Electronic Journal of General Medicine
2020, 17(5), em225
e-ISSN: 2516-3507
https://www.ejgm.co.uk/

Original Article

MODESTUM
OPEN ACCESS

The Hepatoprotective Capacity of Steeping *kersen* Leaves (*Muntingia calabura* L.) on Diabetic Rat

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Citation: Indriawati R. The Hepatoprotective Capacity of Steeping *kersen* Leaves (*Muntingia calabura* L.) on Diabetic Rat. Electron J Gen Med. 2020;17(5):em225. https://doi.org/10.29333/ejgm/7888

ARTICLE INFO

Received: 29 Dec. 2019

Accepted: 21 Feb. 2020

ABSTRACT

Background: Diabetes Mellitus is a degenerative disease that causes many complications in which blood glucose levels and oxidative stress increase that can cause liver damage and characterized by the increase in liver enzymes namely SGOT and SGPT. Kersen (Muntingia calabura L.) is one kind of plants that has antioxidant activity because it contains flavonoids to prevent oxidative stress.

Methods: This study was an experimental research study design with pre and posttest control design. Thirty Sprague-Dawley rats were divided into 5 groups: group 1 (negative control), group 2 (metformin), group 3 (steeping kersen leaves of 250 mg/ 200 gr BW), group 4 (steeping kersen leaves of 500 mg/200 gr BW), and group 5 (steeping kersen leaves of 750 mg/200 gr BW). All groups induced with streptozotocine dose of 65 mg/kg BW and nicotinamide of 230 mg/kg BW for 5 days until the rats were with diabetes mellitus (fasting blood sugar> 135 mg/dl) and then given treatment for 14 days. GOD-PAP enzymatic method used glucose test, whereas a UV-Vis spectrophotometer used SGOT and SGPT test. Data were analyzed using paired t test and One Way Anova.

Results: The results of statistical tests with paired t test showed significant differences in the levels of SGOT and SGPT before and after treatment (p=0.0001). One way Anova test shows that there is significant decrease in each group (p=0.0001).

Conclusion: Steeping kersen leaves most effectively reduce levels of SGOT and SGPT in the dose of 750 mg/200 gr BW.

Keywords: diabetes mellitus, *kersen*, SGOT, SGPT, non-alcoholic fatty liver

INTRODUCTION

Prevalence of Diabetes Mellitus (DM) continues to grow rapidly in developed and developing countries (1,2). Type 2 diabetes mellitus is more common than type 1 diabetes mellitus, which reaches 90-95% of all diabetics (3,4). Type 2 diabetes has become a serious issue at an alarming rate in developing countries. It is predicted that Type 2 diabetes will continue to increase in the next twenty years, and more than 70% of the patients will appear in developing countries, with the majority of them being 45-64 years old (5-7). Type 2 diabetes mellitus is due to reduced tissue sensitivity to insulin (8,9). High levels of blood glucose (hyperglycemia) in diabetes mellitus can cause complications such as cardiovascular disorders, increased lipid accumulation in the liver and in smooth muscle, chronic renal failure, and cancer (10,11).

Chronic hyperglycemia can lead to complications in other organs such as hepatic fatty acids. This hepatic fat is known as NAFLD (Non Alcoholic Fatty Liver Disease) that is closely related to insulin resistance (12). Therapy for DM patients consists of lifestyle improvements supported by oral injection drugs where these drugs are expensive and have side effects such as liver and kidney disorders, bloating, and cardiovascular disorders (13).

One of the plants in Indonesia that has the potential to be an alternative treatment was *kersen* leaves (*Muntingia calabura* L.). The *kersen* leaves contain flavonoids that as antioxidants are useful to protect cells and organs from free radicals, and one of the cells is in liver (14). Therefore, it is necessary to do research to determine effectiveness of the *kersen* leaves on the state of the liver on type 2 diabetes mellitus.

MATERIALS AND METHODS

This study was an experimental research study design with pre and posttest control design. Thirty Sprague-Dawley rats were divided into 5 groups: group 1 (negative control), group 2 (metformin), group 3 (steeping *kersen* leaves of 250 mg/ 200 gr BW), group 4 (steeping *kersen* leaves of 500 mg/200 gr BW), and group 5 (steeping *kersen* leaves of 750 mg/200 gr BW). All groups induced with streptozotocine dose of 65 mg/kg BW and nicotinamide of 230 mg/kg BW for 5 days until the rats became with diabetes mellitus (fasting blood sugar> 135 mg/dl) and then given treatment for 14 days. Blood sampling was

performed 3 times, i.e. before induction with streptozotocine-nicotinamide, after induction of streptozotocine-nicotinamide, and after treatment. The animal inclusion criteria used were Sprague-Dawley strains of male sex, aged ± 8 weeks, and had body weight of ± 150-200 gram. As the mice whose activity is less/ inactive, died during the treatment period, were sick (dull hair appearance, i.e. loss or bald) and lost weight > 10% during adaptation period in laboratory, they were removed from research sample. The independent variable is steeping leaf *kersen* (*Muntingia calabura* L.) with dose of 250 mg/ 200 gr BW, 500 mg/ 200 gr BW, 750 mg / 200 gr BW, while dependent variables were SGOT & SGPT levels (for measuring the liver damage). The controlled variables were the genetic, age, weight, condition of the cage and food factors.

