

BLOOD GLUCOSE LOWERING ACTIVITY OF SWEET BASIL (*OCIMUM BASILICUM* LINN.) LEAVES EXTRACT ON ALLOXAN-INDUCED SPRAGUE-DAWLEY RATS

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ABSTRACT

This study aims to determine the blood glucose-lowering activity of sweet basil (*Ocimum basilicum*) leaves extract in alloxan-induced diabetic rats. The blood glucose lowering activity of Sweet Basil leaf extract was determined through in-vivo analysis of 15 young male Alloxan-induced diabetic Sprague Dawley rats. An oral Glucose Tolerance Test (OGTT) was used to interpret the result. The study was conducted at Esteleydes Animal Laboratory and Research Facility, which started in January 2023 and was completed by the second week of July 2023. The phytochemical screening of the extract revealed the presence of tannins, saponins, alkaloids, glycosides, flavonoids, steroids, and triterpenoids, which may be accountable for the blood glucose-lowering activity of sweet basil (*Ocimum basilicum* Linn.). It has been demonstrated that administering sweet basil leaf extract to diabetic rats induced with alloxan lowers blood glucose levels in all basil leaf extract groups. At a dose of 400 mg/kgBW, the efficacy of sweet basil leaf extract was demonstrated. Acarbose 50 mg vs. Sweet Basil leaf extract 400 mg has no significant difference, stating that the 400mg of Sweet Basil Leaf Extract has the same efficacy as Acarbose 50 mg. Sweet basil leaf extract was proven to have blood glucose-lowering activity in alloxan-induced diabetic Sprague-Dawley rats.

Keywords: *Ocimum basilicum*, Sprague- Dawley rats, Alloxan-induced, Acarbose

INTRODUCTION

Insulin resistance and insulin deficiency are the defining characteristics of a variety of disorders that comprise type 2 diabetes, which has become a global pandemic. According to J Epidemiology Global Health, by 2020, 462 million people, or 6.28% of the world's population, will have type 2 diabetes. In 2017, it killed more than 1 million individuals. By 2030, the global prevalence of type 2 diabetes in all regions will be 7,079 cases per 100,000 people. Moreover, the Philippine Statistics Authority in 2021 stated that diabetes caused 1.5 million fatalities in 2019, with 48% of all diabetes-related deaths happening before the age of 70. This kind of diabetes, which until recently was solely present in adults, is now occurring in children. One of the most prevalent metabolic illnesses is diabetes mellitus. It is primarily distinguished by a lack of glucose homeostasis brought on by a disruption in the metabolism of carbohydrates, fats, and proteins as well as problems in insulin secretion or action, or both. A lack of insulin can prevent some body parts from properly utilizing the glucose present in the bloodstream. Hyperglycemia, or elevated blood glucose levels, are the result of this. Hepatic glucose production is directly related to hyperglycemia. Due to the resultant

malfunction of β -cells, the production and release of insulin are reduced, leading to persistent hyperglycemia. Chronic hyperglycemia is closely linked to various microvascular complications of diabetes mellitus, including retinopathy, nephropathy, and neuropathy, particularly concerning the vascular endothelium. Numerous research has mentioned basilicum. *O. Basilicum* is a popular herb that has both decorative and medicinal uses. The chemical elements that have been identified from the plant include terpenoids, alkaloids, flavonoids, tannins, saponin glycosides, and ascorbic acid. These substances have been linked to lower blood sugar levels. The flavonoid compounds and polyphenols, which are active substances that have pharmacological effects, are vulnerable to oxidation because they are unstable against the effects of temperature and high light levels.

In this study, the researchers determined the blood glucose lowering activity of sweet basil (*Ocimum basilicum*) leaves extract on alloxan induced diabetic rats with certain modifications to better understand the mechanism by which the leaves extract of sweet basil caused the significant decrease in the blood glucose level of the test animals.

LITERATURE REVIEW

Type 2 Diabetes

Type 2 diabetes is a diverse range of disorders characterized by tissue resistance to insulin action and a relative shortage in insulin production (Katzung, 2018). A person can have moderate or severe abnormalities, more resistance, or more significant beta-cell insufficiency. Enough endogenous insulin is present in the bloodstream to prevent ketoacidosis, but not enough to stop hyperglycemia. Khin et al., 2023 stated that beta cells control insulin secretion in response to plasma glucose concentration, which should be within a limited physiological range.

In diabetic patients, however, β -cells cannot secrete sufficient insulin in response to glucose and other secretagogues to satisfy the increased insulin demand. Insulin resistance typically initiates the pathogenesis of T2DM, and an increase in β -cell-insulin secretion compensates for this resistance, thereby maintaining normal blood glucose levels. Nonetheless, the function and mass of β -cells continue declining, exacerbating inadequate insulin secretion. This eventually results in the inability of β -cells to compensate for insulin resistance, resulting in diabetes.

Furthermore, in the study of Goyal, 2023, individuals experienced a diminished response to insulin. At this stage, insulin's effectiveness was impaired, and initially, the body responded by increasing insulin production to regulate glucose levels and maintain homeostasis. However, as time passed, the amount of insulin produced dropped, eventually causing Type 2 Diabetes Mellitus to appear.

