Determination of the efficacy of okra seed powder in aqueous solution as a glucose lowering agent compared to acarbose in STZ diabetic rats

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Abstract

Introduction Okra is reported to have anti-diabetic effects, but the literature shows conflicting results. The experiment aimed to determine the efficacy of three doses of okra seed powder suspension as a glucose lowering agent on streptozotocin-induced diabetic rats and its cellular effects on the liver and pancreas.

Methods Twenty-five Sprague Dawley rats that were given streptozotocin 60 mg/kg intraperitoneally were randomly allocated to one of five treatment groups: okra seed powder at 100 mg/kg, 150 mg/kg and 200 mg/kg, acarbose (positive control) and vehicle only (negative control). The treatments were given as a 1.5 mL oral gavage daily for 21 days. Significant differences in blood glucose were determined between treatment groups in terms of relative change from baseline, using One-Way ANOVA with Dunnett's method with acarbose as the referent group. Repeated measures ANOVA was used to analyze the blood glucose levels across the time point collections (baseline, T1 and T2). Histopathologic changes on the liver and pancreas were described using counts and proportions.

Results Mean blood glucose values increased from baseline to T2 in all treatment groups. Increasing trend was observed only up to T1 in the 150 mg/kg and the 200 mg/kg okra seed treatment groups. Comparing okra treatment groups to acarbose, the percentage increase of mean blood glucose from baseline to T2 was lowest in the 200 mg/kg okra group ($p = 0.040$). The okra-treated rats had no fatty change and a dose-dependent decrease in cellular degeneration in the liver and none for the 200 mg/kg treatment group.

Conclusion The 200 mg/kg okra suspension has a potential lowering effect on blood glucose and a hepatoprotective effect. A longer period of observation with higher doses of okra suspension is recommended to study these effects further.

Key words: Okra, diabetes mellitus, STZ-diabetic rat

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Diabetes mellitus is a condition diagnosed based on either fasting blood glucose levels greater than 125 mg/dL, a random blood glucose level greater than 200 mg/dl, or a glycated hemoglobin glucose level greater than 6.5%.[1] Type I diabetes is caused by total absence of insulin secretion by the beta cells of the pancreas, while Type II is due to a lack of response to insulin or insulin resistance. The rising prevalence of this disease globally, the continued increase of its prevalence in the Philippines, and that diabetes and its complications rank 9th among the leading causes of premature death among Filipinos – despite the availability of free medicines – underscore the importance of treating the disease.[2,3] Reduction of premature death due to non-communicable diseases such as diabetes is included as one of the targets under Sustainable Development

Goal #3 by all member nations of the WHO, including the Philippines.[4]

Diabetes increases the risk of hypertension, ischemic heart disease, stroke and also causes end stage renal disease. These many complications have made the management of diabetes very expensive. The financial burden of the disease is reported to have increased by more than 100% from 2007 to 2017 in the USA.[5] In the Philippines, this is USD 205 million and for Filipino patients, more than 85% of this cost is an out-of-pocket expense.[6,7] The rising cost of pharmacologic treatments for diabetes and the compounded adverse effects of taking multiple medicines emphasize the need to search for possible alternative or complementary medicines.[8]

Many plants, such as bitter melon, onion, cinnamon, and neem have been reported to have a glucose lowering effect involving several mechanisms.[9-12] Okra, also known as "lady's finger", has also been reported by several studies to have promising glucose-lowering activity: the seed, seed coat and whole fruit have been reported to lower blood glucose in animal studies.[13-16] Thanakosai and Phuwapraisirisan were first to report that the okra seeds contain chemicals that inhibit alpha glucosidase, and thus inhibit absorption of simple sugars through the intestinal wall.[17] Sabitha reported that okra seed powder at 100 mg/kg and 200 mg/kg were effective in lowering blood glucose of streptozotocin (STZ) diabetic Wistar rats, and even a dose of 2000 mg/kg did not result in toxic effects.[14] These studies reported on the glucose lowering effect of okra but none compared okra with a standard glucose lowering agent with a similar mechanism of action.[13-16]

In the local literature, the studies of Almeda (2010), Alingod (2015), and Magbitang and Samaniego (2016) involved human subjects, but these reported conflicting results on the effect of okra on diabetes.[18-20] The evidence from the human clinical trials regarding the use of okra as a blood glucose lowering agent is inconclusive because the studies either did not use the same part of the okra fruit, did not use comparable doses of okra based on the animal studies, or did not compare the okra with a drug with a similar mechanism of action. Since no insulin levels were reported in the study of Magbitang and Samaniego, where the subjects involved were healthy people with normal insulin secretion, the absence of an effect by okra on blood glucose levels could not be definitely established.[20] For this reason, it would be necessary to do more animal studies to address these issues.

This research compared the effect of okra seed powder to a drug with a similar mechanism of action when the effect of insulin is controlled or removed by the action of streptozotocin in order to determine if the effects are comparable. The effect of three different concentrations of okra seed powder were compared to the effect of a standard dose of acarbose (positive control) on the blood glucose levels of rats as compared to a group that received only the vehicle solution (negative control). The differences in the mean change of the blood glucose levels of these groups were also compared. The possible adverse effects of the treatments on the liver and pancreas were also determined by identifying the histopathologic changes in these organs and comparing them between groups.

