



Research Article

EFFECT OF ETHANOL EXTRACT OF *FOENICULUM VULGARE MILL* ON INHIBITION OF URIC ACID CRYSTALS FORMATION IN MALE RATS

Muhammad Yanis Musdja *, Dewa Asmara, Ahmad Musir
Department of Pharmacy, State Islamic University, Jakarta, Indonesia

*Corresponding Author Email: yanis.musdja@uinjkt.ac.id

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ABSTRACT

In traditional medicine *Foeniculum vulgare Mill* (fennel) is used for treatment as antioxidant, anti-cancer activity, anti-inflammatory, antifungal, anti-bacterial and estrogenic effects which are probably due to the presence of aromatic compounds such as anethole, estragole and fenshon. some people, used it for the treatment in gout disease. The purpose of this study was to determine the effect of fennel seed in reducing uric acid levels in serum of male rats. The fennel seed was obtained from Balitro Bagor, Indonesia. Preparation of fennel seed extract was done by cold maceration extraction technique using ethanol 70%. To increase uric acid levels in blood serum, the rats were induced by using caffeine with dose 27 mg/200 g b.w. The rats were divided into 6 groups. In positive control, allopurinol 36 mg/200 g b.w was used. To increase uric acid levels in rat blood, caffeine 27 mg/kg b.w was used. Test preparation was suspended in Na-CMC 0.5%. For test preparation 3 doses were used, i.e, 75 mg/200 g b.w, 150 mg/200 g b.w and 300 mg/200 b.w. Increase in blood uric acid levels was done by inducing caffeine 27 mg/200 g b.w in rats. The blood uric acid levels of the rats were measured on the 6th day in order to ensure that caffeine with such doses causes hyperuricemia. On the 7th day, treatment was given to each group per day and caffeine was also given to all groups except the normal group. Measurement of blood uric acid levels were done on the day 9th, 12th and 15th. Statistical analysis on the 15th day with one-way ANOVA and Least Significant Difference (LSD) test showed that all of the test preparation groups of fennel seeds decreased uric acid. Optimum results were obtained on day 15th with decreased uric acid levels for positive control at the doses of 75 mg/200 g b.w, 150 mg/200 g b.w and 300 mg/200 g b.w with 58.63%, 50.84%, 40.25% and 43.69% respectively and significantly different from the negative control ($p \leq 0,05$), while the decrease effect of dose of 75 mg / 200 g b.w with positive control was not significantly different ($p \geq 0,05$).

Keywords: Fennel, *Foeniculum vulgare*, gout, joint pain, rat, uric acid.

INTRODUCTION

Gout is a metabolic remnant of various purine substances found in food. This substance is usually found in certain foods such as liver, gizzard, seafood and others. Normal levels of uric acid in the human body are: In men less than 7 mg/dl, In women less than 6 mg/dl. In the condition of more uric acid in the body, a person easily experiences the deposition of uric acid crystals, especially in the joints. The causes of gout in general can be divided into two, namely: decreased expenditure of uric acid and increased production of uric acid in the body. Signs and symptoms of gout include: Joint pain, the joints experience swelling and redness, joints are difficult to move, changes in joint shape^{1,2}.

The classification of drugs used in gout, based on the mechanism of action, can be divided into:^{3,4}

- Inhibit uric acid synthesis: Allopurinol
- Increase uric acid excretion: Probenecid, Sulphinpyrazone
- Inhibit neutrophil migration into joint: Colchicine
- Inhibit inflammation and pain: Salicylic acid
- Drugs increasing uric acid oxidation: Urate oxidase

Generally, side effect of these uric acid drugs are drowsiness, headache, diarrhea, vomiting, stomach discomfort, nausea, cramping^{2,3,4}.

