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The Effect of Single Type Black Garlic (Allium Sativum L.) Extract on the Cell of Langerhans Islets and the Kidney Tubular Microscopy in Male Wistar Rats (Rattus Norvegicus) Models of Diabetes Mellitus

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ABSTRACT
Diabetes Mellitus cause oxidative stress. Oxidative stress is the main contributor of the pathogenesis of tubular damage. Black garlic (Allium sativum L) contain antioxidant such as polyphenol and flavonoid can regenerate the cell of Langerhans islets and the kidney tubular in rats with diabetes mellitus model. To indentify the effect of black garlic (Allium sativum L.) extract on the cell of Langerhans islets and the kidney tubular microscopy in rats models of diabetes mellitus. This research is True Experimental with post-test only control group design. The samples were divided into 4 groups. Control group was given alloxan 24 mg/0,3 ml, group 1, 2, and 3 were given 24 mg/0,3ml and black garlic (Allium sativum L.) extracts at dose 0,15, 0,3, and 0,6 (ml/200gBW/day). Treatment was given orally per day within 14 days. Statistical analysis used Post Hoc and t-test. Results was found significant of differences ($p<0.05$) in each group of treatment and control group. Pancreas histopathological description of rat showed that there was improvement as indicated by average total of cells endocrine in Langerhans islets and showed there is effect of black garlic extract on the decrease of hydropic degeneration in the kidney tubular cells. Black garlic (Allium sativum L.) extract given effect on the kidney tubular microscopy and Langerhans Islet in Male Wistar Rats (Rattus norvergicus) Models of Diabetes Mellitus
Keywords: Extract, Allium sativum L., black garlic (Allium sativum L.), Langerhans Islet, Kidney tubular, diabetes mellitus.
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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder disease caused by the pancreas that does not produce enough insulin or the body can not use effectively produced insulin (Prabhakar & Doble, 2008; Al-Goblan et al., 2014; Kemenkes, 2014; Chattopadhyay et al., 2020; de Alencar Alves et al., 2020; Shieh et al., 2020; Awuchi et al., 2020). The prevalence of DM in Indonesia in 2014 is about 6.2% of the total population of 258,000,000 (WHO, 2016).

Diabetes mellitus is characterized by an excessive accumulation of glucose levels (Dai et a., 2020; Simon et al., 2020). Glucose can be oxidized before and after binding to the protein, this oxidation can lead to oxidative stress so that the formation of Reactive Oxygen Species (ROS) increases in the mitochondria that will cause tissue damage and endothelial injury. The combination of glycation and glucose oxidation results in the formation of Advanced Glycogen End-products (AGEs). The process of forming AGEs is an irreversible process that lasts long and can cause cell damage to the Langerhans islets and cause endothelial injury resulting in vascular abnormalities in small blood vessels (microangiopathy) kidney, this will lead to damage to glomerular cells and renal tubules, the damage in the form of adhesion, degeneration of cloudy swelling, hemorrhage, infiltration and necrosis (Handani et al., 2015).

ROS levels in the mitochondria need to be normalized to prevent tissue damage due to oxidative stress by increasing levels of antioxidants (Widowati, 2014). Antioxidants are easily oxidized so that free radicals will oxidize antioxidants and protect other molecules in the cells from oxidation damage by free radicals or reactive oxygen (Werdhasari, 2014; Bharti & Singh, 2020; Cai et al., 2020; Tonin et al., 2020) High content of antioxidants in garlic (Allium sativum) has been widely used by the people of Indonesia as one of alternative treatment for DM therapy, beside that garlic (Allium sativum) is also processed into various products, one of it is black garlic which is the process of garlic of the heating process with a temperature of 70 ° C for 21 days. The antioxidant content of black garlic is higher than unprocessed garlic with total polyphenol content of 25.81-58.33 mg GAE/g while raw garlic is 13.91 mg GAE/g (Choi et al., 2014).

