



## The Role of Bay Leaf Extract in Reducing Liver Inflammation in Mice (*Mus Musculus*) Induced by Potassium Oxonate

Evy Silvia Awwalia<sup>1,2</sup>, Hotimah Masdan Salim<sup>3</sup>, Mochammad Faishal Riza<sup>4</sup>, Mulyadi<sup>5</sup>

<sup>1</sup> Department of Internal Medicine, Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, Indonesia

<sup>2</sup> Department of Internal Medicine, RSIS Jemursari Hospital, Surabaya, Indonesia

<sup>3</sup> Department of Biochemistry and Biomolecular Science, Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, Indonesia

<sup>4</sup> Department of Cardiovascular Medicine, Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, Indonesia

<sup>5</sup> Department of Pulmonology, Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, Indonesia

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### CORRESPONDENCE

E-mail: [dr.hotimah@unusa.ac.id](mailto:dr.hotimah@unusa.ac.id)

### A B S T R A C T

Hyperuricemia is characterized by elevated uric acid levels in the blood, often stemming from increased uric acid production or inadequate uric acid excretion, resulting in levels exceeding 7 mg/dL. If left unmanaged, this condition can lead to gout arthritis. Elevated serum uric acid levels have also been linked to liver damage, as evidenced by findings in patients diagnosed with Non-alcoholic fatty liver disease (NAFLD), showing increased serum uric acid levels associated with liver damage. This study investigates the potential role of bay leaf extract in reducing liver inflammation related to uric acid metabolism. This study employed a true experimental approach with a post-test control group design, utilizing 8-week-old *Mus musculus* as experimental animals. The groups were divided as follows: Group 1 (control), Group 2 (Potassium oxonate-PO), Group 3 (PO with administration of bay leaf extract at 75mg/kg body weight), Group 4 (PO with administration of bay leaf extract at 150mg/kg body weight), and Group 5 (PO with administration of bay leaf extract at 300mg/kg body weight). Histological examination of the liver in the PO administration group revealed cell infiltration compared to the control group. However, a significant reduction in damaged hepatocyte cells was observed by administering bay leaf extract in PO+EDS-1, PO+EDS-2, and PO+EDS-3 groups ( $P < 0.05$ ). Bay leaf extract demonstrates hepatoprotective effects in hyperuricemia induced by potassium oxonate.

## INTRODUCTION

Hyperuricemia occurs when the kidneys fail to excrete uric acid, which results in elevated uric acid levels. The high levels of uric acid occur due to the deposition of monosodium crystals resulting from the breakdown of purines or a combination of both. Uric acid is excreted into the kidneys with urine. However, the decreased secretion of uric acid into the renal tubules is due to disturbances in uric acid elimination in the kidneys, leading to increased uric acid levels in the blood (Ningtiyas & Ramadhian, 2016). High uric acid levels can be caused by foods high in purines (>200 mg/100 g) (Kaneko et al., 2014). The clinical manifestations of hyperuricemia typically include pain because uric acid stimulates the production of proinflammatory cytokines interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), interleukin 8 (IL-8), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), which induce leukocyte migration to areas of monosodium crystal deposition, exacerbating the inflammatory response (Mardiana et al., 2012). Allopurinol and febuxostat are anti-hyperuricemia drugs that work by reducing or inhibiting the production of the enzyme xanthine oxidase (Dien et al., 2005). Allopurinol is one of the drugs used to lower blood uric acid levels.

Allopurinol works by inhibiting the enzyme xanthine oxidase to convert hypoxanthine to xanthine, which becomes uric acid. However, allopurinol can cause side effects such as allergic reactions on the skin, fever, and others.

One plant believed to have efficacy in treating uric acid-related diseases by reducing blood uric acid levels is bay leaf extract (*Syzygium polyanthum*). Previous studies have shown that ethanol from bay leaves can lower blood uric acid levels, supported by flavonoid compounds with anti-inflammatory properties (Sinaga et al., 2014). Generally, flavonoid compounds can be found in the leaves or flowers of a plant (Panche et al., 2016). Based on literature (Muhtadi et al., 2012), bay leaf content that has anti-hyperuricemia abilities includes the flavonoids quercetin, myricetin, and flavonol, which can inhibit the action of xanthine oxidase by reducing the production of xanthine oxidase enzyme.

However, the discovery regarding the effect of bay leaves on liver inflammation caused by increased uric acid levels is still uncertain. Therefore, this study aims to determine the role of bay leaf extract in reducing liver inflammation related to uric acid metabolism.

## **METHOD**

This study is a true experimental research with the test control Group Design. The research was conducted at the integrated research laboratory of the Faculty of Medicine, UNUSA, and the pharmacology laboratory of the Faculty of Medicine, UNAIR.

