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The effect of Balinese Purple Sweet Potato (*Ipomoea batatas* L.) tuber ethanolic extract on renal Nox4 level in mice with a high purine diet



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ABSTRACT

Background: TLR4-NADPH Oxidase-4 or TLR4-Nox4 signaling pathway uric acid (UA)-induced oxidative stress is involved in urate nephropathy pathogenesis, which can culminate into renal fibrosis, a common feature of chronic kidney disease (CKD). Based on the hyperuricemic and antioxidant effect of anthocyanin on the kidney, we assumed that Balinese purple sweet potato (*Ipomoea batatas* L.) tuber in its ethanolic extract form (PSPEE) could suppress the activation of renal TLR4-Nox4 signaling. This study was aimed to investigate the renoprotective effect of PSPEE via renal Nox4 downregulation, using a murine model induced with a high-purine diet.

Methods: This study was an experimental laboratory study using a post-test only with a control group design among 38 male Swiss mice. The Male Swiss mice (20-30 g) were randomly allocated into 2

groups, namely, the control group (treated with a high-purine diet for 7 days) and the PSPEE group (treated with a high-purine diet for 7 days and PSPEE for 14 days). The serum uric acid (sUA) level was quantified using the UA phosphatase method. Meanwhile, the renal Nox4 level was quantified using ELISA. Data were analyzed using SPSS version 20 for Windows.

Results: Our study found that the renal Nox4 levels were 18.65 ± 1.67 ng/mL and 15.14 ± 1.66 ng/mL for the control and PSPEE groups. Based on the statistical analysis, there was no statistically significant difference between control and PSPEE groups ($p > 0.05$).

Conclusion: Balinese PSPEE tends to decrease renal Nox4 level in high-purine diet-treated mice, but fails to show a significant effect.

Keywords: Urate nephropathy, *Ipomoea batatas* L., Serum Uric Acid, Nox4

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INTRODUCTION

Hypersaturation of uric acid (UA) in blood or hyperuricemia may cause renal fibrosis, a common feature of chronic kidney disease (CKD).^{1,2} UA has been recognized as damage-associated molecular patterns/DAMPs that can induce inflammation in the kidney.^{2,3} Renal tubular cells constitutively express toll-like receptors (TLRs), mainly TLR4, activated by DAMPs.³ UA has also been known to damage kidney (induces urate nephropathy) via reactive oxygen species (ROS) biogenesis.⁴ UA-induced renal oxidative stress involves TLR4 signaling and upregulates renal expression of nicotinamide adenine dinucleotide 3-phosphate (NADPH) oxidase 4 (Nox4).⁵

Balinese purple sweet potato (*Ipomoea batatas* L.) tuber exhibits a potential nephroprotective effect in its aqueous extract form by upregulating the renal level of superoxide dismutase-2 (SOD2), an important endogenous antioxidant molecule.⁶ Anthocyanins of the purple sweet potato tubers

are assumed to contribute to the nephroprotective effect.⁶

According to the protective effect, we hypothesized that Balinese purple sweet potato tuber ethanolic extract (PSPEE) could suppress signaling TLR4-Nox4, primarily. Based on those mentioned above, this study investigates the nephroprotective effect of PSPEE by downregulating renal Nox4, using mice induced with a high-purine diet.

MATERIALS AND METHODS

Animal Model

Male Swiss mice (n=38; 20-30 g) were acclimatized for 7 days before administering a high purine diet and PSPEE. The mice were nurtured in 50 x 30 x 15 cm plastic cage under a light-dark cycle of 12 hours and humidity of $50 \pm 5\%$ in the Laboratory of Pharmacology and Therapy, Faculty of Medicine, Universitas Udayana (UNUD), Bali, Indonesia. Mice were fed with standard chow as many as 3-5 g/mouse/day; meanwhile, water was given *ad libitum*.

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UA and PSPEE Administration

In this study, mice were fed with high-purine diet boiled chicken liver juice 0,2% b/v, twice a day, for 14 days to induce urate nephropathy. Mice belonging to the treatment group was also supplemented with Balinese PSPEE 60 minutes before administering a high-purine diet. PSPEE preparations were administered orally at the dose of 100 mg/kg BW, once a day, for 14 days. All treatments were applied between 08.00 – 10.00 AM to minimize the effect of circadian rhythm in the mice's body.

