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The potency of Centella asiatica in protecting organs of rats with type 2 diabetes mellitus

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ABSTRACT

Background: Diabetes mellitus is a major public health problem with an increasing prevalence each year. Chronic hyperglycemia causes impaired function and organ damage. *Centella asiatica* is a plant that contains antioxidants with the main component of pentacyclic triterpenes that has been proven to have antioxidant, anti-inflammatory, and antimicrobial activity. This study aimed to analyze the changes in blood glucose level, the weight of liver, kidney, heart, and brain of streptozotocin-nicotinamide-induced male Wistar rats.

Methods: The research consists of four treatments: negative control (P1), positive control (P2), ethanol extract of *Centella asiatica* with a dose of 300 mg/kg of body weight (P3), and ethanol extract of *Centella asiatica* with a dose of 600 mg/kg of body weight (P4). The treatments were conducted within four weeks at the laboratory of PSPG UGM Yogyakarta. The statistical tests used were the analysis of variance (ANOVA) and the Duncan Multiple Range Test (DMRT) to determine differences between control groups and the treatment groups.

Results: The induction of nicotinamide and streptozotocin led to type 2 diabetes mellitus ($p = 0.001$). The administration of ethanol extract of *Centella asiatica* reduced blood glucose level significantly ($p = 0.001$). The administration of ethanol extract of *Centella asiatica* increased the weight of liver significantly ($p = 0.030$). There were significant differences between the intervention group of *Centella asiatica* dose of 300 mg/kg of body weight and 600 mg/kg of body weight compared to positive control.

Conclusion: Ethanol extract of *Centella asiatica* with a dose of 600 mg/kg of body weight can potentially improve hyperglycemia and increase the weight of the liver organ, which is an indicator of liver cell regeneration in the animal model of diabetes mellitus type 2.

Keywords: Blood glucose level, Centella asiatica, Diabetes, Organ weight
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INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia due to abnormalities of insulin secretion, insulin sensitivity, or both.[1] The prevalence of DM in the world is 9% in 2014, while in Indonesia, the prevalence increased from 1.1% in 2007 to 2.4% in 2013. DM is one of the causes of 82% of deaths worldwide because of non-communicable diseases.[2-4]

DM patients experience hyperglycemia causing a response of inflammatory compounds thus detrimental to insulin sensitivity and glucose balance.[5] Hyperglycemia causes liver damage, impaired fat metabolism, changes the structure of the liver, and harms liver function.[6] The condition of chronic hyperglycemia in DM can lead to complications such as renal impairment, neuropathy, retinopathy, gastroparesis, coronary heart disease, cerebrovascular disease and peripheral arterial disease.[7]

Organ weight changes can be used as an indicator of physiologic changes and complications. In vivo studies report that the weight of organs such as the liver, kidneys, heart, pancreas, lungs, and brain changes in diabetes mellitus model.[8,9] The decrease in liver weight is probably due to the catabolic process of glycogenolysis, lipolysis, and proteolysis due to insulin deficiency in the liver cells. Meanwhile, renal weight increases due to glucose overutilization and increased glycogen synthesis.[10] These changes lead to severe microvascular complications of the kidneys and involve a series of metabolic changes in the pathogenesis of diabetic nephropathy.[11] A total of 40% of diabetic patients worldwide experience diabetic nephropathy.[12] On the other hand, the use of drugs in the management of this disease has been associated with side effects that can cause kidney damage.[13] Besides that, oral anti-diabetic drug therapy causes side effects of gastrointestinal disorders such as bloating, nausea, vomiting, diarrhea, disorders, malabsorption of vitamin B12 in the small intestine, and liver dysfunction.[14] Therefore, new therapeutic agents are needed that are low in side effects and toxicity.[15]

