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Effectiveness green okra pudding on glycemic profile of adults with type 2 diabetes mellitus through regulation of TCF4 protein

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ABSTRACT

Introduction: Type 2 Diabetes Mellitus (T2DM) is a common and increasingly prevalent chronic disease worldwide that can lead to numerous complications. This study aimed to evaluate the effect of green okra pudding consumption on glycemic profile, blood pressure, and TCF4 protein levels in adults with T2DM.

Methods: Single blind randomized controlled trial (RCT) was conducted with 28 adult participants aged 19-59 years with T2DM. The treatment groups were divided into a control group and an intervention group. The intervention group received green okra pudding for six weeks with a portion of two cups each weighing 100 g/day, for a total daily consumption of 200 g. The control group received the same amount of pudding without green Okra. Data were analyzed using independent t-test analysis to evaluate the effect of the treatment. SPSS version 26.0 was used.

Results: Green okra pudding had a significant effect on changes in fasting glucose blood. The control group experienced an increase in fasting glucose blood from 184 to 197.83 mg/dl; HbA1c from 8.26% to 9.61%; Systolic decreased from 148.58 to 143.75 mmHg; Diastolic blood pressure increased significantly from 90.25 to 143.75 mmHg and TCF4 decreased from 191.27 to 186.57. In contrast, Green Okra Pudding group experienced in fasting glucose blood a decrease from 275.58 to 268.83 mg/dl; HbA1c decrease from 10.11% to 9.65%; Systolic decreased from 151.25 to 141.75; Diastolic blood pressure decreased from 91.25 to 89.33 mmHg and TCF4 increased from 173.22 to 179.65.

Conclusion: The intervention with Green Okra Pudding showed statistically positive effects on fasting blood glucose, diastolic blood glucose, and TCF4 compared to the control group. Specifically, the group consuming the pudding experienced a decrease in fasting blood glucose and diastolic blood pressure. This indicates that Green Okra Pudding is potentially effective in helping manage fasting blood glucose, diastolic blood pressure, and TCF4 in Type 2 Diabetes Mellitus.

KEYWORDS

Functional foods, malnutrition, non-communicable diseases.

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is one of the most common metabolic disorders worldwide and its development is caused by a combination of two main factors, impaired insulin secretion by pancreatic cells and the inability of insulin-sensitive tissues to respond to insulin[1]. Globally, around 462 million people suffered from T2DM in 2017, equivalent to 6.28% (4.4 million people aged 15-49 years, 15 million people aged 50-69 years and 22 million people aged ±70 years)[2]. The prevalence of T2DM in Indonesia is estimated at 6.2% in 2019 and 10.8% in 2021, placing it in the top 10 countries with the highest prevalence of T2DM and the steepest increase[3]. According to the 2018 Basic

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Health Research (RISKESDAS) in Indonesia, 10.9% of the population aged ≥15 years suffer from DMT2[4].

Hypoglycemic agents are drugs used to lower blood glucose (sugar) levels. They are commonly used in the treatment of diabetes mellitus. Most conventional chemical antidiabetic drugs have limited benefits and are associated with many serious side effects that leave doctors and patients dissatisfied with the control of the disease, along with the high cost of treatment that has led to a growing trend towards herbal therapies[5]. Herbal plants are rich sources of bioactive compounds such as flavonoids, and antioxidants, several studies have described their efficacy and safety in managing blood glucose levels in DMT2[6].

Okra extracts, both green and purple, show antihyperglycemic potential and the ability to increase MDA levels in streptozotocin-induced diabetic rats[7]. Green Okra products can be processed and utilized in various ways such as steamed, boiled, powdered, extracts and okra soaking water[8-14]. Pudding is a type of food that is generally made from boiled or steamed ingredients and is popular with many people of all ages. Okra is a type of vegetable that is rich in fiber, vitamins, and minerals. Okra contains antioxidants that play a role in counteracting free radicals, but has a pungent taste and aroma, so an alternative to processing okra is to process it into pudding, the texture former of pudding can use agar-agar flour[15]. Pudding product research using purple okra with healthy subjects so that it can be used as a potential antioxidant functional food[16], purple okra has the potential as a functional food in increasing the antioxidant status of healthy adults with higher SOD level changes[17].

