Antihyperuricemic effects of extracts from bulbs of bawang merah (Allium cepa L.) and bawang putih (Allium sativum L.) in hyperuricemic mice

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Abstracts

Current antihyperuricemic drugs which inhibit uric acid synthesis, have limited availability. Allopurinol, a drug used primarily to treat hyperuricemia, is still imported from outside Indonesia. However, many bulbous plants in Indonesia contain flavonols (quercetin, morin, kaempferol, myricetin), which have been reported to inhibit xanthin oxidase activity and decrease uric acid level in serum. In this study, extracts from bulbs of bawang merah (Allium cepa L.) and bawang putih (Allium sativum L.) were selected to investigate their antihyperuricemic activity with in vivo assays. Male ddY mice were divided into nine groups: a normal group given a standard lab diet and other eight experimental groups each given high-purine diet from essence of chicken for 7 days. Then, these experimental groups were induced by potassium oxonate, a urate oxidase inhibitor, one hour before extract or allopurinol administration. Seven experimental groups were given ethanol extracts from bulbs of bawang putih (200, 400, 800 mg/kg body weight), ethanol extracts from bulbs of bawang merah (200, 400, 800 mg/kg body weight), and allopurinol (39 mg/kg body weight). Blood samples were collected from heart, and uric acid levels were measured by DCHBS (dichloro-hydroxybenzene sulfonate)
method using spectrophotometer. The effectiveness of ethanol extracts from bulbs of bawang merah to reduce the uric acid levels at 200, 400, and 800 mg/kg body weight were 0.49%, 19.41%, and 39.56% respectively. While the effectiveness of ethanol extracts from bulbs of bawang putih at 400 and 800 mg/kg body weight were 12.29% and 43.24% respectively. The effectiveness of allopurinol to reduce uric acid level was 75.92%, relatively high compared to groups given extracts, due to hypouricemic condition of the mice. In summary, ethanol extracts from bulbs of bawang merah (*Allium cepa* L.) and bawang putih (*Allium sativum* L.) are potential to reduce uric acid levels in hyperuricemic mice especially at high dose (800 mg/kg body weight).

Keywords: antihyperuricemic effect, *Allium sativum, Allium cepa*.

**Background**

Nowadays, prevalence of hyperuricemia has tended to increase globally [1]. The hardship of inflammation caused by hyperuricemia and gout drops the work productivity; thus, the therapy and medication of its pathogenicity requires a high concern. Allopurinol, a drug used primarily to treat hyperuricemia and gout, inhibits xanthin oxidase activity to produce uric acid. To overcome the high prevalence of hyperuricemia, discovery of alternative drugs is urgently needed. A new-discovered drug that has similar mechanism to allopurinol and been approved by FDA is febuxostat, but it is not yet available in Indonesia [2]. The dependency of imported drugs tends to rise the medication price in Indonesia. Therefore,
Indonesia should observe its local resources that have potential to decrease uric acid level in serum.

Based on the study of Mo, et al., some flavonoids exhibited inhibition on xanthin oxidase activity and uric acid biosynthesis [3]. These flavonoids which reduce uric acid level in animal serum were flavonols (quercetin, morin, kaempferol, myricetin), flavon (apigenin), and isoflavon (puerarin). Many bulbous plants (Liliaceae) from Indonesia contain the flavonols. One of the plants, bawang merah, contain not only quersetin (3,5,7,3′,4′-pentahydroxyflavone) as major content, but also contain small amount of kaempferol, isorhametin, and myricetin. Bawang putih and bawang merah have been studied for their effect to reduce high blood pressure, lower cholesterol level and antioxidant. However, antihyperuricemic effect of bawang putih and bawang merah has not been studied yet. Thereby, the aim of this research was to investigate the effect from these bulbous plants in hyperuricemic mice and to give more value for these natural resources.

Methods
The methods were adapted from Haidari, et al. [4]. Male ddY mice were divided into nine groups: a normal group given a standard lab diet and other eight experimental groups each given high-purine diet from essence of chicken for 7 days. Then, these experimental groups were induced intraperitoneally by potassium oxonate, a urate oxidase inhibitor, one hour before extract or allopurinol administration. The ethanol extracts were given to these groups in
three doses. Blood samples were collected from heart, and uric acid levels were measured by DCHBS (dichloro-hydroxybenzene sulfonate) method using spectrophotometer. The uric acid level of the experimental groups were compared to standard-diet normal groups and negative groups (hyperuricemic mice without given test compounds).

**Ethanolic Extraction of Bawang Putih and Bawang Merah.**

Bulbs of Bawang Putih and Bawang merah were extracted using maceration method. Bulbs were macerated in 70% ethanol (1:5 w/v) for 4 hours at 50°C. Then, the macerates were filtered and evaporated by rotary evaporator. The concentrated extracts were dried in oven for 20 hours at 55°C. The dried extracts were stored at air-tight container.