Gotu kola or *Centella asiatica* is a plant containing antioxidants with the main components of pentacyclic triterpenes (asiatic acid, madecassic acid, asiaticoside, and madecassoside). *Centella asiatica* has been proven to have antioxidant, anti-inflammatory, and antimicrobial activity.[16] The ethanol extract of *Centella asiatica* leaf with a dose 300 mg/kg of body weight for four weeks was proven to decrease blood sugar and lipid levels and increase the production of insulin in type 2 diabetes mellitus.[17] A study with lipid emulsion-induced rat showed that the extraction of *Centella asiatica* using 80% ethanol with a dose of 1000 mg and 2000 mg decreased plasma glucose, triglyceride, and total cholesterol levels.[18]

Centella asiatica plant contains antioxidants in the entire part of the plant, from the leaves to the roots.[19] The triterpenoid aglyconic compound in the non-polar encoder is when it binds to 3 molecules of sugar remains small solubility in water and more soluble in ethanol 70%.[20] The extraction process using ethanol was better compared to methanol and water. Ethanol can well-dissolve triterpene agitone compounds, especially phenolic content, asiaticoside, and madecassoside.[21]

The increasing demand for anti-diabetic herbal preparations, particularly for people in developing countries, is the main reason why this study was conducted.[22,23] The aim of this study was to investigate the potency of ethanol extract from the entire part of *Centella asiatica* in reducing blood glucose levels and protecting the organs of male Wistar rats with type 2 diabetes mellitus induced with 65mg/kg of body weight of streptozotocin (STZ) and 230 mg/kg of body weight of nicotinamide (NA).

METHODS

Design, place, and time

This research was an experimental laboratory research, with rats as the animal model. The experimental design was a randomized controlled trial with four treatment groups. The production and analysis of the extract of *Centella asiatica* were done in unit 1 of the Integrated Research and Testing Laboratory Gadjah Mada University Yogyakarta. The breeding and treatment of animal testing were done in the Experimental Animal Development Unit in Pusat Studi Pangan dan Gizi (PSPG) Gadjah Mada University Yogyakarta. This research was conducted from February to August 2017.

Number and collection of sample

The experimental animal model used in this research was male white *Rattus Norvegicus strain Wistar* rats. The rats were eight weeks old and had 200 ± 50 g of bodyweight obtained from the Experimental Animal Development Unit Gadjah Mada University Yogyakarta, which received approval from the Animal Ethics Commission No 129/II/HREC/2017 Dr. Moewardi Hospital. The samples were taken randomly using the *Simple Random Sampling* (SRS) method from an accessible population. The samples must be suitable with the inclusion criteria and the rats must be fit and healthy, proven by physical and initial blood glucose examinations. Twenty rats were divided into four treatment groups, which were the negative control group consisting of normal or healthy rats (P1), the positive control group, which was the diabetic rats induced with nicotinamide-streptozotocin (P2), the ethanol *Centella asiatica* extract with dose 300 mg/kg of body weight group (P3), and the ethanol *Centella asiatica* extract with dose 600 mg/kg of body weight group (P4).

Research Steps

Centella asiatica was obtained from Merapi Farma herbal, Kaliurang, Yogyakarta. The Simplicia was all parts of *Centella asiatica* plant from root to leaves that had been extracted by the remaseration method using 70% ethanol, according to Nugroho et al.[24] and Pramono & Ajiastuti.[20] The rats were first acclimatized for one week to control the condition of the animal testing. The rats were bred in a special room located in hygienic polypropylene cages with five rats per big cage. The cages were given transparent barriers to make rats live in one small cage individually.

The rats were bred in a 27 – 29°C room, with 12 hours lamp on and 12 hours lamp off (the lamp was turned on at 07.00 AM). The feed given was *pellet* feed type AD II and reverse osmosis drinking water was delivered through ad libitum. The initial procedure in making type 2 diabetes mellitus rats was to fast the *Wistar (Rattus norvegicus*) rats for one night. The next step was the injection of 230 mg/kg of body weight of NA dissolved in normal saline intraperitoneal. After fifteen minutes, the rats were injected with a single dose of 65 mg/kg of body weight of STZ dissolved in a buffer of cold citric acid pH 4.5 intraperitoneal. The rats were declared to have hyperglycemia when their blood glucose levels were > 150 mg/dL after three days of post induction.[25]