In traditional medicine, *Foeniculum vulgare Mill* (fennel) has been used for a wide range of ailments related to digestive, endocrine, reproductive and respiratory systems and also used as a galactagogue agent for lactation, On the other hand, some

people also use *F. vulgare* to treat gout⁵. The results of research from several researchers, showed that *F. vulgare* had Pharmacological effects, namely, antiaging, antiallergic, anticolitic, antihirsutism, anti-inflammatory, antimicrobial and antiviral, antimutagenic, antinociceptive, antipyretic, antispasmodicantistress, antithrombotic, anxiolytic, apoptotic, cardiovascular, chemomodulatory action, cytoprotection and antitumor, cytotoxicity, diuretic, estrogenic properties, expectorant, galactogenic, gastrointestinal effect, hepatoprotective, human liver cytochrome P450 3A4 inhibitory, hypoglycemic, hypolipidemic, memory-enhancing property, nootropic, and oculohypotensive activities^{5,6}.

There are about 60 chemical compounds from *F. vulgare* that are known,^{5,8} namely: Scopoletin (1), Bergapten (2), Psoralen (3), Fenchone (4), para-Anisaldehyde (5), (z)-9-Octadecenoic (6), Dillapional (7), Imperatorin (8), Dillapiol (9), Limonene (10), Terpeneol (11), Fenchone (12), 1,8-Cineole (13), Beta-myrcene (14), 5-Methoxypsoralen (15), para-Anisaldehyde (16), Photoanethole (17), trans-Anethole (18), Dianethole (19), (E)-Phytol (20), Phenylpropanoid (21), 2,4- Undecadienal (22), o-Cymene (23) 2-Hydroxy-3-methyl-2-cyclopenten-1-one (24) Neophytadiene (25), 1,3-Benzenediol (26), Methylchavicol (27), Anethole (28), Linoleic acid (29), 1-Methoxycyclohexene (30), Estragole (31), Sorbic acid (32), 3-Methyl-2- cyclopenten-1-one (33), 3,4-Dihydroxyphenethylalcohol-6-O-caffeoyl-beta-D-glucopyranoside (34), Rosmarinic acid (35), Isoquercetin (36), Oleic acid (37), 3-O-Caffeoylquinic acid (38), 4-O-Caffeoylquinic acid (39), 5-O-Caffeoylquinic acid (40), 1,3-O-Di-caffeoylquinic acid (41), 1,4-O-Di-caffeoylquinic acid (42), 1,5-O-Di-caffeoylquinic acid (43), 3, 8-Binaringenin

(44), Quercetin-3-O-glucoside (45), Quercetin-3-O-galactoside (46), Quercetin-3-O-arabinoside (47), Quercetin-3-O-rutinoside (48), Kaempferol-3-O-glucoside (49), Kaempferol-3-O-arabinoside (50), Kaempferol-3-O-rutinoside (51), Quercetin-3-glucuronide (52), Kaempferol-3-glucuronide (53), Undecanal (54), Exo-fenchyl acetate (55), Dillapional (56), cis-Miyabenol C (57), Eriodictyol-7-rutinoside (58), Limonene-10-ol (59), Isorhamnetin-3-O-glucoside (60), Besides that *F. vulgare* contains essential oils with 87 volatile chemical compounds.

Based on the Structure Activity Relationships (SAR) concept, compounds have the same basic chemical structure but differ in functional groups, will have the same pharmacological work but differ in potential efficacy.^{9, 10, 11} When compared based on the SAR of the compounds contained by *F. vulgare* with chemical compounds that are currently widely used to reduce levels of uric acid in the blood, namely Allopurinol, Salicylic acid, Probenecid and Colchicine (Figure. 3), there are a possibility about 7 chemical compounds contained by *F. vulgare* work to reduce uric acid in rats, namely compounds (Figure. 4): Scopoletin has a basic structure similar with Colchicine. Bergapten, psoralen, dillapiona and dillapiol have a basic structure similar with allopurinol. Limonene-10-ol and 1,3-Benzenediol have a basic structure similar with Salicylic acid.

