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Potravinarstvo Slovak Journal of Food Sciences
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Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 13, 2019, no. 1, p. 644-650
https://doi.org/10.5219/1136
Received: 23 May 2019. Accepted: 30 May 2019.
Available online: 28 August 2019 at www.potravinarstvo.com

ISSN 1337-0960 (online)

POTENCY OF OKRA FLOUR (*ABELMOSCHUS ESCULENTUS*) IN IMPROVING ADIPONECTIN LEVEL AND TOTAL ANTIOXIDANT CAPACITY OF HIGH FAT DIET STREPTOZOTOCIN RAT MODEL

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ABSTRACT

T2DM has increase in global-morbidity and mortality. Oxidative stress and adiponectin-levels are important for insulin-resistance and pancreatic-β-cell-dysfunction in T2DM. Okra fruit is rich of quercetin and phytosterol which have positive-effect for T2DM. Research aimed was to study the effect of okra-flour to adiponectin-levels and total-antioxidant-capacity (TAC) in T2DM. Thirty Wistar-rats were divided randomly in five groups. K1 and (X1, X2 and X3)-treated-groups were in T2DM-condition-induced by high-fat-diet-(HFD)-Streptozotochin-(STZ)-nicotinamid-(NA). Healthy-controls-(K2)-group was also used. Okra-flour was given orally for 28 days at doses of 0.1; 0.2 and 0.3 g/Kg-body-weight/d to X1, X2 and X3-groups, respectively. Statistical program was used to analyse the different between pre-post-intervention, and between groups. Correlations between variables were also analysed. The serum-adiponectin and TAC-levels were measured by ELISA and ABTS-methods, respectively. By comparing pre and post-intervention, adiponectin levels of all-intervention-(X1, X2, X3)-group were increase (p = 0.027 for X1 and X2; p = 0.028 for X3), while in the same period the decrease were found in group K1 (p = 0.026) and K2 (p = 0.028). Increase-TAC-levels pre-post-intervention was observed in group all-intervention-groups (p = 0.027), while no change in K1 (p = 0.66) and the decrease in group K2 (p = 0.039). Reduce-fasting-blood-glucose-levels pre-post-intervention were shown in the all-intervention-groups (p = 0.028), while for the K1 groups was increase (p = 0.028). There were significant differences between the five-groups on fasting-blood-glucose-levels, adiponectin and TAC-levels, and X3-group showed the highest adiponectin and TAC-levels. Very-strong-correlations were found between glucose-adiponectin-TAC-levels-post-intervention. Okra-flour make better glucose-adiponectin and TAC-levels in T2DM-conditions. Okra dose of 0.30 g/Kg-body-weight/day is the best in increasing adiponectin and TAC-levels.

Keywords: Okra; T2DM; adiponectin; TAC

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is metabolic disease (metabolic syndrome) characterized by high blood glucose levels. Mechanisms of T2DM include first, reduce ability of pancreatic-β-cells in producing sufficient amounts of insulin, and second, insulin resistance when the body cannot use it effectively so that excess sugar occurs in the blood **(Melton, 2017)**. DM is a global problem due to the increase incidence particularly those caused by obesity. T2DM caused 1.5 million deaths in 2012 in which high blood glucose from normal was responsible for 2.2 million additional deaths as a result of an increased risk of cardiovascular disease and others, with a total of 3.7 million deaths associated with blood glucose levels in 2012. Many of the deaths (43%) occurred under the age of 70. In 2014 as many as 422 million people in the world suffered from DM with a prevalence of 8.5% among the population of adults suffering from T2DM **(WHO, 2016)**.

The relationship between obesity and T2DM occur through 2 mechanisms, namely insulin resistance that occurs due to obesity and failure of pancreatic β cell function. Abnormalities of the body's metabolism where there is a decrease in response or sensitivity from the action of insulin is called insulin resistance. The HFD-inducing-insulin resistance causes an increase in fat oxidation and subsequently increase reactive oxygen species (ROS) levels **(Styskal, Van Remmen and Richardson, 2012; Asghar, 2017)**. The high ROS level can reduce total antioxidant status (TAS) **(Moheildein et al., 2015)**. Insulin resistance is a result of positive energy balance, because positive energy balance increases adipose tissue, and reduces adiponectin production. Adiponectin is part of adipocytokine which

functions to increase insulin sensitivity by increasing GLUT4 translocation and plays a role in suppressing glucose production by inhibiting gluconeogenic enzymes **(Cheng et al., 2014)**. Adiponectin levels can be increased by consuming polyphenols from food **(Kim and Koegh, 2016)**.