Blood glucose level was measured using glucose oxidase phenol aminoantipyrina peroxidase (GOD-PAP) method, which was conducted in the laboratory of Pusat Studi Pangan dan Gizi (PSPG) UGM Yogyakarta. Vein blood sampling was done through the eyes (*medial canthus sinus orbitalis*) using microhematocrit and accommodated into the ependorop 0.5 ml. The measurement of blood glucose was performed three times. The first test

was the initial measurement (day 0) aimed to ensure that the rats were healthy with normal glucose levels (65 - 135 mg/dl). The second test was performed to ensure that the rats had hyperglycemia on the 3rd day after induction. The third test was performed to ensure that the rats with type 2 diabetes mellitus had lower blood glucose levels after having treatment.[26,27]

The rats were terminated after four weeks by putting them into a jar that has been doused with ether on the inside. Necropsy was performed by slashing the skin and abdominal muscles until the abdominal cavity was opened. The kidney, liver, brain, and heart organs were taken and weighed immediately. The weight of organs and blood glucose levels were calculated using a formula from Eleazu et al.[28]:

Percentage of the changes in Blood Glucose Levels (BGL):

$$\frac{\text{(initial BGL-final BGL)}}{\text{(initial BGL)}} \times 100\ \%$$

Data processing and analysis

Data analysis was done by testing data normality (Shapiro-Wilk test) and homogeneity (Levene test) for the requirement of analysis of variance (ANOVA). The initial blood glucose level, the weight of the kidney, liver, heart, and brain underwent ANOVA testing to know the average differences between the groups. After that, the Duncan Multiple Range Test (DMRT) test was performed to determine the differences between the control groups and the treatment groups. All data were analyzed with a 95% credibility level ($p < 0.05$) using the SPSS 20 software.

RESULT

Table 1 shows that NA-STZ induction had a significant effect on blood glucose levels in diabetic experimental animals before the treatment of *Centella asiatica* extract (pre-test) ($p = 0.001$). Blood glucose levels in the pre-test group of P2, P3, and P4 increased and was significantly different compared to the negative control group (P1). The administration of *Centella asiatica* extract had a significant effect on blood glucose levels at post-test in experimental animals with diabetes ($p = 0.001$). Further analysis showed that each group differed significantly from the other groups as shown in Table 1.

Table 2 shows that the administration ethanol extract of *Centella asiatica* had no significant effect on the weight of the kidney ($p = 0.502$), heart ($p = 0.290$), and brain ($p = 0.810$) of rats with type 2 diabetes mellitus. However, the administration of the *Centella asiatica* extract significantly affected the weight of the liver organ ($p = 0.030$). Further analysis showed that the weight of the liver in the positive control group (P2) decreased significantly compared to the negative control group or healthy rats (P1). The intervention group of *Centella asiatica* P3 and P4 differed significantly from the positive control group (P2) as shown in Table 2.

DISCUSSION

The pre-test blood glucose levels in the negative control group (P1) matched the threshold of normal rat blood glucose levels (65-135 mg/dl) according to Kusumawati.[26] Blood glucose levels at pre-test in group P2, P3, and P4 indicated the condition of diabetes according to the standard by Ortiz-Andrade et al.[29] that rats were categorized with hyperglycemia when their blood sugar was > 150 mg/dl.

The modeling of type 2 diabetes mellitus in this study was successful, indicated by increased pre-test blood glucose levels in group P2, P3, and P4 that were significantly different from P1. This is consistent with the review of a study by Ghasemi et al.[25] that the NA-STZ type 2 induced diabetes mellitus model provides a relatively stable effect of hyperglycemia without the need for exogenous insulin to survive. This model is reported to be more suitable for biochemical and pharmacological studies to examine the potential effects of antidiabetes.

