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STUDY ON THE ANTI-DIABETIC ACTIVITY OF SUGAR APPLE (*ANONASQUAMOSA*) ETHANOLIC SEED EXTRACT ON ALLOXAN INDUCED WISTAR RATS (*RATTUSNORVEGICUS*)

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ABSTRACT

Incidence of diabetes mellitus is high all over the world. It has become the third "killer" of mankind after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality.

This study was conducted to evaluate the anti diabetic activity of the Ethanolic seed extract of Sugar apple via intra peritoneal route against hyper glycemic dose of Alloxan. Results of the study showed that there was a decrease in the blood sugar level of rats which was treated with the different concentrations of the extract. However there was no significant difference among the different concentration after 7th day.

KEYWORDS: Acclimatization, Diabetes mellitus, Hyperglycemia, Hypoglycemia, Insulin

INTRODUCTION

It is well known that the incidence of diabetes mellitus is high all over the world, especially in Asia. Diabetes is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from defects in insulin secretion, insulin action or both. It is becoming the third "killer" of mankind after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality. According to the World Health Organization (WHO) the number of diabetics has doubled in the past few years and is expected to double once again by the year 2035. Today, there are 387,000,000 diabetics worldwide**. The Philippines is one of the world's emerging diabetes hotspots. Ranked in the top 15 in the world for diabetes prevalence, Philippines is home to more than 4 million people diagnosed with the disease – and a worryingly large unknown number who are unaware they have diabetes.** The pathogenesis, progress and the possibility of its management by oral administration of hypoglycemic agents have stimulated great interest in recent decades. Numerous therapies designed for the treatment of DM have

proven to be fairly effective, but none is ideal due to undesirable side effects and diminution after prolonged use.

Our aim is to determine if the Methanolic seed extract of sugar apple(*Annonasquamosa*) will demonstrate a significant decrease in the glucose level of diabetic animals with a view to explore its use for the treatment of diabetes mellitus in humans. Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose of diabetes mellitus apart from strep to zotocin. Alloxan has a destructive effect on the beta cells of the pancreas. Alloxan causes a massive reduction in insulin release by the destruction of b-cells of the islets of langerhans, thereby inducing hyperglycemia. Insulin deficiency leads to various metabolic alterations in the animals increased blood glucose.

Anonnasquamosa according to Pinto et al. (2005) states that it was originated in lowland Central America where it is indigenous, and from there it was distributed to Mexico and throughout tropical America. The Spaniards carried seeds from the New World to the Philippines and the Portuguese are assumed to have introduced the sugar apple to southern India.The plant is reported to contain flavonoids which are reported to possess anti-diabetic activity. Phytochemical analysis of leaves of *Annonasquamosal* revealed the presence of flavonoids. According to the study Beneficial Effects of Anonnasquamosa leaf extract in Strep to zotozin induce Diabetic ratsby Kaleem, Medhaetal., (2008)it was observed that there is a significant decrease in blood glucose in *Anonnasquanosa* treated diabetic rats. In addition, the researchers also examined the influence of oral administration of *Anonnasquamosa*extract on the levels of some biochemical parameters and the activities of some enzymes in plasma, liver, and kidney of Strep to zotozin induce diabetic rats.

Diabetes mellitus is one of the most common non communicable diseases globally. According to the Diabetes Atlas (fifth edition) 366 million people have diabetes in 2011, more than 55 million people in the EUR Region have diabetes, 4.6 million deaths are connected with diabetes in 2011 and in 2030 this number is expecting to rise to 552 million especially to people who live in low- and middle-income countries (WHO, 2010). Many middle- and low-income countries have more people under the age of 60 with diabetes compared to the world average. Meanwhile, for high-income countries, a growing population over the age of 60 makes up the largest proportion of diabetes prevalence (IDF, 2012).

OBJECTIVES OF THE STUDY

This study aims to determine the hypoglycemic activity of Ethanolic seed extract of sugar apple (*Annonasquamosa*) in Alloxan induced Wistar rat.