Methods

The proposal for this experiment was approved by the Manila Central University-Institutional Animal Care Use Committee (MCU-IACUC) prior to submission for funding and the necessary permit for its implementation was obtained from the Bureau of Animal Industry.

This research used an experimental design to determine the effects of the independent variable (treatments: okra seed powder at three concentrations, acarbose, and vehicle) on the dependent variable blood glucose expressed as a mean blood glucose change between and within treatment groups at baseline and post treatment at Day 12 (Time 1) and Day 22 (Time 2), and the histopathologic changes in liver and pancreas after a 21-day treatment period as shown in Figure 1.

Following a two-week quarantine period as per IACUC protocol during which time the in-house veterinarian regularly checked for signs of potential illness prior to the experiment (hair coat, nasal passages and eyes), 25 male 8- to 10-week-old Sprague Dawley rats were obtained from the Research and Biotechnology Group of the St. Luke's Medical Center. They were rendered diabetic with a single intraperitoneal injection of streptozotocin (STZ) at 60 mg/kg which selectively eliminated beta cells (Figure 2).[14,21,22] Hyperglycemia was confirmed (FBG > 200 mg/dL) 24 hours after STZ injection by checking the blood glucose levels using the tail vein sampling method.

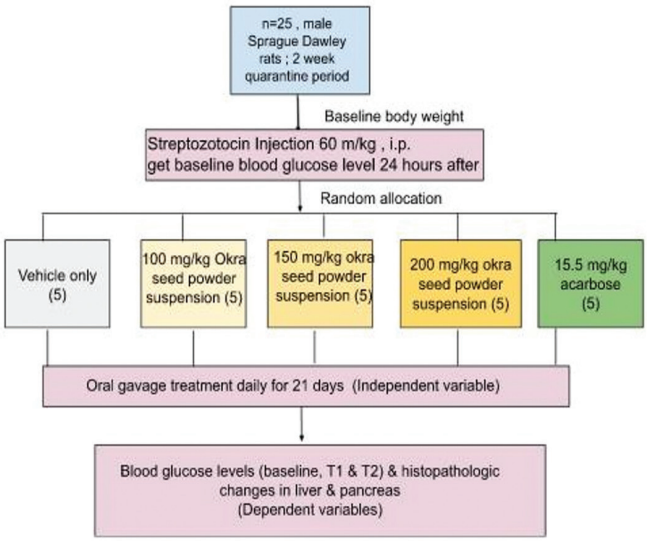

Figure 1. Research protocol

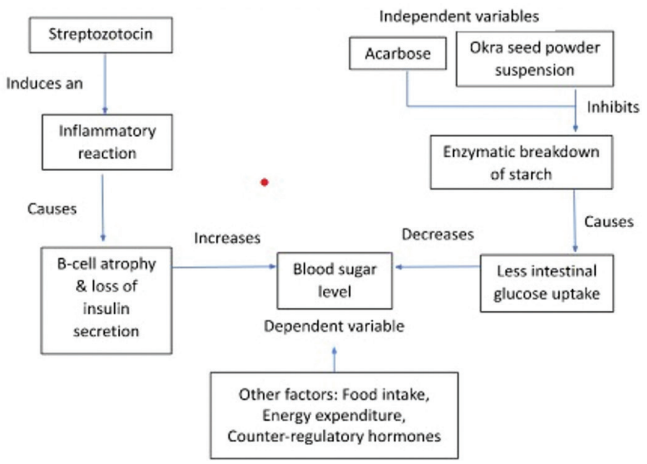

Figure 2. Factors which affect blood glucose

The STZ-diabetic rats were allocated to one of five treatment groups by simple randomization: okra seed powder at three different concentrations (100 mg/kg; 150 mg/kg and 200 mg/kg), negative control (vehicle only), and positive control with acarbose 15.5 mg/kg.[23,24] Each rat was given an oral gavage (maximum volume of 1.5 mL) of the assigned treatment daily for 21 days by a lab technician who was blinded to the treatment group of each rat. The gavage procedure followed the technique described in the Johns Hopkins Animal Care Committee for procedures specific for the rat.[25] The technician who prepared these suspensions was also blinded to the composition of these treatments. Blood glucose was measured using a BTS 350 semi-automatic analyzer (BioSystems SA) with 1 mL of glucose reagent added to 10 uL of serum sample obtained from blood drawn either by tail vein venipuncture at baseline (day 0) and at Day 12 of treatment (T1); and obtained by cardiac puncture on the day of sacrifice, Day 22 (1 day after the 21-day treatment period, T2). Histologic changes in the liver and pancreas at necropsy on Day 22 were assessed qualitatively by a veterinarian pathologist who was blinded to the treatments used on the rats. The research protocol is illustrated in Figure 1.

The rats were housed under standard conditions. Food was standard rat chow, and food and water were available ad libitum. Cages and water bottles were checked daily; food was replenished daily; distilled water was given through water bottles that were also changed daily, and replenished as needed. As per protocol, room temperature was regulated at 20-26ºC; with appropriate ventilation (exhaust fan provided to allow one way flow of air and ensure adequate air exchange and humidity control); light was turned on 12 hours during the day and turned off at night.

