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Antioxidant potency of Okra (*Abelmoschus esculentus* L. Moench) pods extract to Ameliorate Kidney structure and function in diabetic mice

[1,2]Saikhu Akhmad Husen, [1,2]Dwi Winarni, [3]Arif Nur Muhammad Ansori, [1]Raden Joko Kuncoroningrat Susilo, [1]Suhailah Hayaza and [1]*Win Darmanto

[1]Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia
[2]Animal Histology Laboratory, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia.
[3]Doctoral Program in Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

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ABSTRACT

This study was aimed to investigate the ability of okra (*Abelmoschus esculentus* L. Moench) pods extract to reduce plasma blood urea nitrogen (BUN) and creatinine level, and also to ameliorate damaged renal proximal tubular of kidney in diabetic mice. *In vivo*, the antioxidant test was conducted by using male BALB/c mice. Mice were divided into two groups; normal control (KN) and streptozotocin-induced diabetic mice. Streptozotocin (STZ) induction was performed using multiple low-dose of 30 mg/kg BW injected for five consecutive days. Diabetic mice have separated again into three subgroups; diabetic control (KD), diabetic mice treated with acarbose (KA), and diabetic mice treated with fractionation of okra pods extract. Fractionation of pods extract treatment group was categorized based on given; crude extract (CU=100 mg/kg BW), non polar extract (NP=20.04 mg/kg BW), semi-polar extract (SP= 27.39 mg/kg BW) and polar extract (PE=54.10 mg/kg BW). Before and after STZ injection, body weight and fasting blood glucose were measured. Bodyweight and fasting blood glucose were measured at 1st, 7th and 14th day of okra pods treatment. Okra pods extract was given per oral for 14 days. On 15th day, plasma BUN and creatinine levels were measured using Pentra C200 Reader. Interestingly, okra pods extract administration was found to be able to lower plasma BUN and creatinine, and ameliorate damaged renal proximal tubular of kidney in diabetic mice significantly.

Ke ywords *: Blood urea nitrogen, Diabetic mice, Okra pods extract, Plasma creatinine, Renal proximal tubular of kidney*

Introduction

Diabetes mellitus is a global public health problem with high levels of morbidity and mortality (Guariguata *et al*., 2014; Kang *et al*., 2014). In 2017 there were more than 400 million people suffering from diabetes mellitus worldwide and this number is expected to increase to 592 million by 2030. Indonesia is currently ranked fourth in the world with 12.4 million people with diabetes mellitus in the year 2015 and this figure is expected to increase to 21.3 million by 2025 (Arifin *et al*., 2019). Diabetes mellitus is a chronic metabolic disorder of the pancreas with signs of hyperglycemia, as well as disor-

ders of carbohydrate, fat, and protein metabolism (Kim *et al*., 2013; Meles *et al*., 2019; Parvizi *et al*., 2014; Pane *et al.,* 2018). Hyperglycemia in people with diabetes mellitus causes glucose auto-oxidation, activation of protein kinase C, protein glycation, and activation of the polyol metabolic pathway which further accelerates the formation of reactive oxygen species (ROS) or conditions of oxidative stress (Yusoff *et al.,* 2015; Putri and Notobroto, 2020). The presence of ROS causes free radicals in the body to increase. Free radicals can damage various body tissues, including kidney tissue (Ansori *et al*., 2019; Ansori *et al*., 2020; Tacharina *et al*., 2020). Histopathological renal tissue damage has been reported to include hypertrophy of glomeruli, glomerulosclerosis, swelling of epithelial tubules, and tubular epithelial necrosis (Ansori *et al*., 2019). Damage to the proximal tubular cells of the kidney is one of the morphological changes that are typical in the kidney of diabetics, this being a better indicator of disease progression than glomerular damage. Creatinine excretion is the result of two physiological processes, namely glomerular filtration and renal proximal tubular secretion. Creatinine is a creatine metabolite. Creatine is synthesized mainly in the liver and kidney and is present in almost all skeletal muscles that are bound in the form of creatine phosphate, and energy storage compound (Sudjarwo and Koerniasari, 2015; Husen *et al*., 2018).