METHODS

Materials and instruments used are black garlic extract (Allium sativum L.), alloxan, BR-1, aquadest, formalin, 96% alcohol, chloroform, glucometer, modified sonde, and surgical instruments. The test animals used were male wistar rats (Rattus norvegicus) aged 2-3 months, the body weight 150-200 grams with healthy conditions with active movement characteristics, clear eyes, thick fur and slippery, shiny and clean fur.

The Stage of Rat Acclimation

The 24 rats weighing in the range of 150-200 grams were kept for one week in a plastic enclosure with a cover of iron wire. In each cage contains 6 rats. During the process, rats are kept in order for a standard fed feeding requirement of 12gr-20 gr per day, drinking water and a clean enclosure by changing the chaff once in three days (Lucia, 2011).

The Dosing of Black Garlic Extract

Black garlic is extracted by maceration method. The Variations of given dosage are:

1) Dose 1 = 0,15 ml/200gBW/day
2) Dose 2 = 0,3 ml/200gBW/day
3) Dose 3 = 0,6 ml/200gBW/day

The treatments

After the adaptation period, rats were divided into 4 groups. Each group consists of 6 rats. The group is:

1) Group 1 (control +) : Alloxan-induced rats 160 mg/kgBW + BR-1 + aquades.

2) Group 2 (P1): Alloxan induced rats 160 mg/kgBW + black garlic extract 0,15 ml/200gBW/day + BR-1 + aquades.
3) Group 3 (P2): Alloxan induced rats 160 mg/kgBW + black garlic extract 0,3 ml/200gBW/day + BR-1 + aquades.
4) Group 4 (P3): Alloxan induced rats 160 mg/kgBW + black garlic extract 0,6 ml/200gBW/day + BR-1 + aquades.

The effective dose of alloxan used for intraperitoneal diabetic rats was 160 mg/kgBW (Wilmana dan Gan, 2012).

RESULTS

This study looked at renal tubular epithelial cells of male rat (Rattus norvegicus) renals in four fields of view, showing the depictions of renal epithelial cell epithelial degeneration then calculated in amounts using 400x optical-magnification software.

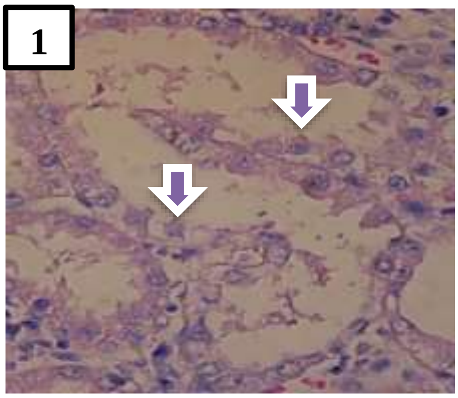

Figure 1: Control (+). Almost all rats renal tubular epithelial cells undergo hydropic degeneration (Marked with purple arrows).

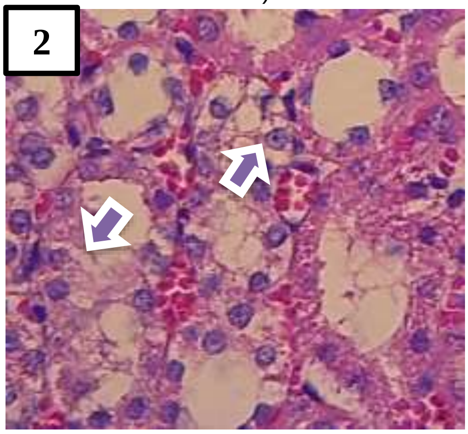

Figure 2: P1. The amount of hydropic degeneration of renal epithelial cells of rats begins to decrease compared to control (+) (Marked with purple arrows).

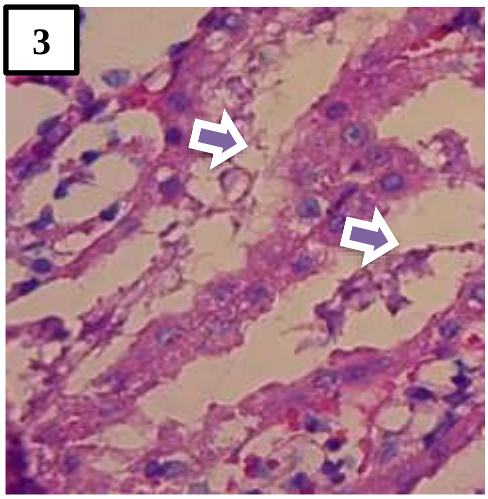

Figure 3: P2. The amount of hydropic degeneration of rat renal tubular epithelial cells is almost the same as P1 (Marked with purple arrow).

