Decreasing of Oxidative Stress of Red Tamarillo (*Solanum Betaceum* Cav.) Extract in STZ-NA-Induced Diabetic Rats

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Abstract: The objectivity of the research investigated tamarillo extract to decreasing of oxidative stress in STZ-NA induced diabetic *Sprague Dawley* rats including its mechanism. The method used complete random designs with six groups of male *Sprague Dawley* rats, every 5 rats. The groups were three groups control such as one healthy rat as healthy control (COS); two diabetes groups: not treated as a negative control (CON) and given metformin drug as a positive control (COP); three diabetes groups another with tamarillo extract treatments such as: given ethanol extract (more anthocyanin) as a DEE; given acetone extract (more carotenoid) as DEE and given both mixtures as DMEEA. The result showed MDA increased and SOD decreased in diabetes control rats compared to healthy control rats. Intervention with ethanol extract, acetone extract and its mixture for 28 days significantly decreased oxidative stress by decreasing of MDA and increasing of SOD. The mechanism of its pathway was by decreasing of malondialdehyde, increasing of superoxide dismutase so could be repairing of pancreas Langerhans islet following improving β-cells function, then increasing of insulin, moreover could decreasing of blood sugar.

1 INTRODUCTION

Oxidative stress implicated an essential in the pathogenesis of diabetes type 2 and its complications (Aouacheri *et al*., 2015). Streptozotocin-nicotinamide (STZ-NA) induced diabetic rats could rise oxidative stress conditions that used to model rats of type 2 diabetes (Szkudelski, 2012; Aboonabi *et al*., 2014). Oxidative stress is the imbalance between reactive oxygen species production and breakdown by endogenous antioxidants. Malondialdehyde (MDA) and superoxide dismutase (SOD) levels are a biomarker of oxidative stress (Aouacheri *et a*l., 2015). Malondialdehyde (MDA) is a marker of peroxidation lipid and SOD is a marker as antioxidant status. Type 2 diabetes is the diabetes mellitus that has characteristic includes hyperglycemia and disordered metabolism lipid, carbohydrate, and protein are caused by disturbing of insulin action or insulin resistance. The prevalence of type 2 diabetes is the highest of all diabetes prevalence (about 90% of all diabetes prevalence). The prevalence of diabetes up to 425 million in 2017, every year always increases and up to 50% undiagnosed (IDF, 2017).

Red tamarillo is a unique fruit because it has anthocyanin and carotenoid compounds together in fruit. The kinds of anthocyanin and carotenoid compounds in red tamarillo had reported, but its utilization was still limited compared to tomatoes and purple eggplants. Red tamarillo fruit had been reported to be used in extract form as a source of antioxidants that have health benefits. Previous research reported the ability of tamarillo extract as a hypocholesterolemic agent (Idris *et al*., 2011; Kadir *et al.*, 2015); and as an antioxidant suppresses oxidative stress in the *in vitro* study (Kou *et al*., 2009). Asvita & Berawi (2016), reported the extract of tamarillo capable decreasing of glucose and cholesterol levels in obese subjects. Puspawati *et al*. (2018) reported in the *in vitro* assay, the tamarillo extract that focusing to the anthocyanin and carotenoid compounds having different polarity were able to inhibit α-glucosidase enzyme, but the *in vivo* assay about the potency decreasing of oxidative stress in type 2 diabetes rats especially in the no obes had

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been not reported, so the aim of research was to determine the ability of the dominant compounds tamarillo extract (anthocyanin and carotenoid) in decreasing of oxidative stress in type 2 diabetes rat induced STZ-NA including its mechanism.

2 MATERIALS AND METHODS

2.1 Tamarillo Extract Preparation

Red tamarillos were collected from farmers in the Dieng Plateau, Wonosobo District, Central Java Province, Indonesia. Tamarillo fruit was 6 months old from flowering (anthesis). The tamarillo extraction was distinguished into two including ethanol extraction and acetone extraction. The ethanol extraction was using mesocarp, endocarp, and seed of tamarillo. The acetone extraction using the mesocarp of tamarillo. The ethanol extraction was using ethanol solvent that made a sour condition with adding citric acid of 3%, so bioactive compounds extracted more anthocyanin. The acetone extract was using acetone solvent and not added citric acid, so be extracted more carotenoid. The methods of ethanol extraction and acetone extraction were using sonication extraction with ultrasonic bath (40 kHz frequency, 100% power, the initial temperature of 27 □, end 29 □). Ethanol extraction was done for 20 minutes, while acetone extraction was about for 30 minutes. Evaporation solvent used a rotary evaporator. The ethanol extract was as the EE, acetone extract was as the AE. The mixture extract included ethanol extract and acetone extract with a ratio 1:1 as MEEA.