Antioxidants bind to free radicals and afterward prevent the adverse effects of free radicals on the body **(Maritim et al., 2002)**. Plants are considered food that is beneficial in many diseases including diabetes in controlling blood glucose levels and preventing long-term complications **(Gallagher, Flatt and Duffy, 2003)**. Phytochemical studies show that polysaccharides, polyphenols, flavonoids, tannins, sterols and triterpenes are the main components of Okra (*Abelmoschus esculentus*) and those components have a variety of biological activities **(Sheu and Lai, 2012)**. Other study show that Okra fruit is rich in bioactive components, such as flavonoids, especially quercetin and phytosterols **(Sa'eed Halilu Bawa, 2016)**. Fresh Okra has flavonoids namely quercetin in amounts 60 – 75% **(Zhang, 2014)**. Okra fruit has phenols and flavonoids that have antioxidant effects and anti-diabetic effects, namely quercetin-3-O-ß-D-glucopyranosyl-(1→6)-ß-D-glucopyranoside and quercetin-3-O-ß-D-4'-O-methyl-ß-D-glucopyranoside **(Zhang, 2014)**.

This study aimed to determine whether intervention of okra fruit flour increase both serum-adiponectin and total antioxidant capacity (TAC)-levels in T2DM-rats induced by high fat diet (HFD) followed by Streptozotochin-(STZ)-nicotinamid-(NA)-injection.

The dosage variations of okra flour given in this study were 0.1; 0.2 and 0.3 g/Kg-body-weight-(BW)/day(d) **(Naeem, Mohammad and Ali, 2018; Sabitha, Ramachandran and Naveen, 2012)**. The intervention of okra fruit flour was carried out for 28 days.

Scientific hypothesis

Okra flour has various dosages which affect adiponectin levels, total antioxidant levels (KAT) and blood sugar levels in DMT2 wistar rats.

MATERIAL AND METHODOLOGY

Okra-flour preparation and Intervention

Okra was bought in the traditional market of Semarang. Choosed okra was those in good condition as indicated by green-colour, about 10 to 20 cm long, notched at the end and had fine feather. The fruit (seeds and skin) was then wash and blend by using a blender machine, then it was dry at low temperature 40 °C in drying cabinet. The dried ingredients are then mashed with mortar or blender. The powder is weighed and put in a plastic bottle and kept away from light and moisture for further use **(Naeem, Mohammad and Ali, 2018)**. The Okra-flour-suspension is given orally using a feeding tube given to experimental –Wistar-rats. The moderate doses were 0.10 and 0.20 g.Kg^{-1}-BW/d. These doses reduced blood glucose levels of T2DM-rat. The high dose was 0.3 g.Kg^{-1}-BW/d, twice the moderate dose **(Naeem, Mohammad and Ali, 2018; Sabitha, Ramachandran and Naveen, 2012)**.

Antioxidant activity test of okra flour

Antioxidant activity test of okra flour was carried out at the Diponegoro University Integrated Laboratory. Analysis of antioxidant activity with DPPH method was referring to the

Figure 1 Okra Fruit (*Abelmoschus esculentus*).

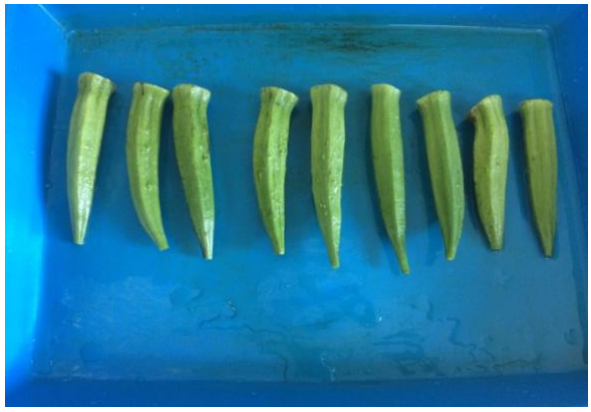

Figure 2 Preparation of Okra flour.

Figure 3 Preparation of Okra flour.

Figure 4 Okra flour.

previous method **(Suica-Bunghez et al., 2016)**. Preparation of DPPH solution was carried out using methanol (0.02 mg DPPH.mL^{-1} MeOH) with violet colour. A total of 0.045 g of okra flour samples were mixed with 1 mL of DPPH solution which was then incubated in a dark place for 1 hour. After that, the mixture is read the absorbance at $\lambda = 517$ nm with a spectrophotometer. The control was made from 0.5 mL of MeOH with 1 mL of DPPH solution. Antioxidant activity (AA%) was calculated by the absorbance control formula minus the absorbance of the sample then divided by the absorbance of the control then multiplied by 100.

