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THE EVALUATION OF POTENTIAL HYPOGLYCEMIC EFFECT OF CAPE GOOSEBERRY *(PHYSALIS PERUVIANA*, FAMILY: SOLANACEAE) LEAVES

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ABSTRACT

Diabetes Mellitus is a medical condition that affects our body' use of its blood sugar and it is important because it fuels a lot of things our brain, our muscles and tissues, and if our body's blood sugar malfunctions, it can make a person experience fatigue, making the person weak. Thus, this study aims to check the potential hypoglycemic effect of Cape Gooseberry. Cape Gooseberry is an indigenous plant found in the Andes, and is suspected to have a hypoglycemic effect on humans. To test its effectivity a total of 16 rats were utilized and each treatment has 4 rats during the experimentation. The study deployed four groups/treatments namely; No Treatment as Control Group, Distilled Water as Negative Control, Glibenclamide as Positive Control and Cape gooseberry. Rats were force fed with glucose solution to increase glucose level before treatments. Results show that the sugar level in the No Treatment as Control Group is significantly higher than the rest of the group/treatment. The glucose level during post inducement is not statistically different with the glucose level in the post-treatment for no treatment as Control Group. The absence of treatment does not lower glucose level. Glibenclamide is a drug known for treating diabetes mellitus type 2 and is a poorly water soluble drug, it proves its consistency in lowering glucose level and was used to compare its effects to Cape Gooseberry, it showed that Cape Gooseberry has a potential hypoglycemic effect. Cape Gooseberry can be a potential substitute to Glibenclamide in lowering glucose level.

KEYWORDS: *Glibenclamide; Rat; Physalis Peruviana; Potential Hypoglycemic effect.*

INTRODUCTION

Diabetes mellitus pertains to a group of diseases that affect your body's use of blood sugar. Glucose is necessary to your health because it's a vital source of energy for your muscles and tissues. Blood sugar is also a fuel for your brain. The underlying cause of diabetes differs by type. But, no matter what type of diabetes you have, it can lead to excess sugar in your blood. Excessive sugar in your blood can lead to serious or fatal health dilemmas. (mayoclinic.org, 2019). There are different types of diabetes, some of which are more prevalent than others. The most common form of diabetes in the general population is type 2 diabetes, which often develops from pre-diabetes. Type 1 diabetes is more common in pediatric patients and gestational diabetes is a form of diabetes that can happen during pregnancy (Diabetes.co.uk). Type 1 diabetes or insulin dependent diabetes is an autoimmune sickness that causes the beta cell producing insulin in the pancreas to be destroyed, stopping the body from producing insulin and adequately regulate blood glucose levels (diabetes.co.uk). Type 2 diabetes is when the body cannot metabolize glucose (a simple sugar). This will result to high levels of blood glucose which over time may damage the body's organs (Diabetes.co.uk). Gestational diabetes occurs when you have hyperglycemia (high blood glucose levels) during pregnancy. (diabetes.co.uk). Predisposing factors diabetes depends on the type of diabetes. Determinant factors for type 1 diabetes are family history, environmental factors, presence of damaging immune system cells (autoantibodies), geography. For type 2 diabetes the risk factors are weight, sedentary lifestyle, family history, race, age, gestational diabetes, polycystic ovary syndrome (PCOS), hypertension, and abnormal cholesterol and triglyceride levels. The predisposing factors for gestational diabetes are age, family or personal history, weight and racial factors. (mayoclinic.org)

MATERIALS AND METHODS

Sample Collection

The leaves of Cape gooseberry (Physalis peruviana) were collected in Taclobo, San Fernando, Sibuyan Island, Romblon. Plastic sack served as the temporary containers for leaves

Extraction Procedure

First, five thousand grams (5000g) of Cape Gooseberry leaves were accurately weighed and used. Then, the leaves were dried using a drying oven at 40 °C,

trituration was done after to pulverize the leaves into powder form. Next, using percolation method, extraction was done. Ethanol was used during this procedure. During percolation, solvents were continuously added. Drug solvent ratio of 1:15 was used. A rotary evaporator was used to obtain a fluid extract. Overall extraction period lasted for 4 days. (Bernal, et al., 2016)

Phytochemical Analysis
Presence of different phyto-constituents was determined in multiple solvents using standard procedures. These phyto-constituents include carbohydrates, phenols, saponins, flavonoids, starch and proteins.

