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Research Article

The Role of *Sechium edule* Fruits Ethanolic Extract in Insulin Production and Malondialdehyde Level in Stz-Induced Diabetic Rat

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ABSTRACT
The objectives of this research were to examine the effect of *Sechium edule* ethanolic extract in insulin and Malondialdehyde (MDA) levels in streptozotocin (STZ) induced diabetic rats. Type 1 diabetic rat were obtained by intraperitonially injected with multiple low dose STZ (MLD-STZ) 20 mg/kgBW for 5 days. The animals were divided into 5 groups: untreated rats in group (K) were considered as negative control, MLD-STZ induced type 1 diabetic rats in group (S) were considered as positive control. In another 3 group (A, B, C) type 1 diabetic rats were orally treated with three doses of *S. edule* ethanolic extract (14, 28, 42 mg/kg BW) for 7 days. The animals were sacrificed in two days after last treatment, serum were collected for measuring of the insulin concentration by ELISA method. Level of MDA on kidney, liver and pancreas were measured by TBA method. The treatment with *S. edule* ethanolic extracts unable to increase of insulin yet but the treatment with 42 mg/kg BW of *S. edule* ethanolic extract showed significantly decreased MDA levels on liver but not significant on kidney and pancreas.

INTRODUCTION
Sechium edule is a plant that belongs to the family of Cucurbitaceae. The morphological characteristics of *S. edule* are herbaceous, perennial, creeper or climbing plant, and the fruits are fleshly-fibrous (Firdous *et al,* 2012). *S. edule* is one of the plants that endemic in Mexico and originally from southern Mexico. Mexican indigenous community used *S. edule* for treating various symptoms such as severe headache, nervousness, and anxiety (Lombardo-Earl *et al,* 2014). In Indonesia, *S. edule* fruits usually used as vegetable in many kinds of food. Nahdi *et al* (2016) reported that Turgo community whose lived in Yogyakarta Province, Indonesia used *S. edule* as medicinal plants to treat high blood pressure. Based on statistical data in horticulture fields, total production of *S. edule* in Indonesia were 357.552 at 2014 (Ministry of Agriculture Republic of Indonesia, 2015) and it considered that Indonesian research can develop alternative drugs from active compound in *S. edule.*

In pharmacological research, *S. edule* were reported as a treatment for various disease such as cardiovascular disease, diabetes mellitus, hypertension, anti-inflammatory, anti-cancer, diuretic, etc (Ordonez *et al,* 2006). *S. edule* contains active compound from secondary metabolites such as flavonoid, tannins, carotenoid, saponins, triterpenes, and alkaloids (Salazar-Aguilar *et al,* 2017). The active compounds from *S. edule* have antioxidant activity as scavenger receptor for free radicals (Albarracin *et al,* 2010; Fidrianny *et al,* 2015). *S. edule* reported has antioxidant activity as antiproliferative in human cancer HeLa cell line (Salazar-Aguilar *et al,* 2017). Mumtaz *et al* (2013) also reported that treatment using aqueous extract from *S. edule* in streptozotocin-induced diabetic nephropathy rat ameliorates the renal tubules architecture (almost intact tubules and glomeruli).

Type 1 Diabetes Mellitus (T1DM) is one type of DM that many children and adolescents suffer. T1DM caused by pancreas has low production of

insulin or does not produce any insulin at all. Type 1 diabetes begins with the inflammation of pancreatic beta cell that occurs due to mononuclear cell infiltration (macrophages, lymphocytes and monocytes), followed by the death of beta cell due to the phagocytosis process by macrophages (Suryohudoyo, 2000). Streptozotocin (STZ) is chemical compound which has ability to destroying pancreatic β cells. STZ can induced the formation of free radicals such as Nitric Oxide (NO) and reactive Oxygen Species (ROS). STZ decreased oxygen consumption by inhibits the krebs cycle in mitochondria and caused pancreatic β cells damage. In other hand, STZ also can caused increasing of Xanthine oxidase which catalysed the formation of superoxide anion. (Siahaan, 2017)

ROS can oxidize polyunsaturated fatty acids (PUFAs) of plasma membranes to form malondialdehyde (MDA) compounds (Ayala *et al,* 2014) which can altered membrane fluidity, increase permeability, cause the loss of membrane integrity, consequently it will decrease the cell viability (Jaggi and Adav, 2015). The increased level of MDA caused of lipid peroxidation is a pathogenesis symptom which has important role for some diseases, such as DM (Suryawanshi *et al,* 2006). The treatment for T1DM generally uses insulin. The treatment using insulin injections cannot repair the damage of the pancreatic beta cells, but it only adds insulin exogenously, thereby causing the person to be insulin dependence throughout his life. There have been many researches on the utilization of medicinal plants as an alternative for chemical drugs for the therapy of DM disease. One of the medicinal plants is the *S. edule.*

The previous research showed that the ethanol extract of *S. edule* can reduce blood glucose levels in Streptozotocin (STZ)-induced DM Wistar rat (Lukiati and Maslikhah, 2014). The ethanol extract of the *S. edule* fruits also could repair β cells pancreatic damage and decreased the pancreas NO level in DM wistar rat (Lukiati *et al,* 2016). This research is a follow-up research with the aim of testing the potential of ethanol extract of *S. edule* to decrease the level of malondialdehyde (MDA) in pancreas, liver and kidney, and increased insulin level of blood serum of DM wistar rats of the induction of streptozotocin (STZ).