**Hyperuricemia Induction on Mice**

A total of 50 male DDY mice (body weight: 20-30 g, age: 2-3 months) were adapted to the new environment for a week and then divided into nine groups (n=6). A normal non-hyperuricemic group was given a standard lab diet and the other eight experimental groups were each given high-purine diet from essence of chicken (28 mL/kg) for 7 days. Then, 250 mg/kg potassium oxonate, dissolved in 0.5% CMC were intraperitoneally administered to each animal in the experimental groups one hour before oral administration of test compounds (allopurinol or extracts).
**Experimental Design**

The eight experimental groups consist of group 1: hyperuricemic mice; group 2: hyperuricemic mice given 39 mg/kg allopurinol; group 3: hyperuricemic mice given 200 mg/kg extracts from bulbs of bawang merah (EBM); group 4: hyperuricemic mice given 400 mg/kg EBM; group 5: hyperuricemic mice given 800 mg/kg EBM; group 6: hyperuricemic mice given 200 mg/kg extracts from bulbs of bawang putih (EBP); group 7: hyperuricemic mice given 400 mg/kg EBP; and group 8: hyperuricemic mice given 800 mg/kg EBP. For seven days, group 3-8 were given the extracts 1 hour after feeding the mice with chicken essence. But for group 2, hyperuricemic mice were given allopurinol only on day 7.

**Blood Collection and Measurement of Uric Acid Level**

After 3 h of potassium oxonate treatment on day 7, mice were anesthetized with diethyl ether and blood was collected from hearts. Plasma was separated from blood cells by centrifugation at 3000 rpm for 10 minutes. Uric acid levels were measured by DCHBS (dichloro-hydroxybenzene sulfonate) method using Biolabo kit and spectrophotometer (RD-60 Semi Auto Biochemistry) at 520 nm.

**Results**

The data of uric acid level from this research was fluctuated because of the biologic variation in each group. However, the data showed a clear pattern (Figure 1). Experimental groups, fed with chicken essence, significantly had higher uric acid levels than normal group (P<0.05). The effectivity of EBM to reduce the uric acid levels at 200, 400, and 800 mg/kg body weight were 0.49%, 19.41%, and
39.56% respectively compared to negative group. While the effectivity of EBP at 400 and 800 mg/kg body weight were 12.29% and 43.24% respectively compared to negative group.

The effectivity of allopurinol to reduce uric acid level was 75.92%. It was higher than the effectivity of EBM and EBP (Table 1). However, the allopurinol groups had 54.63% lower uric acid level than normal, that could be categorized as hypouricemia.

**Discussion**

The search of natural compounds with antihyperuricemic activity to inhibit xanthine oxidase is still ongoing. The exploration of the alternative medicine to hyperuricemia in Indonesia has been organized to overcome the dependency of allopurinol as primer drug. The recurrence pain of hyperuricemia leads individual precaution to retain the normal uric acid level.

The result of this research showed the high potential of EBM and EBP to reduce the uric acid level on hyperuricemic mice. The mice were induced by chicken essence and potassium oxonate. Essence of chicken contains high purine and protein. The data of purine level in chicken essence has not obtained yet; however, in this research, chicken essence together with potassium oxonate, increased the uric acid to 88.43% above normal level.

Potassium oxonate is uricase inhibitor. Uricase is an enzyme in mice which catalyzes uric acid into allantoin. Allantoin dissolve in water that could excreted via urine. Therefore, potassium oxonate was needed to use mice as animal model
in this research that measures the uric acid level in blood plasma. Laksmitawati and Ratnasari on their research showed that the uric acid level in mice leapt the normal level by feeding them chicken essence for 3 weeks [5]. In this research, the uric acid level leapt 88.43% (P<0.05) from the normal level by giving mice chicken essence and potassium oxonate for 7 days.

EBM and EBP at 800 mg/kg body weight are potential to reduce uric acid levels in model-hyperuricemic mice. The reduction effects of uric acid level from mice given extracts were not as high as mice given allopurinol. However, mice given allopurinol at 39 mg/kg body weight showed hypouricemic condition. The hypouricemic effects from allopurinol have not been reported clinically. However, uric acid is endogenous antioxidant that prevents oxidative stress or damage from free radicals. EBM and EBP were highly potential to prevent the hyperuricemic condition without causing the hypo condition.

EBM and EBP contain flavonoids such as kaempferol, isorhametin, and myricetin. Cos, et al. reported that these flavonoids inhibited xanthin oxidase activity in vitro [6]. Xanthine oxidase catalyzes uric acid production. Inhibition of the enzyme could also inhibit the uric acid production. These are probably the basis on how the EBM and EBP could reduce the uric acid level from hyperuricemic condition.
Conclusion

In summary, ethanol extracts from bulbs of bawang merah (*Allium cepa* L.) and bawang putih (*Allium sativum* L.) are potential to reduce uric acid levels in hyperuricemic mice especially at high dose (800 mg/kg body weight).

Acknowledgment

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Tables and figures

![Figure 1. Effect of the ethanol extracts of bawang merah and bawang putih on uric acid level in serum (mean ± SD, n = 6).](image)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Uric acid (mg/dL)</th>
<th>Uric acid compared to negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.16 ± 0.34</td>
<td>-</td>
</tr>
<tr>
<td>Negative</td>
<td>4.07 ± 0.38</td>
<td>-</td>
</tr>
</tbody>
</table>
EBM  200 mg/kg  4.05 ± 0.36  0.49
EBM  400 mg/kg  3.28 ± 0.20  19.41
EBM  800 mg/kg  2.46 ± 0.47  39.56
EBP  200 mg/kg  5.17 ± 0.62  -27.07
EBP  400 mg/kg  3.57 ± 0.36  12.29
EBP  800 mg/kg  2.31 ± 0.46  43.24
Alopurinol       0.96 ± 0.26  75.92

All values are expressed as mean ± SD (n = 6). Independent-sample t-test was used for statistical significance assessment (P<0.05).

References