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POTENTIAL HYPOGLYCEMIC EFFECT OF BINAYUYO (*ANTIDESMA GHAESEMBILLA*, FAMILY: PHYLLANTHACEAE) LEAVES CRUDE EXTRACT – ALLOXAN INDUCED HYPERGLYCEMIC INSTITUTE OF CANCER RESEARCH (ICR) MICE

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ABSTRACT

Plants have been one of the most used natural resources for medical purposes especially in Asia. The world's population (75-80%) relies on plants for their primary health care according to the World Health Organization (WHO). Perceiving the enormous potential, they offer, therapeutic plants have been continually utilized in defining new medications. This study aimed to determine the potential hypoglycemic effect of Binayuyo leaves crude extract (*Antidesma ghaesembilla*, Family: Phyllanthaceae) using Alloxan induced hyperglycemic Institute of Cancer Research (ICR) mice. Binayuyo tree grows with a thick crown that could be measured up to 20 meters tall. Its fruit has a touch of purple. Other parts of the tree were accounted to be used for food and medication. The researchers used the ethanolic crude extract of Binayuyo leaves to be tested for the potential hyperglycemic effect. The crude extract was put to rotary evaporator to discard excess alcohol. After the process, the product was used to mice. The study employed a total of 36 ICR mice wherein 6 mice were allocated per group. There were five groups in the study; Distilled water as the negative control, insulin as the positive control, 500 mg Binayuyo extract, 750 mg Binayuyo extract, and 1000 mg Binayuyo extract. Observations were done in a four-hour period. Results showed that distilled water does not significantly lower the glucose level in 4 hours of observation. Binayuyo shows a potential hypoglycemic effect on the fourth hour of observation compared with the glucose level of post-alloxan. Binayuyo at 750 mg and 1000 mg of concentration has no significant difference with insulin in lowering glucose level.

KEYWORDS: Antidesma ghaesembilla; Alloxan induced; ICR Mice; Binayuyo Leaves; Potential Hypoglycemic Effect.

INTRODUCTION

Plants have been one of the most used natural resources for medical purposes especially in Asia. The world's population (75-80%) relies, partly or entirely, on plants for their primary health care according to the World Health Organization (WHO). Recognizing the tremendous potential they offer, medicinal plants have been constantly used in formulating new drugs. Today, there are almost 150 drugs that are derived from plants or are synthetic modifications or copies of the naturally obtained substances from plants. Seeing the huge benefits of medicinal plants, in 1992 the Philippines' Department of Health (DOH) launched the Traditional Medicine Program. The Traditional Medicine Program, by virtue of Administrative Order No. 12, was set to promote and advocate traditional medicine in the Philippines.[21] Having almost half of the Philippines' land area as forest, the researchers were convinced to use binayuyo, *Antidesma ghaesembilla*, Family: Phyllanthaceae as a plant sample for hypoglycemic effect on type 2 diabetes. Diabetes mellitus is a chronic systemic disease characterized by disorders in[1] metabolism of insulin and of carbohydrate, fat, and protein and[2] the structure and function of blood vessels. It ordinarily appears as one of two recognized clinical pictures – the juvenile (or growth-onset, ketosis-prone) type (Type 1) or the more common adult-onset, ketosis-resistant type (Type 2). The incidence of type 1 and type 2 is 80% obese, maturity-onset diabetes, 10% non-obese, stable adult diabetes, 5% Brittle or labile adult diabetes and 5% Juvenile diabetes. Type 1 diabetes mellitus (5%–10% of cases) is commonly seen less than 35 years old usually develops in childhood or early adulthood and results from autoimmune-mediated destruction of pancreatic β-cells, resulting in absolute deficiency of insulin. Type 2 diabetes mellitus (90% of cases) is characterized by a combination of some degree of insulin resistance and relative insulin deficiency. Due to the high percentage of cases of type 2 DM the researchers aimed

to conduct a study for potential plant alternative medication.[31]

MATERIALS AND METHODS

Sample collection

The leaves of Binayuyo (*Antidesma ghaesembilla*) were collected at Taclobo, San Fernando, Sibuyan Island, Romblon. The collection of leaves was done on June 24, 2019. Plastic sack served as the temporary containers for the leaf samples during the shipment period.