Phytochemical Tests
Presence of alkaloids, saponins, reducing sugar, carbonyls, tannins, terpenoids, flavonoids, phlobatannins, and steroids was also ascertained and analyzed in the extracts (Adetuyi and Popoola 2001; Trease and Evans, 1989; Sofowora, 1982).

Test for Alkaloids
In a test tube, 0.2 gram of accurately weighed plant extract was placed and was warmed with 2% sulphuric acid for two (2) minutes. It was then filtered in another test tube. Few drops of Dragencloff's reagent was added. Appearance of orange red precipitates was monitored as this would have indicated the presence of alkaloids Association, A.P. (2016). Journal of the American Pharmaceutical Association)

Test for Cardiac Glycosides
Keller-Killani Test
In a test tube, 0.5 gram of accurately weighed plant extract was placed. Then, 2 ml of glacial acetic acid that contained a drop of ferric chloride solution was added. After, it was underlayered with 1 ml of concentrated tetra oxosulphate (VI) acid. Appearance of brown ring formation was monitored. (Chapman, 1998)

Test for Terpenoids
Analysis for the presence of alkaloids, terpenoids, reducing sugars, saponins, tannins, carbonyls, flavonoids, phlobatannins and steroids in the extract was done. . (Chapman, 1998)

Test for Reducing Sugars
In a test tube, add 2 ml of accurately weighed crude plant extract. Then, 5 ml of distilled water was added. Filtration was done after. The filtrate was boiled with 3-4 drops of Fehling's solution A and B for 2 minutes. Appearance of orange-red precipitate was monitored as this would have indicated the presence of reducing sugars. (Shubham, 2019)

Test for Saponins
In a test tube, 0.2 gram of accurately weighed plant extract with 5ml of distilled water was boiled. Frothing (appearance of small bubbles) was monitored as this would have indicated presence of saponin. (Shubham, 2019)

Test for Tannins
A minute amount of plant extract was mixed with water and then heated in a water bath. The mixture was filtered, and the ferric chloride was added to the filtrate. Appearance of the dark green solution was monitored as this would have indicated the presence of tannins. (Shubham, 2019)

Test for Carbonyl
In a test tube, 2ml of accurately weighed plant extract was placed while a few drops of 2, 4, dinitrophenylhydrazine solution was added. Shaking of the test tube was done after. Appearance of yellow crystals was monitored as this would have indicated presence aldehydes. (Shubham, 2019)

Test for Flavonoids
In a test tube, 0.2 gram of accurately weighed plant extract was placed and was dissolved with diluted sodium hydroxide. Diluted hydrochloride also added after. Appearance of a yellow solution that turned colorless was monitored as this would have indicated the presence of flavonoids. (Shubham, 2019)

Test for Phlobatannins
In a test tube, 0.5 gram accurately weighed plant extract was placed and dissolved with distilled water. Filtration was done after. Next, the filtrate was boiled with 2% hydrochloric acid solution. Appearance of red precipitate was monitored as this would have indicated the presence of Phlobatannin. (Shubham, 2019)

Test for Steroids
In a test tube, the plant extract was placed. And then, 2ml of acetic anhydride with 0.4 gram of ethanolic extract of each sample, with 2 ml Sulphuric acid was added. Changing of its violet color to blue or green color was monitored as this would have indicated the presence of steroids. (Shubham, 2019)

Summary of Results in Extraction of *P. peruviana*

Table 1

Summary of Results for the Phytochemical Screening.

Tests Performed	Secondary Metabolite Being Tested	Presence (+)/ Absence (-)
	Alkaloids	-
Liebermann-Burchard Test	Unsaturated sterols	-
Salkowski Test	Unsaturated sterols and triterpenes	-
	Flavonoids	+
	Steroids (cardiac glycosides)	+
Kedde Reaction Keller-Killiani Test	Unsaturated lactones 2-deoxysugars	- -
Froth Test	Saponins	-
Borntrager Test	Anthraquinones	+
Modifed Borntrager Test	Anthraquinones	+
Guignard Test	Cyanogenic glycosides	-
Bate-smith Metcalf Test	Leucoanthocyanins	-
Wilstatter "cyaniding"Test	Benzopytrone nucleus	-
Ferric Chloride Test	Tannins Polyphenols	+ -

The table above shows that the crude ethanolic extracts of *Physalis peruviana* possess the following metabolites: flavonoids, cardiac glycosides, anthraquinones and tannins.