Clinical research related to green okra in the form of pudding associated with TCF4 protein regulation against T2DM has never been conducted, so the aim of this study was to determine the effectiveness of green okra pudding on the glycemic profile of adults with T2DM through TCF4 protein regulation.

METHOD

Design, Location, and Time

Interventional research using Single blind randomized controlled trial (RCT). This study was conducted in Kendari in April-November 2024. Screening was conducted in June-September 2024. Green okra pudding intervention was conducted in October-November 2024. Green Okra Pudding was distributed daily. Blood sample analysis (fasting blood glucose) was conducted at the Maxima Kendari Laboratory. TCF4 Protein analysis was conducted at the Institute of Tropical Diseases, Airlangga University. The study has passed the ethical review of the Institute for Research and Community Service, Health Research Ethics Commission, Halu Oleo University Number 1384/UN29.20.1.2/PG.2024, respondents have signed informed consent.

Population and Sample

The number of subjects was determined based on the formula of Chow et al. (2008) using a 95% confidence level. This study used a standard deviation (σ) and an expected average decrease in GDP (fasting blood sugar) (ε) of 24.35 mg/dL and 10.6 mg/dL, respectively, referring to the research of Haryati et al. (2019), so the minimum number of subjects per group was 12 people. In an effort to anticipate subjects who missed observation (drop out), the number of subjects was increased by 20% so that the total number of subjects per treatment group was 14 people. This study consisted of two groups, namely the control and green okra pudding intervention groups, so the total number of research subjects required was 28 people.

Data Collection

The green okra used uses Naila IPB variety seeds grown in Kendari. After being harvested, the okra is blanched and then stored in the freezer until ready to use. The pudding making process with modifications[16,18]. The pudding making process begins with blanching the green okra at a temperature of 90-97°C for 30 seconds. Then, some of the okra is macerated using distilled water (1:3) for 12 hours, and some is pureed using a blender. The next step is to make the pudding mixture by mixing agar powder, sorbitol, milk, and water, then the fine okra and its mucus are added to the pudding mixture. After the pudding mixture is finished cooking, the heat is turned off. After the pudding mixture is warm, it is put into cups to be served. Cooled until set, then ready to be distributed.

Green okra pudding was given as much as 2 cups/day for 6 weeks, so the total cups needed were 2352 pieces. The weight of one cup of pudding was 100 g. The pudding packaging was labeled according to the respondent's coding number. Pudding was given to the subjects every day. Compliance monitoring was carried out every day in the afternoon at 17.00 WITA.

Screening was conducted from June-September 2024. Potential respondents were measured for random blood glucose, if they met the inclusion criteria, namely ≥180 mg/dl, they were directed to fast and undergo further screening. The exclusion criteria in this study were adults with DMT2 accompanied by metabolic syndrome, pregnant, undergoing insulin therapy, experiencing complications, experiencing decreased consciousness, having had ulcers and having a history of smoking. Baseline and endline blood sample collection and testing were carried out by health workers. Subjects were fasted 12 hours before blood sampling in the morning using a vacuum blood collection system from the elbow crease vein so that blood sampling was carried out twice. A blood sample of 15 ml will be taken and placed in a tube that has been labeled with the subject's identity. If a

hematoma occurs as a risk of blood sampling, treatment can be carried out by keeping the blood sampling area clean and compressing it with cold water so that the condition can improve in a few days. Anthropometry was carried out by measuring height using a microtoise and the respondent's weight using a digital scale (GEA).