2.2 *In vivo* Assay

In vivo assay of tamarillo extract was including of ethanol extract, acetone extract and its mixture using *Sprague Dawley* (SD) males rats, weight: 150-200 g. Diabetes rats induced STZ-NA (Szkudelski, 2012). The rats were distinguished into six groups (every 5 rats). They were one as the healthy control (standard feed diet without treatment) as COS, two groups diabetes rats, standard feed without treatment as a negative control (CON) and given the drug metformin as a positive control (COP). Three other groups were diabetes groups, standard feed, given (force-feeding) of ethanol extract (19.3 mg anthocyanin/kg BW) as DEE, given (force-feeding) acetone extract (2.5 mg carotenoid/kg BW) as DEA, and given (force-feeding) its mixture (9.65 mg anthocyanin/kg BW and 1.25 mg carotenoid/kg BW), as DMEEA. The intervention was done for 28 days. The MDA and SOD level of serum and pancreas, histopathology of the pancreas were analyzed. All procedures related to experimental animals were approved by the ethical clearance commission from the LPPT-UGM, Indonesia (Approval No: 00067/04/LPPT/IX/2016).

2.3 Malondialdehyde (MDA) Assay

Malondialdehyde (MDA) is a secondary product that used to estimate indirect lipid peroxidation. This assay employs the quantitative sandwich enzyme immunoassay technique by the ELISA kit. Antibody specific for MDA has been pre-coated into a micro-plate. The MDA kits used Fine Test (*Wuhan Fine Biological Technology* Co., Ltd., Hubei-China). The test sample was supernatant from serum and pancreas. The sample was diluted with a dilution factor of 50 times. The standard concentration: (2000; 1000; 500; 250; 125; 62.5; 31.25, 0) pg/mL. The absorbance test was on the λ450.

2.4 Superoxide Dismutase (SOD) Enzyme Assay

SOD employs the quantitative sandwich enzyme immunoassay technique by ELISA kit. Antibody specific for SOD has been pre-coated into a micro-plate. The SOD kits used Fine Test (ER1347, *Wuhan Fine Biological Technology* Co., Ltd., Hubei-China). The test sample was supernatant from serum and pancreas. The sample was diluted with a dilution factor of 50 times. The standard concentration: (2000; 1000; 500; 250; 125; 62.5; 31.25; 0) ρg/mL Absorbance test was on λ450.

2.5 Histopathology Study

The pancreas samples fixed in the 10% formalin which were sliced about 1 cm thick, and placed into the cassettes, then the cassettes were put into tissue processor machine, which comprises of dehydration with alcohol, clearing with xylene and wax, following with impregnating process automatically for overnight (14 h). The cassettes were embedded in molten paraffin, which later cooled down and formed blocks paraffin. Each block was trimmed then sectioned about 5 mm by using a microtome. Then thin sections were put in the water bath at 45 □ few seconds, fished out, and set on a microscopic glass slide, proceed with hematoxylin and eosin (H&E) staining, and observed under a light microscope for evaluation. (Tatar *et al*., 2012).

2.6 Statistical Analysis

Statistical analysis was performed using SPSS software for windows, version 21 (SPSS, Inc., Chicago, USA). The data were presented as the mean ± S.E.M. (standard error of the mean). The statistical significance of data analysis has been assessed by one-way analysis of variance (ANOVA) and a significant difference among treatment groups was evaluated by Duncan's multiple range test. The results were considered statistically significant at the p-value of less than 0.05 ($p<0.05$).