Research design and Experimental-Animals

This research was a true-experiment study with randomized pre-post test with control group design. Animal used was male Wistar rats, aged 8 – 12 weeks, weighing 150 – 200 g, were acclimatized at the laboratory of the Center for Food and Nutrition Studies at Gajah Mada University, Yogyakarta. The rats were placed in individual stainless-steel cages at regulated temperatures (21 °C). They are housed at cleaned and germ-free place. The rats were fed with 20 g.d^{-1} of the Comfeed II standard-diet (7% fat) during the non-HFD period. They received ad-libitum water during the experiment. Animal care in the laboratory was carried out accordance with the Animal Laboratory Guidelines from the Central Laboratory for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta.

Thirty rats were divided into five groups which were T2DM-control-(K1), T2DM-intervention-(X1, X2 and X3) and healthy-control-(K2)-groups. The T2DM-condition was induced by HFD-STZ-NA. After a week of acclimatization, rats were conditioned on T2DM with oral HFD of 20 g.d^{-1} for 14 days then injected intraperitoneally with STZ (45 mg.Kg^{-1}-rat-BW) and NA (110 mg.Kg^{-1}-rat-BW). Injection of NA was carried out 15 minutes after STZ with the aim of preventing pancreatic cell-apoptosis β **(Ghasemi and Khalifi, 2014)**. T2DM-condition was indicated by fasting blood glucose serum >200 mg.dL^{-1} **(Bao et al., 2015)**. The X1, X2 and X3-groups were then treated with Okra-flour-suspension in doses of 0.1; 0.2 and 0.3 g.Kg^{-1}-rat-BW/d every day for total period of 28 days. The blood sample again was taken at the end of the trial (end of intervention), fasting blood glucose was taken through the retroorbital plexus. Blood samples were collected in a centrifugation tube and centrifuged 4000 rpm in 15 minutes. Adiponectin and TAC levels were analysed by ELISA and ABTS methods respectively. This study was approved by the Health Research Ethics Commission (KEPK) of the Faculty of Medicine Diponegoro University Semarang through ethical clearance No. 06/EC/H/FK-UNDIP/I/2019.

Statistic analysis

Results were expressed as mean ±*SD* (for normally distributed data) otherwise it expressed as median (min-max). Statistical difference was analysed by using one-way analysis of variance (ANOVA) followed by post hoc Bonferroni for normally distributed data, otherwise Kruskal-Wallis test followed by Mann-Whitney-U-test was used (SPSS 21). Spearman's correlative test was used to analyse the relation between variables. Statistical analyses were done by computer. The differences and correlations were considered significant at *p*-value <0.05 and 95% confidence intervals. The strength of correlations was determined by *r*-value.

RESULTS AND DISCUSSION

The data processed in this study was obtained from 30 Wistar-rats, which divided in each group consisting of 6 rats. The experimental animals used before T2DM had an average body weight of 173.25 g and blood glucose levels of 73.11 mg.dL^{-1}, while the T2DM animals after induction had an average body weight of 194.92 g and blood glucose levels of 259.18 mg.dL^{-1}.

Wilcoxon test between body weight before and after intervention showed that the intervention of okra flour increased body weight in the all-treatment-groups (the groups had $p = 0.027$; Table1), while K1-group experienced significant weight loss ($p = 0.027$). This suggested that Okra-flour increased body T2DM-rat-BW. Healthy-control-K2-group was experienced a significant increase in body weight ($p = 0.027$). Statistically difference was also found on the pre-post-intervention change (Δ) of rat-BW among all-groups (Kruskal Wallis test; $p < 0.001$), and then Mann-Whitney U-test between two-groups was performed. The Δ-rat-BW of all-intervention-T2DM-group was higher than T2DM-control-K1-group and this was significant (X1, X2 and X3 had same *p*-value; $p = 0.004$). This demonstrated that Okra-flour recover BW of T2DM-rat. The Δ-rat-BW of X1 and X2-groups were significantly lower than healthy-control-K2-group (X1 and X2 had same *p*-value; $p = 0.004$), while those of X3-group was not different than K2-group ($p = 0.411$). This demonstrated that Okra-flour in dose of 0.3 g/Kg-rat-BW/d normalized BW of T2DM-rat. All of these indicated that Okra-flour increased T2DM-rat-BW in dose dependent manner.

