ORIGINAL ARTICLE

Prevention of Polyuria, Glucosuria, and Increase of Kidney Weight in Diabetes Mellitus Rats by *Centella asiatica* Extract

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ABSTRACT

Introduction: Diabetes mellitus (DM) is a disease that can cause complications in the kidneys. *Centella asiatica* extracts have the potential to inhibit pancreatic, liver and kidney tissue damage. This study was intended to determine the potential of *C. asiatica* extract in inhibiting kidney damage in an animal model of DM. **Methods:** Male Wistar rats were used in 5 treatment groups namely non-DM, DM, and DM with *C. asiatica* extract Dose 1 (250mg / kg), Dose 2 (500mg / kg) and Dose 3 (1000mg / kg). Changes in body weight, blood sugar, serum urea, kidney weight, glycosuria, and urine volume were observed in all treatment groups. **Results:** There were no significant differences between treatment groups on changes in blood glucose concentration, body weight, and serum ureum. However, *C. asiatica* treated group showed significantly lower value of urine volume, glycosuria, and kidney weight compare to those on Non-DM and DM groups. Decrease in blood glucose, although not significantly different, affects glucose urine excretion. **Conclusion:** *C. asiatica* extract has the potential to inhibit kidney damage in rats with DM through prevent the increase of urine volume, glycosuria, and kidney weight.

Keywords: *Centella Asiatica* Extract, Diabetes Mellitus, Kidney damages

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INTRODUCTION

World Health Organization (WHO) reports that DM is an important health problem that in 2014 affected 422 million people in the world. Diabetes-related to hyperglycemia (high blood glucose level) caused by insensitive receptors of insulin hormone and lead to a decrease of insulin production. It also contributes to blindness, kidney failure, heart attack, stroke, and limb amputation (1). Diabetes mellitus (DM) also often triggered severe kidney damage known as diabetic nephropathy (2).

Higher blood sugar levels conditions can disrupt the glucose homeostasis that occurs initially. It further will impair the beta-cells glucose responsiveness to meals by disrupting the first phase of insulin response and causing the blood glucose level to rise. This mechanism together with an excess of fatty acids that are characteristic of obesity and insulin resistance, causes further deterioration of beta-cells function along with further insulin resistance and blood glucose levels rise to DM(3). Hyperglycemia and hyperlipidemia induce oxidative stress and advance glycation end products (AGEs), thereby activating pro-inflammatory cytokines and causing organs damage including kidney damage (4).

C. asiatica extracts have anti-diabetic activity with a mechanism of lowering blood sugar levels. This extract is also known to have the ability to repair damaged tissue in the pancreas, liver, and kidney(5). DM patients can suffer from damage to the kidneys that can be prevented by giving anti-diabetic to control the blood glucose level and thus, protect tissue in the kidneys. This study aims to determine the potential of *C. asiatica* extract in preventing kidney damage in diabetics.

MATERIALS AND METHODS

Male Wistar rats were grouped into treatment and non-treatment groups. Treatment rats were induced by 120 mg / kg BW Nicotinamide (NA) through intra-peritoneal injection and 60 mg/kg BW Streptozotocin (STZ) through intra-peritoneal injection (6). Rats were divided into non-DM groups, DM groups, DOSE 1 (250 mg / kg BW / day), DOSE 2 (500 mg / kg BW / day), and DOSE 3 (1000 mg / kg BW / day). Treatments were given for 8 weeks. Each treatment group was transferred to the metabolic cage after 8 weeks of treatment. Urine volume was observed and taken for examination of glycosuria.

The blood glucose concentrations were measured using spectrophometry based methods in Laboratorium Penelitian and Pengujian Terpadu Universitas Gadjah Mada, Yogyakarta soon after the blood collected in EDTA vacutainer.