Standard Drug (Acarbose)

According to Mustafa (2022), Acarbose was utilized as a treatment for type 2 diabetes for approximately 30 years. It worked by inhibiting carbohydrate digestion in the small intestine, which helped prevent postprandial hyperglycemia. However, over the last 10 to 15 years, incretin-based treatments have become more preferred, resulting in a decline in the use of Acarbose for treating type 2 diabetes compared to its previous popularity. Acarbose was shown to be a α -glucosidase inhibitor in the study cited by the American Chemical Society, which prevented the enzymatic release of glucose from complex carbohydrates in the

stomach. It was a complex oligosaccharide that acted as a membrane-bound intestinal alpha-glucosidase hydrolase and pancreatic alpha-amylase competitive, reversible inhibitor. Intestinal alpha-glucosidase hydrolase's enzymatic activity in the small intestine promotes the hydrolysis of oligosaccharides, trisaccharides, and disaccharides (sucrose and maltose) to produce monosaccharides (glucose and fructose). In the lumen of the small intestine, oligosaccharides are produced due to the breakdown of complex carbohydrates by pancreatic alpha-amylase, an enzyme. Acarbose efficiently lowers postprandial glucose levels by preventing glucose absorption and slowing down carbohydrate digestion (McIver, 2022). Since then, it has been applied in clinical practice as a monotherapy for mild type 2 diabetes or combined with insulin and other antidiabetics for severe and advanced cases (Wang et al. 2021).

Test Animal (Sprague-Dawley Rats)

According to Taconic Biosciences, the Sprague Dawley model was employed in various biomedical fields, encompassing reproductive toxicology, embryonic development, and nutritional studies. Rodents were regarded as superior models for diabetes research compared to various non-mammalian species because their physiology is more similar to humans. While monogenic or single gene-based mutations were often insufficient to cause diabetes in humans, similar observations were found in rodents. For instance, leptin, a hormone produced by adipocytes, plays a role in suppressing hunger, regulating fat storage, and maintaining glucose homeostasis (Zakaria, 2021).

Sweet Basil

The research conducted by Falowo et al., 2019 revealed that sweet basil contains 32 bioactive compounds, including antioxidant and antimicrobial properties and anti-inflammatory, nematocidal, and anticancer properties. Moreover, results from the study of Nadeem et al., 2022 concluded that ethanol extracts of sweet basil (*Ocimum basilicum* Linn.) leaves were found to have greater levels of total flavonoids, and quercetin and rutin, the two flavonoids that are most abundant in sweet basil, have been proven to have anti-inflammatory and cancer-related cytoprotective activities.

Tandi et al., 2021 revealed that, according to the phytochemical screening, flavonoid compounds have the highest quantities of other secondary metabolites. It has also been reported that flavonoids affect the inhibition of α -glucosidase and dipeptidyl peptidase-4 (DPP4). Aside from flavonoids, other constituents have been reported to be associated with antidiabetic activities, such as alkaloids, saponins, and tannins. Furthermore, sweet basil was widely used empirically to reduce blood sugar levels in diabetic patients. It has been reported that basil leaves have antihyperglycemic and liver-protective properties. They stimulate insulin release from the pancreas, inhibit glucose production in the liver, and increase glycogen synthesis. (Widjaja et al., 2019).

METHODOLOGY

The researchers utilized an experimental research approach, manipulating one variable to observe its impact on another. They applied controlled research methods and randomly assigned study participants to test their hypotheses. This method successfully established the relationship between two variables, specifically examining the effectiveness of sweet basil (*Ocimum basilicum*) crude extract and Acarbose in reducing blood glucose levels in rats with

Alloxan-induced type II diabetes. (Cherry, 2022). The study provided a reasonable conclusion and yielded specific results, confirming the hypothesis regarding the effects of these substances on blood glucose in diabetic rats. The fresh leaves of sweet basil (*Ocimum Basilicum*) were obtained from Villasis Fresh Herbs, Pangasinan, and identified at the Institute of Biology, College of Science, University of the Philippines, Diliman. The leaves were weighed and washed before air-drying, then ground into a fine powder before extraction. The collected 430g of powdered sweet basil leaves (*Ocimum Basilicum*) was weighed in a container and macerated using 95% ethyl alcohol for 48 hours with occasional stirring. The mixture was filtered and evaporated to dryness in a water bath until the concentrated extract was collected, weighed, and computed the percentage yield. The extract was transferred to a glass bottle and stored in the refrigerator for further use.

The in vivo analysis was conducted at Esteleydes Animal Laboratory and Research Facility owned by the IACUC Chairman, Dr. Leonardo I. Esteleydes DVM. Also, who was also the veterinarian in charge of supervising the study until it was finished, and under his guidance, the research protocols were followed. Acarbose 50 mg was utilized as the positive control. It was dissolved in distilled water at a 0.5 mg/ml concentration and administered orally in diabetic rats for one day at different intervals. On the other hand, distilled water was utilized as the negative control. The veterinarian conducted the induction of Diabetes via intraperitoneal injection of alloxan monohydrate 150 mg/kg. After two days, the glucose levels of Sprague Dawley Rats were checked using the OGTT, and blood glucose levels of more than 126 mg/dL were considered diabetic. The rats were sugar-overloaded one hour before the treatment.

