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Effect of Skin Melinjo Extract (*Gnetum Gnemon*) On Expression URAT1 and GLUT9 Genes In Hyperuricemia

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Abstract. [Hyperuricemia is defined as a metabolic syndrome disease with markers characteristic of uric acid production that exceed normal limits. Normal uric acid levels range from 3.4-7.0 mg/dl in adult men and 2.4 -6.0 mg/dl in adult women. So if uric acid levels exceed the normal limit, it will cause uric acid can not be fully metabolized and cause hyperuricemia. About 70% of uric acid is excreted through the kidneys. The kidneys are therefore organs that have an important role in maintaining uric acid levels and uric acid homeostasis in general, through complex molecular mechanisms. Reabsorption of uric acid that occurs in the kidneys by URAT1 and GLUT9 has an important role in uric acid levels in the human body, where uric acid is reabsorbed by the kidneys will then be flowed back into the blood so that it affects the levels of uric acid in the human body. Preliminary data showed that ethanol extract of melinjo skin contains 3 main Flavonoid compounds that have a higher Binding affinity value compared to allopurinol, so it has a higher effect in overcoming uric acid levels]

Keyword: [*Hyperuricemia, GLUT9, Skin Melinjo Extract, URAT1*]

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1 . Introduction

Hyperuricemia is defined as a metabolic syndrome disease with markers characteristic of uric acid production that exceed normal limits. Normal uric acid levels range from 3.4-7.0 mg/dl in adult men and 2.4 -6.0 mg/dl in adult women. So if uric acid levels exceed the normal limit, it will cause uric acid can not be fully metabolized and cause hyperuricemia [1]. Where uric acid

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itself is the end product of purine metabolism in the body [2]. Hyperuricemia can occur due to increased purine production or reduced uric acid excretion or a combination of both processes.

Hyperuricemia is considered a metabolic disease that causes gout, chronic nephrosis, and insulin resistance. Hyperuricemia accelerates vasculopathy and the occurrence and development of abnormal glucose tolerance, and is closely related to hypertension, atherosclerosis, coronary heart disease, lipid metabolism disorders, obesity, and sexual dysfunction [3]. Prolonged hyperuricemia will cause gout, where if not treated it will cause damage to the patient's joints and cause tophi gout that can be found in the subcutaneous tissue around the finger joints and in the dorsum. As a result of the disease will be detrimental to sufferers because it causes difficulty activities. Later complications of hyperuricemia in the long term can result in persistent pain and disability due to joint deformities, fractures, or nerve compression [4]. High uric acid levels will cause intense inflammation that displays severe pain and disability, which results in decreased sexual function in men and women [5].

About 70% of uric acid is excreted through the kidneys, while the gastrointestinal tract eliminates 30% [6]. The kidneys are therefore organs that have an important role in maintaining uric acid levels and uric acid homeostasis in general, through complex molecular mechanisms. This function depends on some urate transporter proteins present in the kidneys. These urate transport proteins are located mainly in the proximal tubules of the kidneys, and they are responsible for the excretion and reabsorption of uric acid. Urate transporters that play a role in excretion are ABCG2, while in reabsorption are URAT1 and GLUT9 [7]. Reabsorption of uric acid that occurs in the kidneys by URAT1 and GLUT9 has an important role in uric acid levels in the human body, where uric acid is reabsorbed by the kidneys will then be flowed back into the blood so that it affects the levels of uric acid in the human body.

Currently, one of the ways to treat hyperuricemia is to take drugs. The groups of drugs that can be used for hyperuricemia are drugs that affect uric acid levels (uricostatics) or drugs that stop acute inflammatory (uricosuric) processes. Uricostatic drugs include allopurinol. However, continuous consumption of allopurinol is feared to cause side effects in the form of allopurinol hypersensitivity syndrome (AHS), among them are headache reactions, skin allergies and others [8]. Allopurinol has been associated with severe skin adverse reactions, drug reactions with eonyphilia, toxic epidermal necrolysis, hepatic and renal dysfunction and skin rashes [9].

2 . Content

A. Urid Acid

Uric acid is a product of purine metabolism in humans. Urate Homeostasis depends on the balance between its production in the liver, the process of reabsorption and excretion in the kidneys [10]. About 85% of uric acid comes from synthesis by the human body and 15% comes from food.