All treatment protocols used in this experiment conformed with published and accepted protocols and were accordingly approved by the IACUC. STZ was dissolved in a cold citrate buffer and given intraperitoneally at a dose of 60 mg/kg on Day 0.[22] Okra fruits were purchased from a single online market based in Quezon City, and identified by the Bureau of Plant Industry. The fruits were processed by the Industrial Technology Development Institute of the Department of Science and Technology as follows: fresh seeds were separated from the okra fruit, then oven dried for 16 hours at 60ºC until brittle, then pulverized using a Wiley mill. This process yielded 135 g of seed powder from 10 kg of okra fruit. The following stock solutions were prepared fresh daily by dispersing okra seed powder in 0.2% carboxymethylcellulose: 32.25 mg/mL for the 100 mg/kg group, 48.375 mg/mL for the 150 mg/kg group, and 64.5 mg/mL for the 200 mg/mL group. An appropriate amount (not exceeding 1.5 mL) was withdrawn from the stock solutions daily and gavaged to the rats in the okra treatment groups. The acarbose tablets were ground to a fine powder and were weighed out and dispersed in 0.2% carboxymethylcellulose solution in order to yield a solution containing 5 mg/mL of acarbose. A dose of 15.5 mg/kg, computed to be equivalent to the standard adult human dose, was

given by oral gavage to the acarbose treatment group. The gavage vehicle was a 0.2% carboxymethylcellulose solution used to suspend the okra powder or acarbose powder to be given as an oral gavage. It served as the negative control.

The body weights were determined using an electronic balance. Each rat was weighed at baseline prior to the start of the treatments and every other day during the treatment period; and on the day of euthanasia. Body weights were taken because the oral gavage treatments were based on body weight. Blood samples were obtained to check for blood glucose values at baseline and at T1 of treatment, using the tail vein. Using an immobilization chamber, the tail was exposed and warmed in order to make the lateral tail veins more visible. Under aseptic technique, a sterile scalpel was used to nick the lateral tail vein, and an appropriate volume of blood, not more than 0.5% of the rat's body weight in grams, was collected.[28] The blood was centrifuged to separate the serum. Collection of blood at the day of euthanasia was through cardiac puncture.[29] Blood glucose levels were measured using a BTS 350 semi-automatic analyzer (BioSystems SA).

The rats were sacrificed by CO_2 gas administration in a CO_2 chamber following the NIH Guidelines for euthanasia of rats using carbon dioxide on Day 22 or 1 day after the completion of the treatment period under study.[29] The rats were placed in a CO_2 chamber with flow rate of 7 L/min until the rat stopped breathing and the pupils were dilated. The rat was removed from the chamber after waiting 1-3 minutes following cessation of respiration. Immediately after the rat was euthanized, the blood was collected by cardiac puncture as per NIH guidelines and the liver and pancreas were dissected out, weighed and placed in neutral buffered 10% formalin solution and were subsequently prepared for hematoxylin and eosin staining.[29] Carcass disposal was done in accordance with the approved IACUC protocol of the laboratory. Data was analyzed using SPSS version 23 software. Significant differences in blood glucose were determined between treatment groups in terms of relative change from baseline, using one-way ANOVA with Dunnett's method with acarbose as the referent group. Repeated measures ANOVA was used to analyze the blood glucose levels across the time point collections (baseline, T1 and T2) and $p < 0.05$ was used to indicate statistical significance. Histopathologic changes on the liver and pancreas were described using counts and proportions.

Results

Five rats in each of the five treatment groups completed the experiment. As shown in Table 1, the baseline weights ($p = 0.822$) and blood glucose of the five treatment groups were comparable. The differences in mean blood glucose values between time periods were statistically significant in all treatment groups as shown in Table 2. There was an increasing trend observed in the mean blood glucose values from baseline to Time 2 in the vehicle-only, acarbose, and 100 mg/kg okra seed powder groups. Conversely, this increasing trend was observed up to Time 1 only in the 150 mg/kg and 200 mg/kg okra seed groups, with decreasing levels thereafter. The relative change in the mean glucose values was largest for the acarbose group (46.4% ± 20.5) and least for the 200 mg/kg okra seed group (12.7% ± 9.4) but the differences in this relative change among treatment groups was not significant ($p = 0.087$), as shown in Table 2. The smaller relative change in the 150 and 200 mg/kg okra seed powder suspension groups were reflective of the observed decrease in mean blood glucose values at Time 2. Hence, a potential lowering effect in blood glucose values can be noted in these two doses of okra seed powder suspension. In contrast, the large relative change was reflective of the observed increase in the mean blood glucose values at Time 2 for the acarbose and 100 mg/kg okra seed groups. This was because blood glucose values remained elevated up to the end of the observation period even with treatment (Table 2). However, as shown in the last column of Table 3, using acarbose as the referent group for comparing the relative change from baseline for each of the treatment groups, there was a significant difference was found between acarbose and the 200 mg/kg okra seed powder suspension ($p = 0.040$). This may imply a potential lowering effect of blood glucose values at 200 mg/kg dose of okra seed powder suspension.

There were no significant differences in mean body weights between treatment groups at baseline ($p = 0.822$), Time 1 ($p = 0.215$), and Time 2 ($p = 0.253$). Through time there was a decreasing trend in the mean body weights in all treatment groups except between baseline and Time 1 in the

Table 1. Comparison of baseline weights and blood glucose of the five treatment groups (mean ± SD).

