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The effect of *syzygium polyanthum* (wight) extract in histological changes of kidney in diabetic mice model

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ABSTRACT

Introduction: Diabetes mellitus, or hyperglycemia, is one of the risk factors for atherosclerosis, causing disruption of blood flow through arterial plaque formation and atherosclerosis. Diabetic kidney disease (DKD) is the most common cause of chronic and end-stage renal disease (CKD and ESRD) worldwide and is a stronger predictor of mortality in people with diabetes. Although the concept that the kidney plays a key role in glucose balance is not new, it is only recently that this organ has been viewed as a potential therapeutic target. This study aimed to assess the potential effect of *Syzygium polyanthum* extract in DKD mice model.

Methods: We administrated *Syzygium polyanthum* extract for 2-weeks to alloxan-induced diabetic mice.

Results: Treatment with *Syzygium polyanthum* extract reduced blood glucose levels in dose dependency (2,62mg/20g/day, 5,24mg/20g/day, and 7,86mg/20g/day). Histological analysis showed that *Syzygium polyanthum* extract significantly ($p<0,05$) improved the kidney lesions in low dose, but did not show a significant effect on inflammatory cell at glomerulus.

Conclusion: *Syzygium polyanthum* extract has potential to improve the kidney lesion in low dose and showed therapeutic potential in DKD treatment.

Keywords: *syzygium polyanthum*, DKD, CKD.

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INTRODUCTION

Diabetes mellitus or hyperglycemia is one of the risk factors for atherosclerosis which causes disruption of blood flow due to plaque formation and atheromatous blockages in the arteries.[1] Diabetic kidney disease (DKD) is the most common cause of chronic and end-stage renal disease (CKD and ESRD) worldwide and is a stronger predictor of mortality in people with diabetes. Although the concept that the kidney plays a key role in glucose balance is not new, it is only recently that this organ has been considered a potential therapeutic target. SGLT-1 in the S3 segment is a low-capacity, high-affinity glucose transporter, and SGLT-2, located in the S1 and S2 segments of the PCT, has a low affinity for glucose transport and a promotes the reabsorption of inhibition of this process promotes glucosuria and lowers blood sugar levels.[2,3]

Syzygium polyanthum (Wight) Walp. var. polyanthum leaves are consumed as a traditional Indonesia treatment because there are flavonoids that act as antioxidants.[4] Bay leaves (Syzygium polyanthum) are also widely used for treat hypercholesterolemia and diabetes mellitus.[5] Chemical content in leaves salam, namely tannins, essential oils and eugenol and flavonoids.[6] Mechanism of flavonoids as hypoglycemic thought to inhibit glucose reabsorption from the kidneys and can increase the solubility of blood glucose so that easily excreted in the urine. Bay leaves has the ability as an astringent that is precipitates mucous membrane proteins and forms a layer that protects the intestines, thereby inhibiting glucose intake resulting in the rate of glucose decrease blood.[7]

However, how the mechanism of bay leaves can reduce blood sugar in the histological picture of the kidney is still not clearly known. this study was investigated extract of Syzygium Polyanthum (Wight) in preventing metabolic disease in mice model.

METHODS

Tools and Materials

The tools and material used in this study were animal cage, digital scales, rotavapor, sonde, pipet, object glass, cover glass, microscope, spuit and

glucometer, 2 kg salam leaves, etanol 96%, aquades, alloxan, NaCMC, chloroform, neutral buffered formalin (NBF) 10%, and hematoxylin-eosin (HE).

Syzygyum polyanthum (Wight) Extraction

Syzygium polyanthum leaves with wight 2 kg were used as the simplisia powder, then ethanol 96% is added then allowed to stand in a maserator for 24 hours, then filtered the extract, then added ethanol 96% to stand for 72 hours, then put into a rotavapor until the extract thickens, then mixed with 100ml of Aquades and 1% NaCMC sufficient so that the extract does not settle.

Animal Experiment

Eight-week-old male mice were treated with alloxan (Alox, 120mg/kg) by an intraperitoneal injection. Then, glucose level was evaluated after 48 hours. After 48 hours of alloxan injection, the three groups were treated *Syzygium polyanthum* wight extract (2.62mg/20g/day, 5.24mg/20g/day, and 7.86mg/20g/day) by oral for 14 days.

Blood glucose analyses

Calculation of blood glucose levels of mice used a glucometer (GlucoDR), before the blood glucose level calculations were fasted for 8-12 hours. The blood of the mice was collected through the tail. The blood glucose levels of the mice were calculated at the third, seventh and fourteenth day. Rats are considered diabetic if their blood glucose levels are ≥ 200 mg / dl.