The blood glucose levels were decrease in the end of intervention, and these was significantly difference (X1, X2 and X3 groups had same *p*-value; Wilcoxon test, $p = 0.028$; Table 2), meanwhile those of T2DM-control-K1-group increased significantly ($p = 0.028$). This indicated that Okra-flour reduced glucose-levels of T2DM-rat. The blood glucose level of healthy-control-K2-group was also significantly increase in the end of study ($p = 0.028$), however this change was remained in the normal value. The pre-post-intervention-change (Δ) of blood-glucose-levels was significantly different among five-groups (Kruskal Wallis test; $p < 0.001$). The Δ-glucose-levels of all-intervention-T2DM-group was more apparent than T2DM-control-K1-group and this was significant (X1, X2 and X3 had same *p*-value; Mann-Whitney U-test, $p = 0.004$). This again indicated that Okra-flour reduced glucose-levels of T2DM-rat. The Δ-blood-glucose-levels were significantly different between intervention groups. The Δ-blood-glucose-level of X1 was significantly lower than X2 and X3-groups (X2 and X3 had same *p*-value; Mann-Whitney U-test, $p = 0.004$). Further analysis showed that the Δ-blood-glucose-level of X2 was significantly lower than X3-group ($p = 0.004$). All together these demonstrated that the smallest Δ-blood-glucose-level was found in Okra-flour-intervention-dose of 0.1 g.Kg^{-1}-rat-BW/d, while the biggest change was found in the dose of 0.3 g.Kg^{-1}-rat-BW/d. This indicated that Okra-flour reduced T2DM-rat-glucose-levels in dose dependent manner.

The adiponectin levels were significantly increase at the end of intervention in all treatment-group (Wilcoxon test of

X1 and X2-group had same *p*-value, $p = 0.027$; while X3-groups showed $p = 0.028$; Table 2). This indicated that Okra-flour intervention increased adiponectin levels of T2DM-rats. The adiponectin-level of T2DM-control-K1-group was significantly decrease ($p = 0.026$) at the end of study. Although this was also observed in healthy-control-K2-group, the adiponectin level in K2-group was obviously higher than all T2DM-groups. Kruskal Wallis test showed a significant difference in the pre-post-intervention-change (Δ) of adiponectin-levels among the five groups ($p < 0.001$) (Table 2). Mann-Whitney U-test showed the Δ-adiponectin-levels of all-T2DM-intervention-groups were significantly higher than K1-group ($p = 0.004$). This suggested that Okra-flour increased adiponectin-levels of T2DM-rats. The Δ-adiponectin-levels of X3-group was the higher than X1 and X2 (X1 and X2 had same *p*-vale; $p = 0.004$). The Δ-adiponectin-levels of X2-group was the higher than X1 ($p = 0.004$). These showed that the biggest Δ-adiponectin-levels was in those treated with Okra-flour in dose of 0.3 $g.Kg^{-1}$-rat-BW/d, the middle was those receive 0.2 $g.Kg^{-1}$-rat-BW/d, and the lowest was those treated with 0.1 $g.Kg^{-1}$-rat-BW/d. This indicated that Okra-flour increased T2DM-rat-adiponectin-levels in dose dependent manner.

The pre-post-intervention-TAC-levels were significantly increase in all T2DM-intervention groups (X1, X2 and X3-groups; Wilcoxon test, $p = 0.027$; Table 2), while K1-group showed no significant change of the pre-post-intervention-TAC-levels ($p = 0.066$). This suggested that Okra-flour increase T2DM-rat-TAC-levels. Kruskal Wallis test demonstrated was a significant difference in the pre-post-intervention-change (Δ) of TAC-levels among the five groups ($p < 0.001$) (Table 2). Mann-Whitney U-test showed the Δ-TAC-levels of all-T2DM-intervention-groups were significantly higher than K1-group ($p = 0.004$). This again indicated that Okra-flour increase T2DM-rat-TAC-levels. The Δ-TAC-levels of X1 was significantly lower than X2 and X3 ($p = 0.004$), while no significant different was found between those of X2 and X3-group ($p = 0.091$). This indicated that Okra-flour increased T2DM-rat-adiponectin-levels partly in dose dependent manner.

The Spearman test on all data from all rats at the end of study showed that very strong correlation was found between variables. A very strong positive-correlation was observed between TAC and adiponectin-levels (Table 3), while very strong negative correlation was found between blood-glucose-levels either with adiponectin-levels or TAC-levels. Those correlations were only weak before Okra-flour-intervention began.

