Hypoglycemic Activity of *Abelmoschus* esculentus (Okra) on Streptozotocin-induced Diabetic *Rattus norvegicus* (Sprague-Dawley Rats)

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ABSTRACT

Diabetes mellitus is a metabolic disease that is the sixth leading disorder that has rapid global prevalence and incidence rates. One of its well-known signs and symptoms is hyperglycemia, caused by impaired secretion of insulin or physiologic action in an individual that can lead to renal or cardiac problems. Greater than 60% of these diabetic patients reside in Asia. It has a higher prevalence among underdeveloped countries like the Philippines. A wide range of diabetes medication is now available commercially; however, patients are also at risk of its side effects. In line with the need to produce a nontoxic and affordable alternative to diabetes medication, the researchers investigated the hypoglycemic activity of Abelmoschus esculentus (Okra). Eighteen (18) rats were used in this experiment, that were randomly distributed into six (6) groups. A hyperglycemic agent, Streptozotocin was injected intraperitoneally to increase the blood glucose concentration of Sprague Dawley rats. Treatment was done by administering varying concentrations 100 mg/kg, 250 mg/kg, 500 mg/kg of Okra pure extract. The effectiveness of Okra pure extract is compared to that of hypoglycemic activity of Acarbose, which revealed that 250 mg/kg and 500 mg/kg of Okra pure extract administered orally was more effective hypoglycemic agents in streptozotocin-induced diabetic rats than orally administered Acarbose. Results of the study revealed that 250 mg/kg is the lowest effective dose capable of significantly decreasing blood glucose of diabetic rats. Accordingly, the pure extract of Abelmoschus esculentus (Okra) can be used as an alternative treatment for Diabetes mellitus.

Key words: *Abelmoschus esculentus*, Blood glucose determination, Diabetes mellitus, Streptozotocin, Hyperglycemia

INTRODUCTION

Diabetes mellitus is a chronic non-communicable metabolic disease which affects the lipid, carbohydrate, and fat mechanisms that may lead to many complications such as renal or cardiac problems like stroke when left untreated for a long period of time (Khosrozadeh, Heydari, and Abootalebi, 2016). It has a rapid global progression of prevalence and incidence rates, which, according to Whiting, Guariguata, Weil &, Shaw (2011), is still estimated to increase by 50.7% over the next few years. It has a major impact in third-world countries, such as the Philippines where it is the sixth leading cause of mortality (Tan, 2015). The Philippines, being a developing country, has insufficient means to even provide the basic means of health care for diabetic patients (World Health Organization, 2016). Although there is a wide array of medications in maintaining lowered blood glucose to avoid diabetes-induced complications, some people still search for a cheaper alternative with minimal side effects (Protter, Chakravarty, Jain, and Green 2017). Diabetes may be characterized by either one of these symptoms: nephropathy, hyperlipidemia, neuropathy, retinopathy, and/or ketosis, but the hallmark sign of both Type I and Type II Diabetes is hyperglycemia (Ibegbulem, and Chikezie, 2013).

Commercially available diabetes medications which reduce the effects of hyperglycemia, such as metformin and thiazolidinediones, have possible side effects like the development of kidney complications and increased the of liver disease respectively (Nathan, Buse, Davidson, Ferrannini, Holman, Sherwin, and Zinman, 2009). In line with issues regarding hypoglycemic agents' availability and its side effects, Nathan

et al. (2017) also stated that further research is still needed in the medical field to quest for the possible alternatives for diabetic induced-complications.

Arumugam, Manjula and Paari (2013), stated that medicinal plants are widely used in rural areas due to their abundance to treat diabetes mellitus and they have been proven to be effective hypoglycemic agents. Indigenous plants are also being used to treat diabetes mellitus but their medicinal value needs further research in order to reach the effective therapeutic dose and to gain knowledge about its mechanism of action (Baharvand-Ahmadi, Bahmani, Tajeddini, Naghdi, and Rafieian-Kopaei, 2016).

Abelmoschus esculentus, otherwise known as Okra, lady fingers or gumbo is a green tropical vegetable belonging to the mallow family *Malvaceae*. It grows abundantly and it is always readily available in tropical countries like the Philippines. Fan, Guo, Zhang, Sun, and Yang (2014) indicated that *A. esculentus*' activity exhibits anti-oxidant and anti-hyperlipidemic properties on a molecular level. Khosrozadeh et al (2016) also stated that okra fruit demonstrates a tolerance on the blood glucose level, making it an important dietary element for preventing hyperglycemia. It is also said to be an inhibitor of cholesterol absorption and it decreases the lipid and fat levels in blood significantly.