Specifically, it aims to:

1. Determine the glucose level of the Wistar rats before induction of Alloxan.
2. Determine the glucose level of the Wistarrats after induction of Alloxan.
3. Determine if there is significant difference before and after induction of Alloxan.
4. Determine the glucose level of Alloxan induced Wistar rats after 3rd, 5th and 7th day of induction of the different concentrations of Ethanolic seed extract.
5. Determine if ther is significant difference among the different concentrations of Ethanolic seed extract after 3rd, 5th, and 7th day of induction.

METHODOLOGY

Research Design

Experimental method of research was performed in this study and the statistical method of computing 95% level of significance was used in the computation of ANOVA and Post Hoc Analysis using Duncan Dunnett's test.

Materials

Tools such as Analytical Balance, Beaker, Erlenmeyer flask, Graduated cylinder, Funnel, Evaporating dish, Gluco meter, Glucose, strips, Mortar and pestle, Rotary evaporator, Scissors, Syringe, Vialswere used in the experiment.

Data Gathering Procedures

Processing of Annonasquamosa ethanolic seed extract

Mature sugar apple tree bark were gathered at Amulung, Cagayan. Fresh seeds were utilized for the extraction process. There was no air drying done. Before the extraction, the fresh seeds were washed with distilled water and were cut into pieces. Following this, the solvent (250 ml of ethanol) was added to a round bottom flask, which was attached to a Soxhlet extractor and condenser on an isomantle. Two hundred fifty (250) milligrams of seeds, cut into pieces was loaded into the thimble, which was placed inside the Soxhlet extractor. The side arm was lagged with the glass wool. The solvent was heated using the isomantle and began to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent

reaches the siphon it pours back into the flask and the cycle begins again. The process ran for a total of 8 hours. Once the process has finished, the ethanol was evaporated using a rotary evaporator, leaving a yield of extracted plant material about a total of 25 ml in the glass bottom flask.

Acclimatization of Wistar Rats and Measurement for the baseline sugar level

Rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. A standard Animal feed that contain enough nutrients was given to maintain the health of the rats. Distilled water was also given to the rats.Fasting was started on the first day. On the second day extraction of blood was done by cutting the end tail of each rat by using surgical scissors then blood was placed on Glucose strip to be read by the Glucometer (Accu- check Active). Blood sugar was measured and recorded the same time on the 3rd, 5th, and 7th day of the study.

Induction of Alloxan in Experimental Animals and measuring glucose level after induction

Rats were made diabetic by a single intra peritoneal injection of alloxan monohydrate (150 mg/kg). Alloxan was first weighed individually for each animal according to the body weight and then was solubilized with 0.2ml saline (154mmNaCl) just prior to injection. Induction of Alloxan was done. Two days after alloxan injection, the glucose level of the rats was measured and those with plasma glucose levels of 135 mg/dl was included in the study. Treatment with plant extracts was started 48 h after alloxan injection.

Treatment of Sugar Apple (*Annonasquamosa*) ethanolic seed extract

Treatment of the experimental animals with the different extracts of *Anonnasquamosa* was done. This study has five sets of treatments with three replicates each. Group I served as the Negative control which received no treatment under standard environment conditions. Group II was the positive control and we used human regular insulin (1 unit/kg body weight of rats). Group III-were the treatment of Sugar Apple seed extract in four different concentrations, 25%, 50%, 75% and 100%.

Measurement of Blood Glucose Level after Induction of *Annonasquamosa* ethanolic bark extract

Extraction of blood was done by cutting the end tail of each rat by using a surgical scissors then blood was placed on Glucose strip to be read by the Gluco meter (Accu- check Active). Fasting blood glucose estimation was done the following day after induction of *Annonasquamosa* ethanolic bark extract, on the third day and finally on the fifth day.