According to Borntragers test : About 0.5 gm of the extract was taken into a dry test tube and 5 ml of chloroform was added and shaken for 5 minutes. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red colour in the lower layer indicates the presence of anthraquinone, and our sample showed a pink violet or red color (N.Geetha et al)

DATA FROM EXPERIMENTAION

1. What is the average glucose level for baseline, post-inducement, and post- treatment when data were grouped according to treatment (a. No Treatment as Control, b. Distilled Water as Negative Control, c Glibenclamide as Positive Control, d. and Cape Gooseberry)?

RESULTS:

TABLE 2

Group A. Control Group.

RAT	BASELINE	POST-INDUCTION	POST-TREATMENT
Rat 1	72	108	102
Rat 2	72	106	103
Rat 3	84	73	88
Rat 4	100	74	105
MEAN	**82**	**90.25**	**99.5**
SD	**13.26649916**	**19.36276495**	**7.767453465**

The glucose level during post-inducement is not statistically different with the glucose level in the post-treatment for no treatment as Control Group. The absence of treatment does not lower glucose level.

TABLE 3

Group B. Negative Control.

RAT	BASELINE	POST- INDUCTION	POST- REATMENT
Rat 1	70	246	142
Rat 2	93	207	135
Rat 3	104	205	200
Rat 4	81	226	193
MEAN	87	221	167.5
SD	14.71960144	19.16594202	33.72931465

The table above shows that the glucose level during post-treatment is significantly lower than the glucose level during post inducement. There was a significant decrease in glucose level during post-treatment when distilled water was used

TABLE 4
Group C. Glibenclamide.

RAT	BASELINE	POST- INDUCTION	POST- TREATMENT
Rat 1	71	209	104
Rat 2	99	208	94
Rat 3	93	232	127
Rat 4	97	229	116
MEAN	**90**	**219.5**	**110.25**
SD	**12.90994449**	**12.76714533**	**14.33817748**

The table above shows that the glucose level during post-treatment is significantly lower than the glucose level during post inducement. There was a significant decrease in glucose level during post-treatment when Glibenclamide was used.

TABLE 5
Group D. Cape Gooseberry.

RAT	BASELINE	POST- INDUCTION	POST- TREATMENT
Rat 1	90	229	119
Rat 2	94	212	112
Rat 3	85	201	119
Rat 4	94	213	94
MEAN	**90.75**	**213.75**	**111**
SD	**4.272001873**	**11.52894907**	**11.80395414**

The table above shows that the glucose level during post-treatment is significantly lower than the glucose level during post inducement. There was a significant decrease in glucose level during post-treatment when Glibenclamide was used. This would mean that Cape gooseberry has a hypoglycemic effect.

TABLE:6
Average Glucose Level per Group.

Group	Baseline	Post-Inducement	Post-Treatment
Control	82.00	90.25	99.50
Negative Control	87.00	221.00	167.50
Glibenclamide	90.00	219.50	110.25
Cape gooseberry	90.75	213.75	111.00

The table above shows the average glucose level per treatment. It is observed that there was an increase in glucose level rom baseline to post-inducement across all groups/treatment. However, except for no treatment as control group, the rest of groups/ treatments decrease the glucose after post treatment.

TABLE:7
Test for Significant Difference (Between) for Treatment.

Compared Group		p-value	Significance
Control	Negative Control	**.000***	Significant
	Glibenclamide	.454	Not Significant
	Cape gooseberry	.424	Not Significant
Negative Control	Glibenclamide	**.001***	Significant
	Cape gooseberry	**.002***	Significant
Glibenclamide	Cape gooseberry	.958	Not Significant
**Significant at .05 alpha level*			

Table 7 shows the test for significant difference for Post-Treatment when one group or treatment is compared to another group or treatment.

For Control group compared with Negative control, the computed p-value is less than .05. This would mean that there is significant difference. Hence, the computed glucose level of control group is significantly lower than the negative control group. However, for control group compared with Glibenclamide and Cape gooseberry, the computed p-value is greater than .05. This would mean that there is no significant difference. Hence, the glucose

level obtained after post treatment is statistically not different from obtained glucose level of Glibenclamide and Cape gooseberry after post-treatment.