3 RESULTS

3.1 Malondialdehyde (MDA)

Malondialdehyde (MDA) concentration was measured as a marker of lipid peroxidation, as markers of oxidative DNA damage. Serum and pancreatic MDA levels are highest in diabetes control rats (CON), the lowest in diabetes given a mixture of ethanol and acetone extract of tamarillo (DMEEA). Diet of ethanol extract (EE), acetone extract (EA) and its mixture (MEEA) for 28 days could reduce malondialdehyde (MDA) in the serum and pancreas compare to diabetes control rats ($p<0.05$). All of the extract tamarillo diets showed similar (no significant difference) with the diabetes rats, given metformin or positive control (COP) and healthy control rats (COS). Malondialdehyde (MDA) levels in serum and pancreas of *Sprague Dawley* rats after intervention 28 were shown in Table 1.

Table 1: Malondialdehyde (MDA) levels in serum and pancreas of *Sprague Dawley* rats after intervention for 28 days.

Treatments	MDA serum (μmol/L)	MDA pancreas (μmol/L)
COS	5.58 ± 0.47 bc	3.18 ± 0.24 cd
CON	7.95 ± 0.92 a	7.62 ± 0.71 a
COP	5.86 ± 0.57 bc	3.32 ± 0.59 cd
DEE	6.48 ± 2.32 b	3.46 ± 0.62 c
DEA	6.60 ± 0.34 b	3.92 ± 0.77 b
DMEEA	5.29 ± 0.68 c	2.65 ± 0.10 d

COS=healty control; CON= negative control (diabetes); COP= positive control (diabetes, given metformin drugs); DEE= diabetes, given ethanol extract; DEA=diabetes, given acetone extract; DMEEA= diabetes, given its mixture of ethanol and acetone extract; different alphabet in the back column that similar showed significant difference *(p*<0,05)

3.2 Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) level in the serum and pancreatic of diabetes rats as a negative control showed the lowest levels ($p<0.05$), while the highest in diabetes, given the mixture of ethanol extract and acetone extract (DMEEA). The diet of tamarillo extract (ethanol extract/EE, acetone extract/EA and its mixture/MEEA) for 28 days could increase the SOD level in the serum and pancreatic of diabetic rats. The decreasing was the highest in diabetes, given a mixture of tamarillo extract (DMEEA) and all of the tamarillo extract showed similar to diabetes, given metformin drugs (COP) and healthy rats (COS). Superoxide dismutase (SOD) level in the serum and pancreatic of *Sprague Dawley* rats after 28 days of intervention showed in Table 2.

Table 2: Superoxide dismutase (SOD) levels in the serum and pancreatic of *Sprague Dawley* rats after 28 days of intervention.

Treatments	SOD serum (ng/mL)	SOD pancreas (ng/mL)
COS	5.57 ± 0.07 ab	0.47 ± 0.07 c
CON	3.00 ± 0.03 c	0.19 ± 0.03 d
COP	5.15 ± 0.11 b	0.61 ± 0.11 bc
DEE	5.45 ± 0.08 ab	0.64 ± 0.08 b
DEA	4.63 ± 0.13 b	0.63 ± 0.13 b
DMEEA	6.67 ± 0.17 a	0.81 ± 0.17 a

COH=healty control; CON= negative control (diabetes); COP= positive control (diabetes, given metformin drugs); DEE= diabetes, given ethanol extract; DEA=diabetes, given acetone extract; DMEEA= diabetes, given its mixture of etanol and acetone extract; different alfabet in the back coloum that similar showed significant difference *(p*<0,05)

3.3 Histopathology

Pancreas histopathology was to determine the change of morphology of pancreas tissue, especially in the Langerhans islet. The pancreas of diabetes *Sprague Dawley* rats induced STZ-NA could cause the damage of pancreatic β cell especially in the Langerhans islet, but not all, so implicated as insulin resistance. The illustration in Langerhans islet in diabetes, given ethanol extract (DEE), diabetes, given acetone extract (DEA), diabetes, given mixture of ethanol and acetone extract and diabetes given metformin drugs (COP) after 28 day intervention showed differences in the shape and structure of the with the diabetes control (CON) and approaching healthy control. The Langerhans islet on healthy control sho

wed that the endocrine arrangement spreads on the Langerhans islet with a uniform cell shape, bluish-purple endocrine cell nucleus round, nucleoli visible, pink cytoplasm, whole, and normal endocrine cells. The tamarillo ethanol extract diet in diabetes group (DEE) showed endocrine cell repair on the Langerhans islet, normal endocrine cells appear more and empty cytoplasm without nuclei decreases (Kanter *et al*., 2003). The same was shown in diabetes, given acetone extract (DEA) and diabetes, given a mixture of ethanol and acetone extract (DMEEA). Improvements in the DEA group were seen to be less compared to the DEE and DCEEA groups. This was seen from the lower cell nucleus and the smallest Langerhan islet size among the other groups. Endocrine cell conditions in DEE, DEA and DMEEA were no different from diabetes given metformin (COP). The illustration of Langerhan islet in pancreas after intervention 28 days showed in Figure 1 (A-F) with 400 times

