Tempuyung Leaves (Sonchus arvensis) Ameliorates Monosodium Urate Crystal-Induced Gouty Arthritis in Rats through Anti-Inflammatory Effects.pdf

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Tempuyung Leaves (Sonchus arvensis) Ameliorates Monosodium Urate Crystal-Induced Gouty Arthritis in Rats through Anti-Inflammatory Effects

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Abstract

BACKGROUND: Gouty arthritis, a chronic inflammatory disease characterized by severe pain and swelling in one or more synovial joints, as a result from joint deposition of monosodium urate (MSU) crystals. Tempuyung (Sonchus arvensis) is a plant that has been extensively studied in the role of shedding kidney stones and diuretics. It is presumed that it also has great potential in shedding MSU crystals in the joints.

AIM: This study focused on exploring the anti-inflammatory role of tempuyung extract (ET) on pro-inflammatory cytokines in gout arthritis white rats.

METHODS: The extract of tempuyung was performed to obtain ET. A total of 30 White rats were randomly divided into the following six groups, each containing five rats: Normal control group, MSU group (negative control), MSU + cetuximab group (Cat 0.2 mg/kg), and MSU + ET group (at dose of 25 mg/kg, 50 mg/kg, and 100 mg/kg). Gouty arthritis was induced with 50 ml of MSU solution (20 mg/ml), which was injected into the left ankle joint cavity on day 7. Synovial fluid was evaluated for the examination of Western blotting of tumor necrosis factor α (TNF-α). A portion of synovial tissue was fixed in 4% paraformaldehyde for histopathological examination. Interleukin (IL)-1β levels in the synovial fluid of the joints were examined by IL-1β rat enzyme-linked immunosorbent assay. Statistical analysis was performed with one-way ANOVA followed by post hoc.

RESULTS: The histopathological image of the MSU model group showed a large number of inflammatory cells displaying an inflammatory reaction. This inflammation response decreased in the ET treatment group in dose-dependent manner. ET showed the effect of decreased pro-inflammatory cytokines expression in both IL-1β and TNF-α, as the dose increased.

CONCLUSION: Tempuyung extract possessed an anti-gout arthritis effect in white rats induced by MSU, by reducing the inflammatory response in the synovial joint.

Introduction

Gouty arthritis, a chronic inflammatory disease characterized by severe pain and swelling in one or more synovial joints, as a result from nucleic acid metabolism and joint deposition of monosodium urate (MSU) crystals [1]. In acute gouty arthritis, MSU crystals induce infiltration of leukocyte masses into the joint cavity and phagocytosis by monocytes/macrophages, production of membranolysis, reactive oxygen species (ROS), and the release of the lysosome enzyme. MSU crystals further induce the conversion of pro-caspase-1 to active caspase-1, which then breaks down pro-interleukin (IL)-1β into the active form, IL-1β [2]. Along with chemotaxis factors, IL-1β can activate other pro-inflammatory cytokines, including tumor necrosis factor (TNF-α) and nuclear factor (NF)-κB, which are very important for the initiation and spread of the inflammatory response and contribute to bone erosion and damage [3].

Nonsteroidal anti-inflammatory drugs (naproxen and indomethacin), corticosteroids, and colchicine are currently used as first-line therapy for gouty arthritis [4]. However, these drugs are associated with serious side effects, including kidney toxicity, gastrointestinal toxicity, liver damage, and myelosuppression [5]. Therefore, identifying new agents with better efficacy and reducing toxicity for the treatment of gouty arthritis is needed.

