencit arthritis gout yang hanya diinduksi kristal MSU, (3). BBM: mencit yang diinduksi kristal MSU dan diberi bubuk bawang bombai merah (BBM). Induksi arthritis gout pada kelompok AG & BBM dilakukan dengan injeksi kristal MSU 1 mg/20 mgBB secara intraartikular, dinyatakan berhasil karena terjadi pembengkakan plantar kaki mencit. Pemberian bubuk bawang bombai merah 27 mg/20gBB/hari selama 7 hari pada kelompok BBM. Hari berikutnya dilakukan pengukuran kadar IL-6, TNF-α, dan CRP menggunakan ELISA. Data diuji menggunakan oneway ANOVA dengan tingkat signifikansi 0,05. Rerata kadar IL-6 (95,33±0,99 pg/mL), TNF-α (8,09±0,51 pg/mL), dan CRP (0,93±0,01 ng/mL) di BBM lebih rendah dari AG (139,65±1,01 pg/mL; 16,07±0,27 pg/mL; 2,41±0,02 ng/mL) (p <0,05). Pemberian bubuk bawang bombai merah berpengaruh terhadap biomarker inflamasi mencit yang diinduksi kristal MSU.

Kata kunci: arthritis gout, biomarker inflamasi, bubuk bawang bombai merah

INTRODUCTION

Gouty arthritis is an inflammatory condition caused by a metabolic disorder resulting from the accumulation of monosodium urate (MSU) crystals in the bones and joints.[1] The clinical manifestations of MSU crystal deposition include redness, swelling, warmth, pain, and stiffness in the joints around the metatarsophalangeal joints, knees, and fingers. These are the characteristic clinical signs of gouty arthritis.[2] The accumulation of MSU crystals in gouty arthritis can stimulate an increase in the proinflammatory cytokine tumor necrosis factor-α (TNF-α), which occurs through the activation of nuclear factor kappa-beta (NF-κβ).[3] The inflammatory response is also characterized by elevated levels of the acute-phase protein C-reactive protein (CRP).[4] Another inflammatory marker, interleukin-6 (IL-6), promotes inflammation by activating and proliferating lymphocytes, promoting B-cell differentiation, and increasing leukocyte counts.[5] The Department of Rheumatology and Inflammation Research reports that the global prevalence of gouty arthritis is 6.8 percent, and the prevalence in Indonesia is 4 percent among adults.[6]

One active compound that can help reduce MSU levels is the flavonoid quercetin.[7] Red onions contain 45 mg of quercetin per 100 g of fresh weight, and their quercetin content is higher than that of

many other natural sources.[8] Administration of red onion juice to hyperuricemic rats has been shown to reduce serum uric acid levels and increase total antioxidant status.[9] Red onions may also inhibit the formation of MSU crystals by suppressing the activity of the xanthine oxidase enzyme during the conversion of hypoxanthine to xanthine.[7] Quercetin also acts by suppressing NF-κβ and inhibiting the production of prostaglandin E_2 (PGE_2) and thromboxane B_2 (TXB_2).[10]

Red onion juice has an unpleasant taste when consumed directly. Processing red onions into powder using a freeze-drying method provides a more palatable and convenient alternative. Research on the effects of red onion powder on IL-6, TNF-α, and CRP remains limited, so this study aims to determine the effect of red onion powder on inflammatory biomarkers in mice with gouty arthritis induced with MSU crystals.

MATERIALS AND METHODS

Study Design

This study examined the effect of red onion powder on IL-6, TNF-α, and CRP levels in mice with gouty arthritis using an experimental post-test-only control group design. The research was conducted at the Food and Nutrition Study Center (PSPG) Laboratory, Gadjah Mada University (UGM), Yogyakarta, from July to August 2024.

Experimental Subjects

The experimental subjects consisted of twenty-seven male Balb/C mice aged six to eight weeks and weighing approximately twenty to twenty-five grams. The mice were housed in the PSPG UGM Laboratory in Yogyakarta. They were randomized into three groups:

1. Control group
2. Gout arthritis (GA) group. Mice were induced with MSU crystals only.
3. Red onion powder (ROP) group. Mice were induced with MSU crystals and administered red onion powder at a dose of 27 mg per 20 g BW per day for seven days.

Induction of the Gout Arthritis Mouse Model

All mice underwent the first stage of randomization at a 1:2 ratio. Eighteen mice were induced to develop a gout arthritis model by receiving a subcutaneous injection of 1 mg MSU crystals dissolved in 50 µl PBS into the plantar surface of the left hind paw once daily for three days. The remaining nine mice served as the control group and did not receive MSU injections. Successful MSU induction was indicated by edema and erythema on the plantar surface. These induced mice then underwent a second stage of randomization into the GA

and ROP groups. Mice in the ROP group were administered red onion powder for seven days.