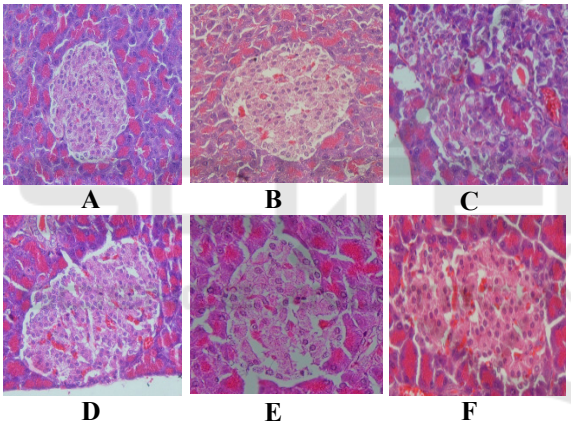

Figure 1: Illustration Langerhans islet in pancreas of *Sprague Dawley* rats (400 x): (A) healthy rat (COS); (B) diabetes (CON); (C) diabetes, given metformin drugs (COP); (D) diabetes, given ethanol extract (DEE); (E) diabetes, given acetone extract (DEA); (F) diabetes, given mixture of ethanol and acetone extract (DCEEA).

Decreasing oxidative stress by reducing MDA levels and increasing SOD levels which causes an improvement of pancreas Langerhans islet, increasing β cell function, further increases insulin production thereby reducing blood sugar. Decreasing oxidative stress can also reduce stress signals that will reduce insulin resistance. Decreasing insulin resistance will increase insulin sensitivity and increase GLUT4 thereby increasing glucose uptake, then lowering blood sugar. The description of the proposed mechanism for reducing blood sugar by the anthocyanin of tamarillo ethanol extract or the carotenoid of tamarillo acetone extract in STZ-NA induced diabetes *Sprague Dawley* rats was shown in the form of a scheme in Figure 2.

4 DISCUSSION

The ability to reduce MDA levels from ethanol extract diets could be caused by antioxidant of anthocyanin compounds, while in acetone extract diets because of the antioxidant carotenoid compounds and in a mixture of ethanol and acetone extract diets because there are antioxidants i.e: anthocyanin and carotenoid together which were like to fresh tamarillo fruit. Puspawati *et a*l. (2018), reported the anthocyanin extract of red tamarillo consisted of anthocyanin total of 386.48 ± 19.82 mg/100 g extract and carotenoid extract of tamarillo consisted of a carotenoid total of 50.80 ± 3.02 mg/100 g extract.

The higher pigment concentrations such as anthocyanin and carotenoid went along with higher antioxidant capacities (Stintzing *et al*., 2002). Prabowa (2019) reported MDA level decreased in rats that oxidative stress condition was due to the presence of antioxidants such as carotenoid and flavonoid. Anthocyanin belongs to flavonoid groups.

The lowest of MDA levels in serum and tissue pancreas of diabetes rats, given the mixture of ethanol and acetone extract indicating that antioxidants of anthocyanin and carotenoid were not contradicting synergism. This phenomenon because the antioxidant could suppress lipid oxidation and free radicals including MDA, so decreasing oxidative status with different functions (Koo & Vaziri, 2003; Tangvarasittichai, 2015; Kim *et al*., 2018). Carotenoid available as antioxidant potency associated with quenching response on oxidative stress. While there was anthocyanin which presented with scavenging pathway (Tangvarasittichai, 2015).

The ability of anthocyanin and carotenoid as antioxidants are influenced by the types of anthocyanins or anthocyanidin and carotenoids. Puspawati *et al*. (2018), reporting the major anthocyanin types in the tamarillo ethanol extract were pelargonidin 3-rutinoside, delphinidin 3–rutinoside and cyanidin 3-rutinoside, while acetone extract found the major carotenoid types were β-carotene, β-cryptoxanthin, zeaxanthin and lutein.

