Exploratory Investigation On The Hypoglycemic Effect Of Abelmoschus Esculentus In Mice

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Abstract: The increased number of glucose in the blood is called Diabetes [7] and is forecasted to be a very major disease by 2030.[7] There is a pressing need to explore better remediation, be it in form of synthetic or naturally occurring chemicals. This paper made an exploratory investigation on the hypoglycemic effect of a common food item known as "okra" or Abemoschus esculentus (EA). Six (6) mice were randomly selected and grouped into 2 groups and were given extracts from the fruit of Abelmoschus esculentus (EA)/okra which was chopped into three pieces and was soak in 250ml potable water overnight. 1st group is the Control Group and the 2nd group is the Treatment Group. Average results of the two (2) groups are determined and recorded upon conducting this experimental research. The Control Group has an average result of 94mg/dl in the 1st test, 99mg/dl in the 2nd test, 94mg/dl in the 3rd test, 101mg/dl in the 4th test, 97mg/dl in the 5th test and 73mg /dl in the 6th test. The Treatment Group has an average result of 87mg/dl in the 1st test, 99mg/dl in the 2nd test, 124mg/dl in the 3rd test, 129mg/dl in the 4th test, 115mg/dl in the 5th test and 88mg/dl in the 6th test. Results indicated that the extract from Abelmoschus esculentus (EA)/okra has hypoglycemic effect and no observable changes in behavior are noticed to the mice. It is recommended to replicate the study with more number of mice and longer observation period.

Index Terms: Abelmuschus Esculentus (AE), Blood Glucose, Diabetes Mellitus, Hypoglycemic Effect.

INTRODUCTION

THE increased number of glucose in the blood is called Diabetes. Diabetes-related complications can be prevented when blood sugar level is carefully controlled. The persons that will be severely affected are those that have heart disease and the elderly people diagnosed with diabetes mellitus. Maintaining blood glucose level within the normal range will prevent the side effects of the diabetes mellitus to develop in the body. Over 180 million people all over the world have diabetes and will likely to double its number in the year 2030 as the World Health Organization has estimated. Type 2 diabetes which is the most common is one of the growing health problems in the world that constitutes major accounts that would range to 85% to 95% cases. Among the risk factor which leads to the development of type 2 diabetes is the lack of physical activity and unhealthy dietary habits that would result to obesity or being overweight that would make the body insulin resistance. Research studies have been made for the efficacy of natural plants and herbals that will treat diabetes mellitus. In the Philippines, herbal medicines are being used to treat this problem.

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Abelmoschus esculentus (AE) is a natural plant that is very potential in the treatment of diabetes mellitus.[7] Okra (Abelmoschus esculentus) also called as lady's finger; is a member of the mallow family that is one of the flowering plants. This plant is cultivated in tropical and with a warm temperature places all over the world but this particular species of plants is still poorly studied.[7] Abelmoschus esculentus is an annual herb that is coarse, erect and has branches that is 0.6 to 1.5 meters high in length and with a long-petioled leaves, orbicular-ovate that has about 25 centimeters or less in length and has a heart-shaped base with 3 to 5 lobed margins. The lengths of its petioles are equal to the blade. It has an axillary and solitary flower with a corolla that is large and yellow to the inside and with a base that is deep purple in color. It has an elongated fruit that is 10 to 25 centimeters in length and with a diameter of 1.5 to 3 centimeters. It has a blunt point and tapering which contains rounded rows that have a kidney shaped seeds. The fruit of this particular plant gives nutritional benefits like protein, niacin, riboflavin, phosphorus, zinc, copper, potassium, vitamins A, B6, C and k, thiamine, magnesium, foliate, calcium, and manganese.[6] Carbohydrate is a kind of glucose; and in human metabolism, glucose is the most important simple sugar. Since called simple sugar or a monosaccharide, it is the smallest unit with the characteristics of this class of carbohydrates. Sometimes it is also called as dextrose. Plants have stored glucose in their saps and it is also found in the bloodstream of humans which is being called as "blood sugar". In plants and animals, glucose serves as an energy source and a fuel which powers cellular machinery. Glycolysis is the process when the glucose in the bloodstream is taken by the cells and breaks down its sugar molecule.[2] Glycoproteins is a special molecule produced from glucose that provides structural benefits to the cells. With its inclusion in the carbohydrates, glucose also plays a structural role in addition to proteins. And with this, enzyme functioning and binding are possible in the human metabolism. The energy from fats although body cells can utilize it in a pinch, glucose is still being relied upon by the brain cells and the red blood cells for its energy needs. Shortage of glucose in the bloodstream can kill these types of cells in a short period of time.[5] Blood has a certain amount of glucose which is referred to as blood glucose level or blood sugar level. It comes from foods that