Petioles and necrotic parts of collected leaves were cut and thrown away. The remaining samples were washed with tap water to eliminate dirt, which may contaminate the crude extract. One kilo of oven-dried leaves (at 90ºC - 100°C for five hours) was crushed and used for extraction.

Extraction Procedure

Approximately 4 kg leaves were extracted twice with 8L of 80% ethanol by maceration for 48 hours. These were then filtered, and the solvent removed by rotary evaporation and lyophilisation.[4]

Phytochemical Screening

The methods used have been so designed as to provide a simple and rapid method for screening with a minimum of equipment, reasonably selective for the kind of constituents under study with provisions for some quantitative measure of evaluation.

Test for Alkaloids

In testing for alkaloids, residue from 35 mL 80% ethanolic extract was dissolved in 3.5 mL of 1% hydrochloric acid with the aid of the 1-2 minute steam bath. The solution was cooled and filtered. The filtrate was added with a few grains of powdered sodium chloride, shaken and refiltered. In the filtrate, 0.5 mL was placed into each of four small test tubes. A few drops of Modified Mayer's reagent was added into the first test tube, Valser's reagent into the second test tube, Wagner's reagent into the third test tube, and Bouchardt's reagent into the last test tube. Formation of precipitate indicates the presence of alkaloid.

Modified Borntrager Test

0.3 g of the plant powder was heated with 10 mL of 0.5 N potassium hydroxide and 1 mL of diluted hydrogen peroxide for 10 minutes. The filtrate was cooled, filtered and acidified with approximately 10 drops of glacial and partition with 10 mL of benzene. The benzene phase was filtered and transferred to a test tube containing 2.5 mL of ammonia. The solution was stirred well. Change in color to pink indicates the presence of anthraquinones.

Screening for flavonoids

Evaporate 30mL of 80% ethanolic extract to dryness on water bath, then cool the residue to room temperature and add 15mL of light petroleum ether. (CAUTION: solvents are inflammable). Mix well and filter. Repeat with additional volumes of petroleum ether as needed until last volume of petroleum ether is colorless. Combine the ethereal filtrates. Then dissolve the defatted residue in 30mL of 50% ethanol after that filter it and place 1-2 mL of the filtrate in each of three test tubes then perform the following test.

Test for leucoanthocyanins: bate-smith and metcalf test

To test tube #1 add 0.5 mL of concentrated hydrochloric acid, then warm it in a steam bath for about 5 mins and observe the color changes then the development of a red – violet color is indicative of the presence of leucoanthocyanins. Then the color formation may be slow but if the color is not immediately apparent, allow the test solution to stand at room temperature for 1 hour before recording the result as negative.

Test for - benzopyrone nucleus: Wilstatter "cyanidin" test

To test tube #2 add 0.5 mL of cone HCl and add 3-4 magnesium turnings. Observe carefully for a color change (green, red etc.) within 10 mins, which is indicative of the presence of flavonols. If definite color is formed, cool and dilute with an equal volume of water and add 1.0 mL of octyl alcohol. Then Shake and allow to separate. The color in the octyl alcohol layer is due to aglycones while the color in the aqueous layer is due to glycosides.

Screening for Tannins and Polyphenols: Ferric Chloride Test

Evaporate 100 mL of 80% ethanolic extract to dryness in a steam bath. Then remove the evaporating dish from the steam bath and add 25mL of hot distilled water to the residue. And mix well with a strirring rod and allow cooling to room temperature spontaneously. Then centrifuge the cooled extract for several minutes and decant the upper half from each tube used. Then add 3-4 drops of 10% sodium chloride solution to the decanted supernatant Precipitation at this point is indicative of salting out reaction probably due to non-tannin components. Then Filter off any precipitate. Add 3mL of filtrate to each of three test tubes. To **tube #1** add 3 drops of 1% gelatin solution, to **tube #2** add same amount of gelatin salt reagent (1% gelatin, 10% sodium chloride); and **tube#3** add several drops of ferric chloride TS. The absence of a reaction with ferric chloride is indicates the absence of tannins and phenolic compounds. A greenish - black color after the addition of ferric chloride correlated with precipitation on the gelatin salt block test indicates the presence of tannins of the catechol type. A blue black color after the addition of ferric chloride correlated with precipitation on the gelatin salt - block test associated with color production after the addition of ferric chloride is indicates the absence of tannins and the presence of other phenolic plant constituents.

