

Research Article

**The Effect of Melinjo (*Gnetum gnemon* L.) Leaves and Melinjo Peel Extracts on Induced-Hyperuricemia Male Rats Model**

**Nia K Sari\*, Andreanus A Soemardji\*\*, Irda Fidrianny\*\***

*\*Indonesian School of Pharmacy*

*Jl. Soekarno- Hatta no.354 (Parakan Resik), Bandung 40266, Jawa Barat Indonesia*

*\*\*School of Pharmacy – Bandung Institute of Technology*

*Jl. Ganesha no. 10 Bandung, Jawa Barat, Indonesia*

*Email: remy.nia@gmail.com*

**Abstract**

*Hyperuricemia is a condition which blood uric acid levels increase, in men are greater than 7 mg/dL and in women over 6 mg/dL. In Indonesia the prevalence of hyperuricemia was 32% in people under 34 years and increases every year. This study aims to assess the effect of melinjo leaves and peel extracts to reduce uric acid levels on hyperuricemia male rats model. Induction was performed by giving high purine diet, crude melinjo seed 4.5 g/kg bodyweight. Ethanol extracts of melinjo leaves and peel were administered in 3 doses. The study showed ethanol leaves extract of melinjo (*Gnetum gnemon* L.) 36 mg/kg bodyweight decreased uric acid levels up to 61.04%, while ethanol peel extract of melinjo 13 mg/kg bodyweight decreased uric acid levels up to 31.25%. We concluded ethanol extracts of melinjo leaves and peel have the effect to decrease uric acid blood levels in hyperuricemia rats.*

**Keywords :** *Gnetum gnemon* L., seed, leaves, peel, antihyperuricemia

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## Kajian Pengaruh Ekstrak Daun dan Kulit Buah Melinjo pada Tikus Jantan Hiperurisemia Biji Melinjo (*Gnetum Gnemon* L.)

Nia K Sari\*, Andreanus A Soemardji\*\*, Irda Fidrianny\*\*

\*Sekolah Tinggi Farmasi Indonesia

Jl. Soekarno- Hatta no.354 (Parakan Resik), Bandung 40266, Jawa Barat Indonesia

\*\*Sekolah Farmasi - Institut Teknologi Bandung

Jl. Ganesha no. 10 Bandung, Jawa Barat, Indonesia

Email: [remy.nia@gmail.com](mailto:remy.nia@gmail.com)

### Abstrak

Hiperurisemia adalah keadaan dimana terjadi peningkatan kadar asam urat darah, pada pria lebih dari 7,0 mg/dL dan pada wanita lebih dari 6,0 mg/dL. Di Indonesia tercatat prevalensi terjadinya hiperurisemia adalah sebesar 32% pada masyarakat usia di bawah 34 tahun dan terus meningkat setiap tahunnya. Penelitian ini bertujuan untuk mengkaji pengaruh ekstrak daun dan kulit buah melinjo (*Gnetum gnemon* L.) pada tikus jantan hiperurisemia. Induksi dilakukan dengan makanan tinggi purin yaitu suspensi simplisia biji melinjo dengan dosis 4,5 g/kg bb selama 9 hari. Ekstrak etanol daun dan kulit buah melinjo diberikan masing-masing dalam 3 dosis. Induksi dengan simplisia biji melinjo 4,5 g/kg bb selama 9 hari dapat meningkatkan kadar asam urat. Penelitian ini mendapatkan bahwa ekstrak daun melinjo 36 mg/kg bb mampu menekan kadar asam urat hingga 61,04%, sedangkan ekstrak kulit buah melinjo 13 mg/kg bb mampu menekan kadar asam urat hingga 31,25%. Dari penelitian ini disimpulkan bahwa ekstrak etanol daun dan kulit buah melinjo dapat menurunkan kadar asam urat.

**Kata Kunci :** *Gnetum gnemon*, L., daun, kulit buah, antihiperurisemia

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**Introduction**

The prevalence of hyperuricemia has increased worldwide. In Indonesia, the prevalence of hyperuricemia is 32% in people under 34 years and increase every year.<sup>1</sup> One factor that influences the high prevalence of hyperuricemia is consumption of “emping”, a kind of snack made from melinjo seeds, and consumption of melinjo seeds in “sayur asem”, a vegetable soup favored by the Indonesian population.<sup>2</sup>

It is important to find natural compounds which can reduce uric acid levels with high effectiveness and guaranteed security.<sup>3,4</sup> Leaves and peel of melinjo are known to contain flavonoids, which can reduce uric acid levels by inhibiting the action of xanthine oxidase enzyme. The flavonoid is a potential compound with antihyperuricemic activity.<sup>5</sup>

This study aimed to assess the effect of melinjo leaves and peel extracts to reduce uric acid levels on hyperuricemia male rats model.