are rich in carbohydrates, which is our human body's source of energy. Insulin is being secreted in the blood by the pancreas to help body use the glucose when the glucose level in the bloodstream rises. Normally, glucose level rises in the blood after a meal and insulin is being released by the pancreas to control the level of glucose in the blood from becoming too high.[1] High glucose levels or high sugar levels occurs when your body is unable to use the glucose in your bloodstream effectively. When you eat, food turns to sugar/glucose. In turn, your pancreas releases insulin to help the sugar move into the muscle cells for energy. When insulin is not working properly or your body does not produce insulin, too much sugar/glucose stays in the blood, creating high glucose levels. The effects are numerous and complex because the sugar/glucose will travel throughout the body affecting all organ system. It also causes neuropathy, coronary disease, affects visions of the eyes, insulin resistance and hyperglycemia. And when sugar/glucose level will become too low it will cause hypoglycemia.[1] Checking the amount of glucose level in the blood if it is within the healthy range, which is not too high or not too low is very important. When sugar/glucose level in the blood is not within the healthy range, it indicates that you have a health problem that needs a treatment. National Diabetes Information Clearinghouse website stated information about the normal blood sugar/glucose level between 70 to 90 mg/dl in a waking or a fasting test and 70 to 140 mg/dl if it is after meals.[3]Mice have identical genes as the humans; its chromosomes are arranged in almost the same and exact order like the humans. They can have heart disease, many cancers, arthritis, immune disorders, depression, obesity and even diabetes. They also have similar system like humans such as neurology, immune defense, metabolism, circulation and digestion.[4] Draft of human and mouse genome sequence have been compared head to head and it is summarized with one word "fourteen". In sixteen (16) genes of human chromosomes, fourteen (14) genes of mice are identical to humans. Human genomes have counterparts with mouse genome that would reach more than 700 genes in all and its genes are grouped together in the same exact order as the mouse genome are being grouped.[4] In other words, genomes of humans and mouse are similarly remarkable, not only in the level of DNA sequences but also in their chromosomal structures. Similarities between this two animal species have been reported for decades but not with the details that the two (2) genome sequences are being lined up. [4]

MATERIALS AND METHODS

The researchers used an experimental procedure to obtain the blood glucose level in mice. This is done to know whether blood glucose level has increased by inducing oral intake of food and treating the mice with Abelmoschus esculentus (AE)/Okra extract, to know whether blood glucose will decrease or return to its normal level.

Plant Material

Pieces of young and fresh fruit of Abelmochus esculentus (AE)/okra fruit were being purchased from the market. Since this edible fruit is cultivated and is abundant in this tropical and warm temperate region of the Philippines, it is being sold abundantly in the markets of this country. This plant is very well known to the people in this country. It is part of the people's everyday food recipes that gives nutrients and nourishment to their body.