Changes in body weight were observed once every week for 8 weeks. Blood sugar was examined before and after the induction of NA and STZ and before the termination process. One day before sacrificed, the animals were kept in the metabolic cage to obtain 24 hours urine. Collected urine was used for glycosuria and urine volume examination. Blood were collected before sacrificed and used for serum ureum examination. The kidney weight was measured after the animals were sacrificed. The blood ureum concentrations were measured using spectrophometry based methods in Laboratorium Penelitian and Pengujian Terpadu Universitas Gadjah Mada, Yogyakarta soon after the blood collected in EDTA vacutainer. The urine glucose concentrations were measured qualitatively using commercial urine strip (UriScan Strip, YD Diagnostics, Korea).

RESULTS

The blood glucose did not significantly difference among group of treatments (Figure 1A). The decrease in blood sugar also did not significantly different among groups of treatment (Figure 1B). The body weight (Figure 2A) and ureum serum (Figure 2B) also did not show any significant diffrent between groups of treatment.

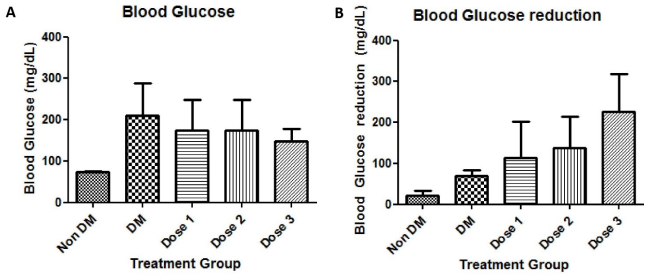

Figure 1: Mean of Blood Glucose (A) and Blood Glucose Reduction (B) after 8 weeks of treatment. Data were presented as mead+SEM. DM= Diabetes Mellitus Group, Non DM= Non Diabetes Mellitus Group, Dose 1=Diabetes Mellitus treated with *C. Asiatica* extract 250 mg/kg BW. DOSE 2=Diabetes Mellitus treated with *C. Asiatica* extract 500 mg/kg BW , DOSE 3= Diabetes Mellitus treated with *C. Asiatica* extract 1000 mg/kg BW

The group with *C. asiatica* extract showed smaller amount of urine compared to those on the DM group (Figure 3A). In addition to small urine volume, *C. asiatica* treated group also showed lower glycosuria values than those in the DM group (Figure 3B). The *C. asiatica* effect on the kidney also shown in the difference of kidney weight compared to DM group without treatment. The kidney weight in C.asiatica treated group is significantly lighter than those in the DM group (Figure 3C).

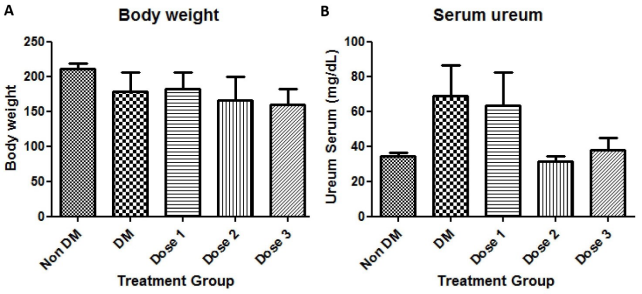

Figure 2: Mean of Body Weight (A) and Serum Ureum Level (B) after 8 weeks of treatment. Data were presented as mead+SEM. DM= Diabetes Mellitus Group, Non DM= Non Diabetes Mellitus Group, Dose 1=Diabetes Mellitus treated with *C. Asiatica* extract 250 mg/kg BW. DOSE 2=Diabetes Mellitus treated with *C. Asiatica* extract 500 mg/kg BW , DOSE 3= Diabetes Mellitus treated with *C. Asiatica* extract 1000 mg/kg BW

