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4-The effect of porang (Amorphophallus muelleri) extract on renal histopathological changes

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ORIGINAL ARTICLE

The effect of porang (*Amorphophallus muelleri*) extract on renal histopathological changes

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ABSTRACT

Diabetes mellitus is a chronic condition defined by elevated blood sugar levels (hyperglycemia). This condition can lead to complications such as nephropathy, which is histologically shown with glomerulosclerosis. Glucomannan, a component of *Amorphophallus muelleri*, offers numerous health benefits, but its direct therapeutic effect on glomeruli remains uncertain. Male Wistar rats which were taken with random sampling (n = 30) were distributed into six distinct groups. All groups, excluding Group N, received 125 mg/kg BW single intraperitoneal dose of alloxan. Group N received a single dose of PBS 125 mg/kg BW. After 7 days, Group K + was induced with acarbose at a dose of 50 mg/70 kg BW (adjusted using a factor of 0.018) orally per day. Groups N and K − induced with 1% CMC Na at 0.2 mL/0.1 kg orally per day. While Group P1, P2, and P3 were orally given *A. muelleri* ethanolic extract orally per day at a dose of 100, 200, and 400 mg/kg BW. The following 50 days of treatment, the Wistar rats were euthanized, and their kidney was preserved for histological slides that were stained with hematoxylin and eosin. The oral administration of *A. muelleri* ethanolic extract in alloxan-induced diabetic rats led to a significant decrease in the average of glomerulosclerosis instances when compared to the K − group. The most effective dose was observed at 400 mg/kg BW per day. *A. muelleri* administration leads to a reduction in glomerulosclerosis occurrences, suggesting its potential as a therapeutic approach for reducing complications probability linked to hyperglycemia.

Key words: *Amorphophallus muelleri* extract, glomerulosclerosis, hyperglycemia

INTRODUCTION

Diabetes mellitus (DM) is a chronic condition defined by elevated blood sugar levels. This condition is caused by insulin resistance or reduced production of insulin.[1,2] DM is a serious health problem with high morbidity and mortality that needs bigger awareness among people.[1,2] Chronic hyperglycemia can lead to various complications. Around 20%–40% of patients with diabetes will develop diabetic nephropathy, and 28% of diabetic nephropathy patients will lead to chronic kidney disease (CKD).[3-5] According to Riskesdas 2018, data revealed a 0.38% prevalence of diagnosed CKD patients among Indonesia's total population, with 19.33% of cases necessitating hemodialysis.[6] Hyperglycemia stimulates the development and storage of advanced glycation end products, polyol pathways, protein kinase C activation, and hexosamine pathways. This

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condition makes reactive oxygen species (ROS), oxidative stress, and endothelial nitric oxide levels rise, and could damage kidney function.[3,7-9] Several techniques have been used to prevent and manage hyperglycemia, such as lifestyle changes and medication interventions.[10]

Widely, rice is the main source of carbohydrates that consumed by the Indonesian population. However, excess rice consumption can lead to hyperglycemia due to its high glycemic index (GI). Foods rich in fiber tend to have a lower glucose absorption, which safer to the blood sugar levels.[11] Indonesia, being rich in various plant varieties, includes Porang or Iles-iles of the taro family *(Araceae)* and the *genus Amorphophallus.* Among these, *Amorphophallus muelleri,* commonly found in Indonesia's tropical climate, has gained popularity as a daily food source and industrial ingredient.[12] The key component within these tubers, utilized for their natural properties in managing and treating diabetes, is glucomannan.[12,13] This study investigated the effect of *A. muelleri* on altering the histopathological kidney structure as a preliminary study of nephroprotective against diabetic nephropathy.

MATERIALS AND METHODS

This study employed various groups for experimentation. Apart from Group N, which served as the control, other groups were induced with alloxan at a dosage of 125 mg/kg BW intraperitoneally in a single administration. Group N, however, was given a single dose of 125 mg/kg BW PBS. After 7 days, the K + group received treatment with acarbose, administered orally at a dose of 50 mg/70 kg BW, adjusted using a conversion factor of 0.018 once daily. Both Groups N and K − were orally administered 0.2 mL/0. 1 kg BW of 1% CMC Na daily. On the other hand, groups P1, P2, and P3 were administered *A. muelleri* extracts at doses of 100, 200, and 400 mg/kg BW orally daily for 50 days. At the conclusion of the treatment period, the rats were euthanized using *diethyl ether* followed by cervical dislocation. Subsequently, a surgical procedure was conducted to extract the kidney organs and prepare histological sampled. The remains of the rats were subjected to cremation.