This kit was based on sandwich enzyme-linked immunesorbent assay technology. Anti TCF4 antibody was pre-coated onto the 96-well plate. The biotin conjugated anti TCF4 antibody was used as the detection antibody. The standards and pilot samples were added to the wells subsequently. After incubation, unbound conjugates were removed by wash buffer. Then, biotinylated detection antibody was added to bind with TCF4 conjugated on coated antibody. After washing off unbound conjugates, HRP-Streptavidin was added. After a third washing, TMB substrates were added to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that turned yellow after adding a stop solution. Read the O.D. absorbance at 450nm in a microplate reader. The concentration of TCF4 in the sample was calculated by drawing a standard curve. The concentration of the target substance is proportional to the OD450 value. The sealed kit can be stored at 2-8°C. This kit is available in two sizes, namely 48T and 96T, and contains various components for ELISA testing. The main components include ELISA Microplates (8x6 for 48T and 8x12 for 96T), Lyophilized Standards (1 vial for 48T and 2 vials for 96T), Biotin-labeled Antibody, and HRP-Streptavidin Conjugate with a volume of 60ul and 120ul. In addition, this kit is also equipped with TMB Substrate (5ml and 10ml), several types of buffers (such as Sample Dilution Buffer, Antibody Dilution Buffer, SABC Dilution Buffer, and Wash Buffer), Stop Solution, and Plate Sealer. For storage after opening, the remaining strips and standards should be placed in a sealed foil pouch with a desiccant. Most liquid reagents and other components should be stored at 2-8°C, while some components such as standards and microplates can be stored at -20°C for 6 months. Finally, the liquid reagent bottle may contain slightly more volume than stated, so users are advised to use the pipette accurately.

Statistical Analysis

The data processing includes coding, editing, entry, cleaning, and analysis. Data processing was carried out with the help of Microsoft Excel 2019 software and IBM Statistical Program for Social Sciences (SPSS) version 26.0. Statistical analysis independent t-test was used to analyze the effect of glycemic profile, blood pressure and TCF4 protein between treatment groups. Analysis paired t-test was used to test the difference in average consumption before and after intervention in each treatment group. Fasting blood glucose was normally distributed ($p>0.05$). This was true for both baseline and endline measurements. Both p-values were 0.498 for baseline data and 0.407 for endline data (Shapiro-wilk).

RESULTS

Characteristics of Respondents

Distribution of respondent characteristic data into two groups, namely the control group and the green okra pudding group with a difference test on variables including age, gender, nutritional status, duration of type 2 diabetes mellitus (DMT2), occupation, education, and income (Table 1). Overall, both groups (control and green okra pudding) were balanced in terms of the distribution of characteristics. This balance is important to ensure that the intervention outcome (green okra pudding) is truly attributable to the treatment given, and not to differences in baseline characteristics between the groups.

Respondent Compliance in Consuming Green Okra Pudding

The level of compliance of respondents in the treatment group (Control and Green Okra Pudding) remained good during the 6-week intervention period with most of the compliance percentages above 90%. The fluctuations that occurred in both groups were influenced by various factors such as feeling bored and not finishing the pudding. Each respondent received 2 cups of pudding without green okra (control)/green okra pudding (intervention) every day for 6 weeks. Distribution was carried out every day to each respondent.

Respondents' Energy and Nutrient Intake

The average energy and nutrient intake of the subjects can be seen in Table 3. The results of the Paired t-test showed that there was no significant difference ($p>0.05$) in the intake of the subjects before and after the intervention in each group. This indicates that there was no change in consumption in all respondents.

The intragroup p-value indicates no significant change over time within the groups. For each parameter listed, there was no statistically significant difference between the two groups (control and Green Okra Pudding).

The Effect of Giving Green Okra Pudding on Glycemic Profile, Blood Pressure and TCF4 Protein Level

The average glycemic profile, blood pressure and TCF4 protein levels based on the treatment group can be seen in Table 4. There was a decrease in fasting blood glucose, HbA1c, systolic, and diastolic, and an increase in TCF4 protein after 6 weeks of intervention in the green okra pudding group. Changes in fasting and diastolic blood glucose before and after treatment showed improvement with a significant decrease ($p<0.05$) in the green okra group. While in HbA1c and systolic TCF4 protein there was an improvement but not significant ($p>0.05$). In TCF4 protein concentration there