It is the limited published literature about the hypoglycemic properties of *Abelmoschus esculentus'* pure extract, specifically in the Philippines, that prompted the researchers to conduct this study. The goal of this scholarly work is to determine the hypoglycemic activity of *Abelmoschus esculentus'* pure extract in male *Rattus norvegicus* rats induced with streptozotocin, and to measure its hypoglycemic activity on varying concentrations. This study also had a comparison between the hypoglycemic activity of okra's pure extract and Acarbose.

The outcome of this experimental study will benefit those patients suffering from diabetes who are seeking for a cheaper alternative treatment that has less side effects and is easily and readily available.

MATERIALS AND METHODS

Newly purchased okra fruits from the market have been washed and cut first, and were purely extracted in MOTS laboratory using an electric blender. Blended okra fruits have been filtered in a kitchen strainer. Afterwards, it went through filtration using a plain-woven fabric cloth for three (3) times, in order to filter the remaining solid parts. The pure extract that has been obtained after approximately one (1) minute was the material used for the treatment group of rats (Jagessar, Rodrigues, Prasad, and Husain, 2018).

The researchers used eighteen (18) male Sprague-Dawley rats which have been acclimated one week prior to experimentation in order for them to adapt to the new environment of the laboratory. These subjects were weighed at the beginning of the experiment for the computation of Streptozotocin dosage.

Upon the computation of right amount of Streptozotocin dosage, the eighteen (18) male Sprague-Dawley rats have all been intraperitoneally induced with Streptozotocin, prepared with 0.1 M citrate buffer. The rats were distributed into six groups, each group with three male rats.

Each group with three rats was treated differently for six days. Group A served as the positive control, group B was the negative control, and group F was the normal group. The remaining groups served as the treatment groups for okra's pure extract at different measurement (Mousavi, 2016). Group A (Positive control) have been treated with 30 mg/kg of Acarbose, a hypoglycemic drug, Group B (Negative Control) was introduced with 1 mL Normal Saline Solution along with their normal feeding regime, the treatment group, have been treated with *Abelmoschus esculentus'* pure extract in various doses. Group C, D, and E received 100 mg/kg, 250 mg/kg, and 500 mg/kg of pure okra extract respectively. Group F (Normal) have been fed normally throughout the experimental stage did not received any treatment.

Spectrophotometer, an instrument used in Clinical Chemistry procedures, facilitated the measurement of blood sugar level of each group of rats. It is based on the principle of light absorption. It differs from colorimetric since spectrophotometry covers ultraviolet region range 200-400 nm (Kumar and Gil, 2018). It was used to assess the chemical substance light absorbance through measuring the light

intensity. As the sample solution was passed through by a beam of light, 0.5 mL of the subjects' whole blood was used in blood glucose measurement. Upon blood extraction, the specimens were centrifuged, and then, serum content was placed in the sample cuvette of the spectrophotometer which utilized glucose oxidase. Glucose oxidase, GOD, is a flavoprotein which has the capability to catalyze β-D-glucose to D-glucono-δ-lactone, through oxidation, and hydrogen peroxide (H_2O_2) through sub-atomic oxygen as an electron acceptor. GOD usually ranges from 130 to 175 kDa in weight. The most well-known diagnostic technique utilized as part of GOD is based upon the mechanism that GOD oxidizes β-D-glucose along with oxygen to β-D-glucono-δ-lactone and H_2O_2, followed by the utilization of H_2O_2 that oxidized a chromogenic substrate in a secondary reaction with horse radish peroxidase. Change in color has been observed and monitored by the spectrophotometer (Bankar, Bule, and Singhal, 2009).