MATERIALS AND METHODS

F. vulgare seeds were purchased from Research Institute for Spices and Medicinal Plants (Balitro) Bogor, Indonesia, then plant authentication was conducted at the Biology research center, Indonesian Institute of Sciences, Bogor, Indonesia.

The male white rats, strain of Sprague-Dawley with 3-4 months old (weight 190-250 g) were purchased from the Faculty of Veterinary Medicine, Bogor Agricultural Institute, Indonesia, The rats were acclimatized for 15 days before the experiment. before the experiment was done, the rats were fasted for 10 hours.

The number of rats per group for experiment was calculated based on Federer's formula:¹²
 $(n-1).(t-1) = (6-1).(4-1) > 15$. Or each group consists of 4 rats.

Simplicia of *F. vulgare* fruit powder was extracted repeatedly by maceration method by using 70% ethanol solvent and shaken occasionally. Maceration was done for 3 weeks, every two days the solvent was replaced and filtered, the liquid extract obtained was dried evaporated by using a vacuum rotary evaporator. The extract that was obtained determined the content of the chemical compounds group by using the Farnsworth method.¹³

The drugs used in this study were calculated as follow;¹⁴ the dose of allopurinol as a positive control used for humans is 200 mg/day and conversion factor from human to rat is 0.018, the pharmacokinetics factor used is 10, so that, dose for rat is $200 \text{ mg} \times 0.018 \times 10 = 36 \text{ mg} / 200 \text{ g b.w}$. The dose of caffeine for humans is 150 mg/day, conversion factor from human to rat is 0.018, the pharmacokinetics factor used is 10. So that, dose for rat is $150 \text{ mg} \times 0.018 \times 10 = 27 \text{ mg}/200 \text{ g b.w}$. The procedure for this study, as shown in table 1.

In this study, the rats were induced with caffeine 27 mg/200 g/b.w. Uric acid levels in rats blood were already high on the sixth day.⁵ On the seventh day, the treatment was given based on each group every day. Caffeine was also administered to all groups except the normal group. Measurement of blood uric acid levels continued on the day, 9th, 12th and 15th.

The measurement level of uric acid in the blood was done by taking the blood on the rats tail and measured with equipment (Easy Touch) by using uric acid strip.

Study on animals were conducted as per ethical guidelines.

Table 1: Grouping of test animals

No	Treatment
1	Normal control group were given solution of Na-CMC 0.5%
2	Negative control were given caffeine 27 mg/200 g b.w in solution of Na-CMC 0.5%
3	For low doses were given caffeine 27 mg/200 g b.w in solution of Na-CMC 0.5% and 175 mg/200 g b.w extract <i>F. Vulgare</i> in solution of Na-CMC 0.5%
4	For middle doses were given caffeine 27 mg/200 g b.w in solution of Na-CMC 0.5% and 350 mg/200 g b.w extract <i>F. Vulgare</i> in solution of Na-CMC 0.5%
5	For high doses, caffeine 27 mg/200 g b.w in solution of Na-CMC 0.5% were given and 700 mg/200 g b.w extract <i>F. Vulgare</i> in solution of Na-CMC 0.5%
6	As positive control were given caffeine 27 mg/200 g b.w and alopurinol 36 mg/200 g b.w in solution of Na-CMC 0.5%

RESULTS AND DISCUSSION

The results of the authentication of plants was carried out by the Center for Biological Research of Indonesian Institute of Sciences, Bogor, Indonesia. Plants used for this research were fennel fruit (*Foeniculum vulgare Mill*).

The maceration result of 500 g of fennel fruit powder yield of 15.18% that was carried out by using 70% ethanol, then was dried by using a vacuum rotary evaporator.