Uric acid most primarily synthesized in the liver, intestines, and other tissues, such as muscles, kidneys and vascular endothelium [11]. The amount of uric acid produced during endogenous (daily synthesis rate of about 300-400 mg) and exogenous (food contribution, about 300 mg) metabolism of purines amounts to a total of 1200 mg in healthy males and 600 mg in females on a purine-free diet [12]. A high concentration of urate in the blood will lead to the formation of sodium urate in the form of crystals. The crystals will collect at a certain point of the area and will then occupy the joint area and also the cartilage which will be cumulative and accumulate [13].

B. Uric acid metabolism

Uric acid is mainly synthesized in the liver, the formation of uric acid begins with the formation of a purine base from the ribose group, which is 5-*phosphoribosyl-1-pyrophosphate* (PRPP) obtained from *ribose-5-phosphate* synthesized with ATP (*adenosine-tri-phosphate*). Furthermore, prep is converted to *inosine monophosphate* (IMP) by the enzyme ATP-ase. IMP is then oxidized to *adenosine monophosphate* (AMP) by the enzyme *adenylosuccinate lyase* and *guanosine monophosphate* (GMP) by the enzyme GMP synthase. Furthermore, AMP, IMP and GMP are converted to adenosine, inosine and guanosine by nucleotidase enzymes. Adenosine is deaminated to inosine, then inosine and guanosine are further converted to hypoxanthine and guanine. Hypoxanthine oxidizes to xanthine by XO (*Xantine oxidase*) and guanine deaminates to xanthine by guanine deaminase. *Xanthine oxidized* by XO will produce the final product is uric acid [14][15].

C. Transporters Affecting Uric Acid Levels

Uric acid formed from purine metabolism in blood plasma will be filtered by the renal glomerulus but with a large percentage of about 90% of the urate will still go through reabsorption back in the proximal tubule [16]. With a pKa of 5.35, uric acid is a negatively charged organic anion urate at physiological pH and requires a transporter to cross cell membranes. Genetic studies indicate that a large number of urate transporters (GLUT9, ABCG2, NPT1/3/4, URAT1, OAT4, MRP4) important for urate homeostasis in humans are expressed in organs that eliminate uric acid [6]. In [7] states that of all urate-nion transporter proteins that are in the proximal tubules of the kidneys, which have a major role in influencing uric acid levels in the body are GLUT9, URAT1 and ABCG2.

D. URAT1

URAT1 is a protein encoded by the *solute carrier family 22 member12 gene* (SLC22A12) which is part of the SLC22 organic transporter URAT1 is also part of OAT. Based on the analysis using Immunohistochemistry, it can be seen that URAT1 is located on the apical membrane of the proximal tubule which was studied based on research that has been carried out in both humans and rats as experimental animals. Now many studies are found regarding URAT1 for the treatment of hyperuricemia. Drugs used as URAT1 inhibitors include uricosuric drugs such as probenecid,

phenobritate and losartan. The mechanism of action of these drugs is to inhibit the reabsorption of uric acid rather than its excretion so that it has an effect on uric acid levels in the blood [17]. In [18] URAT1 (urate transporter 1) is a urate exchanger in the apical region of the proximal renal tubules, being encoded by SLC22A12 (*solute carrier family 22, organic anion/urate transporter, member 12*) gene. It is responsible for the urate reabsorption, therefore having an important key role in maintaining serum UA normal values. [19] The URAT1 protein is specifically localized in the brush border membrane of the proximal tubule. It participates in the apical (luminal) uptake of urate from the primary urine to the proximal tubule cell, thus affecting reabsorption. The in vivo experiments found that URAT1 was a biological target of some uricosuric drugs, including probenecid, indomethacin, 6-hydroxybenzbromarone, and salicylate.

E. GLUT9

The GLUT-9 transporter is encoded by the *Solute Carrier Family 2 gene, Facilitated glucose transporter member 9* (SLC2A9). GLUT9 in Purine and uric acid metabolism functions as a pathway for uric acid exit in the basolateral membrane of the proximal tubule and also functions in the transepithelial direction of uric acid reabsorption [17]. The SLC2A9 gene encodes glucose transporter 9 (GULT9), which expressed in liver, kidney, small intestine and chondrocytes. A large number of studies have found that SLC2A9 has a strong correlation with uric acid and uric acid levels because it is involved in uric acid reabsorption. Therefore, SLC2A9 mutations can mediate the onset of gout and can be targets for gout treatment. The presence of disturbances in the expression of the SLC2A9 gene in the proximal tubule provides information that there is an increase in urate levels that has the potential for hyperuricemia [3][15].

