# "Anti-Hyperglycemic Effect of Serpentina (*Andrographis paniculata)* Leaf Extract in Alloxan-induced Diabetic Mice"

# ABSTRACT

The study evaluated the anti-hyperglycemic effect of Serpentina leaf extract in alloxan-induced diabetic mice. Specifically, it aimed to determine whether there is a significant difference in the blood sugar level of diabetic mice when subjected to the test drug and the control.

Alloxan monohydrate was administered to the test animals with single intraperitoneal injection at a dose rate of 125mg/kg once a day for six days, and on the 7th day, their blood sugar level was evaluated for individual glucose levels. Serpentina leaf extract was administered at doses of 14.1 ml/kg (Treatment 1), 28.2 ml/kg (Treatment 2) and 47 ml/kg (Treatment 3). Metformin was used as the positive control.

The effect was monitored after 3 and 24 hours after oral medication with the test drug and the control. Results showed a mean difference of reduction levels after 3 hours at -44.7, 240.25, 140.5 and 18.75, and at 24 hours at 193.75, 233.75, 264.5 and 93 in the blood sugar levels of test animals treated with Serpentina and the control drug.

The study further reveals that a dose of 28.2 ml/kg Serpentina leaf extract showed significant reduction of blood sugar levels in hyperglycaemic mice sustained after three (3) hours and twenty-four (24) hours post treatment at 0.05 level of significance.

# INTRODUCTION

Hyperglycemia is a wide spread disorder, that is characterized by elevated plasma glucose concentrations resulting from insufficient insulin and insulin resistance. This could lead to the disease called diabetes mellitus. An organ called the pancreas makes insulin. The role of insulin is to move glucose from the bloodstream into muscle, fat, and liver cells, where it can be used as fuel. (American Diabetes Association, Diabetes Care, January 2014.)

Glucose is a sugar that comes from foods, and is formed and stored inside the body. It is the main source of energy for the body's cells and is carried through the bloodstream. While it is true that we need glucose for energy, too much glucose in the blood can be unhealthy. Diabetes mellitus is a serious health problem with continuously increasing number of incidence and mortality. If not cured this will eventually lead to chronic complications causing other diseases, infections and serious organ damage.

Plants represent the richest source of inspiration for the identification of novel scaffold structures that can serve as the basis for drug design. (Schneider & Baringhaus, 2008) Plants with antidiabetic properties, in particular are generating a lot of interest primarily because the number of diabetics worldwide are fast reaching epidemic proportions, with the total number of people with diabetes projected to rise from171 million in 2000 to 366 million in 2030. (World Health Organization)

The treatment of such disease can be done by exploiting the herbal integrity of a plant. The present study evaluates the antihyperglycemic effect of Andrographis paniculata (Serpentina) leaf extract to the induced-diabetic mice. This is to confirm the folkloric claim that the leaves of Serpentina can lower blood glucose levels.

# Materials and Methods

# Collection of Plant Material

Leaves of Serpentina were collected from Cataggamman Nuevo in Tuguegarao City, Cagayan, Philippines. A sample was brought to the National Museum in Manila for authentication.

# Preparation of Plant Extract

Leaves of Serpentina were weighed, washed and dried. Fifty grams (50g) of the leaves were placed in 500ml of 40% ethyl alcohol and allowed to stand for 3 days with frequent agitation, until soluble matter was dissolved. Filtration clarified the combined liquids after straining of the mixture and pressing of the marc. A rotary evaporator processed the filtered extract at 70 degrees Celsius to remove the excess solvent.. The extract was kept in a clean and sterilized container and refrigerated till further use.

# Preparation of Diabetic Mice

The test animals were acclimatized for one week prior to the administration of the different doses of Alloxan monohydrate. Alloxan monohydrate were administered to the 16 normal mice at 125 mg/kg once a day for six days. On the 7th day, the blood glucose level obtained by the tail cut technique was read with accu-check glucometer. An increase of their blood sugar levels (glucose level > 175 mg/dl) was considered diabetic and was used for the study.