Each member of the six groups of Sprague-Dawley rats was carefully monitored according to their weight and blood sugar level. After eight hours of fasting, the baseline results for blood glucose were obtained following blood extraction of 1 mL through cardiac puncture. Steptozotocin was introduced to groups A, B, C, D, and E intraperitoneally in reference to 30mg/kg dosing while Group F was not induced with hyperglycemic drug, leaving it as the normal group. The Sprague-Dawley rats were housed again in their individual cages at room temperature. After 3 days, following 8-hour fasting, blood glucose levels were tested for pre-test results. Upon obtaining high blood glucose level compared to the baseline results, the treatment for the subjects began. Various treatments for six groups were as follows: Group A with Acarbose, Group B with distilled water, Group C with 100 mg/kg of okra's pure extract, Group D with 250 mg/kg of okra's pure extract, Group E with 500 mg/kg of okra's pure extract and Group F with normal feeds for rats. In a span of six days, they were fed thrice a day for every 8 hours. At the last day of treatment, they had undergone fasting for 8 hours prior to blood collection for post-test blood glucose determination. The controlled variable was the gender of the Sprague-Dawley rats to be used. The rats used were all male so that their physiological features were constant, serving as the controlled variable, as compared with the female rats' anatomy. Most streptozotocin-induced diabetic rats are male because they are more resistant to islet-cell toxin than the female rats (Furman, 2015).

Animal experimentation was involved in this study, in the form of inducing Streptozotocin, then Acarbose or *Abelmoschus esculentus* pure extract to Sprague Dawley rats. The researchers have obtained the proof of approval for animal experimentation from the Institutional Animal Care and Use Committee (IACUC). During the experimental stage, the researchers made sure that the entire procedure was done under the careful supervision of the qualified veterinarian who has ensured the safety of the animals especially in the blood collection stage and in the division and testing of control groups. These steps and precautions have been done to make sure that all the procedures that took place were ethical, appropriate, and humane.

In order to determine if there is a significant difference among the control and experimental rats, paired sample t-test was utilized, and the p-values were used to determine if the results between the pre-test and post-test were significant ($P<0.05$).

RESULTS AND DISCUSSION

Baseline results were obtained upon testing the blood glucose absorbed in spectrophotometer prior to induction of Streptozotocin to increase the blood glucose level. Blood glucose of each subject was raised through inducing a hyperglycemic agent and its results are represented under pre-test. Varying treatments were done, and the resulting blood glucose levels are represented under post-test.

Table 1. Hypoglycemic activity of *Abelmoschus esculentus* in varying concentrations

Sample	Baseline	Pre - test	Post - test

Group A (Positive)	21.50	34.80	9.40
	21.00	32.00	20.20
	12.80	33.50	20.90
Group B (Negative)	19.80	31.70	19.10
	22.40	34.70	20.10
	24.20	36.20	6.00
Group C (100 mg/kg)	23.50	35.00	6.10
	22.70	34.50	21.50
	24.10	32.70	14.40
Group D (250 mg/kg)	20.00	33.90	21.80
	21.70	36.20	19.80
	21.50	31.60	6.40
Group E (500 mg/kg)	21.50	44.90	3.70
	17.50	44.30	6.60
	24.80	41.60	6.70
Group F (Normal)	21.50	21.50	1.40
	23.50	25.80	20.20
	24.40	31.50	5.70

Majority of the subjects' post-test results show significant difference to the pre-test results, supporting the researchers' hypothesis. According to Xia et. al (2015), the polysaccharides present in the fruit of *Abelmoschus esculentus* helps in maintaining blood glucose levels in the normal range by modulating the control of glucose absorption in the lumen of the small intestine. According to Dayal, Yannamredy, Singh, Lea &, Ertel, (2012), regular inclusion of *Abelmoschus esculentus* in daily diet (3 times in a week) can provide effective protection against diabetes and diabetic induced hyperglycemia.

Analyzing these statistical results, more specifically for pre-test vs. post-test of group D and E and baseline vs. post-test of group E, the researchers found an evident significance of the hypoglycemic activity of *Abelmoschus esculentus'* pure extract. This means the comparison for the variables rejected the null hypothesis that there is no difference between the means.

Table 2. Comparison of blood glucose levels from different doses of *Abelmoschus esculentus*

Samples	*p*-value (pre-test vs post-test)	Interpretation
Group A (Positive)	0.0638	NOT SIGNIFICANT
Group B (Negative)	0.0751	NOT SIGNIFICANT
Group C (100 mg/kg)	0.0502	SIGNIFICANT
Group D (250 mg/kg)	0.0434	SIGNIFICANT
Group E (500 mg/kg)	0.0023	SIGNIFICANT
Group F (Normal)	0.1039	NOT SIGNIFICANT

Majority of the subjects' post-test results show significant difference to the pre-test results, supporting the researchers' hypothesis that there is a significant difference between the p-value of pre-test and post-test. A study conducted by Prabhune et. al (2017), supports this hypothesis and in this study, they

proved that *A. esculentus* extract produces clinically significant results in lowering the blood glucose levels when compared to palliative, no treatment, and first line diabetic drugs.