	Vehicle only	Acarbose 15.5 mg/kg	Okra 100 mg/kg	Okra 150 mg/kg	Okra 200 mg/kg
Body weight (g)	274.6 ± 62.0	299.2 ± 76.8	308.3 ± 30.3	290.0 ± 34.2	311.2 ± 42.2
Blood glucose (mg/dL)	352.2 ± 69.5	350.2 ± 71.3	343.4 ± 73.1	368.2 ± 24.9	405.0 ± 45.9

Table 2. Comparison of mean blood glucose values from baseline to end of study period.

	Baseline	Time 1	Time 2	p-value[1]
Vehicle only	352.2 ± 69.5	428.0 ± 118.1	471.6 ± 107.5	0.025
Acarbose	350.2 ± 71.3	438.8 ± 89.4	502.8 ± 64.4	0.002
Okra seed powder suspension				
100 mg/kg	343.4 ± 73.1	384.4 ± 129.3	473.0 ± 68.0	0.018
150 mg/kg	368.2 ± 24.9	514.4 ± 51.7	464.0 ± 34.5	0.001
200 mg/kg	405.0 ± 45.9	490.8 ± 37.3	455.3 ± 53.1	0.015

[1]Repeated measures ANOVA

Table 3. Comparison of relative change from baseline in mean blood glucose between treatment groups.

Treatment groups	Relative change from baseline (mean % ± SD)	Relative change from baseline between treatment groups and acarbose§
Vehicle only	33.9 ± 15.1	0.657
Acarbose 15.5 mg/kg	46.4 ± 20.5	--
Okra seed powder suspension		
100 mg/kg	41.7 ± 28.2	0.978
150 mg/kg	26.2 ± 8.6	0.263
200 mg/kg	12.7 ± 9.4	0.040
p-value*	0.087	

*One-Way ANOVA; §Dunnett's test

vehicle-only (negative control) group and between Time 1 and Time 2 in the 100 mg/kg group where an increase was noted. The differences through time were significant in the 150 mg/kg (p = 0.030) and 200 mg/kg (p = 0.012) okra seed groups as shown in Table 4.

There were no significant differences in the means of the percent to body weights ratios of the liver and pancreas across the treatment groups (Table 5). Histopathological examination of the liver specimens revealed absence of hemorrhage, inflammation, and necrosis in all groups. Mild to moderate cell swelling with few to diffuse granular degeneration was noted in 80% of the animals in the negative control group, 40% in the positive control group, and 75%, 20%, and 0% in the 100, 150, and 200 mg/kg okra seed groups, respectively. Mild fatty change was observed in one animal in the negative control group and moderate fatty change was observed in one animal in the positive control group. No fatty changes were observed in any of the okra treatment groups as seen in Table 6.

Figure 3 shows a representative photomicrograph of severe atrophy of the Islet of Langerhans observed in 40% of the rats in the acarbose group. The rest of the 60% in this group showed very slight to slight

Table 4. Comparison of mean body weights between treatment groups (mean ± SD).

Treatment groups	Baseline Mean ± SD	Time 1 Mean ± SD	Time 2 Mean ± SD	p-value±
Vehicle (negative control)	274.6 ± 62.0	307.3 ± 24.8	306.0 ± 28.8	0.428
Acarbose (positive control)	299.2 ± 76.8	290.0 ± 64.4	278.1 ± 49.9	0.169
okra seed powder suspension				
100 mg/kg	308.3 ± 30.3	259.8 ± 48.1	262.9 ± 51.9	0.428
150 mg/kg	290.0 ± 34.2	251.3 ± 17.6	248.1 ± 13.2	0.030
200 mg/kg	311.2 ± 42.2	277.5 ± 27.6	263.8, 50.1	0.012
p-value*	0.822	0.215	0.253	

*One-Way ANOVA
±Repeated measures ANOVA

Table 5. Average body weight (BW), liver and pancreas gross weights.

Treatment Group	Body weight (g)	Liver		Pancreas	
		Gross weight (g)	% to BW ratio	Gross weight (g)	% to BW ratio
Vehicle (Negative control)	306.0	10.52	3.45	0.97	0.32
Acarbose (Positive control)	272.8	9.92	3.63	0.62	0.23
100 mg/kg	283.4	9.87	3.50	0.69	0.24
150 mg/kg	248.1	9.36	3.78	0.78	0.31
200 mg/kg	275.6	9.91	3.59	0.70	0.24
p-value *			0.265		0.659

*One-Way ANOVA

Table 6. Histopathologic findings in the liver of all treatment groups.

Liver No. (%)	Acarbose	Vehicle only	100 mg/kg	150 mg/kg	200 mg/kg	Overall
Hemorrhage						
none	5	5	4	5	4	23 (100)
Cell swelling/granular degeneration						
none	3	1	1	4	3	12 (52)
mild to mild, diffuse	2	2	2	1	1	8 (35)
moderate, diffuse		2	1			3 (13)
Fatty change						
none	3	4	4	5	4	20 (87)
mild, few		1				1 (4)
moderate, diffuse	1					1 (4)
Hepatic necrosis						
none	5	5	4	5	4	23 (100)
Inflammation						
none	5	5	4	5	3	22 (96)

atrophy. Very slight to slight atrophy was also observed in 60%, 75%, 80% and 75% of the negative control, 100 mg/kg, 150 mg/kg, and 200 mg/kg okra seed groups, respectively. The presence of atrophy in the islets of Langerhans is characteristic of the effect of streptozotocin.[30] No degeneration, necrosis and beta cell vacuolization were observed across all specimens in all groups as seen in Table 7.