RESULTS AND DISCUSSIONS

This chapter presents the results and interpretation of the study based on experimental method of research done and the statistical method of computing 95% level of significance was used in the computation of ANOVA and Post Hoc Analysis using Duncan Dunnett's test.

Table 1 shows that there is a significant difference before and after induction of Alloxan Monohydrate.

Table 1 Paired Sample T- test for significant difference in the normal blood glucose level before and normal glucose level after induction of Alloxan

Treatment	Concentration	Mean		Mean difference	T-test value	P- value	Decision
		Before	After				
Ethanolic Seed Extract	25% Extract	85.33	192.67	-107.34	-89.307	< 0.001	Reject Ho
	50% Extract	91.00	198.00	-107.00	-107.000	< 0.001	Reject Ho
	75% Extract	104.33	201.67	-97.34	-36.500	0.001	Reject Ho
	100% Extract	86.33	197.67	-111.34	-66.804	< 0.001	Reject Ho
Positive Control		81.33		NA	NA	NA	NA
Negative Control		101.33	202.33	-101.00	-87.469	< 0.001	Reject Ho

Table 2 shows that after 3rd day of treatment all extracts except the 100% ethanolic seed extract have significant difference with the blood glucose level of wistar rat after induction of alloxan. This implies that these extracts are effective as hypoglycemic agent.

Table 2 Paired samples t- test for Significant Difference in the normal blood glucose level after induction of Alloxan and 3rd day after treatment

Treatment	Concentration	After induction	3rd day after treatment	Mean difference	T-test value	P-value	Decision
Ethanolic seed extract	25% Extract	192.67	189.33	3.333	10.000	.010	Reject Ho
	50% Extract	198.00	191.67	6.333	4.359	.049	Reject Ho
	75% Extract	201.67	175.00	26.667	3.255	.083	Reject Ho
	100% Extract	197.67	188.00	9.667	2.011	.182	Accept Ho
Negative Control			86.67	-84.667		.000	Reject Ho
Positive Control		202.33	171.33	31.000	14.892	.004	Reject Ho

Table 3 shows that after 5thday of treatment, all seed extract concentrations have significant difference with the blood glucose level of wistar rat after induction of alloxan. This implies that these extracts are effective as hypoglycemic agent.

Table 3 Paired samples t- test for Significant Difference in the normal blood glucose level after induction of Alloxan and 5th day after treatment

Treatment	Concentration	After Induction	5th day after treatment	Mean Difference	T-test value	p-value	Decision
Ethanolic Seed Extract	25%Extract	192.67	184.333	8.333	25.000	.002	Reject Ho
	50%Extract	198.00	180	18.000	4.754	.042	Reject Ho
	75%Extract	201.67	163.333	38.333	5.907	.027	Reject Ho
	100%Extract	197.67	175	23.000	15.054	.004	Reject Ho
Negative control			89.67	-64.000		.000	Reject Ho
Positive control		202.33	153	49.333	8.798	.013	

Table 4 shows that there is a significant difference between the glucose level of wistar rat after induction of alloxan and after 7th day of treatment with different concentrations of ethanolic seed extract. This implies that all extracts are effective as hypoglycemic agent after 7th day of treatment.

Table 4 Paired samples t- test for Significant Difference in the normal blood glucose level after induction of Alloxan and 7th day after treatment

Treatment	Concentration	After induction	7th day after treatment	Mean difference	T-test value	P- value	Decision
Ethanolic seed extract	25% Extract	192.67	178.00	14.667	44.000	.001	Reject Ho
	50% Extract	198.00	172.00	26.000	12.490	.006	Reject Ho
	75% Extract	201.67	156.33	45.333	6.775	.021	Reject Ho
	100% Extract	197.67	151.67	46.000	17.386	.003	Reject Ho
Negative Control			101	-25.33		.000	Reject Ho
Positive Control		202.33	126.33	76.000	20.074	.002	

Table 5 shows that 25%, 50%, 100% ethanolic seed extract as well as the negative control has significant difference with the positive control which means that commercially prepared drug is more effective compared to the said concentrations. On the other hand, 75% ethnolic seed extract have no significant difference with the positive control. It only indicates that the said concentration and the commercially available drug have the same level of efficacy in terms of hypoglycaemic property 3rd day after treatment.