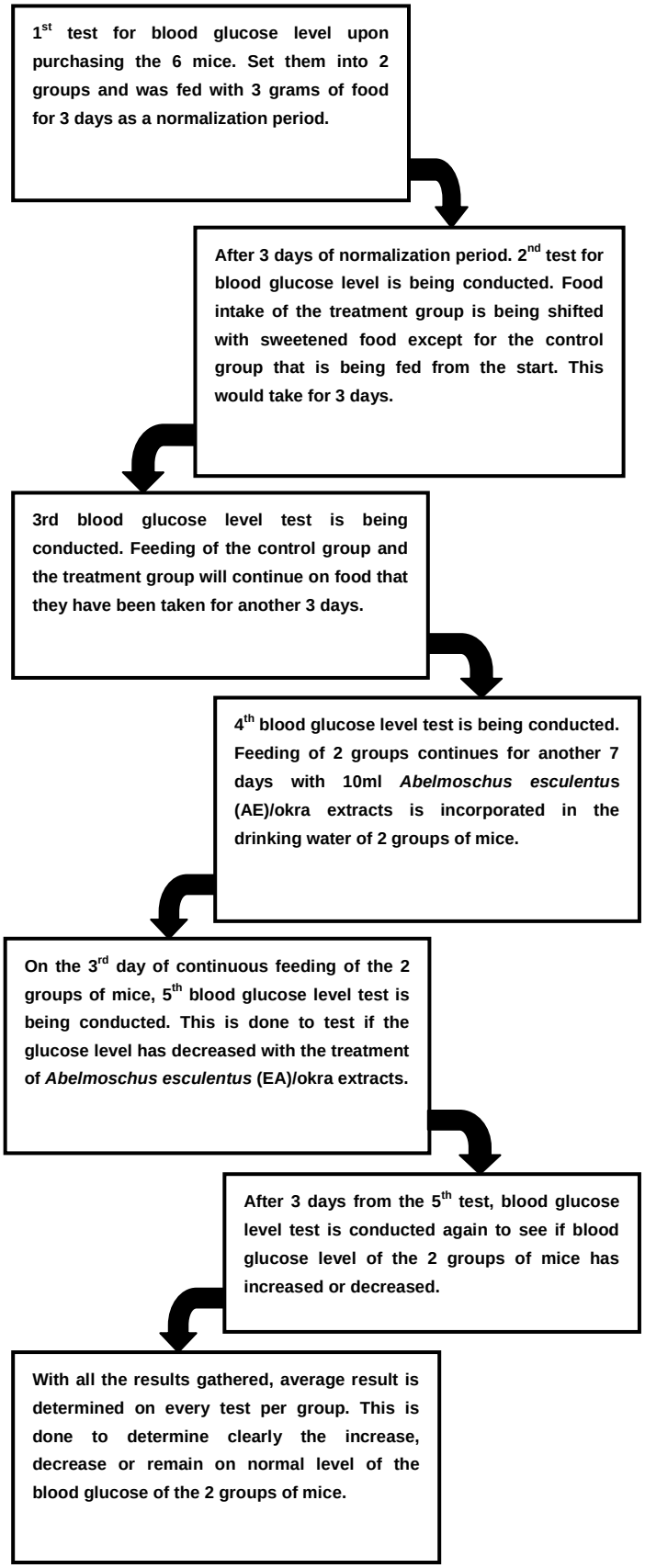
Preparation of the Extract

Three fresh okras were cut into three pieces each and soaked in 250ml potable water overnight in a beaker. On the following day, the okra pieces were removed and the liquid is transferred to a secure container. The liquid at this time has slimy texture. Soaking of okra was done every night for the duration of the study. Thus, the mice have always fresh potable water with okra extract.

Animal Material

Six (6) young adult albino mice of both sexes were obtained and used in this experiment. These animals were obtained from a local pet store and were kept in a normal environmental condition (a 12-h light dark cycle, and 30±5^{o}C, 55±5% humidity) and maintained with its normal diet and has a free access to a drinking water. The animals were then divided into two (2) groups, 1st group is the Control Group and the 2nd group is the Treatment Group. Tail bleeding technique is being performed to these animals in order to obtain a small blood sample (5-10µl) to determine blood sugar/glucose level. A visible vein at the tail of these animals is pricked with a lancet to obtain a small amount of blood sample. Enzymatic reaction is a process in blood chemistry that would specifically determine the glucose in the blood.

DETERMINATION OF BLOOD GLUCOSE LEVEL FLOWCHART



Upon obtaining these six (6) young adult albino mice, RBS (Random Blood Sugar) test were initially performed via glucometer for their blood glucose level and then randomly divided into two (2) groups. 1st group is the Control Group and the 2nd group is the Treatment Group. Every test in this experiment was done 4-6 hours after meal.

CONTROL GROUP

The 1st and 2nd tests were normalization period with a duration of 3 days, the three (3) mice in the Control Group were fed with exact amount of food (3 grams per group everyday that contains 7.68% carbohydrate, 0.96% fiber, 0.96% protein, and 2.4% fat per serving) and measured drinking water (10ml per day); From the 2nd, 3rd and 4th tests, feeding with the same amount of food and giving of measured drinking water were continuously given with 3 days interval in every test; And from 4th, 5th and 6th tests, feeding of the same amount of food also continues but the drinking water was changed with Abelmoschus esculentus (AE)/okra extracts (10ml per day) as their drinking water with an interval of 4 days from the 4th test up to the 5th test and 3 days from the 5th test up to the 6th test.