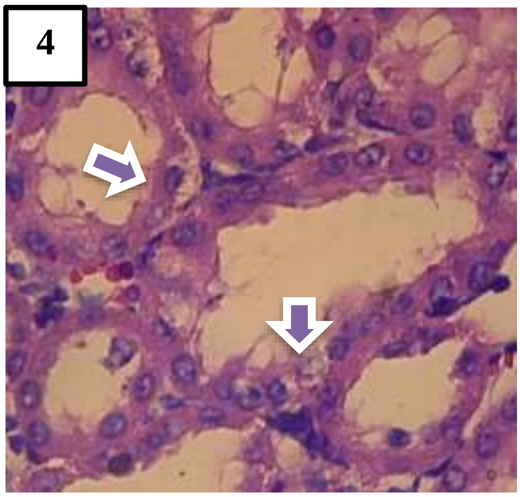

Figure 4: P3. The number of hydropic degeneration of rat epithelial renal tubule cells was fewer than control (+), P1 and P2 (Marked with purple arrow).

Table 1: Data of research results in all groups

Groups of treatment	Degeneration of renal tubular epithelial cells						
	1	2	3	4	5	6	X±SD
K+	80.75	82.00	81.75	80.50	79.50	79.75	80.7 ± 1.01755
P1	62.50	61.75	61.00	63.50	60.00	63.00	61.9 ± 1.30783
P2	60.50	59.25	59.75	58.50	60.50	61.50	60 ± 1.06066
P3	51.50	49.00	48.50	48.75	50.00	49.75	49.5 ± 1.10303

Based on Table 1 above it can be seen on histopathologic observation results with different dose of black garlic extract (Allium sativum L.) in each treatment group giving different effect to microscopic picture of rodus norvegicus renal tubular rats (Rattus norvegicus). The effect of the extract showed that the number of degeneration of rat epithelial tubule cells decreased after the treatment of black garlic extract (Allium sativum L.) at doses of 0.15 ml/200gBW/day, 0.3 ml/200gBW/day, and dose 0.6 ml/200gBW/day compared with the positive control group.

In the rat treatment group with alloxan induction (K +), the average number of rodent epithelial cell epithelium of rats (Rattus norvegicus) was 80.7. In the first group (P1), dosing of black garlic extract (Allium sativum L.) of 0.15 ml/200gBW/day showed an effect of decreasing the number of degeneration of epithelial cells of rat rodus male tubes (Rattus norvegicus) to 61.9 . In the second group (P2), the average number of renal tubular epithelial cell epithelium of male rats (Rattus norvegicus) was 60. In the third group (P3), the average number of rodent epithelial cell epithelium of renal male rats (Rattus norvegicus) is 49.5. This suggests that a positive alloxan-induced control group without black garlic extract (Alliun sativum L.) has a higher rate of renal epithelial cell epithelial degeneration than in the treatment group. In the treatment group with black garlic extract (Allium sativum L.) for 14 days as exogenous antioxidant showed the lowest number of renal tubular epithelial degeneration in treatment group (P3).