Analyzing these statistical results, more specifically for pre-test vs. post-test of group C, D and E and baseline vs. post-test of group E, the researchers found an evident significance of the hypoglycemic activity of *Abelmoschus esculentus'* pure extract. This means the comparison for the variables rejected the null hypothesis that there is no difference between the means.

Evidently, Sprague-Dawley rats in Group C, D and Group E demonstrated significant decrease in the blood glucose level in reference to the control group. As the dosage of *Abelmoschus esculentus* pure extract increases, the blood glucose levels of the Sprague Dawley rats in the post-test significantly decrease. This goes with the findings of Onuoha et al. (2014) stating that increasing concentrations of *Abelmoschus esculentus* pure extract also increases its hypoglycemic effects due to the possible concentration of *okra's* active components present.

These statistical data also manifest that Group C, 100 mg/kg of *Abelmoschus esculentus* administered orally is the lowest effective dose in treating streptozotocin-induced diabetic Sprague-Dawley rats.

There is no significant difference between the pre-test and post-test of Group A (Positive treatment/Acarbose) with a p-value of 0.638. However, the p-value of Group C (100 mg/kg of okra) is 0.0502, group D (250 mg/kg of okra) is 0.0434 and group E (500 mg/kg of okra) is 0.0023 which means there is a significant difference between the pre-test and post-test of group D and group E. This confirms that 100 mg/kg, 250 mg/kg and 500 mg/kg of *Abelmoschus esculentus* administered orally are effective alternative hypoglycemic agent to Acarbose.

In a study conducted by Li, Huang, Ye, Chen, Yu, Yang, and Zhang (2016), the comparison of mulberry twig (Ramulus Mori, Sangzhi) alkaloid tablet and Acarbose yielded results somewhat similar in this study on okra and Acarbose hypoglycemic effect. It is supported with the comparative study results for glycated hemoglobin (HbA1c), 1-hour and 2-hour postprandial and fasting serum glucose levels from baseline until the end of treatment. Interpretation of the experimentation results as HbA1c and 1-hour and 2-hour postprandial of mulberry twig alkaloid tablet and Acarbose were decreased significantly and no significant difference, respectively. On the other hand, for fasting serum glucose levels, results at the end of the treatment were interpreted as both treatments obtained no significant difference. Li, et al. (2016), stated that there is need for enough time periods to reach a drug dosage in order for it to be stable and attain its optimal efficacy.

In a comparative study conducted by Villa- Rodriguez, Aydin, Gauer, Pyner, Williamson, and Kerimi (2017), the researchers concluded that chamomile and green teas yielded a more effective activity in high bolus stress of sugar's absorption and metabolism than Acarbose.

Table 3. Comparison of the hypoglycemic activity of *Abelmoschus esculentus* and Acarbose

Samples	p-value	Interpretation
Group A (Positive)	0.0638	NOT SIGNIFICANT
Group C (100 mg/kg)	0.0502	SIGNIFICANT
Group D (250 mg/kg)	0.0434	SIGNIFICANT
Group E (500 mg/kg)	0.0023	SIGNIFICANT

CONCLUSION

Substitution of synthetic medications with natural hypoglycemic agents may be advantageous for its little or no side effects to human. The capability of *Abelmoschus esculentus* (Okra) pure extract to

significantly reduce blood glucose concentration in Streptozotocin-induced diabetic rats gives support its therapeutic potential and hypoglycemic activity in rats. In assessment of hypoglycemic activity of Acarbose and *Abelmoschus esculentus* (Okra), Acarbose did not demonstrate enough hypoglycemic activity in this experiment wherein there is evident depletion of blood glucose levels. Statistical analysis of different concentration exhibits that 250 mg/kg and 500 mg/kg of *Abelmoschus esculentus* (Okra) pure extract significantly reduced the blood glucose concentration in diabetic rats. Accordingly, it can be said that *Abelmoschus esculentus* (Okra) pure extract has a potential hypoglycemic activity. Therefore, it may serve as an easily accessible alternative hypoglycemic agent in the present selection of antidiabetic drugs.