F. Effects of Skin Melinjo Extract on Hyperuricemia

According to Kato *et al* in [20], Melinjo is one of the Gnetaceae family plants originating from the tropics. People generally consume the young leaves as vegetables for daily meals and seeds as processing material for melinjo chips. Melinjo (Gnetum gnemon L) is a plant species originating from Malaysia and Indonesia, known to have bioactive compounds that can be used in the field of Health. In [21], Preliminary data showed that ethanol extract of melinjo skin contains 3 main Flavonoid compounds that have a higher *Binding affinity* value compared to allopurinol, so it has a higher effect in overcoming uric acid levels. In [22], flavonoids has the potential as an inhibitor of the formation of XOD and *adenosine Deaminase* (ADA) so as to cause a decrease in levels of uric acid.

In [23] Using melinjo skin extract testing techniques with solvents of ethanol fractions. The experimental animals used were mice. Uric acid levels in the blood were known before treatment to be above 5.0 mg / dL where these levels were hyperuricemia levels for experimental animals. Giving ethanol extract to mice can reduce uric acid levels, it is known based on preliminary tests conducted that melinjo skin has metabolite compounds from the flavonoid and saponin groups. A

similar study was also conducted by [24], using melinjo skin microencapsulation system. Preliminary tests conducted for screening of bioactive compounds present in melinjo skin showed the presence of secondary metabolites contained by melinjo skin in the form of flavonoids, alkaloids, tannins, steroids and terpenoids. Evaluation of blood uric acid levels was carried out at intervals of hours after administration of the extract to rats. The results of the study were analyzed using statistical tests explaining that there was a P value that was less than 0.005 and gave the decision that giving melinjo skin microencapsulation to mice could reduce uric acid levels at 4 hours.

Research conducted by [25] used an experimental animal model in the form of hyperuricemia rats by providing interventions with skin extract from melinjo. Experimental animal group in the form of control as a comparison and the implementation of negative control with allopurinol administration to experimental animals. Dosage using allopurinol 90mg / KgBB, while for melinjo skin extract that has been done phytochemical screening has high saponin and plavonoid compounds. The results of skin extract showed that the effectiveness of the extract is better to reach 70% lower uric acid levels compared to allouprinol which is only 50%.

Reference

[1] Aljak. S., Ibrahim. A., and Omer. Musa., "Reference Value for Uric Acid in Sudanese Healthy Adults", S*cholars International Journal of Biochemistry,* vol. 2, pp 26-30, 2019.

[2] Hao. Shinjun., Chunlesi. Zhang., and Haiyan. Song., "Natural Products Improving Hyperuricemia with Hepatorenal Dual Effects", *Hindawi*, vol. 16, pp 1-7, 2016.

[3] Zhang. Yu., Xiaohui. Tun., Zhen. Lin., *et al,* "Fucoidan from *Laminaria japonica* Inhibits Expression of GLUT9 and URAT1 via PI3K/Akt, JNK and NF-κB Pathways in Uric Acid-Exposed HK-2 Cells", vol. 19, pp 1-11, 2021.

[4] Reckendorf. Meyer., and Dahmam., "Hand Involvement In Gout", *Hand Surgery and Rehabilitation,* pp 1-5, 2018.

[5] Sansone. A., "Relationship Between Hyperuricemia With Deposition and Sexual Dysfunction In Males And Females", *Journal of Endocrinological Investigation,* vol. 45, pp 691-703, 2022.

[6] Tan. Philip K., and Jeffrey. N Minner., "Uric Acid Transporter Inhibitors for Gout", vol. 5, no. 2 : 59-74, 2017.

[7] Pavelcova. Katerina., Jana. Bohata., *et al,* Evaluation of The Influence of Genetic Variants of SLC2A9 (GLUT9) and SLC22A12 (URAT1) on The Development of Hyperuricemia and Gout, *Journal of Clinical Medicine,* 9: 1-17, 2020.

[8] Amir. Mellova., dan Julliana. Irem., "Uji Efektivitas Ekstrak Etanol Buah Naga Putih (*Hylocereus undatus*) terhadap Penurunan Kadar Asam Urat Darah pada Mencit (*Mus musculus*), *Jurnal Ilmu Kefarmasian Indonesia,* vol. 16, no 2: 166-171, 2018.