For Negative control compared to Glibenclamide and Cape gooseberry, the computed p-value is less than .05. This would mean that there is significant difference. Hence, the glucose level of negative control obtained after post treatment is significantly higher than the glucose level of Glibenclamide and Cape gooseberry obtained after post treatment.

For Glibenclamide compared with Cape gooseberry the computed p-value is greater than .05. This would mean that there is no significant difference. Hence, glucose level of Glibenclamide obtained after post treatment is not different with the glucose level of Cape gooseberry obtained after post treatment.

TABLE:8
Test for Significant Difference (Within) Between Post-Inducement and Post-Treatment per Group.

Group	p-value	Significance
Control	.383	Not Significant
Negative Control	**.012***	Significant
Glibenclamide	**.000***	Significant
Cape gooseberry	**.000***	Significant
**Significant at .05 alpha level*		

The table 8 shows the Test for Significant Difference (Within) Between Post- Inducement and Post-Treatment per Group. This would mean that we are comparing glucose level between post-inducement and post-treatment for each group.

For Control group, the computed p-value is greater than .05 alpha level. This would mean that there is no significant difference. Hence, there is no difference in glucose level between post-inducement and post treatment for control group or statistically we can say that there is no improvement in the glucose level.

For Negative Control, the computed p-value is less than .05 alpha level. This would mean that there is significant difference. Hence, the glucose level in post- treatment is significantly lower than the glucose in the post-inducement. This would mean that there is an improvement in lowering the glucose level for negative control.

For Glibenclamide, the computed p-value is less than .05 alpha level. This would mean that there is significant difference. Hence, the glucose level in post- treatment is significantly lower than the glucose in the post-inducement. This would mean that there is an improvement in lowering the glucose level using Glibenclamide.

For Cape Gooseberry, the computed p-value is less than .05 alpha levels. This would mean that there is significant difference. Hence, the glucose level in post-treatment is significantly lower than the glucose in the post-inducement. This would mean that there is an improvement in lowering the glucose level using Cape gooseberry.

Assessment of Hypoglycemic Activity

1. General description of animal manipulation methods (including method of conditioning): During acclimation, rats were handled and observed daily for behavioral changes and other symptoms.

2. Dosing method (including frequency, volume, route, method of restrained and expected outcome or effects)

(a) The first group (Group A) served as the control group and received 1 ml normal saline per 100 g of body weight.

(b) The second group (Group B) served as the negative control and received a 4g/kg glucose. No treatment was administered.

(c) The third group (Group C) served as positive control group and received 2.5mg/kg Glibenclamide

(d) The fourth group (Group D) served as a treatment group and received 100mg/kg of Cape gooseberry extract.

3. Specimen or biological agent (blood sample, urine, etc.) collection method (including frequency, volume, route and method of restraint.): blood was collected from the tail vein. The animals were restrained manually with the assistance of a certified animal handler

CONCLUSION

In conclusions, in the absence of any treatment, there is no reduction in the glucose level, although distilled water helps lowering glucose level, this study shows that Glibenclamide and Cape Gooseberry is better than distilled water in lowering glucose level. Glibenclamide proves that it has its consistency in lowering glucose level. Cape Gooseberry shows that it has a potential hypoglycemic effect. Therefore, Cape Gooseberry can be a potential substitute to Glibenclamide in lowering glucose level.

RECOMMENDATIONS

In the light of the conclusions, the following recommendations are hereby recommended: to perform further studies about the other pharmacologic action of Cape gooseberry. Use of other drug as reference standard to test the action of Cape gooseberry and to proceed in the formulation of tablet or capsule form if given a chance after the pandemic. According in the results of the study, the succeeding statements are recommended for further enhancement of the Hypoglycemic Effect of Cape Gooseberry Leaves (Physalis peruviana, Family: Solanaeceae) and for its future improvement. Based on the result and in line with this, the researchers would like to recommend the following.

1. To perform further studies about the other pharmacologic action of Cape Gooseberry (Physalis peruviana, Family: Solanaceae)
2. Use of other drug as reference standard to test the action of Cape Gooseberry (Physalis peruviana, Family: Solanaceae)
3. For other groups, they are welcome to procced or continue the formulation of tablet form after the COVID-19 pandemic.

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