**Methods**

**Plant Material**

Seeds, leaves, and peel of *Gnetum gnemon* L. were taken from the botanical garden of Monoko, Cikampek, West Java, Indonesia. The specimens were identified in Herbarium Bandungense, Sekolah Ilmu dan Teknologi Hayati, Institut Teknologi Bandung, West Java, Indonesia.

**Preparation of sample**

Melinjos were peeled to separate the peels and seeds. Melinjo seeds were dried and mashed for hyperuricemia induction. Leaves and peels of melinjo were extracted by reflux method with a concentration of 96% ethanol. Melinjo leaves and peel extract were concentrated with the rotary evaporator and then evaporated over the waterbath.



Figure 1 Leaves, peel, and seed of Melinjo

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**Preparation of animal test**

**Antihyperuricemic assay**

This study used male Wistar Rats, weighted 180-200 g, aged 6-8 weeks.<sup>6</sup> The animals were procured from animal house, Biofarma Laboratory, Cisarua, Lembang, Indonesia. Animals were kept in light and dark cycle every 12 hours. All animals were fed on a standard balanced diet and provided with water *ad libitum*. All animal experiments were conducted under institutional ethical guidelines for a week.

The assay was performed using ten groups of male Wistar rats and each group consists of five rats. Animals were fasted for 10 hours before day one measurement. In the next morning the animals were measured for the pre-induction uric acid level using a spectrophotometer. All groups except the Group I (non-hyperuricemia) were given a *simplicia* of melinjo seed 4.5 g/kg bodyweight orally to induced hyperuricemia. Within 1 hour after induction, all groups were treated according to the treatment regimen. Group I (non-hyperuricemia) was given Na CMC 0.5% suspension orally; Group II (positive control) was induced without treatment; Group III was given allopurinol 10 mg/kg bodyweight; Group IV was given probenecid 100 mg/kg bodyweight after 9 days of induction; Group V, VI, VII were given ethanol extract of melinjo leaves at dose 18, 36, and 72 mg/kg bodyweight; Group VIII, IX, X were given ethanol extract of melinjo peel at dose 13, 26, and 52 mg/kg body weight.

Assessment of uric acid levels by serum measurement was carried out on day 4, 7, 10, 14, and 18. Induction was stopped after 9 days of induction, and treatment was stopped on day 18. Blood was collected from tail vein, and was centrifuged at 10.000 rpm for 10 minutes. The uric acid level was measured using kit reagent by spectrophotometer (Microlab 300®).

**Statistical analysis**

Analysis between treatment groups was done using one-way analysis of variance (ANOVA) and independent t-test analysis using SPSS software version 21.0. Values with  $p < 0.05$  indicate significant differences.

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Results

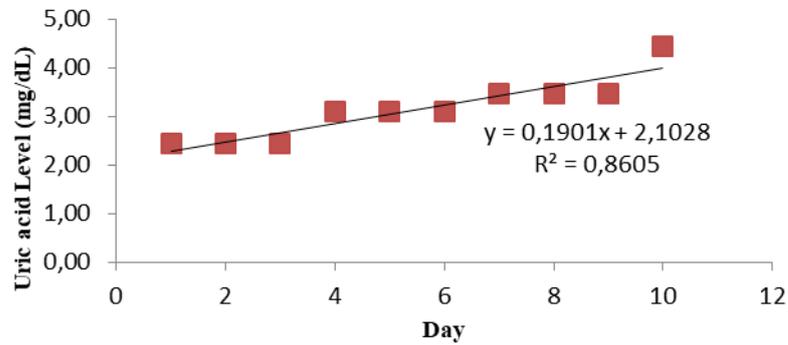


Figure 2 The regression curve of uric acid levels in test control group as measured by spectrophotometer uv-vis