Samples from the kidney were fixed overnight in 4% (v/v) neutral formaldehyde solution and embedded in paraffin. Four µm tissue slices were subjected to hematoxylin-eosin.

Histological analyses

All the animals were sacrificed on the 14th day from each animal was removed, fixed in 10% formalin solution, and processed by the paraffin technique. Sections of 5 µm thickness were cut and stained by hematoxylin and eosin (H&E) for histological examination.

Data Analysis

All results were expressed as mean ± SEM. Data were analyzed using Kruskal-Wallis H test to analyzed the difference of *Syzygium polyanthum* extract effect in each group. Analysis was carried out using SPSS ver. 24. A value of $P < 0.05$ was considered significant.

RESULTS

The Effect of *Syzygium polyanthum* (Wight) in Glucose Level

The results of this study indicate that extract *Syzygium polyanthum* (Wight) is effective decreased glucose level in all dose (2.62mg/20g/day, 5.24mg/20g/day, and 7.86mg/20g/day) because the extract have flavonoid (Figure 1).

The effects of *Syzygium polyanthum* (Wight) in Glomerular Hystology

The results of this study (Figure 2 and **Table 1**) indicate that extract *Syzygium polyanthum* (Wight) repair glomerular damage in low dose (2.62 mg/20gBB/day), this is due to the presence of flavonoids.

The effect of *Syzygium polyanthum* (Wight) in Proximal and Distal Tubule Histology

The results of this study indicated that extract *Syzygium polyanthum* (Wight) is effective in improving the histological picture proximal and distal tubules mice diabetic model in low dose (2.62 mg / 20gBB). The extract *Syzygium polyanthum* (Wight) was effective in repairing the histological picture of mice's proximal and distal tubules because they contain antioxidant flavonoids, which are antioxidants which function as free radical scavengers produced by alloxan, causing damage to the proximal tubules of mice. This pattern is in accordance with previous research conducted by Rohmah dan Munowaratur (2015) where the content of flavonoids in mulberry leaves is effective in the improvement of proximal tubular histology.

DISCUSSION

As we know the mechanism hyperglycemia in mice because of alloxan injection. Alloxan is a compound that is similar to glucose and is an oxidant so that when alloxan is induced into mice, the glucose transporter GLUT 2 in pancreatic β cells will recognize alloxan as glucose and then alloxan will be lowered into the cytosol, alloxan causes damage to pancreatic β cells causing hyperglycemia.[8] The extract of *Syzygium polyanthum* (Wight) contains flavonoids which are antioxidants. As we know flavonoids are one of the phenol group chemicals that can reduce hyperglycemia conditions due to injection of alloxan with anti-oxidants mechanism.[9] This result is in accordance with previous research conducted by Handani, et al., (2015) where the flavonoid content in long beans (*Vigna ungucilata*) is effective

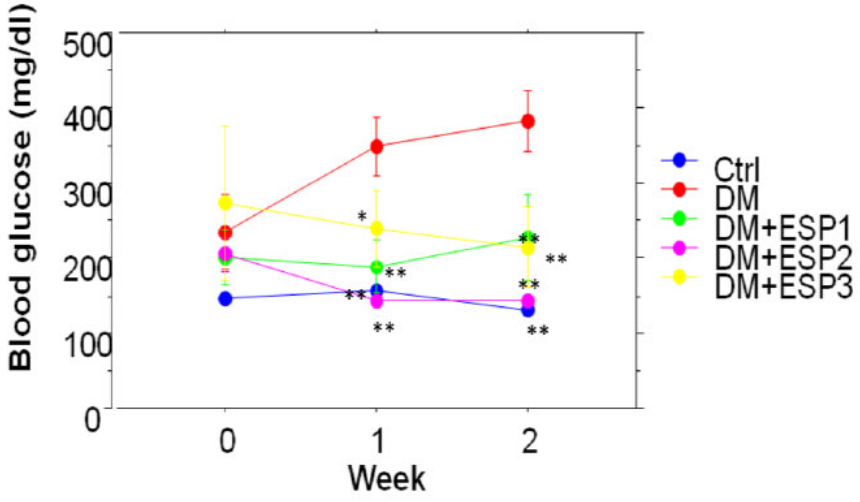

Figure 1. Blood glucose level. n=7 per group. Ctrl; control, DM; diabetes mellitus, DM+ESP1; Low dose (2,62gr/d),DM+ESP2; Mid dose (5,24gr/d), and DM+ESP3; High dose (7,86gr/d). *; P<0.05 and **; P<0.01 vs. DM. All values are mean ± SEM.