The structure of type anthocyanin/anthocyanidin could reduce the potency of scavenging free radical due to low bioavailability, so low absorption in the circulating system and high excretion rate in urine and feces, but opposite with high bioavailability.

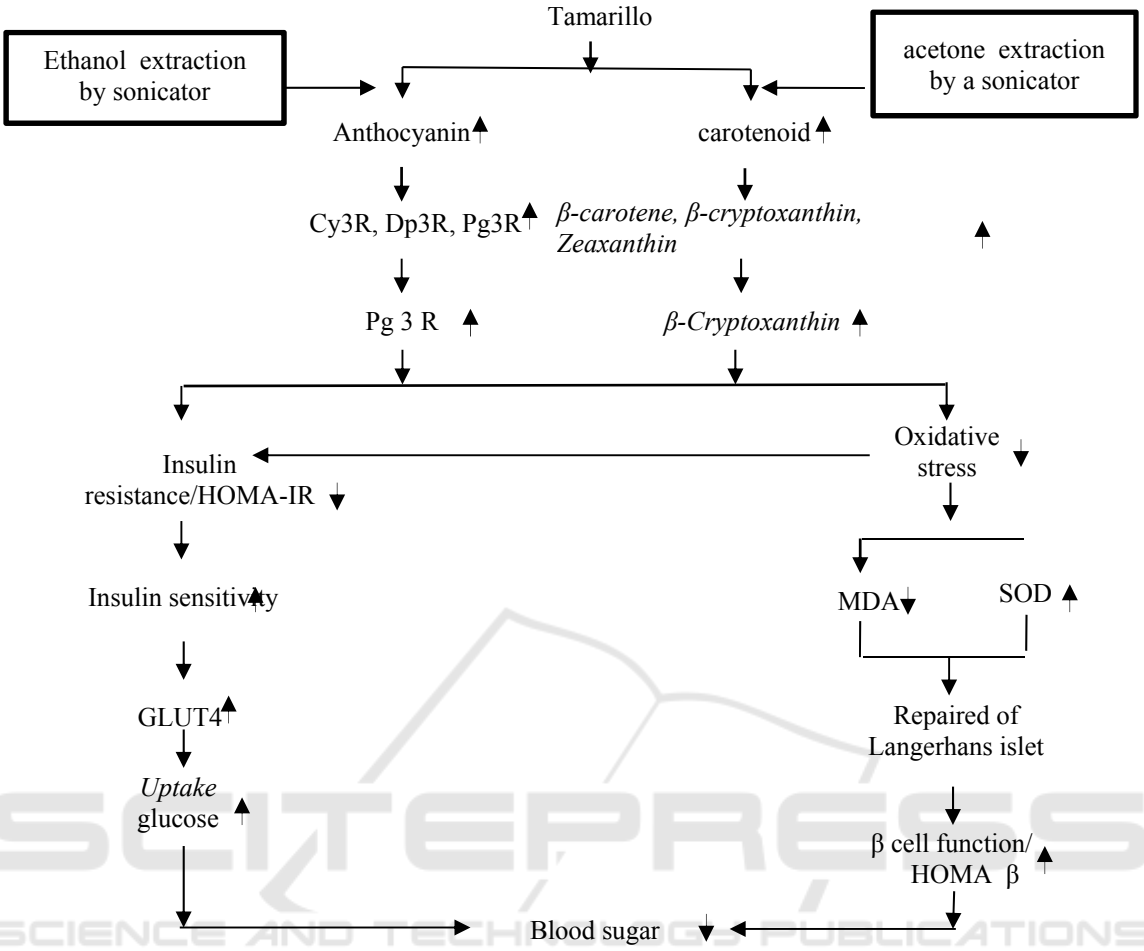

Figure 2: Mechanism of decreasing blood sugar by decreasing oxidative stress of anthocyanin or carotenoid of tamarillo in Sprague Dawley rats.