Okra flour is recovered T2DM-rat-BW. This was based on a significantly higher T2DM-rat BW of Okra-flour-intervention-group of at the end of study than those of before the intervention. This was based on a significantly higher Δ-T2DM-rat-BW in those of Okra-flour-intervention-group than T2DM-control-K1-group, and this effect was dose dependent. The effect of Okra-flour on T2DM-rat-BW was as expected. Reduce BW occurred after STZ-injection during T2DM induction (data not shown).

This is also observed in previous studies observing the effect of STZ injection on BW **(Bermúdez-Pirela et al., 2007; Roopchanda et al., 2015; Rodrigues et al., 2015)**. Other mechanism involve in the reduce BW of T2DM is insulin resistance which increases lipolysis **(Zhou et al., 2013)**.

All together indicates that Okra-flour overcome any mechanism involve in the reduce T2DM-rat-BW.

Okra flour reduced glucose-level of T2DM-rat and increase both serum-adiponectin and TAC-levels. The effect of Okra-flour on blood-glucose, adiponectin-levels and TAC-levels were dose dependent. T2DM-control-K1-group showed increase blood-glucose-levels and reduce both serum-adiponectin and TAC-levels. Healthy-control-K2-group also showed increase blood-glucose-levels. Stress may be a factor for increasing blood-glucose levels. Blood collection in condition which activates stress response in rats through increased adrenaline and non-adrenaline **(Bowe et al., 2014)**.

Table 1 Intake and body weight.

Rat Group	Intake (Rerata ±*SD*) g			
	Pre Intervention	**Post Intervention**	***p***	**Δ**
X1	12.6 ±0.49	13.1 ±0.34	0.051	0.5 ±0.50
X2	12.9 ±0.25	12.7 ±0.69	0.686	-0.1 ±0.82
X3	12.8 ±0.31	12.4 ±0.33	0.153	-0.3 ±0.51
K1	13.9 ±0.16	14.0 ±0.28	0.647	0.07 ±0.36
K2	12.4 ±0.44	13.0 ±0.45	0.136	0.5 ±0.79
p^1	0.002	0.001		0.064
Rat Group	**Body Weight (Median (Min-Max)) g**			
	Pre Intervention	**Post Intervention**	***p***	**Δ**
X1	196.0 (183.0 – 203.0)	211.0 (199.0 – 216.0)	0.027	14.00 (11.00 – 16.00)
X2	199.5 (188.0 – 205.0)	219.5 (208.0 – 226.0)	0.027	20.00 (17.00 – 2 1.00)
X3	185.5 (178.0 – 210.0)	213.5 (205.0 – 236.0)	0.027	27.00 (25.00 – 29.00)
K1	197.0 (185.0 – 210.0)	181.0 (170.0 – 197.0)	0.027	-14.50 (-17.00 – -12.00)
K2	187.5 (175.0 – 193.0)	214.5 (205.0 – 220.0)	0.027	27.50 (26.00 – 30.00)
***p*^{1}**	0.058	0.002		0.000

Note: Δ = change of body weight before and after treatment.

Table 2 Blood serum test.