Portrait of Langerhans Islets

The observed number of endocrine cells on Langerhans islets was calculated using a light microscope with 400x magnification. Each treatment group has 6 preparations according to the number of replications that have been determined. Then each preparation is done 5x repetition for calculation .. The histopathology of Langerhans islets cell number of cells per one field of view. Histopathologic picture thus obtained as follows:

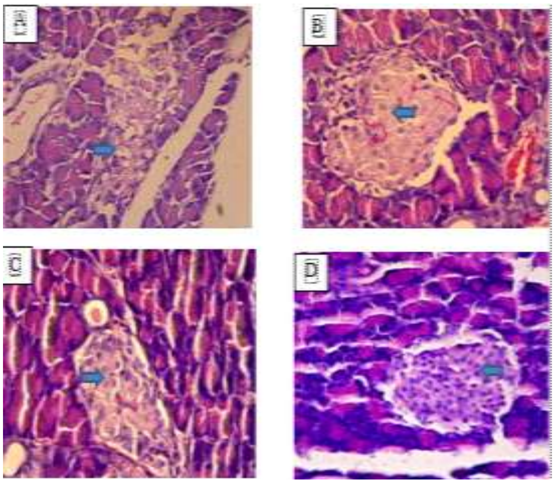

Figure 5: Histopathology of Langerhans Islets, 400x enlargement in white rat (Rattus novegicus strain wistar) male as shown in Figure 5.1 (A) positive control; (B) Dose 0,15 ml/200gBW/day; (C) Dosage 0.3 ml/200gBW/day; (D) Dose 0.6 ml/200gBW/day. Descriptively the percentage of pancreatic beta cell count in dose group 0,6 ml/200gBW/day more than with positive control group.

Table 2: The average number of Langerhans islats cells

Groups	Average number of Langerhans Island						Cell Average	Standard Deviation
	1	2	3	4	5	6		
K+	39.75	41.25	37.30	38.35	40.25	39.40	39.37	± 1.25
P1	53.25	52.00	54.25	53.50	52.00	50.25	52.59	± 1.51
P2	52.20	51.10	50.15	51.00	48.00	49.25	50.28	± 1.43
P3	69.25	68.65	67.00	68.10	70.25	69.50	68.80	± 1.43

From the data above in the positive control group, there were 39,30 ± 1,284 SD cells, the first treatment group with black garlic extract 0.5 ml/200gBW/day of rats was obtained the mean of 52.54 ± 1.511 SD, second treatment with dose 0,3ml/200gBW/day obtained result of 50.28 ± 1.343 SD and third treatment group with dose 0.6 ml/200gBW/day black garlic extract got average equal to 68.39 ± 1.127 SD.

The mean increase in the number of Langerhans endocrine cells from positive controls at a dose of 0.15 ml/200gBW/day, 0.3 ml/200gBW/day and 0.6 ml/200gBW/day were significant and an average decrease occurred. The highest average number of Langerhans Islets cells was found in the group at a dose of 0.6 ml/200gBW/day. So in the research that has been done by researchers, at a dose of 0.6 ml/200gBW/day is what researchers get as a dose of black garlic extract most influential in increasing the number of endocrine cells Langerhans islets in pancreas rats.

DISCUSSION

This study aims to prove the effect of black garlic extract (Allium sativum L.) on the microscopic picture of rats tubule of male wistar rat (Rattus norvegicus) DM model. Rats were adapted for one week (days 1-7) with the same feeding of standard BR-1 feed and aquades for each group. Then the 8th day of the rats was fasted for 12 hours, then given different treatment of each group. rats were divided into four groups: positive control, P1, P2 and P3 with treatment group each given dose of black garlic extract (Allium sativum L.) of 0,15 ml/200grBW/day, 0,3 ml/200grBW/day, dan 0,6 ml/200grBW/day.

Aloxan is a chemical substance to induce experimental animals that produce experimental diabetic conditions (hyperglycemia) rapidly.[3] In hyperglycemic conditions there is an increase in glucose levels. Glucose can be oxidized before binding to proteins, as well as glucose after binding to a protein (glycated protein) can be oxidized to produce Reactive Oxygen Species (ROS). The combination of glycation and glucose oxidation results in the formation of AGEs (advanced glycogen end-products). The process of forming AGEs is an irreversible process that lasts a long time and can cause tissue damage (Sinatra & DeMarco , 1995; Thomas, 1995; Valko et ala., 2006; Rahman, 2007; Widowati, 2008; Korkmaz et al., 2009; Shrivastava et al., 2019; Åkerström et al., 2019).