Discussion

The mean blood glucose values from baseline to Time 2 increased in all treatment groups. However, the increasing trend was observed only up to 12 days of treatment for the 150 mg/kg and the 200 mg/kg treatment groups. Comparing okra treatment groups to acarbose, the percentage increase of mean blood glucose from baseline to T2 was significantly lowest in the 200 mg/kg okra suspension group ($p = 0.040$). There was absence of fatty change and reduced cell swelling and granular degeneration in the liver of rats treated with okra suspension in this study as compared to positive and negative control groups indicating a possible hepatoprotective effect of okra. Compared to positive and negative control groups, okra-treated rats had lesser cellular swelling and degeneration in the hepatocytes, and this was observed to be a dose dependent effect as no liver cellular swelling and degeneration was observed in the histological sections of the livers of the rats treated with 200 mg/kg.

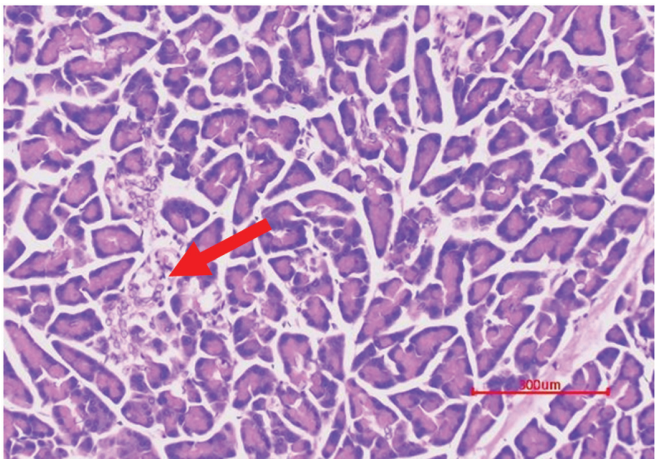

Figure 3. Severe atrophy of the Islet of Langerhans.

Continuous rise in the mean blood glucose levels over the treatment period is evidence that the STZ-treated rats had little ability to lower their blood glucose. This is correlated with the histologic data where low quantity of the islets of Langerhans was observed in 96% (20% had none to almost none and 76% had very few to few Islets), and 64% of the rats had very slight to minimal atrophy while 12% had moderate to severe atrophy. The observed atrophy of islets cells as shown in Figure 3, particularly of the beta cells is a consequence of the STZ treatment.[30] Junod showed a dose-dependent decline in insulin secretion resulting from STZ injection over a period of time, and that 65 mg/kg STZ injection resulted in a significant decline of insulin secretion and resultant

Table 7. Histopathologic findings in the pancreas of all treatment groups.

Pancreas No.(%)	Acarbose	Vehicle only	100 mg/kg	150 mg/kg	200 mg/kg	Overall
Islets of Langerhans						
Atrophy						
none		2	1	1	1	5 (22)
very slight to slight/minimal	3	3	2	4	3	15 (65)
moderate			1			1 (4)
severe	2					2 (9)
Degeneration						
none	5	5	4	5	4	23 (100)
Necrosis						
none	5	5	4	5	4	23 (100)
Beta cell vacuolization						
none	5	5	4	5	4	23 (100)

hyperglycemia in 24 hours.[31] The blood glucose values of all the rats at baseline in this study were consistent with the report of Junod and other researchers who have used STZ to eliminate beta cell function in the study animal.[22,31] The mean blood glucose levels of all groups at baseline were greater than 150 mg/dL and was therefore considered diabetic.[32]

In this study, the histologic examination documented atrophy of the pancreatic islets in the majority of the rats (76%), consistent with the report of Honjo.[30] Given this finding, it is therefore expected that blood glucose in the negative control would have increased from baseline to T1 and T2.

An increasing trend in the mean blood glucose was seen in all the treatment groups, including those that were given an oral hypoglycemic agent acarbose and the okra treatment groups in the three different concentrations. However, the increment in the mean blood glucose values was not as high for the 150 mg/kg and 200 mg/kg okra seed powder groups in contrast to the 100 mg/kg okra seed powder, the negative control and acarbose groups. Therefore, this result shows a potential blood glucose-lowering effect in these two doses of okra seed powder suspension. Post hoc analysis showed that the lower rise in blood glucose at Time 2 for the group treated with 200 mg/kg okra seed powder is statistically significant. The results of the current study further strengthen the findings of local and foreign studies that okra had a glucose lowering effect in human subjects.[13-16,18,19]