Prolonged hyperglycemia can lead to increased levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Increased levels of ROS and RNS can directly oxidize and damage DNA, protein, and lipids. To overcome the high levels of ROS and RNS, more exogenous antioxidants are needed. Antioxidants are substances that can inhibit the negative effects of free radicals, by providing electrons so that damage to lipids, cell membranes, blood vessels, DNA, and other damage caused by reactive compounds, such as ROS and RNS can be prevented (Arudina *et al*., 2017; Purnamasari *et al*., 2019; Roestamadji *et al*., 2017; Sukardiman and Ervina, 2020). One type of antioxidant that still gives hope, to overcome free radicals is quercetin (Sulastri *et al.,* 2018; Sinuraya *et al.,* 2018; Nilamsari *et al.,* 2020), which is a flavonoid compound contained in okra (Arwini *et al.,* 2018). Quercetin compounds have a significant ability to reduce ROS, hydrogen peroxide, and protein oxidation, by donating hydrogen atoms and stabilizing resonant free radicals, which do not easily participate in other radical reactions (Husen *et al*., 2019a; Husen *et al*., 2019b; Husen *et al*., 2019c). In addition to neutralizing free radicals, the antioxidant is expected to reduce oxidative stress, especially in various cells affected by prolonged hyperglycemic conditions, such as hepatocytes in the liver and proximal cells of the kidney tubules (Husen *et al*., 2017; Husen *et al*., 2018; Husen *et al*., 2020).

Based on the background of the problems mentioned above, until now there has been no scientific explanation about the antioxidant potential of various fractions of okra pods extract, which can reduce blood urea nitrogen (BUN) and creatinine blood plasma levels of mice, and do repair damage to proximal cells of kidney tubules in mice diabetics treated with red okra pods extracts. Therefore, this study was aimed to investigate the ability of okra pods extract to reduce plasma blood urea nitrogen (BUN) and creatinine level, and also to ameliorate damaged renal proximal tubular of kidney in diabetic mice.

Materials and Methods

This research is an experimental study conducted at the Molecular Genetics Laboratory, Experimental AnimalsLaboratory, Animal Histology Laboratory, and BiochemistryLaboratory, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia. The research sample used was adult male mice, the type of Mus musculus strain BALB/c, aged 3-4 months, body weight ranging from 30-40 g. The research materials are crude red okra extract and its fractionation (non-polar, semi-polar, and polar), streptozotocin, citrate buffer solution pH 4.5, and phosphate-buffered saline (PBS), solvent CMC extract (carboxymethylcellulose), antidiabetic standard drugs (Acarbose 100 mg/kg BW), lard, anesthetics (ketamine-xylazine), glucose (10% D-glucose in distilled water). The main tool in this study is a mouse cage in the form of a plastic tub with a lid from gauze, drinking bottles, feed containers, husks, microscopes, Petri dish, analytical scales with an accuracy of 4 digits behind the comma, 2-3G injection needles that have been given lead safety at the end, 1 mL insulin injection needle for diabetes induction, Accu-Check glucometer, glucostrips, EasyTouch, blood cholesterol strips, glassware, rotary vacuum evaporator, spectrophotometer (Husen *et al*., 2019a; Husen *et al*., 2019b; Husen *et al*., 2019c).

Before conducting this research, ethics clearness test was conducted at the Faculty of Veterinary Medicine, Universitas Airlangga (2.KE.069.04.2018).

The study sample consisted of 35 male mice, divided into normal control groups (KN) and diabetic groups (induced with streptozotocin). The study sample measured fasting blood glucose levels before and after induction with STZ. Measurement of fasting blood glucose levels was carried out on days 7 and 14 after induction of streptozotocin (STZ). Measurement of blood glucose levels with a glucometer to determine the diabetic condition of mice. Only mice with fasting blood sugar levels of more than 170 mg/dL were used as a group of diabetic mice. The grouping of experimental animals was carried out as follows: nondiabetic mice were used as a normal control group (KN). While the diabetic mice resulting from STZ induction were divided into 2 control groups namely the diabetic control group (KD), the diabetic control group who were given the drug acarbose dose 100 mg/kg BW (KM) and the crude extract treatment group with a dose of 100 mg/kg BW (EK) and fractionation of red okra pods extract. The administration of Non-polar (NP), Semi-polar (SP) and polar (EP) fractionation, refers to the results of conversion with a crude extract dose. NP dose = 20.04 mg/kg BW, SP dose = 28.39 mg/kg BW, while EP dose = 54.11 mg/kg BW. Provision of treatment carried out for 14 days.

On the fifteenth day after the treatment, intracardiac blood sampling was taken and plasma BUN and creatinine levels were measured, through absorbance readings using Pentra C200 (Horiba Medical) at a wavelength of 510 nm. Kidney preparations are stained with Haematoxylin-Eosin staining, then read the variable damage to the proximal cells of the kidney tubules using a light microscope with a magnification of 400X, which is equipped with Grateculae.

Results and Discussion

Data on the reading and calculation of the mean BUN and plasma creatinine levels after administration of crude extracts and various fractions of okra pods extracts on day 15 are presented in Figure 1. While the data on reading and calculation of mean swelling and necrosis of renal tubular cells after administration of extracts of mice after extracting coarse and various fractions of okra pods extract on day 15 are presented in Figure 2. The figure of preparations of kidney tubular cells after administration of crude extracts and various fractions of okra pods extract, presented in Figure 3.