Renal Sampling

Twenty-four hours after the end of the treatment period, the mice were euthanized using ketamine (40 mg/kg BW, IP, according to Iowa IACUC Guideline). Secondary euthanasia (i.e., cervical dislocation) was then applied after ketamine injection. Necropsy and renal harvesting were conducted as fast as possible. The left kidneys were collected and froze in -20° for later analysis.

Nox4 ELISA and Data Analysis

All kidneys were thawed under room temperature during the day of ELISA. The samples were then homogenized using a lysis buffer and tissue sonicator. Renal homogenates were analyzed using the sandwich ELISA method with an anti-mouse Nox4 ELISA kit (Bioassay Technology Laboratory, Shanghai, China [serial no. E4156Mo]), according to manufacturer's protocol. The samples' optical densities were subsequently read and converted into the renal Nox4 level (ng/mL). The data were analyzed statistically using Student's t-test by SPSS version 20 for Windows.

RESULTS

The current study investigates the effect of Balinese PSPEE on renal Nox4 level of high-purine diet-treated mice, as a model of urate nephropathy. Our study found that the Renal Nox4 levels were 18.65 ± 1.67 ng/mL in the control group and 15.14 ± 1.66 ng/mL in the PSPEE group (Table 1). We find that PSPEE ameliorates the renal Nox4 level in these mice but fails to show a statistically significant effect ($p > 0.05$) (Table 1).

DISCUSSION

Nox4 is predominantly expressed in murine and human kidneys (renal tubules, renal fibroblasts, glomerular mesangial cells, and podocytes). Renal Nox4 upregulation has been recognized to affect renal cells adversely, especially in CKD patients.^{7,8} Activation of the innate immune system TLR-4 by UA is known as a molecular event induces Nox4 and Nox4-associated oxidative stress, especially in renal tubular cells.⁵ A previous study by Jeong BY *et al.* proposed that Nox4 could be a potential target of uremic toxicity therapy in CKD cases.⁸ Recently, Nox4-derived ROS has been linked to vascular (aortic) aging or precocious senescence.⁹ Pharmacological modulation of Nox4 expression can thus be viewed as a potential target in anti-aging medicine, especially in the area of renal and vascular aging prevention.

Anthocyanin is ubiquitously present in PSP tuber and other natural products, such as grape and cherry fruit.¹⁰ Anthocyanins in cherry fruits can diminish the risk of recurrent gout attacks induced by UA hypersaturation.¹⁰ This finding has been confirmed *in vivo* by Zhang *et al.*, who found that anthocyanins from PSP tuber cultivar Eshu No.8 from China could normalize renal function test results and inhibit UA biogenesis via the attenuation of hepatic xanthine oxidase (XO) activity.¹¹ A mixture of anthocyanins from bilberry and black currant recently has been studied in a murine model of hyperuricemia.¹² Three-weeks long treatment with these anthocyanins mitigated hyperuricemia's adverse effects on mice liver and kidneys by showing anti-inflammatory and boosting renal clearance of UA, with better overall salutary effects than allopurinol.¹²

Hyperuricemia is clinically associated with CKD and hypertension, two globally-significant pathological entities. A previous study by Oshima N *et al.* showed that UA could stimulate neurons in the *rostral ventrolateral medulla* (RVLM) area of rats, that also involve Nox4.¹³ Another interesting finding is that the Nox inhibitor can eradicate RVLM activation.¹³ This body of evidence signifies that Nox4 plays a significant role in CKD's pathogenesis and its comorbidities, specifically hypertension. Based on previous studies, Balinese PSP is a promising modality of nephroprotection, by acting as renal anti-oxidase under the condition of high purine intake. Another potentially promising effect that must be investigated in the near future is the antihypertensive effect, especially under a hyperuricemic condition regulating blood pressure in RVLM. Although PSPEE lacks a significant impact in our current study, we cannot exclude this preparation's potential as a renoprotective agent,

Table 1. Renal Nox4 Level in Control vs. PSPEE Group

Group	N=38	Renal Nox4 Level (mean±SEM)	P
Control, n (%)	19 (50.0)	18.65 ± 1.67 ng/mL	0.680
PSPEE, n (%)	19 (50.0)	15.14 ± 1.66 ng/mL	

*p-value is considered significant if less than 0.05; PSPEE: purple sweet potato-ethanolic extract

especially in preventing the development of urate nephropathy.