Despite the similarity in the findings of the current study with those of Sabitha, there were also differences.[14] In contrast to the study of Sabitha where the effect of 100 mg/kg and 200 mg/kg okra seed powder was evident in the first week of treatment, in the current study, the effect was only evident and statistically significant after three weeks of treatment.[14] This was seen in the percentage change (elevation in glucose) in Time 2 was also statistically significantly lesser compared to the positive and negative control groups. Although the protocol of Sabitha was very similar to the current study, there were slight differences: 1) the study animals were male Wistar rats in the Sabitha study, and Sprague Dawley rats in the current study, and 2) the okra used could be a different variety and possibly could have been planted in different types of soil, such that the active ingredient in the okra seed powder may actually have a different concentration even if the solutions were similar in concentration (200 mg/kg).[14] Gemede reported that okra contains many flavonoids and antioxidants while Thanakosai and Phuwapraisirisan, in a separate study, reported that the okra seed and peel contain – among the many flavonoids – two flavonol glycosides that have the ability to inhibit glucosidases in the rat intestinal epithelium and thus prevent the absorption of simple sugars.[15,17] Based on the analysis of the okra seed powder done by the Industrial Technology Institute, the okra seed powder used in the current study contained flavonoids. However, the amount was not quantified and chemical composition of these flavonoids was not also identified.

In addition, Sabitha reported that the effect of okra seed powder at 100 mg/kg and 200 mg/kg were similar to the effect of glibenclamide at 5 mg/kg.[14] Glibenclamide is a second-generation sulfonylurea oral hypoglycemic agent used to treat Type II diabetes (non-insulin dependent) because its mechanism of action is to increase insulin secretion by acting on the ATP-sensitive potassium channel receptors.[33] In the current study, as evidenced by the histopathologic examination, absence of islet cells and atrophy of remaining islet cells would have meant a limited capacity to secrete insulin in the study animal that was treated with STZ. Elimination of the insulin secretion capacity as a factor in the experiment would be crucial as insulin secretion is known to lower the blood glucose as it is the main glucose lowering hormone in the body. Thus, the logical comparator to the okra seed powder suspension would be acarbose, an alpha glucosidase inhibitor used as an oral hypoglycemic agent.[26] It is also appropriate to test the effects on an animal model where the insulin secretion capacity of the beta cells is greatly impaired by STZ.[22,30,31] The dose that was used in this experiment resulted in a high degree of islet cell atrophy for almost all of the study rats. Since the results of the current study show that okra seed powder at 200 mg/kg, after three weeks of treatment was able to prevent the high increment of the blood glucose at T2 this is evidence that at this concentration, the absorption of simple sugars was likely to have been limited by the action of the active ingredient in the okra seed powder. Thus, the blood glucose did not increase as much as it did from baseline to T1, in an animal model that had none to very little insulin secretion due to the Islet cell atrophy. This effect was not demonstrated in the acarbose solution given at a dose computed based on the human dose of the drug.[24,26] Therefore, the result points to the possible potential usefulness of okra seed powder suspension at

the 200 mg/kg dose for patients with Type I diabetes as a complementary herbal treatment.

Okra is reported to have hepatoprotective effects, however very few studies have described the mechanism. The absence of fatty change and reduced incidence of cell swelling and granular degeneration in the okra treatment groups compared to the acarbose and vehicle only groups may be attributed to this hepatoprotective effect. In a study by Alqasoumi where liver toxicity was induced in rats through administration of carbon tetrachloride (CCl_4). He demonstrated that rats treated with CCl_4 and okra (250 mg and 500 mg/kg) had minimal inflammation while those treated only with CCl_4 had severe necrosis and inflammation.[34] He further discussed that the possible mechanisms of okra extract to protect liver toxicity produced by CCl_4 in rats might be due to following effects: 1) prevention of lipid peroxidation; 2) hepatocyte membrane stabilization; 3) abolition or inhibition of the cytochrome P450-dependent oxygenase activity and 4) enhancement of non-protein sulfhydryls (NP-SH) and total proteins (TP) concentration in liver tissue possibly due to its antioxidative potential.[34] Wahyuningsih reported that a methanolic extract of okra seed pods at doses of 50 to 100 mg/kg BW given to mice for 19 days following treatment with sodium nitrite by gavage showed hepatoprotective effects of okra demonstrated as a reduced proportion of swollen cells, necrotic cells, number of inflammatory cells, and also reduced levels of ALT and AST in the serum.[35] Although the analysis in the current study was somewhat different, the histologic findings of the liver sections of the rats treated with okra showed no significant cell swelling/granular degeneration in a greater proportion of the rats in the 150 mg/kg and 200 mg/kg groups compared to the acarbose and the vehicle only groups. This may be interpreted also as the presence of hepatoprotective effect. However, the current study did not measure liver function (ALT and AST levels).

The results indicate that the 150 mg/kg and 200 mg/kg okra powder solutions resulted in a lower elevation of glucose after three weeks of treatment as compared to acarbose or vehicle only. In addition, the same concentrations of okra also did not produce any adverse histological changes in the liver. The histological changes seen in the pancreas were expected changes due to the effect of STZ causing eventual death and atrophy of islet cells, especially beta cells as these changes were also seen in the negative control that did not receive any treatment other than the STZ.[36] And because similar atrophy of islet cells was seen in the okra treatment and in the acarbose treatment groups, it can also be inferred that the action of okra or acarbose did not change the effect of STZ on the pancreas.