Induction of Diabetes

Data

Test Sample: Binayuyo leaves extract
Sample concentration: 500 mg/mL in Distilled water
Negative control: Distilled water
Positive control: Insulin pen Injection
pH of Sample: 5.25
Animals used: Male ICR Mice (23-29 grams)

Procedure

1. Animals were acclimatized for seven (7) days.
2. Animal blood glucose level was determined and recorded after sixteen (16) hours of fasting.
3. Diabetes was induced by injecting 110mg/kg Alloxan solution intraperitoneally to test animals.
4. After forty-eight (48) hours, blood glucose was determined and only test animals with blood glucose determination greater than 200mg/dL was used. The resulting blood glucose was used as the baseline.
5. Five (5) groups with five (5) diabetic animals/dose were used:

a. Group 1 – Negative control
b. Group 2 – Positive control (Insulin at 0.5mL/kg/BW)
c. Group 3 – Test Sample at dose of 500 mg
d. Group 4 – Test Sample at dose of 750 mg
e. Group 5 – Test Sample at dose of 1000 mg

6. Blood was extracted from the tail vein, and blood glucose levels were determined and recorded after 1st, 2nd, 3rd and 4th hours post sample and controls administration.
7. Animal weights were also determined and recorded
8. All animals used in the study were humanely euthanized by the in-house veterinarian and properly disposed (Dost Procedure).

RESULTS AND DISCUSSION

Table 1. Average Glucose Level per Group/ Treatment.

Group	Pre-Alloxan	Post-Alloxan	First Hour	Second Hour	Third Hour	Fourth Hour
Distilled Water	134.00	344.50	299.00	244.67	253.67	206.67
Insulin	128.83	348.67	59.50	37.50	20.33	17.33
Binayuyo 500 mg	149.50	342.33	196.50	269.17	226.33	193.50
Binayuyo 750 mg	137.67	342.00	266.00	219.83	196.67	160.83
Binayuyo 1000 mg	112.17	364.33	130.17	212.50	191.83	134.00

Table above shows the average glucose level per group/treatment. The average glucose level in pre-alloxan is the glucose level before alloxan was being induced. The average glucose level in post-alloxan is the glucose level after the alloxan was being induced. The first four hours glucose level is the time of observation after treatments (Distilled water, Insulin, Binayuyo 500 mg, Binayuyo 750 mg, Binayuyo 1000 mg) were induced.

Table 2: Test for Significant Difference in Distilled Water as Negative Control (Within Treatment).

Compared Group		p-value	Significance
Post-Alloxan	First Hour	.551	Not Significant
	Second Hour	.196	Not Significant
	Third Hour	.238	Not Significant
	Fourth Hour	.078	Not Significant
First Hour	Second Hour	.477	Not Significant
	Third Hour	.553	Not Significant
	Fourth Hour	.231	Not Significant
Second Hour	Third Hour	.906	Not Significant
	Fourth Hour	.618	Not Significant
Third Hour	Fourth Hour	.538	Not Significant
**Significant at .05 alpha level*			

Table above shows the Test for Significant Difference in Distilled Water (Within Treatment). This would means that we are checking the significant decrease of glucose level when distilled water is used as a treatment.

For Post-Alloxan compared with first to fourth hour, the computed p-value is greater than .05 alpha level. This would means that there is no significant difference. Hence, the average glucose level in post-alloxan compared to the average glucose level of the first to fourth hour of observation is at the same level or the difference of the glucose level did not significantly change.

For the first hour of observation, compared to second to fourth hour of observation, the computed p-value is greater than .05 alpha level. This means that there is no significant difference. Hence, the average glucose level in the first hour of observation compared to the average glucose level of the second to fourth hour of observation

is at the same level or the difference of the glucose level did not significantly change.