Table 5 Dunnett's POSTHOC pairwise comparison of the glucose level of the Wistar Rats between the treatments and the positive control 3rd day after treatment

Dependent variable	(I) Group	(J) Group	Mean Difference (I-J)	P-value	Decision
Blood glucose level of Wistar rats 3rd day after treatment	25% Ethanolic Seed Extract	Positive Control	18.000	.010	Reject Ho
	50% Ethanolic Seed Extract	Positive Control	20.333	.003	Reject Ho
	75% EthanolicSeed Extract	Positive Control	3.667	.994	Accept Ho
	100%Ethanolic Seed Extract	Positive Control	16.667	.019	Reject Ho
	Negative Control	Positive Control	-84.667	.000	Reject Ho

Table 6 shows that 75% ethanolic seed extract have the same level of efficacy with the positive control. On the other hand 25%, 50%, 100% ethanolic seed extract's glucose level shows significant difference with the postivie control, which means commercially prepared drug is more effective than the said extracts after 5th day of treatment.

Table 6 Dunnett's POSTHOC pairwise comparison of the glucose level of the Wistar Rats between the treatments and the positive control 5th day after treatment

Dependent variable	(I) Group	(J) Group	Mean Difference (I-J)	P-value	Decision
Blood glucose level of Wistar rats 3rd day after treatment	25% Ethanolic Seed Extract	Positive Control	18.000	.010	Reject Ho
	50% Ethanolic Seed Extract	Positive Control	20.333	.003	Reject Ho
	75% Ethanolic Seed Extract	Positive Control	3.667	.994	Accept Ho
	100%Ethanolic Seed Extract	Positive Control	16.667	.019	Reject Ho
	Negative Control	Positive Control	-84.667	.000	Reject Ho

Table 7 shows that all treatment groups show significant difference with the positive group. Therefore, commercially available drug is more effective than any of the treatments after 7th day of induction.

Table 7 Dunnett's POSTHOC pairwise comparison of the glucose level of the Wistar Rats between the treatments and the positive control 7th day after treatment

Dependent variable	(I) Group	(J) Group	Mean Difference (I-J)	P-value	Decision
Blood glucose level of Wistar rats 7th day after treatment	25% Ethanolic Seed Extract	Positive Control	51.667	.000	Reject Ho
	50% Ethanolic Seed Extract	Positive Control	45.667	.000	Reject Ho
	75% Ethanolic Seed Extract	Positive Control	30.000	.000	Reject Ho
	100%Ethanolic Seed Extract	Positive Control	25.333	.000	Reject Ho
	Negative Control	Positive Control	-25.333	.000	Reject Ho

CONCLUSION

Fasting blood sugar levels taken before the induction of Alloxan fell under the normal blood glucose level of rats while the fasting blood sugar levels that were taken after the induction of Alloxan were considered hyperglycemic values for it was beyond the normal range of blood glucose level of rats which is greater than 135mg/dL.

Results of the study showed that there was a decrease in the blood sugar level of rats which was treated with the different concentrations of extract. However there was no significant difference among the different concentration after 7^{th} day.

Results also showed that there was a decrease in the blood glucose of rats from both treated with Human Regular Insulin and the minimum effective concentration but rats treated with commercially available drug showed grater decrease in the blood sugar levels than any of the other extracts resulting to a significant difference between the antidiabetic activity of these two.

RECOMMENDATIONS

1. Lengthening the treatment and observation period to further confirm the anti-diabetic activity of the different concentrations of *Annonasquamosa* Ethanolic seed extracts.
2. Use glucose measuring device for a better blood glucose reading.
3. Use hyperglycemic drug other than Alloxan monohydrate.
4. Utilization of other methods of *Annonasquamosa* extraction.

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