The administration of *Centella asiatica* extract provided a significant effect on post-test blood glucose levels in experimental animals with diabetes. The administration of ethanol extract of *Centella asiatica* with a dose of 300 mg/kg of body weight and 600 mg/kg of body weight decreased blood glucose levels by 37.54% and 51.0% respectively compared to positive control group. The administration of ethanol extract of *Centella asiatica* with a dose of 600 mg/kg of body weight for four weeks can restore blood glucose levels to a normal threshold, although it still differed significantly with the negative control. The results of this study are in line with Kabir et al.[30] which showed that the administration of the water extract of *Centella asiatica* for four weeks with a higher dose of 1000 mg/kg of body weight resulted in lower blood glucose levels. Furthermore, Maulidiani et al.[17] also showed that the administration of ethanol extract from the leaf of *Centella asiatica* with a dose of 300 mg/kg of body weight lead to a decrease in blood glucose levels in the plasma of STZ inducted rats.

Table 1. Changes in blood sugar levels before and after intervention

Group	Pre-test (mg/dl)	Post-test (mg/dl)	Percentage change in blood glucose (%)
P1	75.34 ± 7.55[a]	76.49 ± 7.57[a]	-1.06 ± 0.65[a] (increase)
P2	260.32 ± 7.09[b]	264.85 ± 9.61[b]	-1.73 ± 0.98[a] (increase)
P3	251.33 ± 12.69[b]	166.42 ± 27.99[c]	37.54 ± 13.12[b] (decrease)
P4	256.46 ± 9.91[b]	139.80 ± 11.06[d]	51.00 ± 14.92[b] (decrease)

PI = negative control; P2 = positive control; P3 = ethanol extract of pegagan dose 300 mg/kg BW; P4 = ethanol extract of pegagan dose 600 mg/kg BW; values are presented as means ± SD; n = 5; different notations indicate a significant difference ($p < 0.05$).

Table 2. Organ weights of diabetic and non-diabetic rats

Group	Liver weight (g)	Kidney weight (g)	Heart weight (g)	Brain weight (g)
P1	10.69 ± 1.72[a]	2.52 ± 0.48[a]	0.92 ± 0.15[a]	1.78 ± 0.17[a]
P2	8.52 ± 1.09[b]	2.13 ± 0.32[a]	0.80 ± 0.17[a]	1.75 ± 0.15[a]
P3	10.86 ± 1.22[a]	2.55 ± 0.72[a]	0.77 ± 0.12[a]	1.78 ± 0.12[a]
P4	10.66 ± 0.91[a]	2.30 ± 0.34[a]	0.72 ± 0.20[a]	1.84 ± 0.17[a]

PI = negative control; P2 = positive control; P3 = ethanol extract of pegagan dose 300 mg/kg BW; P4 = ethanol extract of pegagan dose 600 mg/kg BW; values are presented as means ± SD; n = 5; different notations indicate a significant difference ($p < 0.05$); numbers with the same letter (1 column) showed no significant difference ($p > 0.05$).

The ethanol extract of *Centella asiatica* contains asiatic acid, which is a derivative triterpenoids of *Centella asiatica.* Asiatic acid has several pharmacological activities such as antioxidants[31] and hepatoprotectors.[32] Diabetes mellitus patients with chronic hyperglycemia are known to have decreased *Phosphatidylinositide* 3-Kinase (PI3K)/ Akt signal pathway that is required for insulin regulation.[33] A study by Ramachandran & Saravanan shows that asiatic acid increased insulin secretion by increasing the Kinase/ Akt PI3 signal pathway. In addition, the compound also improves glucose response through increased protein GLUT-4 muscle, IR, IRS-1, and IRS-2.[34]

The weight of the kidney, heart, and brain of rats between the treatment groups in this study was not significantly different. The results of this study are supported by the study conducted by Cintra et al.[8] which showed that there was no significant difference in the weight of the brain, heart, and pancreas organs. However, the weight of the liver decreased significantly. This study contradicts Zafar & Naqvi study that reported the induction of STZ 45 mg/kg of BW significantly reduced the organ weights and ratios of the kidneys and pancreas of albino rats.[35] This is probably due to different doses of STZ provision and the addition of NA, which functions as a protective factor.