For the second hour of observation, compared to third and fourth hour of observation, the computed p-value is greater than .05 alpha level. This means that there is no significant difference. Hence, the average glucose level in the second hour of observation compared to the average glucose level of the third and fourth hour of observation is at the same level or the difference of the glucose level did not significantly change.

For the third hour of observation, compared to fourth hour of observation, the computed p-value is greater than .05 alpha level. This means that there is no significant difference. Hence, the average glucose level in the third hour of observation compared to the average glucose level of the fourth hour of observation is at the same level or the difference of the glucose level did not significantly change.

The above analysis shows that distilled water does not significantly decrease glucose level regardless of the time of observation. Although there is a need for more fluid when blood glucose levels are high according to an article entitled "Water and Diabetes published in 2019." However, the results of the study convinced that there is low evidence of the positive effects of water or mineral water in improving glycemic parameters according to the study of according to Naumann et.al. (2017).

Table 3. Test for Significant Difference in Insulin as Positive Control (Within Treatment).

Compared Group		**p-value**	**Significance**
Post-Alloxan	First Hour	.000*	Significant
	Second Hour	.000*	Significant
	Third Hour	.000*	Significant
	Fourth Hour	.000*	Significant
First Hour	Second Hour	.076	Not Significant
	Third Hour	.003*	Significant
	Fourth Hour	.001*	Significant
Second Hour	Third Hour	.162	Not Significant
	Fourth Hour	.102	Not Significant
Third Hour	Fourth Hour	.804	Not Significant
**Significant at .05 alpha level*			

Table above shows the Test for Significant Difference in Insulin as Positive Control (Within Treatment). This means that we are checking the significant decrease of glucose level when insulin is used as a treatment.

Post-Alloxan compared to first to fourth hour of observation, the computed p-value is less than .05 alpha level. This means that there is significant difference in the decrease in glucose level. Hence, the glucose level in post-alloxan is significantly higher than the glucose level in the first to fourth hour of observation when insulin was used as treatment.

For first hour of observation, compared with second hour of observation, the computed p-value is greater than .05 alpha level. This means that there is no significant difference in the decrease in glucose level. Hence, the average glucose level in the first hour of observation compared to the average glucose level of the second hour of observation is at the same level or the difference of the glucose level did not significantly change from first to second hour of observation.

For first hour of observation, compared with third and fourth hour of observation, the computed p-value is less than .05 alpha level. This means that there is significant difference in the decrease in glucose level. Hence, the average glucose level in the first hour of observation is significantly lower compared to the average glucose level of the third and fourth hour of observation when insulin was used.

For the second hour of observation, compared with third and fourth hour of observation, the computed p-value is greater than .05 alpha level. This means that there is no significant difference in the decrease in glucose level. Hence, the average glucose level in the second hour of observation compared to the average glucose level of the third and fourth hour of observation is at the same level or the difference of the glucose level did not significantly change.

For the third hour of observation, compared with fourth hour of observation, the computed p-value is greater than .05 alpha level. This means that there is no significant difference in the decrease in glucose level. Hence, the average glucose level in the third hour of observation compared to the average glucose level of the fourth hour of observation is at the same level or the difference of the glucose level did not significantly change.

The result of the study supports literatures and articles that insulin is a standard treatment to lower glucose level in the body. As cited in an article published in July of 2019 titled *"Diabetes treatment: Using insulin to manage blood sugar."* Insulin therapy is vital for replacing the insulin your body does not produce.

Insulin therapy is often an important part of diabetes treatment.

Table 4. Test for Significant Difference in 500 mg of Binayuyo (Within Treatment).

Compared Group		p-value	Significance
Post-Alloxan	First Hour	.011	Significant
	Second Hour	.182	Not Significant
	Third Hour	.039	Significant
	Fourth Hour	.009	Significant
First Hour	Second Hour	.185	Not Significant
	Third Hour	.582	Not Significant
	Fourth Hour	.956	Not Significant
Second Hour	Third Hour	.431	Not Significant
	Fourth Hour	.168	Not Significant
Third Hour	Fourth Hour	.545	Not Significant
**Significant at .05 alpha level*			

Table above shows the Test for Significant Difference in 500 mg of Binayuyo as (Within Treatment). This means that we are checking the significant decrease of glucose level when 500 mg of Binayuyo is used as a treatment.