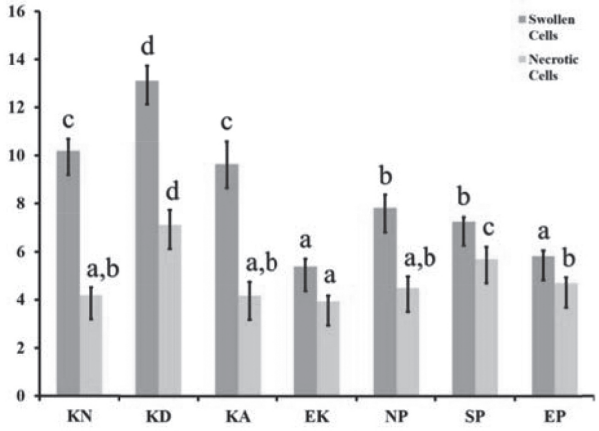

Fig. 2. The effect of okra pods extracts toward the number of tubular cell damages in diabetic mice's kidneys. The different letter indicated a significant difference.

Hyperglycemic conditions cause glucose cannot be processed into energy, so energy must be made

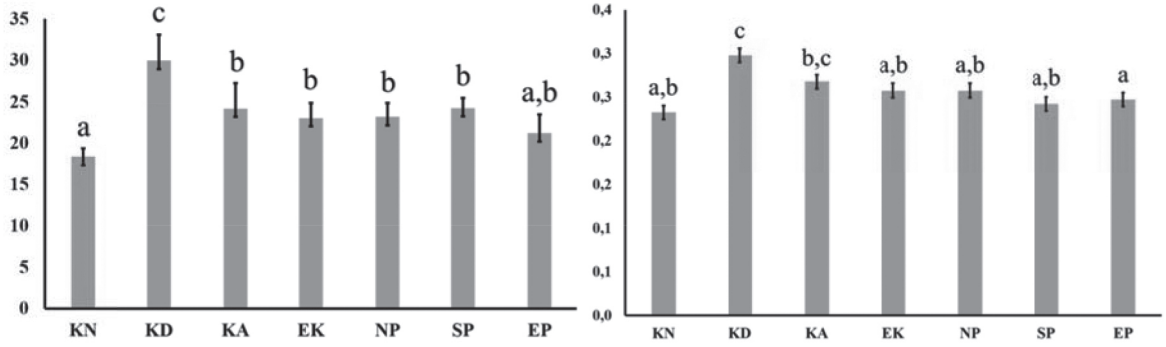

Fig. 1. The effect of okra pods extracts toward plasma BUN (left) and creatinine (right) levels in diabetic mice. The different letter indicated a significant difference.

from other sources such as fats and proteins (Hayaza *et al*., 2019). A condition of hyperglycemia in people with diabetes mellitus causes glucose auto-oxidation, resulting in the activation of protein kinase C, protein glycation, and activation of the polyol metabolic pathway which further accelerates the formation of reactive oxygen species (ROS) or conditions of oxidative stress. The presence of ROS causes free radicals in the body to increase. Free radicals can damage the structure and function of various body tissues, one of which is kidney tissue (Fadholly *et al*., 2019; Fadholly *et al*., 2020; Tacharina *et al*., 2019). Damage to the structure and function of kidney tissue histopathologically has been reported to include hypertrophy of glomeruli, glomerulosclerosis, swelling of the epithelial tubules and apoptosis and necrosis of the epithelial tubules (Ansori *et al*., 2019). In patients with DM, hyperglycemia causes an increase in the production of ROS and RNS due to increased oxidation of NADPH in endothelial tissue. ROS and RNS are highly reactive molecules that can directly oxidize and damage DNA, protein, and lipids and cause oxidative stress. Oxidative stress occurs when there is an imbalance between the number of highly reactive molecules (ROS and RNS) with existing antioxidants (Tacharina *et al*., 2019).

Streptozotocin (STZ), is a free radical that can increase ROS and RNS levels and is very reactive, especially for hepatocytes and renal tubular cells. In the group of mice induced by STZ, showed increased levels of BUN and plasma creatinine in the diabetic control group (KD) significantly different from the normal control groups (KN), KA, EK, NP, and SP. This is because the diabetic condition triggers the formation of ROS through the glucose auto-oxidation pathway, the formation of the advanced glycationend products (AGE), and the polyol pathway mechanism. ROS can trigger lipid peroxidation in cell membranes that cause damage to these cells. This is reinforced by Ansori*et al*. (2019) who states that free radicals can cause lipid peroxidation that can damage the structure of cell membranes, causing damage to the structure and function of renal tubular cells characterized by increased levels of BUN and plasma creatinine in the diabetic control group (KD) compared to all groups. The treatment, except for plasma creatinine levels, did not differ in KD from KA but differed significantly when compared with the other groups. From the results of this