Fifteen (15) Sprague Dawley rats are grouped into three groups. Two groups are treated with Acarbose, and the other is with *Ocimum basilicum* (Sweet basil) crude extract. The acclimatization of the rats was accomplished with the guidance of the animal facility. Before conducting the experimental procedures and treatment, the Sprague-Dawley rats were acclimatized for seven days to adjust to their new environment. The researchers adhered to the standard protocol, which included keeping the cage in an air-conditioned setting with a temperature of 24 + 2 degrees Celsius and relative humidity between 45 and 55%. For illumination, a 12- hour light-dark cycle was utilized. The animal diet consisted of standard rat pellets and potable drinking water. The rats were given food and water ad libitum. The research strictly follows and corresponds to the principle of the Institutional Animal Care and Use Committee (IACUC) recommendations that were strictly followed for animal care.

All groups were sugar-overloaded before the treatments, and the feeding schedule was stopped but continually given with water as necessary. The Oral Glucose Tolerance Test (OGTT) determined the fasting blood sugar level. A drop of blood is placed in a calibrated glucometer to be used for data collection. It was done by pricking the lateral tail vein of the rat using a lancet every 0, 60, 120, 180, and 240 minutes after administering the treatments. The Acarbose was given at a dose of 50 mg/kg weight of rat, the *Ocimum basilicum* (Sweet basil) crude extract was given at a dose of 400 mg/kg weight of rat, and the distilled water, which is the negative control were provided 5 mL/kg weight of rat using the oral gavage with the maximum allowable volume of 10 mL/kg. Five blood collections were done within the whole-time frame of the experimentation. During the blood collection and the treatment, the rats were restrained using the standard tail and neck finger restraining. One-Way Repeated-Measures Analysis of Variance (ANOVA) was conducted to determine the significant difference in the blood glucose-lowering activity within each treatment group. Post Hoc Tukey-HSD (Beta) was performed for the pairwise comparison Between the blood glucose-

lowering activity within each treatment group of Acarbose (positive control), sweet basil leaves extract, and distilled water (negative control) across the different time intervals of administration.

RESULTS

Table 1
Percentage Yield of Collected Sweet Basil Leaves Extract

Weight of Fresh Sweet Basil Leaves	20,000g
Weight of Sweet Basil Leaves extract	21.43g
Percentage yield	0.107%

The percentage yield of collected sweet basil leaf extract is shown in Table 1. The Sweet basil leaves consisted of 20,000g fresh sweet basil leaves as an actual yield, 21.43g basil leaves extract theoretical yield, and a total of 0.107 % percentage yield.

Table 2
Results of Organoleptic Evaluation on the Collected *Ocimum Basilicum* (Sweet Basil) leaves extract

PHYSICAL TEST PARAMETERS	SWEET BASIL LEAVES EXTRACT
Color	Dark Green
Odor	Strong Grassy Herbal Smell
Appearance	Thick dark green with a syrup consistency
Taste	Bittersweet
pH	7

The results of the organoleptic evaluation of the collected sweet basil (*Ocimum basilicum* Linn.) leaves are presented in Table 2. The collected leaves extract was observed to be dark green in color with a thick syrupy consistency, a strong, grassy herbal odor, and a bitter-sweet taste. The pH is 7, indicating that the crude extract has neutral pH.

Table 3
Results of the Solubility Behavior of the Collected *Ocimum Basilicum* (Sweet Basil) leaves extract

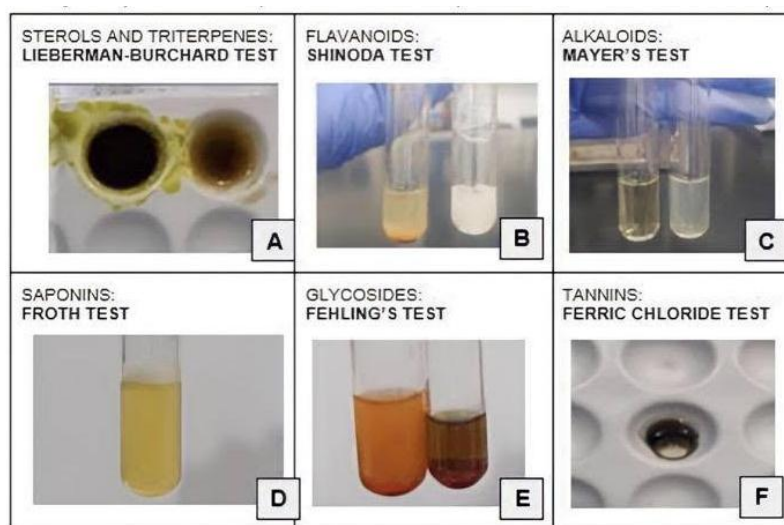
SOLVENT	SWEET BASIL LEAVES EXTRACT	
	Trial 1	Trial 2
Polar Solvents		
Distilled Water	Practically Insoluble	Practically Insoluble