Post-alloxan glucose level compared to first to fourth hour, the computed p-value is less than .05 alpha level (except for second hour). This means that there is a significant difference. Hence, there was a significant decrease of glucose level in the first, third and fourth hour of observation from the glucose level of post-alloxan.

For first hour of observation, compared with second, third and fourth hour of observation vis-à-vis, the computed p-value is greater than .05 alpha level. This means that there is no significant difference. Hence, there was no significant difference in the decrease of glucose level in the first to fourth hour of observation or the change of glucose level is at the same level of glucose.

The results of the study shows that there is a significant decrease in the glucose level from the time of post alloxan inducement down to the fourth hour of observation using a 500 mg of Binayuyo extract. The results can say that 500 mg of Binayuyo extract has a hypoglycemic effect. This result is supported by the study of Gargantiel & Ysrael, (2014), which indicates that Binayuyo extract showed a significant hypoglycemic potential.

Table 5: Test for Significant Difference in 750 mg of Binayuyo (Within Treatment).

Compared Group		p-value	Significance
Post-Alloxan	First Hour	.256	Not Significant
	Second Hour	.072	Not Significant
	Third Hour	.034*	Significant
	Fourth Hour	.010*	Significant
First Hour	Second Hour	.487	Not Significant
	Third Hour	.299	Not Significant
	Fourth Hour	.119	Not Significant
Second Hour	Third Hour	.726	Not Significant
	Fourth Hour	.376	Not Significant
Third Hour	Fourth Hour	.589	Not Significant
**Significant at .05 alpha level*			

Table above shows the Test for Significant Difference in 750 mg of Binayuyo (Within Treatment). This means that we are checking the significant decrease of glucose level when 750 mg of Binayuyo is used as a treatment.

Post-alloxan glucose level compared to first and second hour of observation, the computed p-value is greater than .05 alpha level. This means that there is no significant difference. Hence, there is no significant decrease of glucose level in the first and second hour of observation from the glucose level of post-alloxan. However, post-alloxan glucose level compared to third and fourth hour of observation, the computed p-value is less than .05 alpha level. This means that there is significant difference. Hence, there is significant decrease of glucose level in the third and fourth hour of observation from the glucose level of post-alloxan.

For first hour of observation compared with second, third and fourth hour of observation vis-à-vis, the computed p-

value is greater than .05 alpha level. This means that there is no significant difference. Hence, there is no significant difference in the decrease of glucose level in the first to fourth hour of observation or the change of glucose level is at the same level of glucose.

The results of the study shows that there is a significant decrease in the glucose level from the time of post alloxan inducement down to the fourth hour of observation using a 750 mg of Binayuyo extract. The results can say that 750 mg of Binayuyo extract has a hypoglycemic effect.

Table 6. Test for Significant Difference in 1000 mg of Binayuyo (Within Treatment).

Compared Group		Significant	Significance
Post-Alloxan	First Hour	**.016**	Signifcant
	Second Hour	.109	Not Significant
	Third Hour	.070	Not Signifcant
	Fourth Hour	**.018**	Significant
First Hour	Second Hour	.378	Not Significant
	Third Hour	.507	Not Significant
	Fourth Hour	.967	Not Significant
Second Hour	Third Hour	.824	Not Significant
	Fourth Hour	.400	Not Significant
Third Hour	Fourth Hour	.534	Not Significant
**Significant at .05 alpha level*			

Table above shows the Test for Significant Difference in 1000 mg of Binayuyo as (Within Treatment). This means that we are checking the significant decrease of glucose level when 1000 mg of Binayuyo is used as a treatment.

Post-alloxan glucose level compared to first and fourth hour, the computed p-value is less than .05 alpha level (except for second hour). This means that there is a significant difference. Hence, there was a significant decrease of glucose level in the first and fourth hour of observation from the glucose level of post-alloxan. However, post-alloxan glucose level compared to second and third hour of observation, the computed p-value is greater than .05 alpha level (except for second hour). This means that there is no significant difference. Hence, the change in the glucose level of post alloxan compared with second and third hour of observation is not that different or significant.