Exploration of natural materials as a new modality in the management of gout arthritis is a strategy, considering that Indonesia is the country with the world second largest natural capital. [6]. Various plants with therapeutic potential are numerous and easily found in Indonesia tempuyung (Sonchus arvensis) is a plant often found in Indonesia. This plant has been extensively studies in connection with the role of this plant in shedding kidney stones and diuretics. Kidney stones are composed of various compounds, in which calcium and uric compounds are the main compounds making up kidney stones. With the potential that tempuyung possess in shedding kidney stones, it is presumed that
it also has great potential in shedding MSU crystals in
the joints. This potential is further strengthened by
the diuretic ability of tempuyung which can accelerate
the clearance of MSU crystals. Tempuyung contains
flavonoid compounds (catechin, myricetin, routine,
quercitin, kaempferol, orientin, and hyperoside); phenol
acid (cinamic acid); terpenoids (carotenoids and
saponins); alkaloids; and vitamins B and C. Flavonoids
possesses the potential as antioxidants, where these
compounds act as electron donors to prevent the
destructive reactivity of ROS. ROS is the initiator of
inflammation caused by MSU deposition in the joints,
which causes complaints and disorders of gout arthritis.
Tempuyung with antioxidant potential is very promising
to be investigated to reduce the inflammation in the
condition of gout arthritis, with the potential to shed MSU
crystals and increase MSU clearance [5], [7], [8].

This study was the first to explore the effects of
tempuyung extracts (ET) on the inflammatory response
in the joint of the experimental animal: white rat (Rattus
norvegicus) induced by giving MSU crystals. This study
focused on exploring the anti-inflammatory role of ET on
pro-inflammatory cytokines in gout arthritis white rats.

Methods

Animal preparation

A total of 30 male Wistar rats (weight 200 ±
20 g) were obtained from Eureka Research Laboratory
(Palembang, Indonesia). Experimental animals were
placed in cages under controlled conditions (12 h
of light/dark cycles with temperatures of 22 ± 1°C
and humidity of 40–60%), with feeding ad libitum. All
animal treatments and experimental procedures were
approved by the Ethics and Humanities Commission of
the Faculty of Medicine, Sriwijaya University (No.231/ kptkunsri-rsmh/2019).

Tempuyung extract preparation

Simplisia of tempuyung was obtained from the
Institute for Research of Traditional Medicine,
Tawangmangu, Central Java, Indonesia. The extraction
of tempuyung was done using maceration method. A total
of 500 g of simplisia were macerated with 96% ethanol
for 72 h. Then, the separation was performed between
pulp and macerate. The macerate was then evaporated
with a rotary evaporator (Heidolph), to obtain ET.

MSU crystal synthesis

Uric acid (0.8 g) was dissolved in 155 ml
Aquabidest containing 5 ml NaOH (1 M), and the pH
was adjusted to 7.2 with HCl. Gout solution was cooled
and stirred at room temperature, and stored overnight
at 4°C for crystal formation. Next, the precipitate was
filtered from the solution, dried at 70°C for 4 h, ground
into a fine powder, sieved with a 200 mesh metal filter,
stereilized by heating at 180°C for 2 h and stored in
sterile conditions. Before administration, MSU crystals
were suspended in saline-buffered phosphate saline
(phosphate-buffered saline; pH 7.2) at 20 mg/ml [9].

Gouty arthritis animal model

After 1 week of adaptation, rats were randomly
divided into the following six groups, each containing
eight rats: Normal control group, MSU group (negative
control), MSU + colchicine group (Col; 0.28 mg/kg), and
MSU + ET group (at dose of 25 mg/kg, 50 mg/kg, and
100 mg/kg). Before MSU administration, rats in the Col
group, as a positive control group, were given orally with
0.28 mg/kg/day Col for 7 days (Dexa Medica, Indonesia).
Animals in the ET treatment group were given ET once
a day for 7 days, and the doses used were 10, 50, and
100 mg/kg, respectively. The control group and the
model group were given the same volume as 0.9% saline
(10 ml/kg) 7 days. Furthermore, gouty arthritis was
induced on day 7. 1 h after treatment. Rats were
anesthetized by injecting 10% intraperitoneal chloral
hydrate (3.5 ml/kg), after which each rat in the treatment
group was given 50 ml of MSU solution (20 mg/ml),
which was injected into the left ankle joint cavity. Each
animal in the control group received 50 μl injection of
saline in the left ankle joint cavity. Mice were euthanized
by intraperitoneal injection of 10% chloral hydrate,
then synovial fluid was evacuated, which was then centrifuged
at 10,000 rpm for 10 min, 25°C and the supernatant
was stored at −20°C for IL-1β analysis by enzyme-
linked immunosorbent assay (ELISA). Meanwhile, the
synovial tissue of the joint was evacuated, in which homogenization and centrifugation were partially carried
out so that a supernatant was obtained and put in a
maize solution (Sigma-Aldrich) and stored at −20°C, for
the examination of Western blotting of TNF-α. A portion
of synovial tissue was fixed in 4% paraformaldehyde buffer
for histopathological examination of synovial tissue.