Table 1. Comparison of Glomerular Damage Rates.

Groups	Damage Degree (Modus)	Evaluations	Uji *Kruskal-Wallis H*
K-	Mild	Enlargement of the glomerulus +, narrowing of the capsular space +, the grains of erythrocytes +	19,325 p = 0,001 (< 0,05)
K+	Moderate		
P1	Mild		
P2	Moderate		
P3	Moderate		

*Information: Mild (≤25%), moderate (25-50%), severe (≥50%).

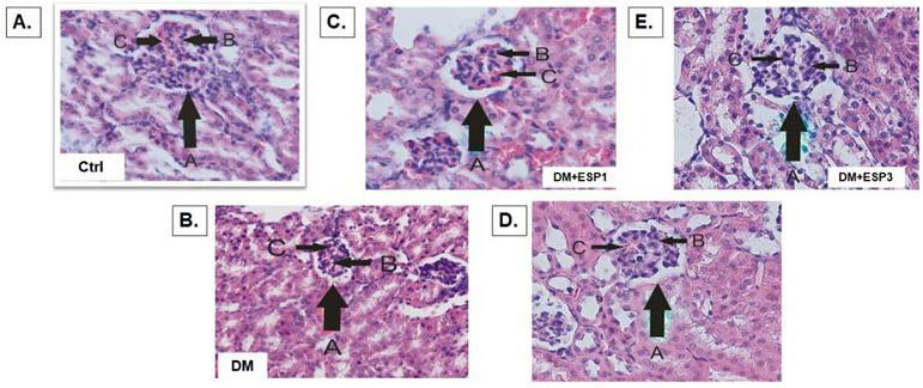

Figure 2. Histological of glomerular. Histological examination (H&E stained) (400x) of glomerular in diabetic kidney group. n=7, per group. A. Ctrl; control, B. DM; diabetes mellitus, C. DM+ESP1; Low dose (2,62gr/d), DM+ESP2; D. Mid dose (5,24gr/d), and E. DM+ESP3; High dose (7,86gr/d)., A; Glomerular Enlargement, B; (Narrowing of capsular space), C; (Inflammatory Cells).

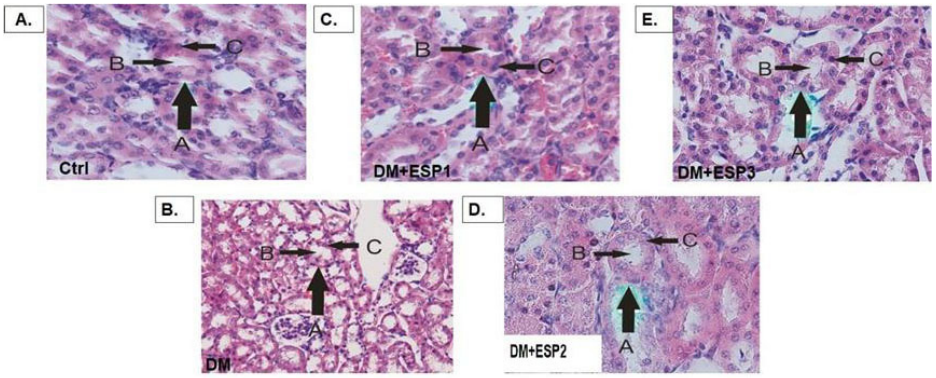

Figure 3. Proximal and distal tubules histology. Histological examination (H&E stained) (400x) of proximal and distal tubules of diabetic kidney. n=7, per group.A. Ctrl; control, B. DM; diabetes mellitus, C. DM+ESP1; Low dose (2,62gr/d), D. DM+ESP2; Mid dose (5,24gr/d), and E. DM+ESP3; High dose (7,86gr/d)., A; (Tubulus shape), B; (Lumen), C; (Hidrofik degenaration).

in reducing blood glucose in diabetic mice model.[10]

Flavonoids is a phenol class of chemicals that can protect the body from free radicals through anti-oxidant mechanisms.[11] Anti-oxidants are substances that are able to repair damaged cells and are able to ward off free radicals.[12] These results are in accordance with previous studies conducted by Handani et al (2015) where the content of flavonoids in long beans (*Vigna ungucilata*) is effective against the histologic improvement of mice glomerulus.[10]