REFERENCES

Bankar, S. B., Bule, M. V., Singhal, R. S., &, Ananthanarayan, L. (2009). Glucose oxidase—an overview. *Biotechnology advances, 27(4), 489-501.*

Dayal B, Yannamreddy VR, SinghPA, Lea M, H. Ertel N (2012). Bioactive compounds from okra seeds: potential inhibitors of advanced glycation end products. *ACS Symposium Series. In: Emerging Trends in Dietary Components for Preventing and Combating Disease.*

Fan, S., Guo, L., Zhang, Y., Sun, Q., Yang, B., &, Huang, C. (2013). Okra polysaccharide improves metabolic disorders in high-fat diet-induced obese 18 C57BL/6 mice. *Molecular Nutrition &, Food Research*

Furman, B. L. (2015). Streptozotocin- induced diabetic models in mice and rats. *Current protocols in pharmacology*, 5-47.

Ibegbulem, C. O., &, Chikezie, P. C. (2013). Hypoglycemic properties of ethanolic extracts of Gongronema latifolium, Aloe perryi, Viscum album and Allium sativum administered to alloxan-induced diabetic albino rats (Rattus norvegicus). *Pharmacognosy Communications, 3(2), 12.*

Jagessar, R. C., Rodrigues, A., Prasad, K., Husain, A., Kanhai, V., &, Bernarai, B. (2018). An Investigation Of The Hypoglycemic Effect Of The Aqueous Extract Of The Fruits Of Psidium Guajava, Averrhoa Bilimbi And The Peel Of Tamarindus Indica In Normoglycemic Guinea Pigs. *World Journal Of Pharmacy And Pharmaceutical Sciences.*

Khosrozadeh, M., Heydari, N., &, Abootalebi, M. (2016). The Effect of Abelmoschus Esculentus on Blood Levels of Glucose in Diabetes Mellitus. *Iranian journal of medical sciences, 41*(3), *S63.*

Kumar, D. S., Tony, D. E., Kumar, A. P., Kumar, K. A., Rao, D. B. S., & Nadendla, R. (2013). A review on Abelmoschus esculentus (Okra). *Int. Res J Pharm. App Sci, 3(4), 129-132.*

Kumar, V., & Gill, K. D. (2018). Photometry: Colorimeter and Spectrophotometer. In Basic Concepts in Clinical Biochemistry: A Practical Guide (pp. 17-20). Springer, Singapore

Li, M., Huang, X., Ye, H., Chen, Y., Yu, J., Yang, J., & Zhang, X. (2016). Randomized, double-blinded, double-dummy, active-controlled, and multiple-dose clinical study comparing the efficacy and safety of mulberry twig (Ramulus Mori, Sangzhi) alkaloid tablet and Acarbose in individuals with type 2 diabetes mellitus. Evidence-Based Complementary and Alternative Medicine, 2016.

Mousavi, L., Salleh, R. M., Murugaiyah, V., &, Asmawi, M. Z. (2016). Hypoglycemic and hypoglycemic study of Ocimum tenuiflorum L. leaves extract in normal and streptozotocin-induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine*, *6*(12), 1029-1036.

Onuoha, N., Iroegbu, L., & Uwaezuoke, N. (2017). Anti-diabetic effects of okra [Abelmoschus esculentus (L.) Moench] fruits in alloxan-induced diabetic rats. Biokemistri, 29(2).

Prabhune, A., Sharma, M., &, Ojha, B. (2017). Abelmoschus esculentus (Okra) potential natural compound for prevention and management of Diabetes and diabetic induced hyperglycemia. *International Journal of Herbal Medicine, 5, 65-68.*

Protter, A. A., Chakravarty, S., Jain, R. P., &, Green, M. J. (2017). U.S. Patent No. 9,550,782. Washington, DC: U.S. Patent and Trademark Office.

Villa- Rodriguez, J. A., Aydin, E., Gauer, J. S., Pyner, A., Williamson, G., & Kerimi, A. (2017). Green and chamomile teas, but not Acarbose, attenuate glucose and fructose transport via inhibition of GLUT2 and GLUT5. *Molecular nutrition & food research*, *61*(12), 1700566.

Whiting, D. R., Guariguata, L., Weil, C., &, Shaw, J. (2011). IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes research and clinical practice, 94(3), 311-321.*