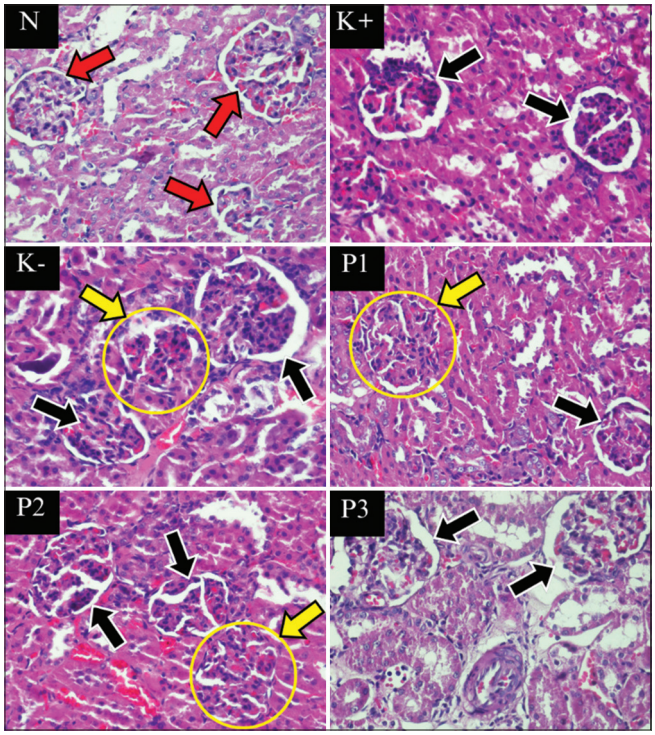

Figure 1: Microscopic Picture *of Hematoxylin-Eosin* Staining of Rat Kidney Glomerulus Between Groups. Normal glomerulus (➡), early-stage glomerulosclerosis (➡), glomerulosclerosis (⇨). Yellow circle was red blood cells

Samples

Male Wistar *Rattus norvegicus* strains weighing 100–150 g and aged approximately 2 months were procured from the Animal Laboratory of the Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia. These animals were provided with a certificate of conformity, authorizing their usage in experimental research. The rats were housed in ventilated cages under controlled conditions encompassing room temperature, humidity, and lighting. The cages were equipped with wire top covers and contained natural zeolite.

Preparation of *Amorphophallus muelleri*

A total of 3 kg *of A. muelleri* was macerated with 9 L of 96% ethanol over the course of three cycles of 24 h each, maintained at room temperature, and stirred daily. The resulting macerate was then subjected to evaporation using a rotary evaporator at a temperature of 50°C and a speed of 45 rpm, yielding a concentrated extract.

Histopathological examination of kidney glomerulus

Tissue preparations underwent hematoxylin and eosin staining. The assessment involved quantifying the instances

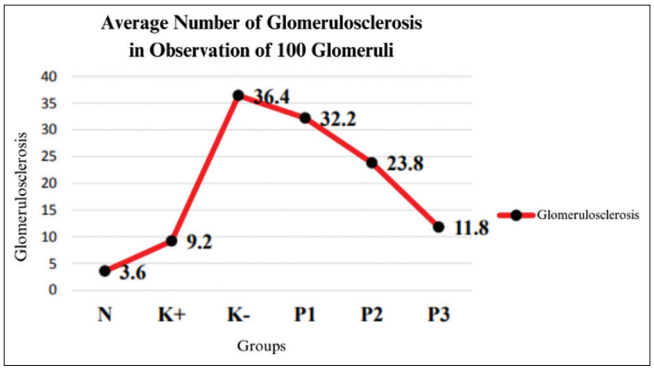

Figure 2: Graph of average number of glomerulosclerosis in observation from 100 Glomeruli among groups. Based on the comparison of the average number of glomerular sclerosis, it was found that groups K+, P2, and P3 were closest to normal. There was a significant decrease in the number of glomerulosclerosis in groups P1, P2, and P3 compared to the K − group

Table 1: Mean number of glomerulosclerosis

Group	$\bar{x}$±SD
N	3.60±1.517
K+	9.20±3.421
K−	36.40±1.342
P1	32.20±2.588
P2	23.80±3.962
P3	11.80±2.588