The materials used in this study were *kersen* leaf (*Muntingia calabura* L.) obtained from the laboratory of food and nutrition center of University of Gadjah Mada, while streptozotocine and metformin obtained from pharmacies, rats fasting plasma, nicotinamide, 0.9% NaCl, 0.1 M citric buffer, aquadest, SGPT reagent kits, and SGOT reagent kits available in the laboratory. The tools used in this study included digital balance for weighing rats, feeding tube to provide sedation to rats, syringes for blood glucose collection, gloves, masks, pots to boil water, strainers, stoves, animal cages, centrifuge, UV-Vis spectrophotometer, micro capillary tube, and vortex.

The study began with prepared cage, measured the body weight of rats, and dividing them into 5 groups randomly. The rats were adapted for 7 days. On the 7th day, they were weighed for determining the dose of streptozotocine and nicotinamide, and the first blood samples were taken for measurement of fasting blood sugar, SGOT, and SGPT levels. The rats were induced with nicotinamide of 230mg /kgBW, and 15 minutes later there was continued induction of streptozotocine of 65 mg /kg BW on the 8th day (15,16). The second sample was taken 5 days after the induction of streptozotocin-nicotinamide with fasting blood glucose, SGOT, and SGPT. The rats were declared with diabetes mellitus if the blood glucose levels > 135mg /dl (17). After the mice were declared Diabetes Mellitus, the mice were weighed again for deciding the dose of treatment. Furthermore, the preparation was done for the steeping of *kersen* leaves. The *kersen* leaves used were the dark green ones, with no rolls and insect bites. The leaves were taken from UGM food and nutrition center laboratory's yard, dried in the sun to dry (brownish), then brewed with boiling water until the color resembles to tea. Before it was given to mice, the steeping water was filtered so that it was separated from the leaves.

The treatments were given in accordance with the respective group for 14 days, i.e. the negative control group was given only aquadest, the positive control group was given with of metformin of 0.9 mg / 200 gr BW, the treatment group 1 (P1) was given with cotton leaf dose of 250 mg / 200 gr BW, 2 (P2) was given cotton leaf dose of 500 mg / 200 gr BW, and treatment group 3 (P3) was given *kersen* leaf dose of 750 mg / 200 gr BW. After 14 days of treatment, GDP, SGOT, and SGPT were measured again. The data were analyzed using paired t test for the difference before and after induction, and One Way Anova test to know the significance of the difference between the study groups, followed by Post Hoc test and Tuckey mean test.

Table 1. Average fasting blood glucose levels before and after induction of streptozotocin-nicotinamide by paired t-test

No	Fasting Blood Glucose Level (mg/dl) ± SD		p
	Before STZ	After STZ	
Negative Control	60.73 ± 2.26	213.32 ± 5.71	0.0001
Positive Control	59.47 ±1.62	206.82 ± 1.91	
P1(250 mg *kersen*)	62.24 ± 1.72	211.00 ± 4.26	
P2(500 mg kersen)	59.97 ± 1.91	207.52 ± 2.22	
P3(750 mg *kersen*)	58.83 ± 2.08	211.84 ± 3.18	

Table 2. Average SGOT levels before and after induction of streptozotocin-nicotinamide by paired t-test

No	SGOT Level (IU/l) ± SD		p
	Before STZ	After STZ	
Negative Control	38.19 ± 0.50	76.95 ± 2.18	0.0001
Positive Control	37.38 ± 0.43	78.65 ± 2.38	
P1(250 mg *kersen*)	38.03 ± 0.39	71.04 ± 0.39	
P2(500 mg *kersen*)	38.52 ± 1.55	71.85 ± 1.73	
P3(750 mg *kersen*)	38.35 ± 0.43	77.67 ± 2.03	

Table 3. Average SGPT levels before and after induction of streptozotocin-nicotinamide by paired t-test

No	SGPT Level (IU/l) ± SD)		P
	Before STZ	After STZ	
Negative Control	18.36 ± 0.36	38.03 ± 0.66	0.0001
Positive Control	18.36 ± 0.36	37.06 ± 0.50	
P1(250 mg *kersen*)	18.20 ± 0.66	37.78 ± 0.56	
P2(500 mg *kersen*)	18.85 ± 0.56	37,86 ± 0.61	
P3(750 mg *kersen*)	19.50 ± 0.36	38.59 ± 0.90	

Table 4. The difference in the mean decrease in SGOT levels by one way ANOVA test

Groups	The mean SGOT decreased ±SD (mg/dl)	p
Negative Control	1.13 ± 0.66	0.0001
Positive Control	-37.22 ± 1.95	
P1 (250mg *kersen*)	-7.93 ± 2.14	
P2 (500mg *kersen*)	-14.56 ± 3.36	
P3 (750mg *kersen*)	-31.23 ± 2.67	

RESULTS

There were statistically significant differences in blood glucose levels in the five groups after the induction of STZ-NA (**Table 1**). The overall sample of mice was expressed as type 2 diabetes mellitus with fasting blood glucose levels > 135 mg /dl.