For first hour of observation, compared with second, third and fourth hour of observation vis-à-vis, the computed p-value is greater than .05 alpha level. This means that there is no significant difference. Hence, there was no significant difference in the decrease of glucose level in the first to fourth hour of observation or the change of glucose level is at the same level of glucose.

Table 7. Test for Significant Difference in Post-Alloxan (Between Treatment).

Compared Groups		p-value	Significance
Insulin	Binayuyo 500 mg	.925	Not Significant
	Binayuyo 750 mg	.921	Not Significant
	Binayuyo 1000 mg	.815	Not Significant
Binayuyo 500 mg	Binayuyo 750 mg	.996	Not Significant
	Binayuyo 1000 mg	.743	Not Significant
Binayuyo 750 mg	Binayuyo 1000 mg	.739	Not Significant
**Significant at .05 alpha level*			

The table above shows the Test for Significant Difference in Post-Alloxan (Between Treatment). This shows the glucose level before treatment or after inducing alloxan to increase glucose level.

Across compare groups the computed p-value is greater than .05 alpha level. This means that there is no significant difference. Hence, the glucose level before treatment and after inducing alloxan is at the same level or the difference in glucose level is statistically not significant. This is a very good indicator that glucose level is controlled at the same level after inducing alloxan.

Table 8. Test for Significant Difference in First Hour of Observation (Between Treatment).

Compared Groups		p-value	Significance
Insulin	Binayuyo 500 mg	.040	Significant
	Binayuyo 750 mg	.003	Significant
	Binayuyo 1000 mg	.273	Not Significant
Binayuyo 500 mg	Binayuyo 750 mg	.281	Not Significant
	Binayuyo 1000 mg	.303	Not Significant
Binayuyo 750 mg	Binayuyo 1000 mg	.041	Significant
**Significant at .05 alpha level*			

The table above shows the Test for Significant Difference in the glucose level in the first hour of observation between insulin and Binayuyo at different concentrations.

For Insulin compared to Binayuyo 500 mg of concentration and 750 mg of concentration in the first hour of observation, the computed p-value is less than .05 alpha level. This means that there is significant difference. Hence, the glucose level of insulin is significantly lower than the glucose level of Binayuyo 500 mg of concentration and 750 mg of concentration. However, for insulin compared with Binayuyo 1000 mg of concentration, the computed computed p-value is greater than .05 alpha level. This means that there is no significant difference. Hence, the difference in the glucose level of insulin and Binayuyo 1000 mg of concentration is statistically not significant.

For Binayuyo 500 mg of concentration compared with 750 mg of concentration and 1000 mg of concentration, the computed p-value is greater than .05 alpha level. This means that there is no significant difference. Hence, the difference in the glucose level of Binayuyo 500 mg compared with Binayuyo 750 mg and 1000 mg of concentration is statistically not significant.

For Binayuyo 750 mg of concentration compared with 1000 mg of concentration, the computed p-value is less than .05 alpha level. This means that there is significant difference. Hence, the glucose level of 1000 mg of concentration of Binayuyo is significantly lower than 750 mg of Binayuyo concentration.

Table 9. Test for Significant Difference in Second Hour of Observation (Between Treatment).

Compared Groups		p-value	Significance
Insulin	Binayuyo 500 mg	.008	Significant
	Binayuyo 750 mg	.031	Significant
	Binayuyo 1000 mg	.038	Significant
Binayuyo 500 mg	Binayuyo 750 mg	.543	Not Significant
	Binayuyo 1000 mg	.485	Not Significant
Binayuyo 750 mg	Binayuyo 1000 mg	.928	Not Significant
**Significant at .05 alpha level*			

The table above shows the Test for Significant Difference in the glucose level in the second hour of observation between insulin and Binayuyo at different concentrations.

For insulin compared to the rest of Binayuyo concentrations, the computed p-value is less than .05 alpha level. This means that there is significant difference. Hence, the glucose level of insulin is significantly lower than the glucose of the Binayuyo regardless of concentration in the second hour of observation.

For Binayuyo 500 mg of concentration compared to Binayuyo 750 mg of concentration and Binayuyo 1000 mg of concentration vis-à-vis, the computed p-value is greater than .05 alpha level. This means that there is no significant difference. Hence, regardless of Binayuyo concentration, the difference of glucose level is not significant or they are at the same level of glucose level in the second hour of concentration.