The formation of AGEs has an effect on endothelial cell damage (endothelial injury). Glycated proteins and AGEs modified proteins can lead to oxidative stress, both of which can release O2, H2O2 directly and can activate phagocytes. Cells such as macrophages, monocytes and endothelium are able to recognize AGEs through cell-surface receptors (RAGE = receptor for AGE). Under normal circumstances RAGE causes macrophages to recognize and ingest glycosylated cells (AGEs-modified erythro-cytes) (Widowati, 2008).

From the results of this study, the positive control group had the highest average number of hydropic degeneration cells in renal tubules, which was 80.7. This is because the alloxan induced into the rat body is a chemical compound that can damage the kidneys by oxidizing cells in the kidney.[3] The epithelium of the kidney tissue is a part of the kidney that is sensitive to toxic substances. Toxic substances that usually enter the kidneys through the bloodstream, can cause changes in renal epithelial cells in the form of degeneration (Schnellman et al., 2008; Yulinta, 2013; Al-Harbi et al., 2020; Li et al., 2020).

The alloxan-induced pancreatic rats in histopathologic observation suggests the presence of severe necrosis and cell degeneration, with the abundance of empty space on Langerhans islets. The occurrence of necrosis and degeneration is caused by the induction of alloxan in rats that leads to the formation of free radicals that can damage biomelecules such as lipids, phospholipids and carbohydrates which are important components in cell membranes and DNA in the cell nucleus. Free radical activity will cause cell membranes become damaged and degenerate to necrosis by marked by pale cytoplasm and damaged nucleus (Wu et al., 2019; Möller et al., 2019; Yaribeygi et al., 2019; Nechaeva et al, 2020; Saleem et al., 2020; Andrew et al., 2020).

Previous research proves that polyphenol antioxidants are able to reduce oxidative stress and prevent endocrine cell destruction on Langerhans islets by inhibiting the chain reaction of superoxide conversion into superoxide hydrogen by donating hydrogen atoms to bind to free radicals and dispose of them through excretion systems. Flavonoids are reported to have antidiabetic activity capable of regenerating endocrine cells of the langerhans islets by capturing free radicals such as ROS so as to improve the state of damaged tissue and inhibit the inflammatory process (Widowati, 2008).

Based on the results of this study, it can be concluded that alloxan administration can affect the kidney tubular microscopy (Rattus norvegicus) in the form of hydropic degeneration cell, while giving black garlic extract (Allium sativum L.) can reduce the number of hydropic degeneration cells tubulus and increase the mean endogenous cells of the Langerhans islets male wistar rats (Rattus norvegicus).

CONCLUSION

Black garlic extract (Allium sativum L.) at a dose of 0.15 ml/200grBB/day was an effective dose that began to influence between three doses used to repair kidney tubule damage and increasing the number of Langerhans island cells in DM model male wistar (Rattus norvegicus) mice. Possessed antihyperglycemic therapy, and antioxidant effects. This means that black blood is a blood glucose-lowering agent for diabetic patients. The results of this study indicate that, in diabetic treated extracts, the pancreatic islets were significantly increased compared to controls, so production and secretion of insulin were increased. The increased insulin results in a decrease of blood glucose. According to histological results, we can conclude that the mechanisms of black garlic are extracted for decrease of blood glucose are Langerhans repair islets.

From the results above, further research is needed on the use of black garlic extract (Allium sativum L.) in improving hydropic degeneration of tubular epithelial cells and the number of Langerhans island cells using different extraction methods. In addition, further research is needed to examine the effect of black garlic extract (Allium sativum L.) on other histopathological markers of the kidneys such as necrosis, inflammation, etc. From the results of this study, it is necessary to develop further research on other antioxidant activities such as SOD, CAT and GSH to examine the effects of endogenous antioxidants on microscopic images of kidney tubules and islets of Langerhans DM model wistar rats (Rattus norvegicus). Further research with variations in the dosage of black garlic extract (Allium sativum L.) in searching for optimal effects in improving microscopic picture of renal tubules and increasing number of Langerhans island endocrine cells male wistar mice (Rattus norvegicus) DM model also needs to be done

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