Methanol	Slightly Soluble	Slightly Soluble
NSS	Practically Insoluble	Practically Insoluble
Non-Polar Solvents		
Methylene Chloride	Very Soluble	Very Soluble
Xylene	Very Soluble	Very Soluble
Hexane	Very Soluble	Very Soluble

As shown in Table 3, the sweet basil (*Ocimum basilicum* Linn.) was very soluble in non-polar solvents (Methylene Chloride, Xylene, and Hexane). On the other hand, the crude extract was practically insoluble in polar solvents (Distilled water and NSS) and slightly soluble in methanol.

Table 4
Results of Phytochemical Screening Performed on the Collected *Ocimum Basilicum* (Sweet Basil) leaves extract.

Phytochemical Constituents	Name of Test Performed	Expected Results	Leaves	
			Visible Results	Interpretation
Sterols	Lieberman-Burchard Test	Reddish brown coloration in the interface	Reddish brown coloration in the interface	(+) Positive
Triterpenes		Greenish color that turns blue on standing	Greenish color that turns blue on standing	(+) Positive
Flavonoids	Shinoda Test	Intense yellow coloration	Yellow coloration	(+) Positive
Alkaloids	Mayer's Test	Yellowish or white precipitate	White precipitate	(+) Positive
Saponins	Froth Test	Froth in 10 minutes	Froth in 10 mins	(+) Positive
Glycosides	Fehling's Test	Reddish- brown precipitate	Reddish- brown precipitate	(+) Positive
Tannins	Gelatin Test	White precipitate	White precipitate	(+) Positive

**Legend:**

A. Liebermann-Burchard Test

B. Shinoda Test

C. Mayer's Test

D. Froth Test

E. Fehling's Test

F. Ferric Chloride Test

Figure 1. *Ocimum basilicum* Extract Phytochemical Screening Results

Table 4 shows the specific phytochemical constituents present in Sweet Basil Leaves extract, including the test conducted and the visible results after the test, which will specify if certain phytochemicals are present. These phytochemical constituents are all responsible for blood glucose-lowering activity in Alloxan-induced Sprague Dawley rats. This means that chances are high that Sweet Basil leaves have antidiabetic activity.

Results obtained supports the study conducted by Tandi et al., 2021 that Basil leaf extract may reduce blood sugar and manage diabetes because of its hypoglycemic impact the presence of saponins, alkaloids, glycosides, tannins, flavonoids, steroids, and triterpenoids has been observed.

Table 5
Results of Blood Glucose Level After Administration of Alloxan Solution

Rat No.	Baseline Fasting Blood Sugar	Weight	After 2 days of Alloxan-Induction	Weight
1	65 mg/dL	108 g	180 mg/dL	110 g
2	68 mg/dL	100 g	134 mg/dL	102 g
3	96 mg/dL	122 g	535 mg/dL	118 g
4	62 mg/dL	124 g	HIGH (600 mg/dL or higher)	116 g
5	92 mg/dL	100 g	376 mg/dL	94 g
6	92 mg/dL	114 g	566 mg/dL	108 g
7	68 mg/dL	96 g	496 mg/dL	84 g
8	90 mg/dL	120 g	137 mg/dL	120 g
9	79 mg/dL	114 g	345 mg/dL	114 g

10	76 mg/dL	96 g	138 mg/dL	90 g
11	60mg/dL	102 g	546 mg/dL	98 g
12	69 mg/dL	108 g	438 mg/dL	110 g
13	82 mg/dL	118 g	433 mg/dL	116 g
14	75 mg/dL	122 g	157 mg/dL	120 g
15	91 mg/dL	118 g	134 mg/dL	122 g

To increase the blood glucose level of the test animals, alloxan solution was administered via intraperitoneal injection to the Sprague-Dawley rats two days prior to the treatment. As shown in table 5, the baseline fasting blood sugar including the initial weight of the test animals were recorded, then after 2 days, it was observed that there was significant increase in blood glucose to peak levels in all the rats, to make sure that the rats were sugar-overloaded one hour before the treatment. Data presented revealed that all the representative fifteen (15) Sprague-Dawley rats produced significant increase in the blood glucose level with considerable increase in their body weights. The same data showing increased blood glucose level were used to determine whether administration of the sweet basil leaves extract would cause the anticipated lowering activity. Thus, all the alloxan treated Sprague-Dawley rats are considered diabetic control group, and treatment was promptly initiated right after the identification of diabetes in the rats.