TREATMENT GROUP

The 1st and 2nd tests were normalization period with a duration of 3 days, mice in the Treatment Group were fed with exact amount of food (3 grams per group everyday that contains 7.68% carbohydrate, 0.96% fiber, 0.96% protein, and 2.4% fat per serving) and measured drinking water (10ml per day); From the 2nd test, 3rd test and 4th test, giving of food is shifted to sweetened food (3 grams per day that contains 5.5% carbohydrate,0.66% fiber, 0.44% protein, 1.43% fat and 3.08% sugar per serving) and with measured drinking water (10ml per day) with an interval of 3 days in every test as to increase their blood glucose level; And from the 4th, 5th and 6th tests, feeding of the same amount of sweetened food continues but the drinking water was changed with Abelmoschus esculentus (AE)/okra extracts (10ml per day) as their drinking water with an interval of 4 days from the 4th to 5th test and 3 days from the 5th to 6th test. In obtaining a sample of blood from these six (6) mice, sterile gloves were worn to prevent the researcher from any blood borne diseases that are present on the blood of the mice.

DATA ANALYSIS

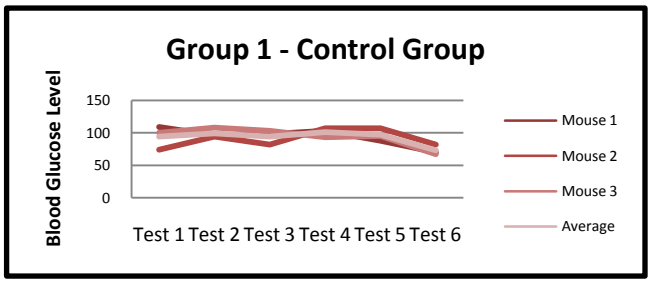
Table 1 – Test results of the Control Group

Table 1 shows test results of each mice with the average results in the Control Group

Group 1	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Mouse 1	109mg/dl	96mg/dl	98mg/dl	103mg/dl	88mg/dl	70mg/dl
Mouse 2	74mg/dl	94mg/dl	82mg/dl	107mg/dl	107mg/dl	82mg/dl
Mouse 3	100mg/dl	108mg/dl	103mg/dl	93mg/dl	95mg/dl	67mg/dl
Ave. Results	94mg/dl	99mg/dl	94mg/dl	101mg/dl	97mg/dl	73mg/dl

Figure 1 – Control Group

Figure 1 shows the graph of the blood glucose level of each mouse with the average results in the control group



In table 1 and figure 1 shows the results of each mouse with the average results of blood glucose level of mice in the Control Group. In the 1st test as an initial test upon obtaining the mice up to the 2nd test is the normalization period. In these tests the results are within the normal limits of the blood glucose level; And from the 2nd, 3rd and 4th tests, The results are still within the normal limits of the blood glucose level; And from the 4th, 5th and 6th tests, the results of blood glucose from the mice in this tests slightly decreases from its previous normal results but didn't really decrease beyond the normal range of the normal glucose level.

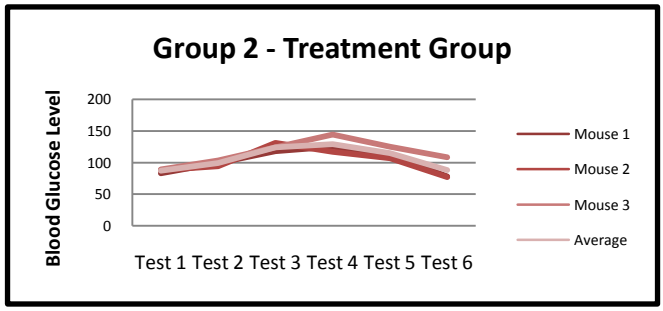
Table 2 – Treatment Group

Table 2 shows test results of each mouse with the average results in the Treatment Group

Group 2	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Mouse 1	83mg/dl	99mg/dl	118mg/dl	125mg/dl	113mg/dl	78mg/dl
Mouse 2	88mg/dl	94mg/dl	131mg/dl	117mg/dl	107mg/dl	77mg/dl
Mouse 3	89mg/dl	103mg/dl	124mg/dl	144mg/dl	125mg/dl	108mg/dl
Ave. Results	87mg/dl	99mg/dl	124mg/dl	129mg/dl	115mg/dl	88mg/dl