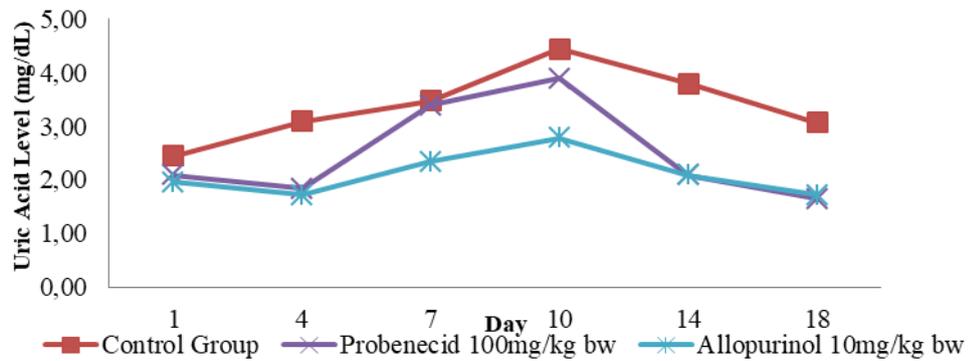
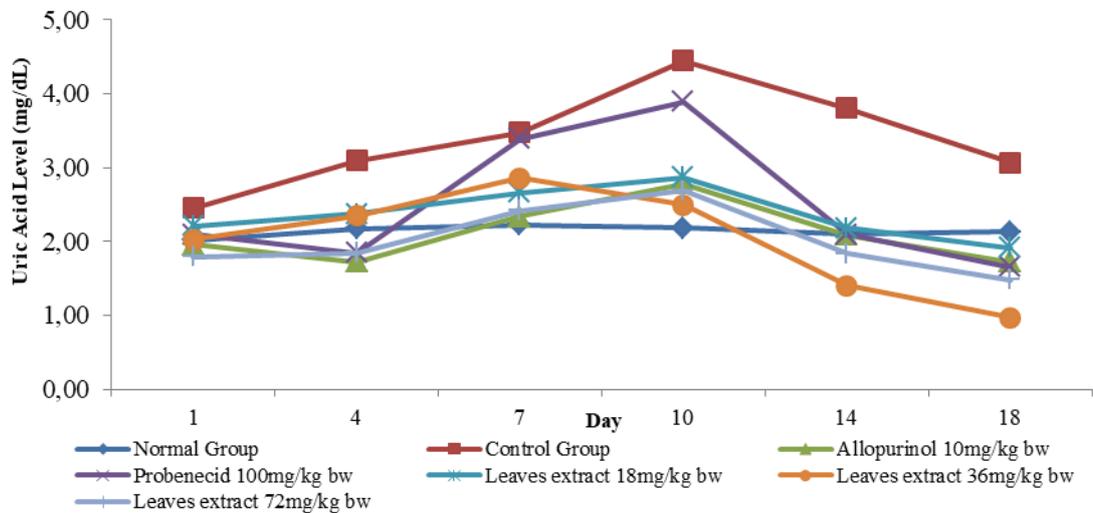


Figure 3 Graph of uric acid levels by treatment of comparative drug therapy as measured by a spectrophotometer uv-vis

Suspension of *simplicia* melinjo seeds was able to significantly increase uric acid levels in male rats blood by 81.2% as measured by spectrophotometer on day 10, compared to the normal group ( $p < 0.05$ ). The allopurinol and probenecid groups were both significantly different from positive control group ( $p < 0.05$ ), on day 4 for allopurinol and day 13 for probenecid.

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**Figure 4 Graph of uric acid level with treatment of melinjo leaves extract measured by a spectrophotometer uv-vis**

The dose of melinjo leaf extract 18 mg / kg bodyweight can significantly reduce uric acid levels ( $p < 0.05$ ) against the positive control group starting on day 4. The level of uric acid in hyperuricemia group of rats given dose of melinjo leaf extract of 36 mg / kg bodyweight and 72 mg / kg bw were significantly reduced ( $p < 0.05$ ) compared to positive control group starting day 6, whereas allopurinol group did not show any significant difference. Thus melinjo leaves extract of 18 mg, 36 mg, and 72 mg / kg bodyweight can reduce uric acid level in the same potency as allopurinol. On day 11 melinjo leaves extract of 36 mg / kg bodyweight also showed a significant difference to probenecid ( $p < 0.05$ ).

Melinjo leaves extract decreased uric acid level after reaching maximum increase (100% hyperuricemia) until last day of treatment, at dose 18 mg/kg bodyweight equal to 33,45%, at dose 36 mg/kg bodyweight equal to 61,04%, and at a dose of 72 mg/kg bodyweight of 44.7%. Dose 36 mg/kg bodyweight leaves extract of melinjo is the most effective dose to decreased uric acid level.