The induction of diabetes to test animals was adopted from the study of Rumengan et al 2019, diabetes mellitus was induced in fasted rats by a single intraperitoneal injection of freshly prepared Alloxan monohydrate dissolved in sterile normal saline, administered at a dose of 150 mg/kg body weight. After three days, measurements of fasting blood glucose levels confirmed the presence of diabetes mellitus. Rats with fasting blood glucose levels exceeding 126 mg/dL were categorized as diabetic and used in the subsequent experiments. Following the diagnosis of diabetes in rats, treatment was started right away. After receiving treatment, fasting blood sugar levels were checked every hour. All blood samples were taken at regular intervals from the rats' tail arteries.

Table 6
Results of blood glucose lowering activity of the Treatment Groups on Alloxan-induced diabetic Sprague-Dawley rats

Rat No.	Weight	Blood Glucose Level After Alloxan-Induction	Weight	Blood Glucose Level After 4 hours of Administration	Decrease in Blood glucose level
Positive Control (Acarbose 50mg)					
1	110 g	180 mg/dL	108 g	124 mg/dL	56 mg/dL
2	102 g	134 mg/dL	104 g	121 mg/dL	13 mg/dL
3	118 g	535 mg/dL	124 g	100 mg/dL	435 mg/dL
4	116 g	HIGH (600 mg/dL or higher)	98 g	288 mg/dL	312 mg/dL
5	94 g	376 mg/dL	100 g	127 mg/dL	249 mg/dL
Experimental Control (Sweet Basil Leaves extract 400mg)					
6	108 g	566 mg/dL	110 g	323 mg/dL	243 mg/dL
7	84 g	496 mg/dL	80 g	255 mg/dL	241 mg/dL

8	120 g	137 mg/dL	116 g	107 mg/dL	30 mg/dL
9	114 g	345 mg/dL	98 g	137 mg/dL	208 mg/dL
10	90 g	138 mg/dL	90 g	123 mg/dL	15 mg/dL
Negative Control (Distilled water)					
11	98 g	546 mg/dL	98 g	556 mg/dL	(+) 10mg/dL
12	110 g	438 mg/dL	106 g	485 mg/dL	(+) 47mg/dL
13	116 g	433 mg/dL	118 g	468mg/dL	(+) 35mg/dL
14	120 g	157 mg/dL	118 g	199 mg/dL	(+) 42mg/dL
15	122 g	134 mg/dL	118 g	141 mg/dL	(+) 7mg/dL

Results of blood glucose lowering activity of the treatment groups on Alloxan induced diabetic Sprague-Dawley rats showed that the administration of acarbose 50mg, sweet basil extract 400mg, and distilled water treated groups displayed considerable change in the blood glucose levels after 4 hours of treatment. The treated groups with acarbose 50mg exhibited significant decrease in the blood glucose levels ranging from 13mg/dL to as high as 435mg/dL in rats' number 1 to 5, while those treated with sweet basil leaves extract 400mg showed substantial decrease of 15mg/dL to 243mg/dL in rats' number 6 to 10 based on the decrease in their blood glucose level after Alloxan-induction. This may infer that the sweet basil treated group showed comparable blood glucose lowering activity with the acarbose 50mg treated group. Data demonstrated that both the acarbose 50mg and sweet basil leaves extract 400mg treated groups confirmed their blood glucose lowering activities after the treatment. The observed decrease in blood glucose levels at this dosage suggests that it may be attributed to the inhibition of carbohydrate metabolizing enzymes, which restricts glucose absorption, as well as the promotion of hepatic glucose mobilization.

The supporting research by Lazzaroni E. et al., 2022, suggests that acarbose, the compound under consideration is a multifaceted oligosaccharide that functions as a competitive and reversible inhibitor of pancreatic alpha-amylase as well as membrane-bound intestinal alpha-glucoside hydrolase. It facilitates the hydrolysis of complex carbohydrates into oligosaccharides within the small intestine. Additionally, the intestinal alpha-glucosidase hydrolase is responsible for the conversion of oligosaccharides, trisaccharides, and disaccharides (such as sucrose and maltose) into monosaccharides (specifically glucose and fructose) within the brush border of the small intestine. Acarbose retards the process of carbohydrate digestion, hence decelerating the absorption of glucose., decreasing postprandial glucose blood concentrations. On the other hand, the study's findings supporting the blood sugar-lowering properties of sweet basil leaves supported the theory by Widjaja et al. in 2019 that sweet basil leaves are frequently used to lower blood sugar in diabetic patients. Basil leaves are said to promote insulin release from the pancreas, the inhibition of hepatic glucose production and the promotion of glycogen synthesis, all of which have been linked to antihyperglycemic and liver-protective characteristics. Eziana et al., 2017 also discovered that the extract prevented maltose from being produced by α -amylase. Aspartate aminotransferase and alanine aminotransferase levels were also lowered in the diabetes treatment groups by the sweet basil extract.

Finally, the negative control (distilled water) treated group showed contradictory results with the positive control and experimental control showing decreased blood glucose levels, since the test animals represented by rats' number 11 to 15 were already observed to have increased blood glucose levels without any intervention, the test animals were all detected to have developed hyperglycemia over-time as shown by the heightened blood glucose levels.