The mechanism of glomerular damage in mice in this study occurred because of the injection of alloxan in mice which caused blood sugar levels in mice to be high.[8] High blood sugar levels trigger the production of free radicals or reactive oxygen species (ROS) and will form AGE's products that are very high in cells. Which will cause a reaction between glucose and protein which will increase glycosylation products by the non-enzymatic process of protein between the precursor dicarbonyl (intracellular glucose derivatives with amino from intracellular and extracellular proteins). According to Chin (2005) AGE's production can damage cells, because it disrupts the structure of intracellular and extracelular proteins such as collagen.[13]

In the results of this study (Figure.3 and Table 2), the proximal and distal tubular histological damage was caused by hyperglycemia. According to Sing (2010) the hyperglycemia period causes an increase in the workload of proximal tubular cells in glucose reabsorption which then induces hypertrophy of proximal tubular cells, thickening of the tubal basement membrane and tubular dilatation. In the advanced stages of tubular atrophy and peritubular fibrosis.[3] Damage from the proximal tubule occurs due to the formation of high free radicals in the body, resulting in damage to the proximal tubules and endogenous antioxidants in the body unable to neutralize these free radicals. The level of free radicals that are too high from the level of antioxidants will trigger oxidative stress. According to King (2004) states that hyperglycemia induces an increase in the production of reactive oxygen species (ROS) such as superoxide (O2-), hydrogen peroxide (H2O2), nitric oxide (NO) and decreased levels of endogenous antioxidants. An imbalance in the level of free radicals and antioxidants causes oxidative stress which causes lesions in the proximal tubules.[12]

Kidney is an organ that plays a role in maintaining homeostasis plasma glucose levels around 90-100 mg / dL in a 24hour period. About 180g of glucose is filtered by the kidneys every day and almost all (99%) of glucose filtration results are reabsorbed into the circulation through sodium glucose co-transporters (SGLTs)

Table 2. Comparison of Proximal and Distal Tubular Damage Rates.

Groups	Damage Degree (Modus)	Evaluations	Uji *Kruskal-Wallis H*
K-	Normal	Hydrophilic degeneration, indistinct tubular lumen.	25,127 *Signifikansi* = 0,001 (< 0,05)
K+	Moderate		
P1	Mild		
P2	Moderate		
P3	Moderate		

*Information: Mild (≤25%), moderate (25-50%), severe (≥50%).

which transport sodium and glucose into cells using the sodium gradient produced by the Na-K-ATPase pump on the basolateral cell membrane. There are two transporters that play a role in glucose reabsorption, namely GLUTs and SGLTs. SGLTs itself consists of two types namely SGLT1, with low capacity, high-affinity transporters located primarily in the small intestine and proximal tubules of the kidney; and SGLT2, a high-capacity, low-affinity transporter that is especially present at the beginning of the proximal tubule (segments 1 and 2), contributes to 90% glucose reabsorption. The remaining 10% of glucose is reabsorbed by SGLT1 in the more late tubules (segment 3) or distal tubules. Glucose is then passively transported by glucose transporter-2 (GLUT2) in accordance with the concentration gradient into the interstitial space. Renal threshold levels for glucose (RTg) are plasma glucose concentrations above the SGLT capacity to be saturated and urinary glucose excretion arises. This glucose reabsorption capacity is increased in diabetes due to upregulation of SGLT2 and GLUT2 in the proximal tubule, causing hyperglycemia and decreased glucosuria (Chao, 2014). SGLT2 itself lies in the S1 and S2 segments of the proximal tubule and plays a role in reabsorbing glucose back to plasma.[2]

CONCLUSION

Syzygium polyanthum (Wight) extract effective decreased glucose levels in all dose (2.62mg/20g/day, 5.24mg/20g/day, and 7.86mg/20g/day) and significantly (p<0,05) improved the glomerular, proximal and distal tubular lesions in low dose (2.62mg/20gBB/day). Further studies are needed to assess the effective dose, side effect and toxicity of *Syzygium polyanthum* (Wight) extract.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTION

All authors similarly contribute to the think about from the investigate concepts, information acquisitions, information investigation, factual investigations, changing the paper, until detailing the consider comes about through publication.

ETHICAL CONSIDERATION

This research has passed the ethical test of the Health Research Ethics Commission of the Universitas Nahdlatul Ulama Surabaya with certificate number 2382/EC/KEPK/UNUSA/2021.

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