CONCLUSION

From the laboratory finding, it can be inferred that the administration of Balinese PSPEE for two weeks tends to decrease renal Nox4 level in high-purine diet-induced mice, but fails to show a significant effect. Further studies should be done in the near future to confirm the renoprotective effect of Balinese PSPEE and its molecular pharmacodynamics.

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CONFLICT OF INTEREST

The authors declare that there is no competing interest regarding the manuscript.

ETHICS CONSIDERATION

Ethics Committee, Faculty of Medicine, Universitas Udayana, Bali, Indonesia, has provided authors the Ethical Clearance prior to the study being conducted.

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AUTHOR CONTRIBUTION

All of the authors equally contribute to the study from the conceptual framework, data gathering, data analysis until reporting the results of study through publication.

REFERENCES

1. Johnson RJ, Nakagawa T, Jalal D, Sánchez-Lozada LG, Kang DH, Ritz E. Uric acid and chronic kidney disease: which is chasing which?. *Nephrol Dial Transplant*. 2013;28(9):2221-2228.
2. Kim IY, Lee DW, Lee SB, Kwak IS. The role of uric acid in kidney fibrosis: experimental evidences for the causal relationship. *Biomed Res Int*. 2014;2014:638732.
3. Gill R, Tsung A, Billiar T. Linking oxidative stress to inflammation: Toll-like receptors. *Free Radic Biol Med*. 2010;48(9):1121-1132.
4. Li Z, Sheng Y, Liu C, Li K, Huang X, Huang J, et al. Nox4 has a crucial role in uric acid-induced oxidative stress and apoptosis in renal tubular cells. *Mol Med Rep*. 2016;13(5):4343-4348.
5. Milanesi S, Verzola D, Cappadona F, Bonino B, Murugavel A, Pontremoli R, et al. Uric acid and angiotensin II additively promote inflammation and oxidative stress in human proximal tubule cells by activation of toll-like receptor 4. *J Cell Physiol*. 2019;234(7):10868-10876.
6. Satriyasa BK. Aqueous extract of purple sweet potato tubers decreases MDA and increases SOD2 in kidney of diabetic rats. *Bali Med J*. 2016;5(3):388-390.
7. Gorin Y. Nox4 as a potential therapeutic target for treatment of uremic toxicity associated to chronic kidney disease. *Kidney Int*. 2013;83(4):541-543.
8. Jeong BY, Lee HY, Park CG, Kang J, Yu SL, Choi DR, et al. Oxidative stress caused by activation of NADPH oxidase 4 promotes contrast-induced acute kidney injury. *PLoS One*. 2018;13(1):e0191034.
9. Canugovi C, Stevenson MD, Vendrov AE, Hayami T, Robidoux J, Xiao H, et al. Increased mitochondrial NADPH oxidase 4 (NOX4) expression in aging is a causative factor in aortic stiffening. *Redox Biol*. 2019;26:101288.
10. Zhang Y, Neogi T, Chen C, Chaisson C, Hunter DJ, Choi HK. Cherry consumption and decreased risk of recurrent gout attacks. *Arthritis Rheum*. 2012;64(12):4004-4011.
11. Zhang ZC, Su GH, Luo CL, Pang YL, Wang L, Li X, et al. Effects of anthocyanins from purple sweet potato (*Ipomoea batatas* L. cultivar Eshu No. 8) on the serum uric acid level and xanthine oxidase activity in hyperuricemic mice. *Food Funct*. 2015;6(9):3045-3055.
12. Qian X, Wang X, Luo J, Liu Y, Pang J, Zhang H, et al. Hypouricemic and nephroprotective roles of anthocyanins in hyperuricemic mice. *Food Funct*. 2019;10(2):867-878.
13. Oshima N, Onimaru H, Matsubara H, Uchida T, Watanabe A, Takechi H, et al. Uric acid, indoxyl sulfate, and methylguanidine activate bulbospinal neurons in the RVLM via their specific transporters and by producing oxidative stress. *Neuroscience*. 2015;304:133-145.



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