The observed results of SGOT, and SGPT were shown in **Tables 2** and **3**. The induction of streptozotocin-nicotinamide can damage the liver as evidenced by the increase in SGOT and SGPT (**Tables 2** and **3**).

The paired t test result showed a significant increasing of the blood glucose, SGOT, and SGPT levels before and after the induction of STZ (p=0.0001). The results of the data before and treatment were tested using statistical analysis paired sample t-test to show a significant decrease in levels of GDP, SGPT enzymes and SGOT enzymes.

Tables 4 and **5** showed that One-way ANOVA of the SGPT and SGOT levels demonstrated a significant decreasing (p=0.0001; p=0.0001). The SGPT levels after treatment in all treatment groups decreased, except in the control group. Post

Table 5. The difference in the reduction in average SGPT levels by the one way ANOVA test

Groups	The mean SGPT decreased ±SD (mg/dl)	p
Negative Control	1.78 ± 0.66	0.0001
Positive Control	-14.32 ± 1.05	
P1 (250 mg *kersen*)	-2.18 ± 1.00	
P2 (500 mg *kersen*)	-5.50 ± 0.58	
P3 (750 mg *kersen*)	-9.30 ± 0.99	

Hoc test results showed the most effective decrease in SGOT levels was 750 mg / 200 gr BW with a 31 difference, 23 IU / L, and the most effective reduction in SGPT levels was the group of 750 mg / 200 gr BW with a decrease of 9.30 IU / L.

DISCUSSION

The mechanism of action streptozotocine to pancreatic β cells is through DNA damage. DNA damage will inhibit secretion and synthesis of the insulin. The limited production of mitochondrial ATP subsequently results in a drastically reduced nucleotide of pancreatic β cells. Meanwhile, nicotinamide and pyridine-3-carboxamide are vitamin B3 (niacin) derivation with an antioxidant capacity that reduces the cytotoxic action of STZ. Nicotinamide (NA) is a catcher of oxygen-free and NO radicals, and provides NAD^+. NA also increases the regeneration and growth of β-cell cells and inhibits apoptosis. Data from the literature concludes that the mechanism of nicotinamide protection against pancreatic beta cell destruction is caused by streptozotocine, through 2 mechanisms, namely PARP-1 inhibition, and an increase in NAD^+, in which other mechanisms play less role (16). A study conducted by Bayrasheva et al. (18) proves that white rats induced streptozotocine dose of 65 mg / kg Bw and nicotinamide 230 mg/kg will have Diabetes Mellitus within 5 days (18,19). Another study conducted by Nagarajan (20) proves that the white rats induced streptozotocine dose of 60 mg / kg BW and nicotinamide of 120 mg /kg BW show symptoms of diabetes such as hyperglycemic, polyuria and glukosuria. The effective dose of streptozotocine is 65 mg / kg BW and the autotinamide is 230 mg / kg BW (21).

There are statistically significant difference in the five groups (**Tables 2** and **3**). This proves that if blood glucose levels increase then levels of SGOT and SGPT also increases. SGOT is another enzyme that aids in producing protein. It catalyzes the reductive transfer of an amino group from aspartate to α-ketoglutarate to yield oxaloacetate and glutamate. The liver injury can cause elevated SGOT. Normal SGOT level are 7-40 U/L in human and 54 g/dL in rat. The SGPT normal levels are 5-50 U/L in human and 18 g/dL in rat (22,23). The increased levels of SGOT and SGPT are due to insulin resistance (reduced sensitivity of tissue to insulin) and oxidative stress in type 2 diabetes mellitus. Oxidative stress can lead to oxidative damage including tissue damage and dead cell. With the formation of reactive oxygen species (ROS) and lipid peroxidation due to oxidative stress can lead to the formation of xenobiotic that will induce the occurrence of hepatocyte liver cell death. Liver cells are the main tissue that is the target of increased concentration of free radicals because the liver is a place of metabolic processes of xenobiotic compounds. Membrane damage to liver cells results in the increased activity of liver enzymes in the blood (21,24,25).

Insulin resistance is an important factor underlying nonalcoholic fatty liver (26). Other factors that can induce non-alcoholic fatty liver include oxidative stress, defective mitochondrial function, pro inflammatory cytokines produced by visceral adipose tissue, and high-carbohydrate diets that trigger the synthesis of de-novo free fatty acids in the liver (27). A study by Hanley et al (2004) finds that SGPT and SGOT levels are closely correlated with the incidence of type 2 diabetes mellitus. Increase in SGOT and SGPT can help hepatocellular injury (23). The *kersen* leaves contain flavonoids that as antioxidants are useful to protect cells and organs from free radicals, and one of the cells is in liver (14).

CONCLUSION

The steeping *kersen* leaves are effective in reducing SGOT and SGPT levels in Diabetes Mellitus rats. It has the potential as a hepatoprotective in diabetes mellitus.

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