MATERIALS AND METHODS

S. edule Ethanolic Extraction

Fifty kilograms of *S. edule* fruits were obtained from Materia Medica Batu, then were dried into powder. Nine hundreds gram *S. edule* powder then pass through ethanolic extraction. The extraction of *S. edule* was carried out by repeated maceration method of the *S. edule* powder simplicia using 95% ethanol solvent. The maceration was done for 3 times until the extract in transparent colour. First maceration was done for 3x24 hours, the second was 1x24 hours, and the third was 1x24 hours. The liquid extract was concentrated with a rotary evaporator at room temperature (Lee *et al,* 2007) and final result was 400 mg of pasta *S. edule* extract.

STZ Induced Diabetic Rats and Experimental Design

Twenty five male Wistar rats (150±200 gram, 2 months old) were obtained from CV. Karunia Jasa Pratama, Malang. The animals were housed in standard cage and given free access for food and water. The animals were acclimatized for 7 days. The animals were divided into five groups i.e:

1) K = healthy rats group as the control
2) S = STZ-induced DM rats not treated with *S. edule* ethanolic extract
3) A = STZ-induced DM rats + *S. edule* ethanolic extract 14 mg/kgBW
4) B = STZ-induced DM rats + *S. edule* ethanolic extract 28 mg/kgBW
5) C = STZ-induced DM rats + *S. edule* ethanolic extract 42 mg/kgBW

The doses of the extract used was calculated based on the simplicia use of human, which is 5-7 grams, and converted to rats = x 0.018 (weight of the rats wistar strain is 200 gram) (Studiawan and Santosa, 2005). Three different doses of the extract were used in this research based on previous research (Lukiati and Maslikhah, 2014).

After acclimatized, at 8th day, the DM rats were obtained by injecting MLD-STZ (dose 20 mg/ kgBW) intraperitonialy (ip) for 5 days respectively (Aulanni'am *et al,* 2005), then the rats were incubated for 14 days. DM rats were determined by blood glucose level when it more than 200 mg/dL (Hussain, 2002). After in DM state, the animal treated with *S. edule* extract at 23rd day for 7 days. At 32nd day, the blood glucose was observed and the animal were sacrificed. The serum were collected and pancreas, liver, and kidney were removed to observed the MDA level. This research was approved by Institutional Ethic Committee of Brawijaya University (No: 61-KEP-UB).

Measurement of Insulin Level

The serum which collected at 32nd day were used to observe insulin level. Measurement of insulin leveln accordance with the protocol on Insulin ELISA Rat KIT. Creating a standard curve of insulin with a concentration of 0.15; 0.30; 0.60; 1.25; 2.5; 5.0; 10 ng/ml measured at a wavelength of 450 nm and 630

nm. The serum was added with the standard solution incubated for 30 minutes and then the absorbance was measured. The results were then plotted on the standard curve which had already been made to determine the insulin level (Sarode *et al,* 2016).

Measurement of MDA Level

The pancreas, liver, and kidney which collected at 32nd day were used to MDA level analysis. The analysis of MDA level used Thiobarbituric acid (TBA) method (Ghanbari et al, 2016). The standard curve for MDA level measurement was in accordance with the protocol of KIT Rat anti MDA polyclonal antibody. Each sample (kidney, liver, pancreas) of 1.8 grams was cut into small pieces and crushed in cold mortar, added 1 mL of NaCl 0.9%, then the homogenate was moved into a micro tube and centrifuged at 8000 rpm for 20 min and supernatant was taken. Each supernatant of 100 µL was added with 550 µl aquades, 100 µL TCA, 250 µL HCl 1 N, and 100 µL Na-Thio, the solution was homogenized, and then centrifuged at 500 rpm for 10 min. The supernatant was taken and incubated in a water bath, 100^{0}C in temperatures for 30 minutes. The absorbance of the sample was measured at maximum wavelength for the TBA test (533 nm) and plotted on the standard curve that had been made to calculate the sample level.

Statistical Analysis

Statistical analysis of measurement results in insulin and MDA levels using one-way ANOVA and LSD post hoc test, *P* value *<0.05* indicated significantly different.