Figure 2 – Treatment Group

Figure 2 shows the graph of the blood glucose level of each mouse with the average results in the treatment group



In table 2 and figure 2 shows the results of each mouse with the average results of blood glucose level of mice in the Treatment Group. In the 1st as an initial test upon obtaining the mice up to the 2nd test is the normalization period. In these tests the results are within the normal limits of blood glucose level; And from the 2nd, 3rd and 4th tests, results of blood glucose in mice slightly increases in each and every tests; And from the 4th, 5th and 6th tests, the results in these tests decreases in each and every tests conducted but didn't really decrease beyond the normal range of the normal glucose level.

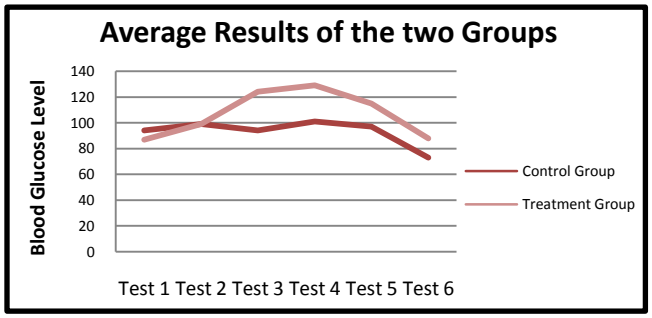
Table 3 – Average Results

Table 3 shows average results of Control Group and Treatment Group

Experimented Groups	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Control Group	94mg/dl	99mg/dl	94mg/dl	101mg/dl	97mg/dl	73mg/dl
Treatment Group	87mg/dl	99mg/dl	124mg/dl	129mg/dl	115mg/dl	88mg/dl

Figure 3 – Average Results

Figure 4 shows all the average results from the control group and treatment group



In table 3 and figure 3 shows the average results of the blood glucose level of both the Control Group and the Treatment Group. In the 1st as an initial test upon obtaining the mice up to the 2nd test is the normalization period. The results in these tests are within the normal range of the blood glucose level; And from the 2nd test, 3rd test and 4th test, the results of blood glucose level in the Control Group is still within the normal level but the blood glucose level in the Treatment Group slightly increases in each and every test ; And from the 4th test, 5th test and 6th test, the blood glucose level of the Control Group and the Treatment Group slightly decreases in each and every tests conducted but didn't really decrease beyond the normal range of the normal glucose level.

FINDINGS

Average results of the Control Group is 94mg/dl in the 1st test, 99mg/dl in the 2nd test, 94mg/dl in the 3rd test, 101mg/dl in the 4th test, 97mg/dl in the 5th test and 73mg /dl in the 6th test. Average results in the Treatment Group is 87mg/dl in the 1st test, 99mg/dl in the 2nd test, 124mg/dl in the 3rd test, 129mg/dl in the 4th test, 115mg/dl in the 5th test and 88mg/dl in the 6th test. With these average results, determination of blood glucose level shows reaction in the shifting of food in the Treatment Group and the hypoglycemic effect of Abelmoschus esculentus (AE)/okra extract treatment on both the Control Group and the Treatment Group. These findings

were similarly related to the research framework study of Indah Mohd Amin. That STZ induce diabetic mice and with the Control Group mice were given Abelmoschus esculentus (AE)/okra extract that results in decreasing of the blood glucose level of mice after its administration and as expected. [11]

CONCLUSION

The researchers conclude that Abelmoschus esculentus (AE)/Okra extract has a hypoglycemic effect that helps decrease blood glucose level as it is being showed in the results from the tables and graphs. Its properties can be a remedy to manage diabetes mellitus. The results that are being showed in the tables and graphs are evidence to help scientist conduct more scientific researches to uncover more nutritious properties of the AE fruit that will be benefited from these findings.

RECOMENDATION

Based on the results that are being showed from the tables and graphs. There was significant decrease of blood glucose level of the mice when given with AE extracts. To really appreciate the blood glucose level in mice that increase and decrease, food intake should be increased and the duration of experiment or testing should be lengthen and that testing should start when the mice is already 5 weeks old as it is in the standard age for testing. It is also better to breed your own mice for the experiment so that you can control the food that is being fed to the mice and at the same time, have an environment that is conducive to their health.

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