[9] Stamp. Lisa., and Murray. L Barclay, "How to Prevent Allopurinol Hypersensitivity Reactions?", *Rheumatology,* 57: 135-141, 2018.

[10] James. Armachius., Hengming Ke., Ting. Yao., Yousheng. Wang., 2021, "The Role of Probiotics in Purine Metabolism, Hyperuricemia and Gout: Mechanisms and Interventions", *FOOD REVIEWS INTERNATIONAL*, pp 1-17, 2021.

[11] El Ridi, R., and Tallima, H., "Physiological functions and pathogenic potential of uric acid: A review", *Journal of Advanced Research*, vol. 8, 2017.

[12] Benn. Caroline L., Pinky. Dua., Rachel. Gurrell., *et al*, " Physiology of Hyperuricemia and

Urate-Lowering Treatments", *Hyperuricemia Physiology and Treatmen,* vol. 5, no. 160, pp 1-28, 2018.

[13] Kalembang J, Oka AA and Widiana G., " The Relationship Between Urine Specific Gravity Urine Ph And Blood Uric Acid Levels To The Type Of Urinary Stiries Of Patients With Urolithiasis At Sanglah Hospital Bali, Indonesia". *Intisari sains medis*. vol. 11, no 2: 566-570, 2020.

[14] Maiuolo. J., Francesca. Oppedisano., Santo. Gratteri., *et al*, "Regulation Of Uric Acid Metabolism and Excretion", *International Journal of Cardiology,* 1-7, 2015.

[15] Yin. Hui., Na. Liu., and Jie. Chen., The Role of The Intestine in The Development of Hyperuricemia, *Frontiers in Immunology*, vol. 13, 2022.

[16] Fitzgerald. John D., Nicola. Dalbeth., *et al*, 2020 American College of Rheumatology Guideline for The Management of Gout, *Arthritis Care & Research*, pp 1-7, 2020.

[17] Mandal. A., and David., Mount., "Molecular Physiology of Uric Acid Homeostasis The Molecular Physiology of Uric Acid Homeoostatic", *Annu Rev Physiol*, 77: 323-345, 2015.

[18] Gherghina. Mihai-Emil., Ileana. Peride., *et al,* "Uric Acid and Oxidative Stress—Relationship with Cardiovascular, Metabolic, and Renal Impairment", *International Journal of Molecular Sciences,* 1-16, 2022.

[19] H. J. Shin, M. Takeda, A. Enomoto., *et al*., "Interactions of urate transporter URAT1 in human kidney with uricosuric drugs", *Nephrology*, vol. 16, no. 2, pp. 156–162, 2011.

[20] Muadifah. Afidatul., dkk, "Studi Aktivitas Ekstrak Etanol dan Sediaan Gel Daun Melinjo (*Gnetum Gnemon* L) Sebagai Antibakteri Terhadap Staphylococcus *Aureus*", *Chempublish Journa*l, vol. 4, no. 2 : 89-100, 2019.

[21] Harahap. Armansyah., "Efek Pemberian Ekstrak Eanol Kulit Melinjo (*Gnetum gnemon*) Terhadap Ekspresi Gen XDH, ABCG2 dan Kadar Asam Urat Pada Tikus Wistar Model Hiperurisemia", Thesis, Univ. Sumatera Utara, Medan, 2022.

[22] Jiang. L., Gong. X., Ji. M., Wang. C., Wang. J., and Li. M., "Bioactive compounds from plant-based functional foods: A promising choice for the prevention and management of hyperuricemia", *Foods,* vol. 9, no. 8, 2020.

[23] Khisti. Tsabita., dan Rika. Nilapsari., "Efek Ekstrak Etanol Kulit Melinjo terhadap Penurunan Kadar Asam Urat pada Hiperurisemia", *In Proceeding of Medical Education,* vol. 3, no. 2,pp 506–510, 2017.

[24] Dhimas A Advistasari Y dan Bekti, "Aktivitas Antihiperurisemia Ekstrak Kulit Melinjo (*Gnetum gnemon* L) Secara in vivo", *Jurnal Poltekgal Journal parapemikir,* vol. 9, no. 1, pp 1-6, 2020.

[25] Hasan AE, Husnawati and Setiyono A, "Efektivitas Ekstrak Kulit Melinjo (G*netum gnemon*) Pada Penurunan Kadar Asam Urat Paad Tikus Putih (*Rattus novergicus*) Hiperu Risemia", *Curr Biochem*, vol. 7, no. 1, pp 21-28, 2020.