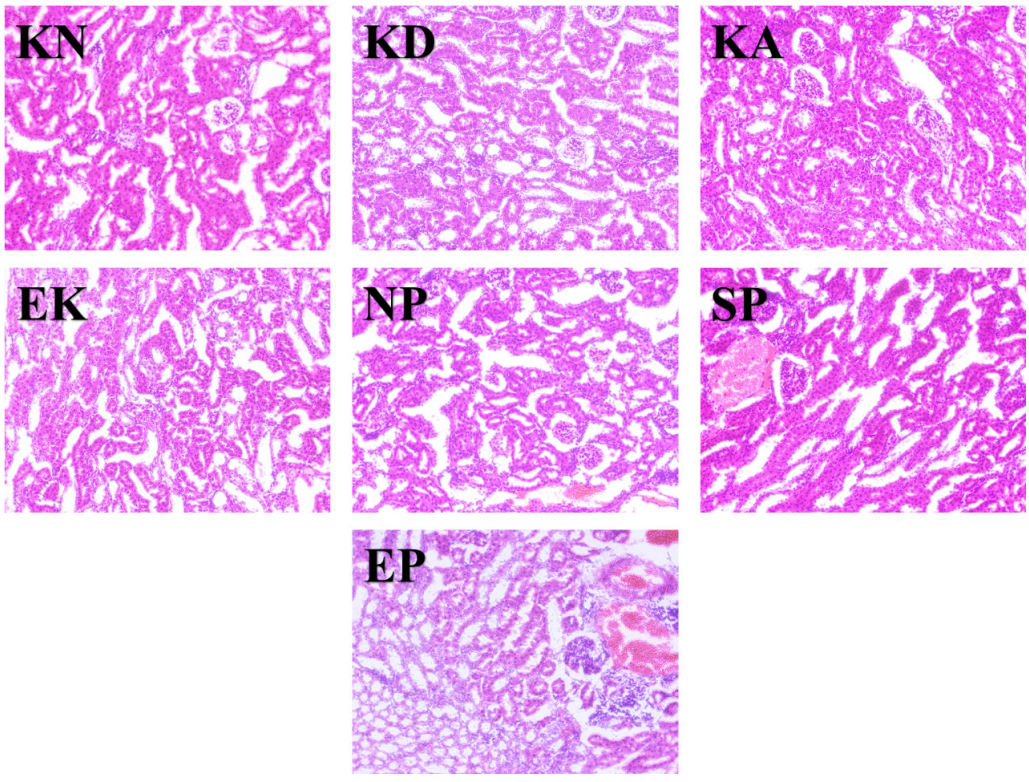

Fig. 3. The histological structure of damages of kidney tubular cells in diabetic mice.

study, it can be said that the kidneys are organs that play a role in regulating body balance, maintaining body fluids, and regulating the disposal of metabolic waste and toxic substances such as urea, uric acid, ammonia, creatinine, inorganic salts, and also medicinal compounds. Drugs that are not needed by the body (Husen *et al*., 2019a; Husen *et al*., 2019b; Husen *et al*., 2019c).

Increased creatinine levels in the blood can be caused by kidney damage mainly due to glomerular filtration disorders, acute tubular necrosis, glomerulonephritis, and tubular apoptosis (Husen *et al*., 2018). The results of research conducted by Parvizi*et al*. (2014) showed that diabetic rats (*Rattus norvegicus*) who had been injected with STZ, had elevated serum creatinine levels compared to the normal group. This is because of the result of damage to kidney histological structure that occurs in rats (*Rattus norvegicus*) diabetic so that the work of the kidneys in eliminating creatinine is disrupted. Ansori *et al*. (2019) state that serum creatinine levels increase to approximately twice normal if there is a decrease in kidney function by up to 50%. The kidneys are not dependent on insulin for glucose absorption, so an increase in blood glucose levels in diabetic conditions will result in high glucose at intracellular levels and is faced with severe and sustained hyperglycemia. Increasing the amount of glucose filtered by the glomeruli under hyperglycemia will increase the workload of proximal tubular cells. In addition, proximal tubular cells cannot reduce glucose transport levels to prevent excess intracellular glucose in hyperglycemic conditions. This is supported by Hayaza *et al*. (2019) which states that hyperglycemia is associated with increased ROS production and produces conditions of oxidative stress that are thought to play a key role in the pathogenesis of this disorder.

Conclusion

From the results of this study, it can be concluded that the administration of okra pods extract can significantly reduce BUN and plasma creatinine levels and is able to repair proximal tubular cell damage, especially swelling and necrosis in the kidney organs of mice. Therefore, it is advisable to always do counseling to the wider community, about the benefits of the okra pods extract in tackling diabetes mellitus, especially in improving the structure and function of liver cells.

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