### **Experimental Materials**

Potassium oxonate (PO) (Sigma-Aldrich Co., MO, USA), a uric oxidase inhibitor, was applied to induce acute hyperuricemia.

### **Preparation of *Syzygium polyanthum* Extract (Wight.)**

Bay leaves were macerated using 96% ethanol in a ratio of 7.5 times the weight of the test material. Maceration was carried out for five days, stirring once a day. After five days, each macerate was filtered using a flannel cloth. Each macerate was then evaporated using a rotary evaporator at 70°C until a sufficient filtrate was obtained. The filtrate was poured into a porcelain dish and further heated with a water bath at 70°C until a thick extract was formed.

### **Experimental Animals**

This study used *Mus musculus* experimental animals divided into five groups: Group 1 was the control (Control), Group 2 was hyperuricemia (K2 PO), Group 3 was hyperuricemia, and *Syzygium polyanthum* Wight extract 75mg/kg body weight, Group 4 was hyperuricemia and *Syzygium polyanthum* Wight extract 150mg/kg body weight, and Group 5 was hyperuricemia and *Syzygium polyanthum* Wight extract 300 mg/kg body weight given for two weeks.

## Histological Analysis

All sacrificed animals on day 14 were removed, fixed in 10% formalin solution, and processed using paraffin. Sections with a thickness of 5  $\mu\text{m}$  were cut and stained with hematoxylin and eosin (H&E) for histological examination.

## Statistical Analysis

All results are expressed as mean  $\pm$  SEM. An unpaired student's t-test was performed to compare the parameters of the two groups. Dose-response curve comparisons were made using two-way repeated measures, ANOVA, and Tukey's posthoc test for intergroup comparisons. A P-value  $<0.05$  was considered significant.

## RESULT

### The Effect of Bay Leaf Extract on Hepatic Histological Features

Observations on hepatic histology in this study were conducted using hematoxylin and eosin staining and viewed under a light microscope. The histological changes in the liver in the PO administration group in this study were significantly pronounced compared to the control group. Cell infiltration and hepatocyte cell necrosis were observed (Figure 1).

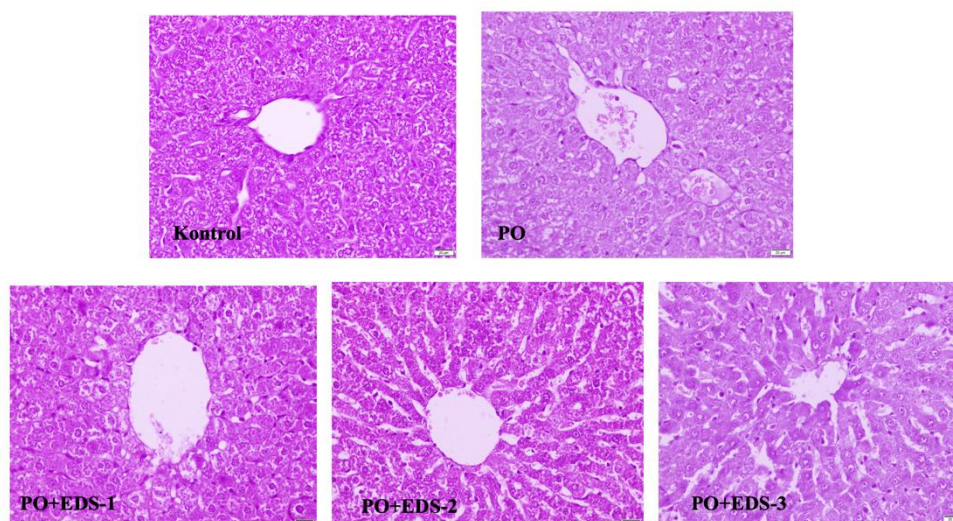


Figure 1. Hepatic histology of PO; potassium oxonate group, PO+EDS-1; potassium oxonate and bay leaf extract dose 75 mg/kg BW group, PO+EDS-2; potassium oxonate and bay leaf extract dose 150 mg/kg BW group, PO+EDS-3; potassium oxonate and bay leaf extract dose 300 mg/kg BW group

### The Effect of Bay Leaf Extract on Hepatocyte Cells

Histological observations of the liver were conducted in five different fields of view at 400x magnification. In each field of view, 20 cells were randomly counted, and 100 liver cells were observed in one preparation. These observations were then recorded, and the percentage of damage was calculated. In Figure 1, hepatocyte cell necrosis was observed and subsequently quantified. There was a significant

increase in hepatocyte cell damage in the PO group compared to the control group ( $P < 0.01$ ). However, the administration of bay leaf extract in the PO+EDS-1, PO+EDS-2, and PO+EDS-3 groups significantly decreased hepatocyte cell damage ( $P < 0.05$ ) (Table 1 and Figure 2).