Anthocyanin with a high bioavailability efficiently reduces cellular lipid peroxidation, hence reducing the risk of many diseases (Khoo *et al*., 2017). Matsumoto *et al*. (2001) and reported delphinidin 3-rutinoside and cyanidin 3-rutinoside from blackcurrant belongs high bioavailability. They could be directly absorbed into the circulating system after 30 minutes of consumption of anthocyanin mixture from red fruit, the absorbed anthocyanins were not metabolized into the aglycones or any other metabolite forms in the human body, but not overall. There are their glycosylated forms are excreted into urine and feces (Miyazawa *et al*., 1994; Matsumoto *et al*. 2001). Stintzing *et al*. (2002) reported the glycosylated B-ring structure of anthocyanin contributes to the high antioxidant activity, where ortho hydroxylation and methoxylation substantially increase the antioxidant activity. The Anthocyanidins had higher antioxidant activity than their glycosides, which are to be expected because the aglycons are very unstable and highly reactive. Tangvarasittichai (2015) reported anthocyanidin type was like in tamarillo extract such as pelargonidin, delphinidin and cyanidin have antioxidant functions by scavenging free radicals.

The carotenoid including β-carotene, β-cryptoxanthin, zeaxanthin and lutein types are reported to suppress lipid oxidation so that they can reduce MDA (Murillo & Fernandez, 2016). The β-cryptoxanthin work by quenching free radicals decreases MDA levels (Adela & Momeu, 2008).

Table 1 also showed a decrease in serum MDA levels in diabetes rats, given mixture of ethanol and acetone extract (DMEEA) lower than in the pancreas MDA could be caused the anthocyanin or carotenoid in the serum more in their original form, while the pancreas has undergone metabolism into their aglycone (Fernandes *et al*., 2014).

The antioxidant function of anthocyanin is influenced by the type of aglycone associated with

their hydroxyl group. Tangvarasittichai, (2015) reported the cyanidin, pelargonidin, and delphinidin have the ability to scavenging free radicals better than their original forms. The ability of anthocyanin is due to the presence of hydroxyl groups in C3', C4'-ortho-dihydroxyl and 3 hydroxyl. Pelargonidin aglycone could reduce MDA levels in STZ-NA induced rats to normal conditions (Lucioli, 2012). This was similar to Puspawati *et al*. (2018), who reported tamarillo had the highest type of pelargonidin between cyanidin and delphinidin. So, the pelargonidin in tamarillo had potent to reduce MDA levels in type 2 diabetes.

Suzuki *et al.* (2011) reported that β-cryptoxanthin was the highest type of carotenoid in tamarillo that reduces oxidative stress with high levels of hyperglycemia. This shows that tamarillo extract, especially a mixture of ethanol and acetone extract of tamarillo can reduce MDA levels better than the individual form.

The superoxide dismutase (SOD) enzyme is one of the antioxidant enzymes that are responsible for neutralizing superoxide radicals (O2*) to be more stable hydrogen peroxide (H_2O_2). The role of SOD is the most critical role among antioxidant enzymes in reducing the effects of oxidative stress (Sari *et al.,* 2005). Low serum and pancreatic SOD levels in diabetes no treatment (CON) were associated with high levels of MDA. High levels of MDA could cause the body's antioxidant system such as SOD would increase but following to decrease. This condition was caused by oxidative stress which leads to oxidative damage such as interference or damage to the SOD enzyme.

Szkudelski (2001) reported induction STZ-NA in rats could free radical cause oxidative stress which leads to damage to β cells, but not overall. Kamble *et al*. (2015), reported type 2 diabetes characterized by oxidative stress could lead to oxidative damage such as protein damage and could reduce the expression of antioxidant enzymes such as SOD.

The ability of tamarillo ethanol extract can be caused by the type of anthocyanin belonging to the flavonoid group. Magalingam *et al*. (2013) reported the flavonoid compounds to have the ability to activate the antioxidant enzyme gene so that it can increase its activity. The activation mechanism by maintaining the bioavailability of NO, so that it does not change into NOO- so that it can induce antioxidant transcription factors, namely nuclear factor E2-related factor-2 (Nrf-2) binds to ARE (antioxidant response element) which will regulate antioxidant gene formation such as SOD. This enzyme in the production process is triggered by levels of endothelial NO synthetase (eNOs) which is positive regulators of mRNA to produce SOD(Levonen et al., 2007). Stimulation of the Nrf2/ARE pathway is fundamental for the induction of antioxidant defense enzymes and the modulation of the intracellular GSH in response to stress (Liu et al., 2018). Flavonoid bound with the antioxidant enzymes and caused direct activation of these enzymes, where any of these mechanisms will result in increased activity of the antioxidant enzyme (Magalingam *et al*., 2013). Other mechanisms can be through abilities as antioxidants that directly suppress free radicals thereby suppressing the occurrence of oxidative stress and oxidative damage (Kamble *et al*., 2015). These compounds help in scavenging the species that initiate the peroxidation, breaking the autoxidative chain reaction, quenching •O2-, and preventing the formation of peroxides (Gaschler & Stockwell, 2017) The most effective antioxidants are those possessing the ability to interfere with the free radical chain reaction (Wojtunik-kulesza, *et al*. 2016)