Table 10. Test for Significant Difference in Third Hour of Observation (Between Treatment).

Compared Groups		p-value	Significance
Insulin	Binayuyo 500 mg	.012	Significant
	Binayuyo 750 mg	.029	Significant
	Binayuyo 1000 mg	.033	Significant
Binayuyo 500 mg	Binayuyo 750 mg	.699	Not Significant
	Binayuyo 1000 mg	.654	Not Significant
Binayuyo 750 mg	Binayuyo 1000 mg	.950	Not Significant
**Significant at .05 alpha level*			

The table above shows the Test for Significant Difference in the glucose level in the third hour of observation between insulin and Binayuyo at different concentrations.

For insulin compared to the rest of Binayuyo concentrations, the computed p-value is less than .05 alpha level. This means that there is significant difference. Hence, the glucose level of insulin is significantly lower than the glucose of the Binayuyo regardless of concentration in the third hour of observation.

For Binayuyo 500 mg of concentration compared to Binayuyo 750 mg of concentration and Binayuyo 1000 mg of concentration vis-à-vis, the computed p-value is greater than .05 alpha level. This means that there is no significant difference. Hence, regardless of Binayuyo concentration, the difference of glucose level is not significant or they are at the same level of glucose level in the third hour of concentration.

Table 11.Test for Significant Difference in Fourth Hour of Observation (Between Treatment).

Compared Groups		**p-value**	**Significance**
Insulin	Binayuyo 500 mg	.019	Significant
	Binayuyo 750 mg	.052	Not Significant
	Binayuyo 1000 mg	.109	Not Significant
Binayuyo 500 mg	Binayuyo 750 mg	.646	Not Significant
	Binayuyo 1000 mg	.405	Not Significant
Binayuyo 750 mg	Binayuyo 1000 mg	.706	Not Significant
**Significant at .05 alpha level*			

The table above shows the Test for Significant Difference in the glucose level in the third hour of observation between insulin and Binayuyo at different concentrations.

For Insulin compared to Binayuyo 500 mg of concentration, the computed p-value is less than .05 alpha level. This means that there is significant difference. Hence the glucose level in Insulin is significantly lower than Binayuyo 500 mg of concentration. However, for insulin compared with to Binayuyo 750 mg and 1000 mg of concentration, the computed p-value is greater than .05 alpha level. This means that there is no significant difference. Hence the glucose level of insulin, Binayuyo 750 mg and 1000 mg is statistically not different.

CONCLUSION

Based on the experimentation conducted, the Distilled water is not a good treatment in lowering glucose level. Insulin as a standard drug established a consistency in lowering glucose level. The 500mg, 750mg and 1000mg of Binayuyo extract has a potential hypoglycemic effect. The 750mg and 1000 mg of Binayuyo Extract can be a potential substitute of insulin.

As shown in the results of the study that there is a significant decrease in the glucose level from the time of post alloxan inducement down to the fourth hour of observation using a 750 mg of Binayuyo extract. The results can say that 750 mg of Binayuyo extract has a hypoglycemic effect.

RECOMMENDATIONS

In the light of the conclusions, the following recommendations are hereby recommended:

1. Formulate Binayuyo supplements at 750mg and 1000 as a potential hypoglycemic effect.
2. Use different solvents or other concentration to crude extract from Binayuyo (*Antidesmaghaesembilla*) leaves;
3. Perform more extensive studies, such as toxicity testing, chemical analysis, and drug formulation of the crude extract from Binayuyo (*A.ghaesembilla*) leaves;
4. Study other plant parts of Binayuyo (*A.ghaesembilla*) especially its fruit and roots;
5. Perform the hypoglycemic test in long duration of time using the crude extract of Binayuyo (*A.ghaesembilla*) leaves;
6. Compare different Standard Drugs like oral hypoglycemic agents with the crude extract of Binayuyo (*A.ghaesembilla*) leaves.
7. Continue studying hypoglycemic activity of Binayuyo (*A.ghaesembilla*) with greater or more than 1000 mg concentration of the crude extract.

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