Table 1. Distribution of respondent characteristics

Variabel		Control group		Green Okra Pudding Group		Total	
		n	%	n	%	n	%
Sex	Male	3	10,71	1	3,57	4	14,28
	Female	11	39,28	13	46,42	24	85,72
Nutritional status	Underweight	1	3,57	1	3,57	2	7,1
	Normal	7	25	6	21,42	13	46,42
	Overweight	5	17,85	1	3,57	6	21,42
	Obesity 1	1	3,57	5	17,85	6	21,42
	Obesity 2	0	0	1	3,57	1	3,57
	Mean±SD	21,86±3,231		23,43±4,014			
Long time suffering from DM	< 1 year	3	10,71	1	3,57	4	14,28
	1-3 years	4	14,28	3	10,71	7	25
	4-5 years	0	0	5	17,85	5	17,85
	>5 years	7	25	5	17,85	12	42,85
Work	Civil Servant/Teacher	0	0	1	3,57	1	3,57
	Self-employed	3	10,71	3	10,71	6	21,42
	Housewife	9	31,14	10	35,71	19	67,85
	Farmer	2	7,14	0	0	2	7,14
Education	Senior High School	13	46,42	13	46,42	26	92,85
	Bachelor	1	3,57	1	3,57	2	7,14
Income	1-2 Million	14	50	13	46,42	27	96,42
	>2 Million	0	0	1	3,57	1	3,57
	Total					28	100

*n=number of respondents; SD=Standard deviation; DM=Type 2 diabetes mellitus; PNS=Civil Servants; SMA=Senior High School; Chi square test (age, gender, duration of DM between control group and green okra pudding group) data is not normally distributed; Oneway ANOVA (groups >2) is normally distributed. Nutritional status: Thin (BMI <18.5 kg/m2), Normal (BMI 18.5-22.9 kg/m2); Overweight (BMI 23-24.9 kg/m2); Obesity I (BMI 25-29 kg/m2); Obesity II (BMI ≥30 kg/m2) (WHO, 2000).

was an increase after treatment but not significantly. Improvement in systolic and TCF4 protein occurred in the control group after treatment but not significantly ($p>0.05$).

Fasting blood glucose levels before and after treatment, there was a decrease in fasting blood glucose levels of -6.75 ± 152.86 after consuming green okra pudding and an increase in fasting blood glucose levels of 13.83 ± 56.26 in the control group. When compared between the control group and the green okra pudding group, there was a significant decrease in fasting blood glucose levels ($p<0.05$) in the green okra pudding group. Giving boiled okra has been proven to be effective in reducing fasting blood glucose in DMT2 patients, giving green okra by boiling can significantly reduce fasting blood glucose[13].

Table 2. Respondents' Energy and Nutrient Intake

Parameters	Control Group (Mean±SD)	Intragroup p-value	Green Okra Pudding Group (Mean±SD)	Intragroup p-value	Intergroup Comparison (p-value > 0.05)
Energy (Cal)	815.77±148.42	0.819	862.90±521.69	0.348	No Significant Difference
Carbohydrate (g)	121.38±40.08	0.706	129.78±68.93	0.168	No Significant Difference
Protein (g)	27.66±6.31	0.651	31.02±14.51	0.634	No Significant Difference
Fat (g)	23.03±20.36	0.871	25.44±29.11	0.651	No Significant Difference
Fiber (g)	3.12±1.67	0.692	2.90±2	0.145	No Significant Difference
Sodium (mg)	1563.21±957.10	0.739	1765.82±1788.6	0.333	No Significant Difference
Potassium (mg)	523.4±193.03	0.787	583.69±300.20	0.765	No Significant Difference

Table 3. Effect of Treatment on Glycemic Profile, Blood Pressure, and TCF4 Protein

Parameter	Control Group	Green Okra Pudding Group	*p**
	Mean±SD	Mean±SD	
Fasting Blood Glucose			
Before (mg/dl)	184±107.76	275.58±145.21	0.040
After (mg/dl)	197.83±145.98	268.83±140.92	
Δ (mg/dl)	13.83±56.26	-6.75±152.86	
p-value	0.413	0.881	
HbA1c (%)			
Before (mg/dl)	8.26±2.76	10.11±2.17	0.223
After (mg/dl)	9.61±2.88	9.65±2.70	
Δ (mg/dl)	1.35±2.78	-0.46±2.14	
p-value	0.121	0.467	
Systolic			
Before (mmHg)	148.58±23.78	151.25±23.13	0.959
After (mmHg)	143.75±20.71	141.75±21.09	
Δ (mmHg)	-4.83±15.18	-9.5±14.24	
p-value	0.294	0.041	

Data are presented as mean ± SD; *p= Analysis of difference test using independent t-test. HbA1c= Hemoglobin A1c; TCF4= Trancription Factor 4; Δ = delta change before and after intervention.