Group	Hepatocyte cell damage
Control	98,25±10,27##
PO	258,75±12,5***
PO+EDS-1	210±18,25**#
PO+EDS-2	156.25±14,36**#
PO+EDS-3	109,5±12.15##

Table 1. Effect of Bay Leaf Extract on Hepatocyte Cell Damage. PO; potassium oxonate group, PO+EDS-1; potassium oxonate and bay leaf extract dose 75 mg/kgBW group, PO+EDS-2; potassium oxonate and bay leaf extract dose 150 mg/kgBW group, PO+EDS-3; potassium oxonate and bay leaf extract dose 300 mg/kgBW group. Data represent mean  $\pm$  SD. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  compared to the control group. # $P < 0.05$  and ##  $P < 0.01$  compared to the PO group.

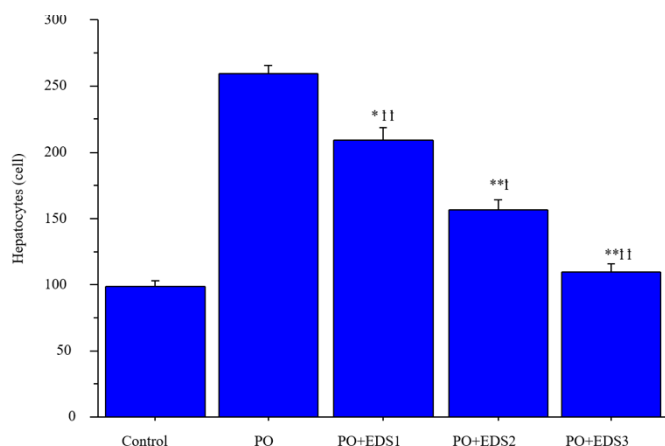


Figure 2. Hepatocyte Cell Damage. PO; potassium oxonate group, PO+EDS-1; potassium oxonate and bay leaf extract dose 75 mg/kgBW group, PO+EDS-2; potassium oxonate and bay leaf extract dose 150 mg/kgBW group, PO+EDS-3; potassium oxonate and bay leaf extract dose 300 mg/kgBW group. Data represent mean  $\pm$  SD

## DISCUSSION

Hyperuricemia can occur due to the accumulation of uric acid in the body, leading to increased purine levels. Purines in the body can be converted into endogenous uric acid by xanthine oxidase enzymes in the blood. This mechanism causes elevated uric acid levels in the blood and prevents maximal excretion. Flavonoids can reduce uric acid levels by inhibiting the activity of xanthine oxidase enzymes (Khalid et

al., 2019). This study found that administering methanol extract from bay leaves reduced hepatocyte cell damage induced by potassium oxonate in hyperuricemia.

The decrease in damaged hepatocytes in this study suggests that the higher the dose of bay leaf extract administered, the lower the decrease in hepatocyte cell damage, particularly at a 300 mg/kg BW dose. This may be influenced by the contents of bay leaves, including fluorettin, quercetin, and myricetin (Muhtadi et al., 2012). Flavonoid content in bay leaves also acts as an antioxidant, inhibiting xanthine oxidase enzyme activity and inhibiting uric acid formation (Harismah, 2016).

Flavonoids such as apigenin, kaempferol, luteolin, fluorettin, quercetin, and myricetin have been found to inhibit xanthine oxidase activity by producing hydrogen peroxide and superoxide anions during the oxidation of hypoxanthine to xanthine and uric acid (Harismah, 2016). The highest inhibition of xanthine oxidase activity is seen in flavonols and planar flavonoids with a 7-hydroxyl group. Hydroxyl groups from chrysin and luteolin at C-5 and C-7 of the flavonoid skeleton have potent inhibitory effects on xanthine oxidase activity (Tungmunnithum et al., 2018)

Quercetin compounds in bay leaf ethanol extract are believed to inhibit xanthine oxidase activity. The inhibition of xanthine oxidase reduces endogenous uric acid production, lowering uric acid levels in the blood (Darussalam & Rukmi, 2019). Additionally, a study has stated that bay leaf extract can reduce serum IL-6 and TNF- $\alpha$  levels in hyperuricemia patients, with the delta value of bay leaf extract reducing uric acid greater than allopurinol, although statistically, it did not show significant differences. The decrease in TNF- $\alpha$  values corresponds to the clinical condition, where bay leaf extraction as a uric acid reducer can reduce pain in hyperuricemia patients (Choi et al., 2014). Furthermore, previous research has found that methanol extract of bay leaves has a hepatoprotective effect in diabetes mellitus models (Salim et al., 2022). Inhibition of xanthine oxidase and reduction of inflammatory mediators by bay leaf extract administration are some of the factors contributing to the reduction of damaged hepatocyte cells and its role as hepatoprotective in the hyperuricemia mode.