At the end of a 21-day treatment period, the mean blood glucose levels were significantly higher for all treatment groups. However, there was a lower increase in mean blood glucose levels in the 150 and 200 mg/kg okra seed treatment groups that was significantly much less compared to acarbose and vehicle only groups and was statistically significant in the 200 mg/kg group. There was no histologic evidence of hepatocellular damage for all okra treatment groups and a dose-dependent decrease in cellular degeneration most evident in the 200 mg/kg group indicating a hepatoprotective effect of okra seed powder. Thus, 200 mg/kg okra suspension has a potential blood glucose lowering effect and a hepatoprotective effect.

It is recommended that an analysis of the specific concentration of known flavonoids found in okra seed powder be made using different sources of the okra fruit from different parts of the country in order to identify which areas yield the okra seed powder with highest concentration of these compounds. Moreover, the period of observation be lengthened to see if 200 mg/kg okra suspension can produce a lowering of blood glucose, but using a lower dose of STZ to prevent early demise of the STZ diabetic rats. In addition, since higher doses of okra had a hepatoprotective effect, it is recommended to study the effect of higher doses of okra suspension to see if higher doses may produce a glucose lowering effect that may be seen at an earlier time point. Lastly, studies of the efficacy of okra seed powder at least at 200 mg/kg concentration or higher, may be used in an animal model of Type II diabetes mellitus. It may also be used for initial clinical trials for Type I diabetic patients, as it was shown to produce an effect on glucose absorption at the 200 mg/kg concentration of okra seed powder, without any adverse effects on the liver.

References

1. American Diabetes Association. Classification and Diagnosis of Diabetes: Standards of Medical Care 2021. Diabetes Care [Internet]. 2021; 44(Suppl. 1): S15–S33. Available from: https://doi.org/10.2337/dc21-S00

2. World Health Organization. Global Report on Diabetes [Internet]. 2016. Retrieved on June 20, 2018. Available from: apps.who.int/iris/bitstream/10665/204871/1/9789241565257eng.pdf
3. Philippine Statistics Authority. Deaths in the Philippines, 2016 [internet]. Philippine Statistics Authority. Retrieved from: https://psa.gov.ph › People › Vital Statistics on July 20, 2018.
4. World Health Organization. Transforming our World: The 2030 Agenda for Sustainable Development. World Health Organization [Internet]. 2015. Retrieved on June 8, 2022. Available from: https://sdgsun.org/publications/transforming-our-world-2030-agenda-sustainable-development-17981
5. Riddle MC, Herman WH. The cost of diabetes care — an elephant in the room. Diabetes Care [Internet]. 2018 May; 41(5): 929-32. Retrieved from: https://doi.org/10.2337/dci18-0012
6. Paz-Pacheco E, Jimeno C. Diabetes care in the Philippines. Journal of the ASEAN Federation of Endocrine Societies [Internet]. 2015 Nov; accessed: 29 July 2018; 30(2): 118. Available from: <http://asean-endocrinejournal.org/index.php/JAFES/article/view/267/667.
7. Tan GH. Diabetes care in the Philippines. Ann Global Health [Internet]. 2016; 81(6): 863–9. doi: http://doi.org/10.1016/j.aogh.2015.10.004
8. Zhang L, Chen Q, Li L, et al. Alpha-glucosidase inhibitors and hepatotoxicity in Type 2 diabetes: A systematic review and meta-analysis. Sci Rep [Internet]. 2016 Sep 6; 6: 32649. doi: 10.1038/srep32649
9. Chuang CY, Hsu C, Chao CY, Wein YS, Kuo YH, Huang CJ. Fractionation and identification of 9c, 11t, 13t-conjugated linolenic acid as an activator of PPARalpha in bitter gourd (Momordica charantia L.). J Biomed Sci [Internet]. 2006 Nov; 13(6): 763-72. doi: 10.1007/s11373-006-9109-3
10. Sasa M, Komoda T, Awata T, Katayama S. Activating effect of momordin, extract of bitter melon (Momordica charantia L.), on the promoter of human PPARdelta. J Atheroscler Thromb [Internet]. 2009; 16(6): 888-92. doi: 10.5551/jat.2790
11. Ülger TG, Çakiroglu FP. The effects of onion (Allium cepa L.) dried by different heat treatments on plasma lipid profile and fasting blood glucose level in diabetic rats. Avicenna J Phytomed [Internet]. 2020 Jul-Aug; 10(4): 325-33.
12. Nazarian-Samani, Z, Sewell RDE, Lorigooini Z, et al. Medicinal plants with multiple effects on diabetes mellitus and its complications: A systematic review. Curr Diab Rep [Internet]. 2018; 18: 72. Available from: https://doi.org/10.1007/s11892-018-1042-0
13. Bontilao ED, Digman KM, Fernandez EL, et al. The effect of 100% Abelmoschus esculentus Linn. (okra) fruit extract on alloxan-induced hyperglycemic male Mus musculus (mice). 2007. Thesis. [Abstract] Retrieved from Herdin Record #: R07-CDU-13071713492197
14. Sabitha V, Ramachandran S, Naveen KR, Panneerselvam K. Antidiabetic and antihyperlipidemic potential of Abelmoschus esculentus (L.) Moench in streptozotocin-induced diabetic rats. J Pharm Bioallied Sci [Internet]. 2011 Jul-Sep; 3(3): 397–402. doi: 10.4103/0975-7406.84447
15. Gemede HF, Ratta N, Haki GD, Woldegiorgis AZ, Beyene F. Nutritional quality and health benefits of okra (Abelmoschus esculentus): A review. J Food Process Technol [Internet]. 2015; 6: 458. doi:10.4172/2157-7110.100045
16. Shahreza, FD. Hibiscus esculentus and diabetes mellitus. J Nephropharmacol [Internet]. 2016; 5(2): 104–5. Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5297561/
17. Thanakosai W, Phuwapraisirisan P. First identification of α-glucosidase inhibitors from okra (Abelmoschus esculentus) seeds. Nat Prod Commun [Internet]. 2013 Aug; 8(8): 1085-8.
18. Almeda CND, Aludo RGC, Azcona JMA, et al. A comparative study on the effectiveness of Momordica charantia (ampalaya) leaves decoction versus pounded Hibiscus esculentus (okra) seed tea in lowering capillary blood glucose levels among selected Type II diabetic patients in Cebu City. 2010. Thesis. [Abstract] Retrieved from Herdin Record #: R07-CDU-13020115022716
19. Alingod A, Allam JC, Gordon K, Macatuggal JP, Ramos J, Vergara J. Slipping through diabetes: Unshrouding the hypoglycemic capabilities of okra (Abelmoschus esculentus). Thesis. St. Louis University School of Health Sciences Bulletin vol 1. June 2012-March 2015. [Abstract] Retrieved from Herdin Record #: R02-USL-16052613365962
20. Magbitang MA, Samaniego IM. Randomized open-label study comparing the effect of Abelmoschus esculentus water vs placebo in postprandial blood glucose on normal human subjects. The Filipino Family Physician. 2016 Oct-Dec; 54(4): 151-9. [Abstract] retrieved from Herdin Record #: PCHRD17020608395439
21. Arifin WN, Zahiruddin WM. Sample size calculation in animal studies using resource equation approach. Malays J Med Sci [Internet]. 2017 Oct; 24(5): 101-5. doi: 10.21315/mjms2017.24.5.11
22. Furman BL. Streptozotocin-induced diabetic models in mice and rats. Current Protocols [Internet]. 2021; 1: e78. doi: 10.1002/cpz1.78
23. Johnson PD, Besselsen DG. Practical aspects of experimental design in animal research. ILAR Journal [Internet]. 2022; 34(4): 202-6. Retrieved from https://doi.org/10.1093/ilar.43.4.202
24. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm [Internet]. 2016 Mar; 7(2): 27-31. doi: 10.4103/0976-0105.177703
25. Johns Hopkins University Animal Care and Use Committee. Policies and guidelines. Available from: http://web.jhu.edu/animalcare/procedures/rat.html#anesthesia
26. Acarbose (Glucobay Monograph). Retrieved from: https://www.bayer.ca/omr/online/glucobay-pm-en.pdf