Preparation of Red Onion Powder

Fresh red onions were washed and sliced, then processed into juice at a 1:1 ratio. The juice was frozen and subsequently freeze-dried to obtain red onion powder. The powder was administered orally using an oral gavage at a dose of 27 mg/20 g BW once daily for seven days.

Measurement of Inflammatory Biomarkers

Specimens were collected from the orbital vein at the medial canthus of the mice at the end of the study. The inflammatory biomarkers CRP, IL-6, and TNF-α were measured using ELISA.

Statistical Analysis

The mean levels of IL-6, TNF-α, and CRP were analyzed using one-way ANOVA with a significance level of $p < 0.05$, followed by Tamhane's post hoc test for CRP and LSD post hoc tests for IL-6 and TNF-α. Data analysis was performed using SPSS version 26.0.

RESULTS AND DISCUSSION

Mouse Plantar Size

The plantar size of the gouty arthritis model mice and the control mice was measured after MSU injection. The measurements showed that the mice in the gouty arthritis model group injected with MSU crystals had a larger plantar size than the control group, with a mean of 1.79±0.05 mm (Figure 1).

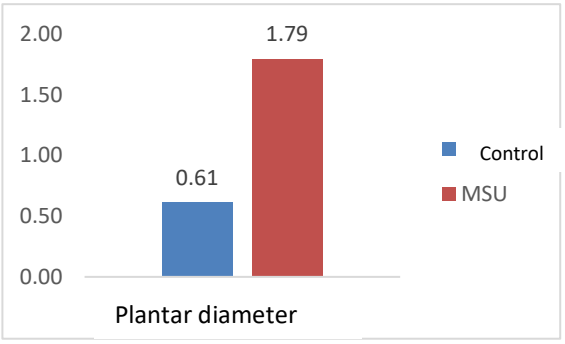

Figure 1 Plantar diameter of mice in the control and gouty arthritis groups.

The results obtained are consistent with previous studies demonstrating that induction with 1 mg of MSU crystals dissolved in 50 µl PBS for 3 days can increase the plantar diameter of mice.[11] The accumulation of MSU crystals triggers the

production of IL-1β and the activation of the nucleotide-binding oligomerization domain-, leucine-rich repeat-, and pyrin domain-containing protein 3 (NLRP3) inflammasome, which leads to the release of inflammatory cytokines such as IL-6, TNF-α, and IL-8, resulting in inflammation and subsequent edema.[7]

Effect of Red Onion Powder on CRP, IL-6, and TNF-α Levels

Table 1 shows that MSU crystal induction significantly increased CRP, IL-6, and TNF-α levels. The AG group had significantly higher levels of CRP, IL-6, and TNF-α compared with the other groups, while the control group had the lowest levels, followed by the BBM group. The one-way ANOVA test showed $p < 0.05$, indicating that at least two groups differed significantly in their CRP, IL-6, and TNF-α levels.

Table 1 Mean (±SD) levels of CRP, IL-6, and TNF-α

	CRP (ng/mL)	IL-6 (pg/mL)	NF-α (pg/mL)
Control	0,78±0,001	76,12±0,54	6,07±0,20
AG	2,41±0,02	139,65±1,01	16,07±0,27
ROP	0,93±0,01	95,33±0,99	8,09±0,51
P value	<0,001^	0,001^	<0,001^

^one-way ANOVA

Effect of Red Onion Powder on CRP Levels

CRP levels in the AG group (2,41±0,02 ng/mL) were the highest, showing that gouty arthritis induced with MSU crystals triggered an inflammatory response. MSU crystal induction increases uric acid levels, which may elevate acute-phase proteins. Subcutaneous injection of MSU crystals in rats closely resembles the pathophysiology of human gouty arthritis, as shown by the formation of tophi-like structures under the skin and subcutaneous edema.[12]

The lowest CRP levels were found in the control group. Since the control group was not induced with MSU crystals, these mice did not develop gouty arthritis. Mice that were not induced with MSU crystals consistently showed lower CRP levels.[13]

The CRP levels in the BBM group were nearly comparable ($p<0.05$) to those of the control group, which was not induced with MSU crystals. Red onion powder contains quercetin, a type of flavonoid known for its anti-inflammatory properties. Quercetin inhibits the activity of cyclooxygenase enzymes COX-1 and