Screening examination results of the groups of chemical compounds of fennel fruit extract are shown in Table 2

Table 2: Results of the analysis of the content of the chemical compounds groups of fennel fruit extract with the Harborne method

Chemical groups	Analysis results
a. Alkaloid	-
b. Flavonoid	+
c. Saponin	+
d. Steroid/triterpenoid	-
e. Tannin	-
f. Quinon	-
g. Essential oil	+
h. Qoumarine	-

The results of measurements of the average levels of uric acid in the blood of test animals during the experiment were shown in Table 3 and Figure 1.

Table 3: Results of measurements of mean blood uric acid levels of test animals during the experiment (mg/dl)

Time (days)	Normal Control	Negative Control	Positive Control	Low Dose Extract	Medium Dose Extract	High Dose Extract
0	1.65	1.48	1.30	1.28	1.43	1.35
6	1.50	2.90	2.78	2.95	3.23	2.93
9	1.43	3.33	2.45	2.65	2.65	2.83
12	1.50	3.55	1.78	2.58	2.20	2.40
15	1.43	3.85	1.15	1.45	1.93	1.65

The results of statistical tests, one-way ANOVA and LSD for the 6th day showed that blood uric acid levels in all groups of test animals were significantly different ($p \leq 0.05$) with normal control groups. As shown in Table 2 and figure 1. That all animals induced by caffeine had increased levels of uric acid (hyperuricemia). On the 9th day the work effect of the test preparation had not worked well, this was indicated by ANOVA and LSD tests which were still significantly different from normal group test animals ($p \leq 0.05$) and not significantly different from negative group animals or animals suffering from hyperuremia because it was induced by caffeine

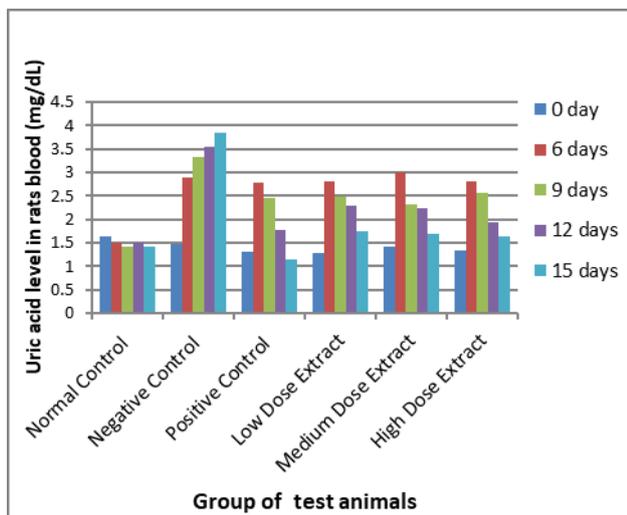


Figure 1: The Chart of measurement of mean blood uric acid levels of test animals during the experiment (mg/dl)

On the 12th day, medium-dose, high-dose and positive-control test work was effective, this was indicated by the results of one-way ANOVA statistical tests and LSD that were not significantly different from normal animal groups ($p \geq 0.05$) and significantly

Table 3: Average percentage of increase and decrease of uric acid levels on the test rat group

Time (days)	Normal Control	Negative Control	Positive Control	Low Dose Extract	Medium Dose Extract	High Dose Extract
9	-7	43	-33	-32	-130	-22
12	0	65	-100	-50	-77	-85
15	-7	95	-163	-105	-130	-115

As we know, that based on Structure Activity Relationship (SAR), pharmacological effects of a new chemical compound, can be predicted from its molecular structure using data from similar compounds that are similar. This is because similar compounds usually have similar physical and biological properties. In other words there is a relationship between the structure and pharmacological activity of compounds that resemble their chemical structure. Structure-activity relationships (SAR) are key to many aspects of drug discovery, ranging from primary screening to lead optimization. the development of a chemical series involves optimizing multiple physicochemical and biological properties simultaneously.^{9, 15, 16}

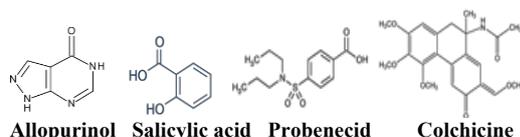


Figure 3: Chemical compounds are widely used to reduce uric acid levels in gout

different against negative group animals ($p \leq 0.05$) or animals suffering from hyperuremia because they were induced by caffeine.