SD: Standard deviation

Table 2: Hypothesis test of total glomerulosclerosis

Group	*P*
One-way ANOVA	
N: K+: K−: P1: P2: P3	0.000*
LSD	
N: K+	0.004*
N: K−	0.000*
N: P1	0.000*
N: P2	0.000*
N: P3	0.000*
K+: K−	0.000*
K+: P1	0.000*
K+: P2	0.000*
K+: P3	0.146
K−: P1	0.023*
K−: P2	0.000*
K−: P3	0.000*
P1: P2	0.000*
P1: P3	0.000*
P2: P3	0.000*

*Significant ($P<0.05$). Table 1 displays the histopathological changes in kidney glomeruli as quantified by the average occurrences of glomerulosclerosis in rat kidneys. The K− group exhibited the highest average number of glomerulosclerosis (36.40±1.342), while the P3 group had the lowest average (11.80±2.588). The average glomerulosclerosis in the K+ and P3 groups closely resembled that of the N group. It is noteworthy that all treatment groups demonstrated a lower glomerulosclerosis count compared to the K− group [Table 1]. The normality of the data was confirmed using the Shapiro–Wilk test and the homogeneity test using Levene's test yielded a *P* value of 0.427 ($P>0.05$). This indicates a normal distribution of the data. Subsequently, the data underwent the one-way ANOVA test, resulting in a *P* value of 0.000 ($P<0.05$), signifying a statistically significant difference among the groups. Further analysis with the LSD *post hoc* test yielded a *P* value of 0.146 ($P<0.05$), indicating significant differences among most groups, except for the K+ and P3 groups. LSD: Least significant difference

of glomerulosclerosis. This analysis followed a systematic pattern, and three anatomical pathologists conducted the readings using a Nikon ECLIPSE Ci Series Microscope with × 400. Alterations in the histopathological structure of the glomerulus were observed through the examination of glomerulosclerosis in 100 glomeruli per preparation. Glomerulosclerosis was identified based on the presence of one or more of the following histological characteristics: thickening of the glomerular wall leading to Bowman space constriction, the formation of circular eosinophilic protein accumulations that culminate in Kimmelstiel–Wilson nodules [Figure 1].

Statistical analysis

Comparative analysis was conducted employing the one-way ANOVA test in combination with the Mann–Whitney and Kruskal–Wallis tests. *Post hoc* analysis was performed using the least significant difference method. In addition, correlation analysis was undertaken using Spearman's rank correlation coefficient (rho).

Table 3: Correlation between glomerulosclerosis levels and random blood sugar levels after treatment

Variable	Significant	*r*
Glomerulosclerosis levels and random blood sugar levels after treatment	0.000*	0.579

*Significant ($P<0.05$). Table 3 presents the results of Spearman's rho analysis, revealing a strong positive correlation between variables with a correlation coefficient (*r*) of 0.579

RESULTS

Results of analysis of kidney glomerular histopathological changes

Table 1 shows that the histopathological changes in kidney glomeruli as quantified by the average occurrences of kidney glomerular histopathological changes (glomerulosclerosis) in rat kidneys. The K negative (placebo) group exhibited the highest average number of glomerulosclerosis (ẋ36.40±1.34). While, the P3 group had the lowest average (ẋ11.80±2.59) [Figure 2]. The average of glomerulosclerosis in the K positive (acarbose) and P3 groups closely resembled to the N group. The normality of the data was confirmed using the Shapiro–Wilk test and the homogeneity test using Levene's test yielded a *P* value of 0.427 ($P>0.05$). This indicates a normal distribution of the data. Subsequently, the data underwent the one-way ANOVA test, resulting in a *P* value of 0.000 ($P<0.05$), signifying a statistically significant difference among the groups. Further analysis with the LSD* *post hoc* test yielded a *P* value of 0.146 ($P<0.05$), indicating significant differences among most groups, except for the K+ and P3 groups, as seen in Table 2. *LSD: Least significant difference.

Correlation between the two variables

Table 3 shows, using Spearman's rho analysis, *Amorphophallus muelleri* extract gave a strong positive correlation in preventing glomerulosclerosis (correlation coefficient (r) of 0.579).