## CONCLUSION

Based on the conducted research, it can be concluded that the methanol extract of Bay Leaf (*Syzygium polyanthum* (Wight) Walp) influences reducing damaged hepatocytes and acts as a hepatoprotective agent in potassium oxonate-induced hyperuricemia in experimental animals.

## REFERENCES

Choi, Y.-J., Shin, H.-S., Choi, H. S., Park, J.-W., Jo, I., Oh, E.-S., Lee, K.-Y., Lee, B.-H., Johnson, R. J., & Kang, D.-H. (2014). Uric acid induces fat accumulation via generation of endoplasmic reticulum stress and SREBP-1c activation in hepatocytes. *Laboratory Investigation*, *94*(10), 1114–1125. <https://doi.org/10.1038/labinvest.2014.98>

- Darussalam, M., & Rukmi, dwi kartika. (2019). *The Role Of Boiled Water Of Syzygium Polyanthum Leaves In Decreasing Hyperuricemia Levels*. September.
- Dien et al., 2013, Guillermo Garcia-Manero Shao-Qing Kuang, Susan O'Brien, Deborah Thomas, and Hagop Kantarjian, H. Y., & Jones, R. (2005). 基因的改变 NIH Public Access. *Bone*, 23(1), 1–7. <https://doi.org/10.1002/acr.21773>.2012
- Harismah, K. and C. (2016). PEMANFAATAN DAUN SALAM (*Eugenia polyantha*) SEBAGAI OBAT HERBAL DAN REMPAH PENYEDAP MAKANAN. *WARTA LPM*, 19(2), 110–118.
- Kaneko, K., Aoyagi, Y., Fukuuchi, T., Inazawa, K., & Yamaoka, N. (2014). Total Purine and Purine Base Content of Common Foodstuffs for Facilitating Nutritional Therapy for Gout and Hyperuricemia. *Biological and Pharmaceutical Bulletin*, 37(5), 709–721. <https://doi.org/10.1248/bpb.b13-00967>
- Khalid, M., Saeed-ur-Rahman, Bilal, M., & HUANG, D. (2019). Role of flavonoids in plant interactions with the environment and against human pathogens — A review. *Journal of Integrative Agriculture*, 18(1), 211–230. [https://doi.org/https://doi.org/10.1016/S2095-3119\(19\)62555-4](https://doi.org/https://doi.org/10.1016/S2095-3119(19)62555-4)
- Mardiana, Kartini, A., & Widjasena, B. (2012). Media Medika. *Pemberian Cairan Karbohidrat Elektrolit, Status Hidrasi Dan Kelelahan Pada Pekerja Wanita*, 46(14), 6–11.
- Muhtadi, Andi Suhendi, Nurcahyanti W., dan E. S. (2012). THE POTENTIAL OF SALAM LEAVES (*Syzygium polyanthum* Walp.) AND BLACK CUMIN SEED (*Nigella sativa* Linn) AS A CANDIDATE STANDARIZED HERBAL MEDICINE OF URIC ACID. *FARMACON*, 3(1), 30–36.
- Ningtiyas, I. F., & Ramadhian, M. R. (2016). Efektivitas Ekstrak Daun Salam untuk Menurunkan Kadar Asam Urat pada Penderita Arthritis Gout. *Medical Journal of Lampung University*, 5(3), 105–110.
- Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, 5, e47–e47. <https://doi.org/10.1017/jns.2016.41>
- Saigal, R., & Agrawal, A. (2015). Pathogenesis and clinical management of gouty arthritis. *Journal of Association of Physicians of India*, 63(DECEMBER2015), 56–63.
- Salim, H. M., Kurnia, L. F., Bintarti, T. W., Handayani, H., & Shimabukuro, M. (2022). Hepatoprotective Effects of Methanol Extract of *Syzygium polyanthum* L. Leaves (Salam) on High Fat Diet. *Tropical Journal of Natural Product Research*, 5(12), 2092–2095. <https://doi.org/10.26538/tjnpr/v5i12.8>
- Sinaga, A. F., Bodhi, W., & Lolo, W. A. (2014). Uji Efek Ekstrak Etanol Daun Salam (*Syzygium polyanthum* (Wight .) Walp) Terhadap Penurunan Kadar Asam Urat Diinduksi Potasium Oksonat. *PHARMACON Jurnal Ilmiah Farmasi*, 3(2), 141–145.
- Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines (Basel, Switzerland)*, 5(3), 93. <https://doi.org/10.3390/medicines5030093>