RESULTS AND DISCUSSION

The research results of the potential *Sechium edule* ethanolic extract on the insulin level in serum of DM rats are presented in Figure 1.

Based on statistical analysis, the insulin level in serum between groups were not significantly difference. The average of insulin level in K group was 1127,3 ng/mL, in S group was 1479,5 ng/mL, in A group was 1131,8 ng/mL, in B group 1054,5 ng/mL, and in C group was 1181,8 ng/ml. Patil and Kothavade (2018) reported that the serum insulin level in diabetic rat which treated with 0.9% NaCl (vehicle group) was 32.65 ng/mL. Based on data showed that treatment using *S. edule* ethanolic extract for 7 days still unable to increase insulin level yet. Another research administration using mango (*Mangifera indica*) peel ethanol extract for 60 days at doses 100 mg/kgBW can increase insulin level significantly in T1DM rat compared with untreated/ diabetic group (Gondi and Rao, 2015). Antioxidant effect from *Aloe barbadensis* gel and skin ethanolic extract also showed increased insulin level until 20% on 28 days treatment compared with diabetic control rats group (Moniruzzaman *et al,* 2012). Hemmati *et al* (2016) reported that the level of insulin after treatment using *Berberis vulgaris* fruits ethanolic extract on STZ-induced diabetic rats were significantly increased compared with diabetic control rats group. Ethanol extract of *Berberis vulgaris* that has similar result with glibenclamide as a common drug for DM disease was higher than 100 mg/kgBW. These reports indicated that the reason *S. edule* ethanolic extract still unable to increase insulin level caused by the administration were done in 7 days only and also low doses of *S. edule* ethanolic extract.

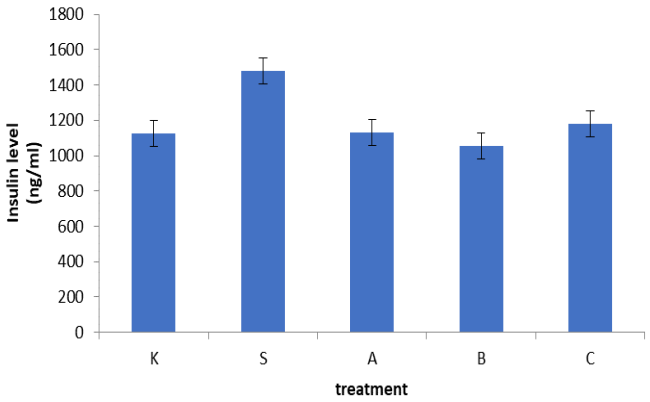

Figure 1. Potential of *S. edule* ethanolic extract on the levels of insulin in the blood serum of DM rats. K, DM rats. K, normal rats. A: DM rats + *S. edule* ethanolic extract dose 14 mg/kgBW. B: DM rats + *S. edule* ethanolic extract dose 28 mg/kgBW. C: DM rats + *S. edule* ethanolic extract dose 42 mg/kgBW

The potential of *S. edule* ethanolic extract results on the MDA level in the liver, kidney, and pancreas of DM rats are presented in Figure 2.

Based on statistical analysis, the MDA levels in liver were significantly different, meanwhile the MDA levels in the kidney and pancreas were not significantly difference. The MDA level liver, kidney, and pancreas of DM rats is quite high at 2400 ng/mL, 1900 ng/mL and 1942 ng/mL. Anjani et al. (2018) reported that the average of MDA level in the liver of streptozotocin-induced diabetes rats was 227.25±3.07 ng/mL while the average of MDA level in normal rats were 148.00±2.22 ng/mL. After giving treatment with *Sechium edule* ethanolic extract, the MDA levels in each organ has change. The results showed that *Sechium edule* ethanolic extract can reduce the MDA levels in the liver of DM rats significantly, but not significantly in the kidney and pancreas. The results of LSD test showed that a dose of 42 mg/kgBW was an effective dose to reduce MDA levels in liver. Gondi and Rao (2015)

reported that mango (*M. indica*) peel ethanol extract also decreased MDA level significantly in T1DM rat model compared with untreated group Treatment using *B. vulgaris* fruits ethanol extract in low doses (25 mg/kgBW) also showed decreased MDA level significantly compared with diabetic control group (Hemmati *et al,* 2016). Treatment using *A. barbadensis* gel and skin ethanolic extract in streptozotocin-induced T2DM rats significantly decreased level of MDA compared with diabetic control group (Moniruzzaman *et al,* 2012).