The ability of tamarillo acetone extract can be caused by the presence of carotenoid compounds that function as antioxidants. The antioxidant function directly suppresses free radicals by breaking chain reactions into more stable products, which can reduce oxidative stress levels and oxidative damage as in the SOD enzyme (Kamble *et al*., 2015).

The highest SOD levels in DMEEA show that anthocyanin and carotenoid compounds in the extract can work in synergy to increase antioxidant levels. This research is in line with that reported by Kadir *et al.* (2015), the tamarillo extract diet derived from all parts of tamarillo fruit given to *Sprague Dawley* rats on a high-fat diet can increase SOD.

The illustration in diabetes, not treatment (CON) was due to too STZ-NA induction, lesion of the pancreatic tissue, endocrine cell degeneration on the Langerhans islet, picnosis (endocrine cells constrict/shrink), then necrosis, disappear, only the cytoplasm appears empty and contains glycogen deposits, enlarges without nucleus or vacuolization. Necrosis is cell death that occurs after the blood supply is lost or the presence of toxins characterized by vacuolization (cell swelling), protein denaturation and organelle damage. Besides necrosis, there is a pattern of apoptotic cell death. Apoptosis occurs in undesirable cell conditions eliminated in physiological conditions and irreparable damage to mutations (pathological conditions)(Tatar et al., 2012).

Normal endocrine cell enhancement in diabetes, given ethanol extract (DEE), diabetes given acetone extract (DEA) and diabetes given mixture of ethanol and acetone extract (DMEEA) was caused by the

antioxidant properties of anthocyanin and carotenoids which protect cells from oxidative damage due to oxidative stress and the presence of anthocyanin and carotenoids will play a role in increasing cell proliferation. The ability as an antioxidant is also shown by the results of increased SOD and decreased MDA. The repairing pancreatic Langerhans islets in DEE, DEA, and DMEEA were not different from what happened in diabetes given metformin drug (COP). Metformin can repair the pancreatic Langerhans islet, not because of its antioxidant properties but through its ability to increase insulin sensitivity, thereby increasing GLUT4 levels and blood glucose uptake to the tissues causing blood sugar to decrease. This condition causes endocrine cells to regenerate by mitosis or proliferation (Song, 2016; Rena *et al.,* 2017).

Figure 2 went through starts from tamarillo in the form of ethanol extract and acetone extract. Ethanol extract predominantly contained anthocyanin, while acetone extract was more dominant containing carotenoids. From the dominant type of anthocyanin i.e: pelargonidin 3-rutinoside and the dominant of carotenoid type i.e: β-cryptoxanthin which were antioxidant function. They could suppress oxidative stress by reducing MDA levels, increasing SOD levels. That condition would cause cell repair such as β-cells on the pancreas Langerhans islet, so the β cell could increase to producing insulin which was used to reduce blood sugar in STZ-NA induced diabetes rats. Decreasing of oxidative stress also could reduce insulin resistance, so the insulin sensitivity increased, further GLUT4 activity increased, followed by the increase of glucose uptake, then also decreasing of blood sugar.

5 CONCLUSION

Tamarillo extract that caused synergism of anthocyanin and carotenoid compounds with different polarity properties could decrease oxidative stress in STZ-NA induced diabetes rats by decreasing malondialdehyde (MDA), increasing the level of superoxide dismutase (SOD) and repairing of Langerhans islet of pancreas rats. Its mechanism of decreasing oxidative stress with decreased MDA, increased SOD, so impaired of Langerhans islet, moreover improved function of beta cells/HOMA-β following improved β-cells function, moreover increased insulin level than would decrease blood sugar. Other hand decreasing oxidative stress also implied decreasing HOMA IR/insulin resistance, so improved insulin sensitivity, moreover increasing of GLUT activity, then increasing of uptake glucose following to decreasing of blood sugar.

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