COX-2, which are produced in response to inflammation.[14] It also suppresses NF-κB, a key transcription factor involved in inflammation. Inhibiting cyclooxygenase activity and suppressing inflammatory signaling reduces the formation of proinflammatory cytokines, which prevents an increase in acute-phase proteins such as CRP. Therefore, quercetin demonstrates pharmacological anti-inflammatory effects.[15]

Effect of Red Onion Powder on IL-6 Levels

The IL-6 level in the AG group (139,65±1,01 pg/mL) was the highest among all groups. The increase in IL-6 in the AG group occurred because MSU crystal induction in gouty arthritis triggers interleukin release from monocytes, which leads to neutrophil accumulation that contributes to the inflammatory process.[16] The increase in IL-6 occurs because MSU crystal induction promotes crystal accumulation, resulting in NLRP3 inflammasome oligomerization and dysfunctional activation. This elevates IL-6 levels and triggers a cascade of inflammatory mediators, which ultimately activates the endothelium, recruits leukocytes, and contributes to the progression of inflammation.[13,17]

IL-6 levels in the BBM group were lower than those in the AG group ($p<0.05$) and were comparable to those in the control group ($p<0.05$). The anti-inflammatory effect of red onion powder is attributed to its flavonoid content, particularly quercetin. Quercetin inhibits NLRP3 inflammasome activation triggered by MSU crystal induction, preventing caspase-1 activation, which converts pro-interleukin-1β into bioactive IL-1β. IL-1β stimulates the release of inflammatory cytokines such as IL-6.[18] By blocking this mechanism, quercetin reduces inflammatory responses. It also modulates T-cell activation and suppresses inflammatory cytokines, which decreases IL-6 production.[19,20]

Effect of Red Onion Powder on TNF-α Levels

TNF-α levels in the AG group (16.07±0.27 pg/mL) were the highest among all groups. The elevated TNF-α levels indicate an ongoing inflammatory process. This finding is consistent with previous research showing that MSU crystal induction increases the severity of gouty arthritis and causes joint damage, including erosion. Immunohistochemical analyses have also demonstrated significantly higher expression of the proinflammatory cytokine TNF-α in MSU-induced gout models.[21]

Increased TNF-α levels are associated with inflammation in gouty arthritis. MSU crystals are recognized by

NLRP3 receptors, forming inflammasomes with adaptor proteins, apoptosis-related proteins containing CARD (ASC), and pro-caspase-1. Pro-caspase-1 is cleaved to produce caspase-1. Caspase-1 then cleaves the pro-IL-1β precursor to generate active IL-1β after stimulation with MSU crystals.[22]

The decrease in TNF-α levels is attributed to the effects of red onion powder, which contains high levels of flavonoids, particularly quercetin, known for its anti-inflammatory and antioxidant properties.[18] Quercetin suppresses the NF-κβ pathway and inhibits the production of prostaglandin E2 (PGE2) and thromboxane B2 (TXB2).[10] It suppresses NF-κβ activation by inhibiting NF-κβ transcription and preventing the degradation of the inhibitor of nuclear factor kappa β (Ikβ). NF-κβ is found in the cytoplasm associated with the Ikβ protein. Upon signal reception, the NF-κβ, MAPK, and TNF receptor-associated factor (TRAF) genes activate Inhibitor-κβ kinase (IKK). Quercetin can act on these genes, which leads to the suppression of IKK activation. This suppression, in turn, limits NF-κβ activation and reduces the activation of cyclooxygenase 2 (COX2).[23] In addition to quercetin, other compounds in red onions, such as thiosulfate and cepaenes, also exert anti-inflammatory effects. They inhibit polymorphonuclear leukocyte chemotaxis and reduce arachidonic acid production by inhibiting COX and LOX enzymes. This inhibition prevents the formation of leukotrienes, TXB_2, and PGE_2, ultimately lowering the production of proinflammatory cytokines such as IL-1α, IL-4, and TNF-α.[18]

This study used a single dose. As a result, the CRP, IL-6, and TNF-α levels in the red onion powder group did not reach values comparable to those of the control group. Further studies are therefore needed to identify the optimal dose that may produce levels more closely resembling those of the control group. Another limitation of this study is that the success of MSU crystal induction was evaluated solely through plantar edema measurements using a digital caliper. A gold standard assessment, such as joint fluid aspiration, would be required to confirm that the mice truly developed gouty arthritis.

CONCLUSION

Red onion powder has an effect on inflammatory biomarkers in a mouse model of gouty arthritis. This effect is reflected in the reduction of CRP, IL-6, and TNF-α levels.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in the preparation of this article.

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