Table 7

Summary of ANOVA F-test for the significant difference in the blood glucose-lowering activity within each treatment group of acarbose (Positive control), sweet basil leaves extract, and distilled water (Negative control) across the different time intervals of administration

Source	SS	df	MS	F-ratio	p-value	Interpretation / Decision
Between treatments	164316.13	2	82158.067	F= 6.18021	p-value is .014288	Significant at p<.05
Within treatments	159524.8	12	13293.73			Reject Ho
Total	323840.93	14				

Results of ANOVA F-test in table 7 showed that significant differences existed between the blood glucose-lowering activity of the different treatment groups of acarbose (positive control), sweet basil leaves extract, and distilled water (negative control) across the different time intervals of administration as indicated by the computed F-value of 6.18021 with a p-value of .014288 being lower the .05 level of significance at 2 degrees of freedom between treatments, and 12 degrees of freedom within treatments, therefore, the hypothesis postulated in chapter 1 was hereby rejected. Data implied that although, the treatment groups of acarbose (positive control) and sweet basil leaves extract (experimental group) exhibit comparable blood glucose lowering activity, the treatment groups between acarbose and distilled water (negative control) as well as sweet basil leaves extract and the negative control significantly differ in terms of their blood glucose lowering activity, therefore, since not all these variables did not meet similar expected results, it instigated discrepancies in the overall blood glucose lowering of the concerned variables.

Table 8

Pairwise Comparison {(Post Hoc Tukey-HSD (Beta))} Between the blood glucose-lowering activity within each treatment group of acarbose (Positive control), sweet basil leaves extract, and distilled water (Negative control) across the different time intervals of administration.

Paired Variables	Weighted Mean		HSD _{.05} = 194.5422	Q _{.05} = 3.7729	p-value	Interpretation	Decision
positive control (Acarbose 50mg) vs Sweet basil leaves extract 400mg	152	189	37.00	0.72	.86918	Not Significant at p>.05	Acarbose 50mg has comparable blood glucose lowering with Sweet basil leaves extract 400mg
positive control (Acarbose 50mg) vs Negative control (distilled water)	152	390.20	238.20	4.62	.01718	Significant at p<.05	Acarbose 50mg has better blood glucose lowering
Sweet basil	189	390.20	201.20	3.90	.04256	Significant at	Sweet basil

leaves extract 400mg vs Negative control (distilled water)						p<.05	leaves extract 400mg has better blood glucose lowering
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Data on table 8 shows that the positive control acarbose 50mg, (M=152) exhibit better blood glucose lowering activity than the experimental control, sweet basil 400mg leaves extract (M=189) as indicated by the computed p-value of .89791, which is greater than .05 level of significance. Further analysis revealed that HSD.05 of 37 is lower than the true value of HSD.05 of 222.2988, while the Q.05 of 0.63 is even lower than the true Q.05 value of 3.7729, hence, no significant difference existed between these variables indicating that the 400mg sweet basil extract has comparable blood glucose lowering with that of acarbose 50mg.

Between the positive control (Acarbose 50mg) and the Negative control (distilled water), both the computed HSD.05 equivalent to 238.20 and Q.05 of 3.7729 are greater than their standard values of 4.62 194.5422, which gave a p-value of .01718 lower than .05 level of significance, thus, significant difference existed between these variables indicating that acarbose 50mg has exhibit blood glucose lowering, whereas, the negative control, distilled water, was observed to cause higher blood glucose level in the test animals.

Lastly, sweet basil leaves extract 400mg exhibit blood glucose lowering, as shown by the computed HSD.05 of 201.20, and a Q.05 of 3.90, which are both found greater than their standard values. Therefore, it may be implied by these findings that sweet basil leaves extract 400mg exhibit blood glucose lowering while the negative control, distilled water, was similarly observed to cause an even increase blood glucose level in the test animals.

Therefore, it may be concluded that the blood glucose-lowering effects of 400 mg of sweet basil leaf extract are equivalent to 50 mg of acarbose, the positive control.

DISCUSSION

Sweet basil (*Ocimum basilicum* Linn.) leaves extract contains phytochemical constituents, including triterpenoids, steroids, alkaloids, glycosides, tannins, flavonoids, and saponins. The extracted plant produces a thick, sticky, dark green substance with a syrupy consistency and a strong, grassy, herbal odor. It also has a bittersweet taste and is insoluble in water. It has been demonstrated that administering sweet basil leaf extract to diabetic rats induced with alloxan lowers blood glucose levels in all basil leaf extract groups. At a dose of 400 mg/kgBW, the efficacy of sweet basil leaf extract was demonstrated in Table 6. There is evidence that quercetin and basil leaf extract have antihyperglycemic properties. The specific flavonoid present in the plant has the highest levels that are accountable for the activity of reducing blood glucose levels to the study by Tandi J. (2021). The mechanism of action of Flavonoids in lowering blood glucose is by improving glucose tolerance by reducing the postprandial rise in blood sugar level, likely through enhanced insulin sensitivity or 48 increased glucose uptakes by skeletal muscle and adipose tissue Al-Ishaq, R (2019).