Histopathology examination

Synovial tissue that has been fixed with a buffer of
4% paraformaldehyde was dehydrated using alcohol
and xylene, then paraffinized and cut as thick as 5 μm using
a rotary microtome (Leica). The result of the slice was then
placed on glass object and stained with hematoxylin and
eosin. Histopathological changes in synovial tissue were
analyzed under a microscope (Olympus).

ELISA of IL-1β

IL-1β levels in the synovial fluid of the joints
were examined by IL-1β Rat ELISA (Cloud Clone),
based on the manufacturer’s protocols. Briefly, 50 µl standard diluent or serum samples were added to wells that had been coated with anti-IL-1β and incubated at 37°C for 30 min. After the plates were washed, 100 µl of biotinylated antibody solution was added and incubated for 30 min at 37°C. After washing 3 times, 50 µl avidin peroxidase complex solution was added and incubated for 15 min at 37°C. After washing, 50 µl of the tetramethylbenzidine color solution was added and incubated in the dark for 15 min at 37°C. Finally, 50 µl stop solution was added to stop the reaction and optical density values were measured using an ELISA reader (Bio-Rad), at wavelength of 450 nm.

Western blot of TNF-α

This process began with the stage of protein extraction. Joint synovial tissue was put into radioimmunoprecipitation assay buffer (Sigma-Aldrich) equipped with phenylmethylsulfonyl fluoride on ice for 5 min. After centrifugation at 12,000 rpm for 10 min at 4°C, the supernatant was collected as total protein lysate. Cytoplasmic protein and nucleus were extracted from synovial tissue using a protein extraction kit (Sigma-Aldrich), based on the manufacturer’s protocol. Briefly, synovial tissue was cut into small pieces and homogenized with a protein extraction agent. After incubation on ice for 15 min and centrifugation at 5000 rpm for 5 min at 4°C, the supernatant was collected as a partial cytoplasmic protein, while the pellet was re-extracted again in the extraction buffer. After incubation on ice for 15 min and centrifugation at 12,000 rpm for 5 min at 4°C, the supernatant was combined with the cytoplasmic protein above. The pellet was then extracted again in an extraction buffer and shaken violently for 30 min at 4°C. After centrifugation at 12,000 rpm for 10 min at 4°C, protein was obtained. Total protein concentration was quantified using the bicinchoninic acid protein assay (Sigma-Aldrich) kit, according to the manufacturer’s protocol.

A total of 40 µg of protein extract was separated at sodium dodecyl sulfate polyacrylamide gel electrophoresis 10%. Furthermore, the protein that had been deposited was transferred to the polyvinylidene difluoride (PVDF) membrane and blocked with 5% nonfat milk in Tris-buffered saline with Tween 20 for 1 h at room temperature. The membrane was incubated overnight at 4°C with the rabbit polyclonal TNF-α 1:700 primary antibodies (Cloud Clone). Next, incubation was performed with secondary antibodies: horseradish peroxidase-conjugated goat anti-rabbit 1:5000 for 45 min at 37°C. Visualization of the results of blotting with chemiluminescence (Bio-Rad) was performed. Standardization of blotting results was done by blotting on β-actin.

Statistical analysis

All data were presented as mean ± standard deviation and all statistical analyses were performed with the SPSS 25 (IBM) program. One-way ANOVA followed by post hoc analysis was carried out to assess differences in the mean expression levels of each protein. p < 0.05 was determined as an indication that there were significant differences in the mean levels.