Rat Group	Blood Glucose Level (Median (Min-Max)) (mg.dL^{-1})			
	Pre Intervention	**Post Intervention**	***p***	**△**
X1	253.8 (251.09 – 262.04)	144.9 (138.55 – 152.61)	0.028	-109.34 (-115.83 – -105.89)
X2	262.2 (258.03 – 264.60)	128.1 (124.50 – 129.32)	0.028	-134.29 (-135.36 – -132.33)
X3	262.4 (258.76 – 268.25)	108.4 (100.40 – 116.47)	0.028	-155.71 (-160.62 – -149.88)
K1	257.3 (250.73 – 262.04)	258.8 (253.01 – 263.05)	0.028	1.96 (0.68 – 2.39)
K2	73.1 (68.98 – 76.64)	74.7 (71.08 – 77.11)	0.028	1.52 (0.39 – 2.10)
***p*^{1}**	0.000	0.000		0.000
Rat Group	**Adiponektin Level (Median (Min-Max)) (mg.L^{-1})**			
	Pre Intervention	**Post Intervention**	***p***	**△**
X1	3.1 (2.49 – 4.19)	9.4 (8.72 – 9.94)	0.027	6.23 (5.47 – 6.42)
X2	3.2 (2.21 – 3.72)	12.3 (11.50 – 12.90)	0.027	9.12 (9.04 – 9.31)
X3	3.2 (2.02 – 4.38)	13.8 (13.40 – 14.60)	0.028	10.58 (9.69 – 11.90)
K1	3.3 (2.68 – 3.91)	3.1 (2.30 – 3.62)	0.026	-0.23 (-0.48 – -0.19)
K2	16.1 (15.30 – 17.40)	15.6 (15.00 – 16.50)	0.028	-0.45 (-0.90 – -0.10)
***p*^{1}**	0.006	0.000		0.000
Rat Group	**TAC Level (Median (Min-Max)) (mmol.L^{-1})**			
	Pre Intervention	**Post Intervention**	***p***	**△**
X1	0.2 (0.15 – 0.44)	0.9 (0.74 – 1.18)	0.027	0.59 (0.44 – 1.03)
X2	0.3 (0.15 – 0.44)	1.6 (1.62 – 1.91)	0.027	1.33 (1.18 – 1.61)
X3	0.2 (0.15 – 0.59)	1.9 (1.76 – 2.21)	0.027	1.69 (1.17 – 2.06)
K1	0.4 (0.15 – 0.59)	0.2 (0.15 – 0.29)	0.066	-0.15 (-0.44 – 0.00)
K2	2.2 (2.06 – 2.35)	2.0 (1.91 – 2.21)	0.039	-0.15 (-0.29 – 0.00)
***p*^{1}**	0.005	0.000		0.000

Table 3 Spearman correlative test before and after intervention.

Variable	Pre Intervention		Post Intervention	
	r	***p***	***r***	***p***
Kadar adiponektin dan KAT	0.424	0.019*	0.919	0.000*
Kadar adiponektin dan glukosa darah	-0.582	0.001*	-0.980	0.000*
KAT dan kadar glukosa darah	-0.405	0.026*	-0.930	0.000*

Note: *p-value <0.05 = significant.

This then contributes in the increase of blood-glucose-levels **(Bowe et al., 2014)**. The stress effects on blood-glucose-levels, therefore may occur in the present study. The very strong correlations between blood-glucose, adiponectin, and TAC-levels after Okra flour intervention in T2DM-rats, strengthen the notion that Okra flour influence mechanisms contribute in regulating blood-glucose, adiponectin, and TAC-levels. Mechanisms which may explain these present finding have been studied. Serum adiponectin levels correlate with insulin resistance in T2DM. Adiponectin plays a role in maintaining insulin sensitivity through direct and indirect mechanisms. Adiponectin decreases glucose production in the liver by inhibiting gluconeogenic enzymes in the AMPK pathway and adiponectin also upregulates IRS-2 expression to strengthen insulin in the liver. Adiponectin increases GLUT 4 translocation to the skeletal muscle plasma membrane, so that glucose uptake increases **(Cheng et al., 2014)**. Conditions of hyperglycemia in T2DM can interfere with antioxidant status because the condition of hyperglycemia can produce excessive ROS and can disrupt the body's defence system. Low antioxidant status and high production of ROS can trigger oxidative stress **(Zhou et al., 2013; Dornellas et al., 2015)**. ROS is toxic because it has high reactivity to enzymes, so it can damage tissue **(Skovso,**

2014). In uncontrolled T2DM conditions, there is an increase in NADPH activity. Increased NADPH activity can increase the production of superoxide radical anions which indicates that there is an increase in oxidative stress **(Lim et al., 2018)**. The antioxidants contained in food contribute to giving hydrogen atoms to radical compounds taken from hydroxyl groups, thereby forming stable phenoxyl hydroxyls **(Nagarchi et al., 2015)**. Total phenol content in food directly correlated with KAT in mice tested **(Nagarchi et al., 2015)**. Further Okra flour study in more detail mechanisms involve will complete the present finding.

CONCLUSION

The administration of Okra flour with various doses tested, proved to significantly reduce blood glucose levels, increase adiponectin and TAC levels in T2DM wistar rats. The administration of Okra flour with a dose of 0.30 $g.kg^{-1}$ BB per day is most effective in reducing blood glucose levels, increasing adiponectin and TAC levels in DMT2 wistar rats.

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Acknowledgments:

The authors wish to Department of Nutrition Science, Faculty of Medicine Diponegoro University,Semarang, Indonesia, laboratory of food technology Diponegoro University, Semarang, Indonesia and laboratory of the Center for Food and Nutrition Studies at Gajah Mada University, Yogyakarta, Indonesia for entirely supporting this research.

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