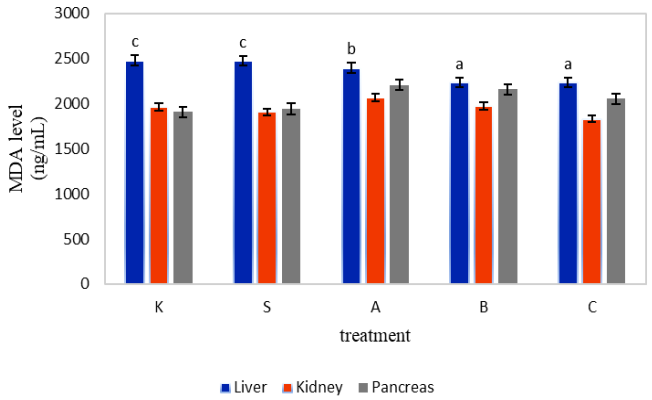

Figure 2. Potential of *S. edule* extract on the MDA level in the liver, kidney, and pancreas of DM rats. K, normal rats. S, DM rats. A, DM rats + *S. edule* ethanolic extract dose 14 mg/kgBW. B, DM rats + *S. edule* ethanolic extract dose 28 mg/kgBW. C, DM rats + *S. edule* ethanolic extract dose 42 mg/kgBW. Blue colour as liver, orange colour as kidney, grey colour as pancreas. [a,b] significance different (*P<0.05*)

STZ as an exogenous NO donor can increase some reactive oxygen species (ROS), such as superoxide radicals ($O_2^{\bullet-}$), hydroxyl radicals ($OH^{\bullet-}$), and hydrogen peroxide (H_2O_2) (Szkuldelski, 2001), causing methylation DNA of Langerhans beta cell thus causing cell damage (Akinola *et al,* 2013). Increased ROS causes oxidative stress of the STZ-induced rats, resulting in increased levels of MDA in the liver, kidney, or pancreas organ. *S. edule* ethanolic extract can reduce levels of MDA in DM rats, especially in the kidneys. *S. edule* ethanolic extract can reduced the levels of MDA because it contains flavonoid as active compounds and has antioxidant activity (Aini *et al,* 2014). The *S. edule* ethanolic extract was proven to reduce inducible nitric oxide (iNOS) in DM rats (Lukiati *et al,* 2016). iNOS is an enzyme which plays role in NO generation. NO is a reactive nitrogen species (RNS) group that has similar function as well as ROS. Both of RNS and ROS were free radicals which can mediate for several metabolic degenerative included diabetes mellitus (Soskic *et al,* 2011).

Antioxidants are compounds that can donate their electrons, in general antioxidants biologically function as an oxidant scavenger and free radicals (Dauani *et al,* 2013; Sharma *et al,* 2013). The action of the antioxidant not only depends on the dose and duration of use but also it is influenced by the type of antioxidant and its environment (Amri *et al,* 2016). Flavonoid compounds have many double bonds on aromatic rings, so it is a very effective antioxidant compound (Vermerris and Nicholson, 2006). The chemical structure of flavonoid compounds as antioxidants was caused by the presence of: (a). hydroxyl group 3 ', 4' *(ortho* -dihydroxy) in the B ring flavonoids, (b). 2.3 double bonds conjugated with the 4-oxo group (1.4-piron group) on the C ring and (c). hydroxyl groups at positions 3 and 5 (Zhang, 2005). Rasyid et al. (2012) reported that chocolate bean extract contained flavonoid can reduce MDA level in order to prevent myocyte damage. Another research reported that administration with licorice flavonoid supplement for 14 days can decrease MDA concentration in serum of obese dogs Kawasumi *et al,* 2014). Flavonoid were contained *S. edule* ethanolic extract has ability to decrease MDA level in DM rats and as a result it can reduce free radicals in the body.

The low levels of insulin in rats exposed to STZ are due to the STZ toxic effect that damages the insulin receptor accompanied by the damage of pancreas beta cells (Akinola *et al,* 2013), so that the glucose entering the pancreas beta cells cannot be responded by insulin receptors. The damage of insulin receptors and pancreas beta cells causes the blood glucose cannot be absorbed into the cells to be used and converted into energy, resulting in high levels of glucose in the blood. The *S. edule* ethanolic extract was proven to successfully repair the damage of pancreas beta cells as insulin-producing glands (Lukiati *et al,* 2016). This research shows that the treatment of *S. edule* ethanolic extract up to a dose of 42 mg/kgBW for 7 days was able to increase insulin production of DM rats, but the increase is not statistically significant. The treatment of the extracts probably requires more than 7 days in order to significantly increase the insulin production.

CONCLUSION

The decreasing of free radicals resulting on improve metabolism in DM rats. *S. edule* had been able to decrease MDA level in liver. Meanwhile, *S.edule* ethanolic extract still can not decrease the MDA level in kidney and pancreas. The treatment using *S. edule* ethanolic extract for 7 days with those doses unable able to increase the insulin production in DM rats. Furthermore, the research should be continued with higher doses of *S. edule* ethanolic extract to determine the effective doses for DM treatment.

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