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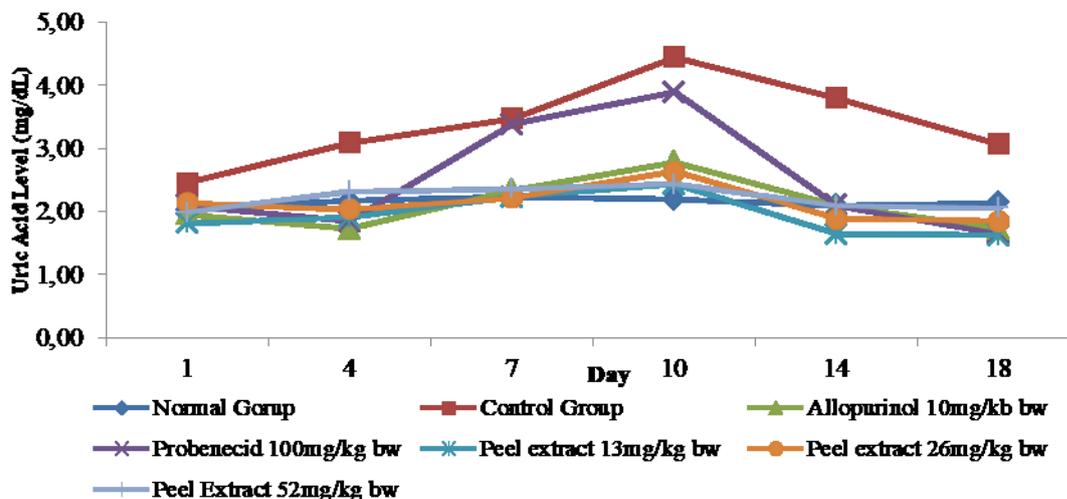


Figure 5 Graph of uric acid level with treatment of melinjo peel extract measured by a spectrophotometer uv-vis

The dose of melinjo peel extracts 26 mg / kg bodyweight can significantly reduce uric acid levels ( $p < 0.05$ ) for positive control group starting on day 3. Melinjo peel extract of 13 mg / kg bodyweight and 52 mg / kg bodyweight significantly reduced uric acid levels ( $p < 0.05$ ) towards positive control group starting on day 4. On the day 11 melinjo peel extract at a dose of 13, 26, and 52 mg / kb bodyweight showed a significant difference to allopurinol ( $p < 0.05$ ).

Peel of melinjo extract decreased uric acid level after reaching maximum increase (100% hyperuricemia) until last day of treatment, at dose 13 mg/kg bodyweight equal to 31,25%, at dose 26 mg/kg bodyweight equal to 30,03% , and at dose of 52 mg/kg bodyweight equal to 15.63%. Dose 13 mg/kg bodyweight peel extract of melinjo is the most effective dose to decreased uric acid level.

**Discussion**

A higher level of blood uric acid is called hyperuricemia and can cause the precipitation of uric acid in the joint, inducing pain and Gout arthritis. Hyperuricemia is a result of increased metabolism (overproduction) of uric acid, or reduction of secretion in the urine (under expression), or a combination of both.<sup>7</sup> Consumption of food containing high purine will increase the risk of hyperuricemia.<sup>3</sup>

Allopurinol is the only drug approved for use in inhibiting uric acid synthesis through the inhibition of xanthine oxidase.<sup>8,9,10</sup> The major side effects of allopurinol are skin rash, leukopenia, and occasional gastrointestinal toxicity.<sup>3,7</sup>

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Probenecid acts by inhibiting the reabsorption of uric acid in proximal tubules and consequently reduces the level of uric acid in the blood.<sup>7</sup> Comparison with probenecid was used to evaluate the ability of extract the lower level uric acid reabsorption in the kidney that can be seen by the increase of uric acid level in the urine.<sup>7</sup>

The group with allopurinol did not reach peak of uric acid levels compared with positive control group. The group with probenecid reach peak of uric acid levels that approached the positive control group, because the probenecid comparison drug was only given on day 10. The group with allopurinol did not show a drastic increase in uric acid levels, but gradually decrease. In group with probenecid the decrease after induction was seen to be drastic. This is in line with the comparison of allopurinol as preventive and probenecid as curative. After reaching peak levels, in 4 days allopurinol reduced uric acid levels by 24.8%, while probenecid 45.6%.

Melinjo leaves and peel are known to contain flavonoids, which can reduce uric acid levels by inhibiting the action of the xanthine oxidase enzyme, so flavonoids are compounds that have the potential as antihyperuricemia.<sup>5,11,12</sup> The inhibitory activity of xanthine oxidase from ethanol extract of melinjo rinds has been tested in vitro at 45.46% compared to the activity of allopurinol 27.28%.<sup>5</sup>

### Conclusion

Ethanol extracts of melinjo (*Gnetum gnemon* L.) leaves and peel has the effect to decrease uric acid blood levels in hyperuricemia rats.

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