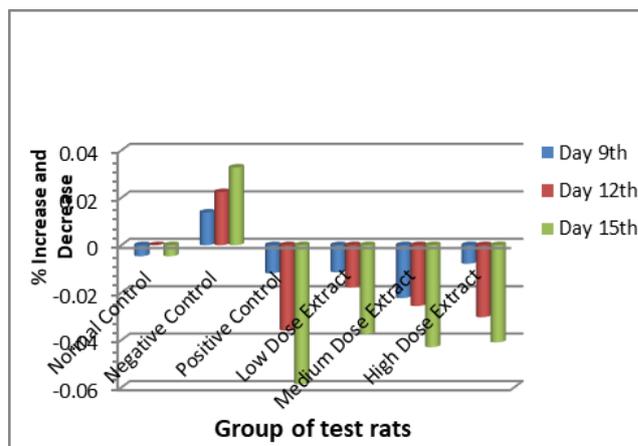


Figure 2: The chart of average percentage of increase and decrease of uric acid levels on the experimental rats groups

The work of all test preparations and positive controls on the 15th day were all effective in reducing uric acid levels, this was indicated by the results of one-way ANOVA test and LSD which were not significantly different from normal controls ($p \geq 0.05$) and were significantly different from the groups negative or animals suffering from hyperuremia because it was induced by caffeine ($p \leq 0.05$).

As mentioned above, that up to now there have been found around 60 chemical compounds in *F.vulgare*, there are about 7 chemical compounds from 60 chemical compounds that have basic chemical formulas similar to drugs for gout which are often used today. The chemical structure of the drugs for gout is shown in the figure 3.

If we observe some structural forms of chemical compounds present in *F. vulgare*, such as Scopoletin, Bergapten, Psoralen, Dillapional and Dillapiol, it is possible that these compounds have pharmacological effects similar to Allopurinol due to the proximity of their chemical structure. Besides that there is also the possibility that Limonen and 1,3-Benzenediol have a pharmacological effect similar with Salicylic acid due to the proximity of their chemical structure. Then it also does not rule out the possibility of Bergapten and Psoralen compounds have pharmacological effects similar with Colchicin or Limonen and 1,3-Benzenediol have pharmacological effects similar with Probenecid. One more possibility is that the synergy of the chemical compounds of *F. vulgare* has a pharmacological effect as anti-gout.

Based on, Structure Activity Relationships (SAR) are relations between the molecular structure and biological or physicochemical activity of chemicals or in pharmacology, chemical compounds that have the same chemistry and differ in functional groups, will have the same properties but differ in potential efficacy.^{9,10, 17.}

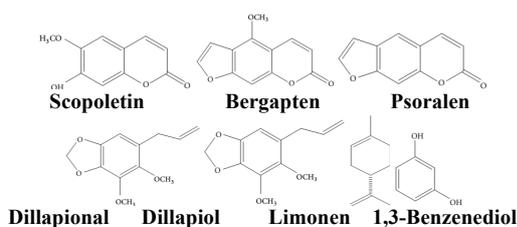


Figure. 4: The chemical compound content of *F. vulgare* which is likely to reduce uric acid levels in gout

CONCLUSION

Fennel fruit extract (*F. vulgare*) showed the effect of reducing blood uric acid levels in male white rats which were induced by caffeine, low doses (75 mg / 200 g BB). Thick extract of fennel fruit decreases blood uric acid levels which are quite significant and different significant negative control ($p \leq 0.05$) on day 15 and not significantly different from Allopurinol as positive control ($p \geq 0.05$).

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