Table 3 continuation. Effect of Treatment on Glycemic Profile, Blood Pressure, and TCF4 Protein

Parameter	Control Group	Green Okra Pudding Group	*p**
	Mean±SD	Mean±SD	
Diastolic			
Before (mmHg)	91.86±16	91.25±9.24	0.022
After (mmHg)	143.75±20.7	89.33±12.82	
Δ (mmHg)	53.50±18.38	-1.91±9.35	
p-value	0.000	0.493	
TCF4			
Before	191.27±113.36	173.22±52	0.712
After	186.57±77.09	179.65±42	
Δ	-4.69±88.54	6.42±35.45	
Change (%)	-2.45%	+3.71%	
p-value	0.871	0.580	

Data are presented as mean ± SD; *p= Analysis of difference test using independent t-test. HbA1c= Hemoglobin A1c; TCF4= Trancription Factor 4; Δ = delta change before and after intervention.

DISCUSSION

The presence of two different p-values—one significant and one insignificant—does require careful explanation to avoid misinterpretation. This indicates that the two p-values answer different statistical questions. Intragroup p-value (p=0.881), this value comes from a statistical test comparing before-and-after results in the same group, namely the Green Okra Pudding group.with a p-value of 0.881 (well above 0.05), the average decrease from 275.58 to 268.83 mg/dl is not considered significant. This means the observed change could simply be random fluctuations. Then, the Intergroup P-value (p=0.040) is derived from a statistical test comparing the differences between the two groups, namely the Green Okra Pudding group and the control group. With a p-value of 0.040 (below 0.05), it indicates a significant difference between the two groups. The control group experienced an increase (+13.83 mg/dl), while the Green Okra Pudding group experienced a decrease (-6.75 mg/dl). This trend difference (increase vs. decrease) is considered statistically significant. The crucial finding of this study was not that okra pudding caused a significant decrease in blood sugar in the participants, but rather that it significantly prevented the rise in blood sugar. This suggests that green okra pudding has an important protective or stabilizing effect that cannot be ignored.

HbA1c levels in the control group and the green okra pudding group were not significantly different. However, in the green okra pudding group, there was an improvement in HbA1c so that green okra pudding has the potential to improve HbA1c levels in T2DM. This can occur because HbA1c reflects average fasting blood glucose control for 2-3 months, so a period of 6 weeks is not long enough to show significant changes in HbA1c even though fasting blood sugar levels decrease. Thus, although green okra pudding is effective in lowering blood sugar levels, evaluation of its effects on HbA1c needs to be carried out over a longer period of time to obtain significant results[9].

Systolic blood pressure before and after treatment between the two groups did not show any significant difference changes. Although the control group and the green okra pudding group experienced a decrease. This may be due to several factors such as the amount of administration, processing method, duration of administration, and type of okra. Diastolic pressure before and after treatment between the two groups showed significant differences. There was a drastic increase in the control group, green okra pudding managed to maintain/maintain diastolic much better than the control group. The most crucial aspect of diastolic blood pressure is the stark and significant difference in results between the control group and the group given Green Okra Pudding. While the control group experienced a significant and significant increase

in their diastolic blood pressure (from 91.86 to 143.75 mg/dl, with a p-value of 0.000), the Green Okra Pudding group showed the opposite trend, a slight decrease (from 91.25 to 89.33 mg/dl). The intergroup p-value of 0.022 proves that the difference in results between the two groups is statistically significant and not due to chance. This indicates that Green Okra Pudding effectively prevented the spike in diastolic blood pressure that occurred in the control group.