Results

To observe the effects of ET histopathologically on the synovial and capsule of the knee joint, an evacuation and analysis with H and E staining was performed. Figure 1 shows that when compared with the control group, the histopathological image of the MSU model group showed a large number of inflammatory cells depicting an inflammatory reaction. This inflammation response decreased in the ET treatment group with increasing dose (dose-dependent manner). The ET group with a dose of 100 mg/kg BW possessed the same effect as the positive control group, colchicine.

![Figure 1: Effect of tramiprosate extracts (ET) on mononuclear cells](https://www.id-press.eu/mjs/index)

Based on the theory that pro-inflammatory cytokines, namely, IL-1β and TNF-α, are the main cytokines that play a role in the initiation and propagation of MSU-induced gout arthritis. Table 1 shows that MSU-induced gout arthritis exhibited increased levels of IL-1β and such results are shown in Figure 2, which showed that TNF-α levels showed an increase in the model group. ET showed the effect of decreased pro-inflammatory cytokines expression in both IL-1β and TNF-α, as the dose increased.

| Table 1: Level of IL-1β in synovial fluid |
|------------------------------------------|
| Group          | IL-1β (pg/ml) | p value* |
|----------------|---------------|----------|
| Control        | 25.76±3.41    | 0.00     |
| Model          | 47.8±10.14    | 0.00     |
| ET 25          | 84.4±17.21    | 0.00     |
| ET 50          | 358.3±21.43   | 0.00     |
| ET 100         | 278.2±16.85   | 0.00     |
| ET 150         | 154.2±16.12   | 0.00     |

* VS Model; ANOVA, post hoc Bonferroni; p<0.05. IL, interleukin; ET, Tramiprosate extract.
Discussion

Gout arthritis is an inflammatory disease that results from the deposition of MSU crystals in the joints. The underlying mechanism is the activation of the inflammatory cascade caused by MSU crystals, which has been investigated for several years, and a number of studies have shown that pro-inflammatory cytokines, including IL-1β and TNF-α, and transcription factors, NF-κB, are important in the initiation and propagation of gout arthritis caused by MSU crystals [10], [11], [12], [13]. In the pathogenesis of gouty arthritis, NF-κB signals can stimulate the production of genes that encode pro-inflammatory cytokines. Conversely, overexpression of TNF-α and IL-1β can directly activate the NF-κB pathway, which leads to a positive feedback loop, which further strengthens the inflammatory response and causes joint injury [14], [15], [16], [17]. Usually, NF-κB binds to the inhibiting protein, IκB, and is localized in the cytoplasm. Certain stimuli, including MSU crystals, can cause IκB degradation and NF-κB translocation into the nucleus where it regulates the transcription of various target genes [18], [19], [20], [21], [22], [23], [24]. In this study, serum levels of IL-1β and TNF-α increased significantly in response to MSU crystals.

Exploration of natural materials as a new modality in the management of gout arthritis is a necessity given the absence of optimal management of this disorder. Tempuyung is one of the medicinal plants with potential that can be optimized for the treatment of gouty arthritis. This study showed that tempuyung possessed the ability to reduce the inflammatory response caused by the induction of uric acid crystals in the joints. This study showed that ET reduced the expression of pro-inflammatory cytokines, IL-1β, and TNF-α. It is known that recruitment and infiltration of neutrophils into joint fluid and synovium are a major feature of gouty arthritis. After activation of monocytes and neutrophils, these cells actively phagocytize MSU crystals, which in turn triggers an inflammatory caspase response. Therefore, inhibiting inflammatory cell infiltration can be an effective therapeutic strategy against gouty arthritis. Histopathological results in this study showed that the dose of ET 100 mg/kg significantly weakened the infiltration of inflammatory cells into synovium induced by MSU crystals and increased synovial hyperplasia, even the ET 100 mg/kg BW effect was comparable to the effects of colchicine. These findings indicate that ET was able to play a role as anti-gouty arthritis which was induced by MSU crystals and synovial damage.

Conclusion

Tempuyung extract possessed an anti-gout arthritis effect in white rats induced by MSU, by reducing the inflammatory response in the synovial joint.

Authors’ Contributions

The authors equally contributed in design, data compiling and analysis, and the composing of the manuscript.

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