Acarbose 50 mg vs. Sweet Basil leaf extract 400 mg has no significant difference, stating that the 400mg of Sweet Basil Leaf Extract has the same efficacy as Acarbose 50 mg. The

comparison of Acarbose 50 mg vs. the negative control group (Distilled water) showed a significant difference indicating the effectiveness of Acarbose in treating type 2 diabetes. While the comparison of Sweet Basil leaf extracts 400 mg vs. negative control group (Distilled water) showed a significant difference, indicating that sweet basil leaf extract exhibits blood glucose lowering activity in alloxan-induced diabetes Sprague-Dawley rats.

CONCLUSION

Sweet basil leaf extract was proven to have blood glucose-lowering activity in alloxan-induced diabetic Sprague-Dawley rats and blood glucose-lowering activity differed significantly within each treatment group of Acarbose (Positive control), sweet basil leaves extract, and distilled water (Negative control) across the different time intervals of administration.

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REFERENCES

Acarbose - American Chemical Society. (n.d.). American Chemical Society. <https://www.acs.org/molecule-of-the-week/archive/a/acarbose.html>

Al-Ishaq, R. K., Abotaleb, M., Büsselberg, D., Kajo, K., & Büsselberg, D. (2019). Flavonoids and their Anti-Diabetic Effects: Cellular mechanisms and effects to improve blood sugar levels. *Biomolecules*, 9(9), 430. <https://doi.org/10.3390/biom9090430>

- Altay M. Acarbose is again on the stage. *World J Diabetes* 2022; 13(1): 1-4 [PMID: 35070055 DOI: 10.4239/wjd.v13.i1.1]
- Akmal, M. (2022, August 12). *Alpha glucosidase inhibitors*. StatPearls - NCBI Bookshelf. <https://www.ncbi.nlm.nih.gov/books/NBK557848/>
- Dhanya, R. (2022). Quercetin for managing type 2 diabetes and its complications, an insight into multitarget therapy. *Biomedicine & Pharmacotherapy*, 146, 112560. <https://doi.org/10.1016/j.biopha.2021.112560>
- Ezeani, C., Ezenyi, I. C., Okoye, T. C., & Okoli, C. O. (2017). *Ocimum basilicum extract exhibits antidiabetic effects via inhibition of hepatic glucose mobilization and carbohydrate metabolizing enzymes*. *Journal of Intercultural Ethnopharmacology*, 6(1), 22. <https://doi.org/10.5455/jice.20161229054825>
- Falowo, A. B., Mukumbo, F. E., Idamokoro, E. M., Afolayan, A. J., & Muchenje, V. (2019). *Phytochemical Constituents and Antioxidant Activity of Sweet Basil (Ocimum basilicum L.) Essential Oil on Ground Beef from Boran and Nguni Cattle*. *International Journal of Food Science*, 2019, 1–8. <https://doi.org/10.1155/2019/2628747>
- F M Rumengan et al (2019) Antihyperglycemic capacity of basil (*Ocimum basilicum* L.) leaves extracts coated with the marine fish scales derived nanochitosan <https://iopscience.iop.org/article/10.1088/1757-899X/567/1/012023/pdf>
- Goyal, R. (2023, May 8). Type 2 diabetes. StatPearls - NCBI Bookshelf. <https://www.ncbi.nlm.nih.gov/books/NBK513253/>
- Katzung B.G.(Ed.), (2018). *Basic & Clinical Pharmacology*, 14e. McGraw Hill. <https://accessmedicine.mhmedical.com/content.aspx?bookid=2249§ionid=175215158>
- Kennard, M. R., Nandi, M., Chapple, S., & King, A. J. (2022). The glucose tolerance test in mice: Sex, drugs, and protocol. *Diabetes, Obesity and Metabolism*, 24(11), 2241–2252. <https://doi.org/10.1111/dom.14811>
- Khalil, H. E., Abdelwahab, M. F., Emeka, P. M., Badger-Emeka, L. I., Thirugnanasambantham, K., Ibrahim, H. M., Naguib, S. M., Matsunami, K., & Abdel-Wahab, N. M. (2022). Ameliorative Effect of *Ocimum forskolei* Benth on Diabetic, Apoptotic, and Adipogenic Biomarkers of Diabetic Rats and 3T3-L1 Fibroblasts Assisted by In Silico Approach. *Molecules*, 27(9), 2800. <https://doi.org/10.3390/molecules27092800>
- Khin PP, Lee JH, Jun H-S. Pancreatic Beta-cell Dysfunction in Type 2 Diabetes. *European Journal of Inflammation*. 2023;21. doi:10.1177/1721727X231154152
- McIver, L. A. (2022, September 21). Acarbose. StatPearls - NCBI Bookshelf. <https://www.ncbi.nlm.nih.gov/books/NBK493214/>
- Nadeem HR, Akhtar S, Sestili P, Ismail T, Neugart S, Qamar M, Esatbeyoglu T. Toxicity, Antioxidant Activity, and Phytochemicals of Basil (*Ocimum basilicum* L.) Leaves Cultivated in Southern Punjab, Pakistan. *Foods*. 2022 Apr 26;11(9):1239. doi: 10.3390/foods11091239. PMID: 35563962; PMCID: PMC9102432.
- National Institute of Diabetes and Digestive and Kidney Diseases. (2021, January 10). *Acarbose. LiverTox - NCBI Bookshelf*. <https://www.ncbi.nlm.nih.gov/books/NBK548181/#:~:text=Acarbose%20is%20an%20alpha%20glucosidase,clinically%20apparent%20acute%20liver%20injury>.
- Nguyen, V. T. et al 2021 IOP Conf. Ser.: Mater. Sci. Eng. 1092 012083 Studies on chemical, polyphenol content, flavonoid content, and antioxidant activity of sweet basil leaves (*Ocimum basilicum* L.) <https://iopscience.iop.org/article/10.1088/1757-899X/1092/1/012083/pdf>