The average TCF4 level decreased slightly from 191.27 to 186.57 mg/dl, which is equivalent to a decrease of 2.45%. However, the intragroup p-value of 0.871 indicates that this decrease is not statistically significant. This means that the change may be due to random fluctuations and not the effect of the absence of treatment. TCF4 levels increased slightly from 173.22 to 179.65 mg/dl, which is equivalent to an increase of 3.71%. Similar to the control group, the intragroup p-value of 0.580 indicates that this increase is not statistically significant. Although there appeared to be opposing trends between the two groups (a decrease in the control group and an increase in the Green Okra Pudding group), neither of these changes reached statistical significance.

The concentration of TCF4 protein in the control group and the green okra pudding group, before and after treatment, showed no significant difference. However, in the control group there was a change/decrease in TCF4 concentration and vice versa in the green okra pudding group. If there is an increase in TCF4 in the blood, this is thought to reflect the release of protein from tissues that are stressed or damaged, which is actually a pathological sign. This condition is thought that antioxidants are effective in reducing oxidative stress, which is one of the pathophysiological components of T2DM. However, antioxidant intervention does not directly affect TCF4 expression or function because it does not change the genetic makeup or core signaling pathways that regulate TCF4. Changes in TCF4 protein expression may not be sensitive to short-term antioxidant administration or at doses used in certain clinical studies. TCF4, on the other hand, is more related to genetic predisposition and core molecular mechanisms that regulate beta cell function and glucose metabolism. Changes in TCF4 levels or activity may be more influenced by genetic factors or other specific signaling pathways than just by the level of oxidative stress. Changes in TCF4 protein expression may not be sensitive to antioxidant intervention in the short term or at levels that can be significantly detected in certain clinical studies. Variations in the gene itself may be more dominant in determining protein levels or function than antioxidant interventions. Antioxidants do not change a person's genetic makeup. In summary, although antioxidants play an important role in reducing oxidative stress that is part of the pathophysiology of diabetes, the main mechanism of TCF4 in diabetes mellitus involves genetic regulation and complex cellular signaling pathways (Wnt pathway) that may not be directly or significantly affected by antioxidant interventions. The effects of TCF4 on diabetes are more intrinsic and genetic[19].

Diabetes mellitus is a common chronic disease that is increasingly prevalent worldwide and can cause a number of dangerous complications. The Wnt (Wingless-related integration site) signaling pathway is important for the onset and development of diabetes. Wnt3a is a typical Wnt ligand that can increase β-catenin stability, control TCF7L2 expression, promote β-cell proliferation, and reduce apoptosis[20].

Wnt signaling plays a role in regulating the biological functions of adipose tissue. The relationship between the Wnt signaling pathway and T2DM was first documented in 2006[21]. The identified genetic polymorphisms are in the gene encoding transcription factor 7-like2 (TCF7L2), which produces the TCF4 protein, an important transcription factor in the Wnt signaling pathway. TCF7L2 is one of the strongest candidate genes found to be associated with T2DM to date. The TCF4 protein is a major transcriptional effector of the Wnt/β-catenin signaling pathway, which is an important developmental pathway regulating adipogenesis[22]. Dysregulation of the Wnt signaling pathway plays a role in the occurrence and progression of T2DM, directly affecting pancreatic β-cell differentiation and proliferation, as well as insulin secretion and action[23].

STUDY LIMITATIONS

There are several limitations in this research. The sample size was limited and the intervention duration was not long enough to see changes in HbA1c. In addition, measuring respondents' consumption using the 2x24-hour recall method which relies on memory may not reflect food intake accurately.

CONCLUSION AND RECOMMENDATION

Green okra pudding significantly affected changes in fasting and diastolic blood glucose before and after treatment in the control group and the green okra pudding group ($p<0.05$), but did not affect changes in HbA1c levels but there was an improvement ($p>0.05$). In the concentration of TCF4 protein, there was an increase in the green okra pudding group and vice versa in the control group. So that green okra pudding based on the results of this study is good for controlling fasting blood glucose in DMT2. Further research is needed with a larger sample size considering variables not measured in this study, analyze the effect of green okra pudding on other parameters such as lipid profiles in adults with T2DM using local varieties.

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