DISCUSSION

Based on the analysis of histopathological changes in kidney structures, specifically the occurrence of glomerulosclerosis, the K − group exhibited the highest average result with a significant average of 36.40 instances of glomerulosclerosis. This outcome was in contrast to the other groups. The elevated level of glomerulosclerosis in the K − group can be attributed to its induction with a single intraperitoneal dose of alloxan (125 mg/kg BW) and subsequent administration

of 1% CMC Na (0.2 mL/0.1 kg BW) orally per day for 50 days. Alloxan is recognized for its ability to induce DM, leading to kidney microvascular complications, notably glomerulosclerosis.

Multi-comparison analysis revealed that K + and P3 groups showed no significant differences. This outcome can be attributed to the administration of *A. muelleri* extract at a dose of 400 mg/kg BW orally per day and acarbose therapy at a dose of 50 mg/70 kg BW (converted using a factor of 0.018) orally per day. These treatments seem to induce changes in histopathological features that resemble those observed in Group N.

This finding aligns with a study by Zhao *et al.*, where glucomannan from *Amorphophallus konjac* improves glomerular histopathology in diabetic rats.[14] Another parameter indicative of kidney health is urea level. Meo *et al.*'s research demonstrated that glucomannan treatment for 30 days led to lower average urea levels compared to untreated DM groups. This suggests that glucomannan offers more favorable effects on the kidneys than untreated DM patients.[15]

The current study indicates that administering *A. muelleri* extract at a dose of 400 mg/kg BW yields the most favorable kidney effect. Nonetheless, even at doses of 100 and 200 mg/kg BW, observable changes in histopathology still occur when compared to the K − group.

The correlation analysis between random blood sugar levels and changes in the histopathological structure of the kidney, specifically total glomerulosclerosis, revealed a highly significant value of $P = 0.000$. The correlation coefficient (r value) calculated using Spearman's rank correlation test was 0.579. This strong positive coefficient suggests a robust relationship between random blood sugar levels and the occurrence of glomerulosclerosis. Notably, a reduction in random blood sugar levels corresponds to a decrease in glomerulosclerosis and vice versa.

This study provides evidence that the utilization of porang tubers under hyperglycemic conditions can offer protection against kidney damage. Hyperglycemic states often lead to an elevated production of ROS and instigate a pro-inflammatory cytokine response. This cascade of events can lead to tissue disruption. The administration of *A. muelleri* extract is believed to exhibit its effect through multiple mechanisms. One such mechanism involves the porang tuber's role as an antidiabetic agent, which aids in preventing prolonged hyperglycemia due to its glucomannan content. In addition, the extract contains antioxidant phytochemicals that effectively counteract free radicals and inhibit inflammatory pathways. This leads to suppression of pro-inflammatory cytokine production.[16,17] This pathway may be the potential mechanism of *A. muelleri* as nephroprotective agent; however, it needs further investigation.

Limitation of study

This study investigated *A. muelleri* extract as a potential therapeutic agent for diabetic nephropathy. While the study offers promising insights, it has some limitations: (1) this is *in vivo*, limiting scope extrapolation to human conditions. The variations in physiology and immune responses were not considered; (2) despite highlighting the positive impact on nephroprotective activity, safety profile, and potential side effects or adverse reactions of combining *A. muelleri* with oral antidiabetic, particularly in humans, requires further investigation.

CONCLUSION

The administration of *A. muelleri* to Wistar strain *R. norvegicus* resulted in a notable reduction in the occurrence of glomerulosclerosis. In addition, a robust negative correlation was observed between the administration of *A. muelleri* and the extent of glomerulosclerosis. Therefore, based on this research, *A. muelleri* may be considered as an additional alternative for lowering blood sugar levels in diabetes to prevent hyperglycemic conditions that may complicate into diabetic nephropathy.

Ethics of study

This study adhered to the ethical principles governing the treatment of experimental animals, encompassing the 3Rs (Reduction, Replacement, and Refinement) and 5Fs (Freedom from pain, injury, or disease; Freedom from hunger and thirst; Freedom from fear and distress; Freedom from discomfort; and Freedom to express natural behavior). The study protocol obtained approval from the Health Research Ethics Committee (KEPK) of Widya Mandala Catholic University, Surabaya, Indonesia (Approval No. 0255/WM12/KEPK/MHS/T/2022).

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Nil.

Conflicts of interest

There are no conflicts of interest.

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