The provision of *Centella asiatica* extract significantly affected the weight of the liver organ. The results of this study are in line with the studies by Zafar & Naqvi and Cintra et al. which showed that STZ induced diabetes mellitus models can significantly reduce liver weight compared with the group of healthy rats.[8,35]

Diabetes is associated with microvascular and macrovascular diseases affecting several organs such as the liver, heart, brain, and kidney.[36] Changes in organ weights can be used as indicators of some physiological changes.[8,9] Our research shows that the condition of type 2 diabetes mellitus decreased the weight of the liver. However, the weight of the kidneys, heart, and brain was not significantly different. Reduced liver weight in the control group diabetes is likely due to changes in the structure of the liver caused by the induction of STZ. The induction of STZ 35 mg/kg of BW leads to histopathological changes in diabetic rats, i.e., the disorientation of cellular structures with degeneration such as glycogen deposition, fat changes, and nucleus cells experiencing pignosis.[37] The results of this study are supported by the study of Noorafshan et al. it was found that the liver weight decreased by 15%, which was associated with a decrease in the volume of hepatocytes, nucleus, and sinusoid in rat models of diabetes mellitus.[38]

The ability of ethanol extract of *Centella asiatica* in preventing the decrease in liver weight can be linked to its ability to lower blood sugar levels. In this study, reduced blood sugar levels increased the liver weight back to normal conditions. The increase in liver weight was likely caused by the ability of *Centella asiatica* to normalize the process of glycolysis. The liver is an organ that plays an important role in glucose homeostasis, glycolysis, glycogenesis, and gluconeogenesis. The process will be affected when the condition of diabetes mellitus occurs. Net glucose uptake by the liver depends on the activity of glucokinase and glucose-6-phosphatase. The diabetic condition causes a decrease in the activity of glucokinase and glucose-6-phosphatase that almost doubled.[39] An in vivo study indicated that the content of asiatic acid in *Centella asiatica* has the role of antidiabetic through increased glycolysis pathway to restore the activity of key enzymes such as hexokinase, glucose-6-phosphate dehydrogenase (G6PDH), fructose 1,6-bisphosphatase and pyruvate kinase.[40] The process of glycolysis produces pyruvate, which then goes into the citric acid cycle. Furthermore, the process of decarboxylation becomes acetyl coenzyme A. This cycle provides NADH for the oxidative phosphorylation process to form the ATP required to generate energy.[39] If the process of glycolysis runs normally, then it is expected that

the cell regeneration process will occur. According to Mc-Queen, one of the factors that played an important role in the regeneration of liver cells was the availability of sufficient energy in hepatocytes cells.[41]

Increased liver weight due to the provision of *Centella asiatica* extract may also be associated with the effects of hepatoprotective. *Centella asiatica* contains the antioxidant triterpenoid group, saponin, and aglikon.[42] An antioxidant-rich plant can serve as a hepatoprotector that helps reduce liver damage in diabetic patients.[43] A study conducted by Kumar et al. stated that the provision of *Centella asiatica* juice for 28 days decreased markers of liver function damage in paracetamol-induced animals study.[44] In addition, a study by Choi et al. reported that the administration of aqueous extract of *Centella asiatica* with a dose of 200 mg/kg of body weight had a hepatoprotective effect in dimethylnitrosamine-induced rats, which was due to the reduction of necrosis of periportal degeneration intralobe and focal necrosis with fibrosis of the liver tissue on the result of histology.[45] Based on the description above, the results of this study indicate that the administration of ethanol extract from the entire part of *Centella asiatica* with a dose of 300 mg/kg and 600 mg/kg of body weight for 28 days can regenerate liver cells through the weight gain indicator.

CONCLUSION

The ethanol extract from the entire part of *Centella asiatica* with a dose of 600 mg/kg of body weight can lower blood sugar levels and increase the weight of the liver to normal conditions. The model of type 2 diabetes mellitus with NA-STZ induction did not cause severe changes in the kidneys, brain, and heart. *Centella asiatica* can be an alternative to herbal preparations that may potentially improve hyperglycemia and regenerate liver cells through the indicator of liver weight.

It is suggested that the ethanol extract from the entire part of *Centella asiatica* in NA-STZ induction on rats should be continued to histopathology and immunohistochemistry studies. It is expected to determine biomolecular liver tissue damage markers.

CONFLICT OF INTERESTS

The authors in the study state that there is no conflict of interest in regards to this research.

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