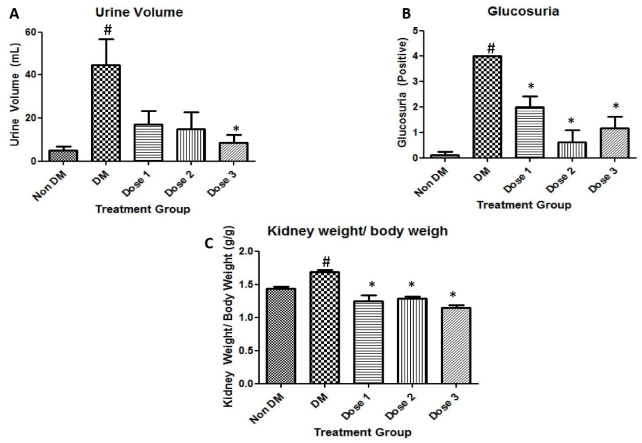

Figure 3: Mean of Urine Volume (A), Glucosuria (B), and Ratio of Kidney Weight/Body Weight (C) after 8 weeks of treatment. Data were presented as mead+SEM. DM= Diabetes Mellitus Group, Non DM= Non Diabetes Mellitus Group, Dose 1=Diabetes Mellitus treated with *C. Asiatica* extract 250 mg/kg BW. DOSE 2=Diabetes Mellitus treated with *C. Asiatica* extract 500 mg/kg BW , DOSE 3= Diabetes Mellitus treated with *C. Asiatica* extract 1000 mg/kg BW. *p<0.05 vs DM, #p<0.05 vs Non DM

DISCUSSION

The difference of blood glucose levels and decrease of blood glucose levels between groups in this study did not statistically significant even though the group with *C. asiatica* treatment showed lower blood glucose compared to those on DM group. Based on the previous study *C. asiatica* has anti-hyperglycemic activity without causing hypoglycemia (7,8). *C. asiatica* has been shown the ability to inhibit glucose absorption by inhibition of intestinal saccharidase, inhibition of α-amylase and increase of glucose-fiber binding (7). Weight loss is the sign of hyperglycemia and uncontrolled level in diabetes. Therefore we also measure the bodyweight of the rats. There were no significant different in term of bodyweight of the rat before it was sacrificed. We

suggest that the hyperglycemia in our DM model is not high enough to cause loss of bodyweight.

The examination of kidney effect of *C. asiatica* was done by measuring ureum serum concentration, urine volume, glycosuria and kidney weight. Observation of serum ureum concentration in the serum did not show significant differences although the serum ureum in DOSE 2 and 3 of *C. asiatica* appears to be lower compare to those on DM group. It might indicate that there is no disturbances in the kidney function, so that serum urea levels are not different between all groups of treatment including between DM and nonDM groups.

An increase in blood glucose is followed by an increase in blood osmolality that then result in increasing circulating blood volume. The increase of circulating blood volume will increase the glomerolus filtration rate (GFR). If damage occurs to the kidneys it can cause excessive filtration as well as a reduction in degradation and reabsorption in the kidney tubules (9). Filtered blood with high glucose concentration will reach kidney tubule and if the glucose concentration is higher than the glucose reabsorption threshold of the kidney tubule, the glucose will be excreted in the urine and cause glucosuria (10). The increase in GFR causes kidney inflammation and hypertrophy, which then result in an increase of kidney weight. DM can affect the kidneys, causing swelling and disrupting GFR that ends in sclerosis and kidney failure (2,9). DM characterized by an increase in kidney weight, urine volume and the discovery of glucose content in urine (10). Glycosuria or glucosa content in urine is the result of glomerular filtration in excess of glucose concentrations than the ability of the kidney tubules to reabsorb because there is an increase in plasma glucose and/or impaired renal glucose absorption capacity (11).

The DM group showed higher urine volume, glucosuria level and kidney weight compared to those on Non-DM group. Meanwhile, *C. asiatica* treated group showed lower urine volume, glucosuria and kidney weight compare to those on DM group. It indicates that *C. asiatica* treatment in DM can prevent the increase of urine volume and glycosuria and the increase of kidney weight. We suggest that *C. asiatica* might has an nephroprotective effect in DM. However the mechanism is independent of blood glucose level regulation. Further study needed to be done to find *C. asiatica* nephroprotective mechanism of action in DM.

CONCLUSION

Administration of *C. asiatica* extract in animal model of DM did not improve blood glucose level. However, the administration of *C. asiatica* extract could have an effect in preventing the development ofkidney damage in DM.

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