27. Drugs.com (n.d.). Acarbose [Drug Information]. https://www.drugs.com/monograph/acarbose.html
28. University of California - Davis. IACUC Guide 31. Blood collection: Volumes, frequencies and sites. [Internet]. 2019. Retrieved from https://research.ucdavis.edu/wp-content/uploads/IACUC-31.pdf
29. National Institutes of Health - Office of Animal Care Use. Guidelines for Euthanasia of Rodents Using Carbon Dioxide [Internet]. 2019 Retrieved from https://oacu.oir.nih.gov/system/files/media/file/2021-06/b5_euthanasia_of_rodents_using_carbon_dioxide.pdf
30. Honjo K, Doi K, Doi C, Mitsuoka T. Histopathology of streptozotocin-induced diabetic DBA/2N and CD-1 mice. Lab Anim [Internet]. 1986 Oct; 20(4): 298-303. doi: 10.1258/002367786780808695
31. Junod A, Lambert AE, Stauffacher W, Renold AE. Diabetogenic action of streptozotocin: Relationship of dose to metabolic response. J Clin Investigation [Internet]. 1969; (48): 2129-39. Retrieved from: https://dm5migu4zj3pb.cloudfront.net/manuscripts/106000/106180/JCI69106180.pdf
32. Wang Z, Yang Y, Xiang X, Zhu Y, Men J, He M. Estimation of the normal range of blood glucose in rats. Wei Sheng Yan Jiu [Internet]. 2010 Mar; 39(2): 133-7, 142. Chinese. PMID: 20459020. [Translated Abstract] Retrieved from: https://pubmed.ncbi.nlm.nih.gov/20459020/
33. Rendell M. The role of sulphonylureas in the management of Type 2 diabetes mellitus. Drugs [Internet]. 2004; 64(12): 1339-58. doi: 10.2165/00003495-200464120-00006
34. Alqasoumi SI. 'Okra' Hibiscus esculentus L.: A study of its hepatoprotective activity. Saudi Pharm J [Internet]. 2012 Apr; 20(2): 135-41. doi: 10.1016/j.jsps.2011.10.002
35. Wahyuningsih SPA, Sajidah ES, Atika BND,Winarni D, Pramudya M. Hepatoprotective activity of okra (Abelmoschus esculentus L.) in sodium nitrite-induced hepatotoxicity. Veterinary World [Internet]. 2020; 13(9): 1815-21.
36. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res [Internet]. 2001; 50(6): 537-46.