- Nguyen, V. H., Nguyen, N., Thi, N. Q. N., Thi, C. Q. N., Truc, T. T., & Nghi, P. T. (2021). Studies on chemical, polyphenol content, flavonoid content, and antioxidant activity of sweet basil leaves (*Ocimum basilicum* L.). IOP Conference Series, 1092(1), 012083. <https://doi.org/10.1088/1757-899x/1092/1/012083>
- Purushothaman, B., Suganthi, N., & Shanmugam, K. (2020). Qualitative and Quantitative Determination of Various Extracts of *Ocimum basilicum* L. Leaves. Journal of Natural Remedies, 20(1), 53–60. <https://doi.org/10.18311/jnr/2020/24113>
- Republic of the Philippines (2022) 2022 causes of Deaths in the Philippines. https://psa.gov.ph/sites/default/files/attachments/crd/pressrelease/1_Press%20Release_2022%20Cause%20of%20Death%20Statistics%20as%20of%2031%20March%202022_VSD%20Draft_JRV_CRD_rev_VSD%20updated_JRV_CRD_clean%20file-signed.pdf
- Rumengan, F., Mandey, L., Citraningiyas, G., & Luntungan, A. (2019). *Antihyperglycemic capacity of basil (Ocimum basilicum L.) leaves extracts coated with the marine fish scales derived nanochitosan*. IOP Conference Series: Materials Science and Engineering, 567, 012023. <https://doi.org/10.1088/1757-899X/567/1/012023>
- Shahrajabian, M. H., Sun, W., & Cheng, Q. (2020). Chemical components and pharmacological benefits of Basil (*Ocimum basilicum*): a review. International Journal of Food Properties, 23(1), 1961–1970. <https://doi.org/10.1080/10942912.2020.1828456>
- Tambun, R., Alexander, V., & Ginting, Y. (2021). Performance comparison of maceration method, soxhletation method, and microwave-assisted extraction in extracting active compounds from soursop leaves (*Annona muricata*): A review. IOP Conference Series, 1122(1), 012095. <https://doi.org/10.1088/1757-899x/1122/1/012095>
- Tandi, J., Handayani, T., & Widodo, A. (2021). *Qualitative and Quantitative Determination of Secondary Metabolites and Antidiabetic Potential of Ocimum basilicum L. LEAVES EXTRACT*. Rasayanjournal, 14(0974–1496). http://rasayanjournal.co.in/admin/php/upload/3109_pdf.pdf?fbclid=IwAR00TeExYhL AkX-PizbirD_4_7As7zg3PSPIvEvWVgxb39MMkqDPkboxw_oI
- Teofilović, B., Tomas, A., Martić, N., Stilinović, N., Popović, M., Čapo, I., Grujić, N., Ilincic, B., & Rašković, A. (2021). *Antioxidant and hepatoprotective potential of sweet basil (Ocimum basilicum L.) extract in acetaminophen-induced hepatotoxicity in rats*. Journal of Functional Foods, 87, 104783. <https://doi.org/10.1016/j.jff.2021.104783>
- Wang Z, Wang J, Hu J, Chen Y, Dong B, Wang Y. A comparative study of acarbose, vildagliptin and saxagliptin intended for better efficacy and safety on type 2 diabetes mellitus treatment. Life Sci. <http://www.ncbi.nlm.nih.gov/pubmed/33460667>
- Washington State University. (n.d.). <https://lcme.wsu.edu/research-organisms/sweet-basil/>
- Widjaja, S. S., Rusdiana, R., Savira, M., & Amelia, R. (2021). *Antihyperglycemic, Endothelial protection and Toxicity study of Basil Leaves Extract on Diabetic Rats*. Open Access Macedonian Journal of Medical Sciences, 9(A), 589–594. <https://doi.org/10.3889/oamjms.2021.6520>
- Zakaria, Z., Ahmad, M. N., & Qinna, N. A. (2021). Animal Models in Type 2 Diabetes Mellitus Research: Pros and Cons. Jordan Journal of Agricultural Sciences, 17(4), 425–440. <https://doi.org/10.35516/jjas.v17i4.95>
- Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: a comprehensive review. Chinese medicine, 13, 20. <https://doi.org/10.1186/s13020-018-0177-x>