# Administration of the Test drug and the Control

The test animals were divided into 4 treatments with 4 replicates. Treatment 1(treated with 14.1 ml/kg of serpentina leaf extract), Treatment 2 (treated with 28.2 ml/kg of serpentine leaf extract), Treatment 3 (treated with 47 ml/kg of serpentine leaf extract) and Treatment 4 (treated with metformin as the positive control). The leaf extract was administered orally once and effects on blood glucose levels were measured 3 hours and 24 hours post medication.

# Evaluation of biochemical parameter

Blood glucose was measured with Accu-check glucometer using the tail prick method. The researchers extracted blood samples before an initial 3 hours fasting and 6 days after alloxan administration. Blood glucose levels > 175 were considered hyperglycemic. Test animals with hyperglycemia were treated with the test extract and the control and their glucose levels were measured 3 hours and 24 hours thereafter.

# Statistical analysis

The paired t-test was used to compare the anti hyperglycemic effect of the test extract before and after test at 5% level of significance.

# Results

Analysis of the blood glucose profile of mice medicated with Alloxan showed a significant difference before and after administration of the drug as seen in Table 1.

**Table 1: Initial and Final Blood Glucose Levels of Mice After Medication with Alloxan**

| | MEAN | STANDARD DEVIATION |
|---|---|---|
| BEFORE | 105.1250 | 27.91 |
| AFTER | 398.69 | 157.32 |

The mean difference of the blood glucose levels after 3 and 24 hours post treatment are shown in Table 2.

Table 2. Mean Difference of Blood Glucose Levels after Treatments

| | 3hours | | | | | 24 hours | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | N | SD | MD | T-value | p-value | N | SD | MD | T-value | p-value |
| **T1 14.1ml/kg** | 4 | 171.48 | -44.75 | -0.52 | 638 | 4 | 169.14 | 193.75 | 2.29 | .106 |
| **T2 28.2ml/kg** | 4 | 240.25 | 69.45 | 6.918 | 006 | 4 | 99.66 | 233.75 | 4.691 | .018 |
| **T3 56.4ml/kg** | 4 | 183.83 | 140.5 | 1.52 | .226 | 4 | 241.44 | 264.5 | 2.19 | .116 |
| **T4(+)** | 4 | 37.50 | 18.75 | 1 | .391 | 4 | 271.36 | 93 | 0.68 | .544 |

Blood glucose levels were reduced with administration of metformin at a rate of 447 ml/kg with a mean difference of 18.75 after three (3) hours and a mean difference of 93 after twenty four (24) hours. Administration with Serpentina leaf extract reveals a mean difference of reduction to -44.75, 69.45 and 140.5. The reduction was further observed after 24 hours of treatment with a mean difference obtained at 193.75, 233.75 and 264.5 respectively.

# Discussions

Hyperglycemia is the hallmark of diabetes and as a very common chronic disease, it is reported that diabetes mellitus is becoming the third “killer” of humans along with cancer, cardiovascular and cerebrovascular diseases.

Hyperglycemia happens when there is no insulin in the blood, not enough insulin in the blood, or the insulin in the blood is not working properly(www.digestground.com/hyperglycemia-facts). The condition needs to be treated immediately. Once diagnosed, it is usually regulated by means of various commercially available drugs for its treatment.

However, prominent side-effects of such drugs are the main reason for an increasing number of people seeking alternative therapies that may have less severe or no side effects. Some of the most serious known side effects are tiredness, difficulty in breathing, irregular heartbeat, vomiting or diarrhea, and kidney complications, to name a few. (Panigrahi, B. et al, 2015)

In this study, we investigated the anti-hyperglycemic activity of ethanolic extract of Serpentina leaves which has been used by locals for the traditional management of diabetes with claims of efficacy. Our data showed that the reduction of blood glucose level of mice after three (3) and twenty four (24) hours post treatment revealed a significant difference at a dose of 28.2 ml/kg Serpentina leaf extract. The foregoing findings only corroborates reports about the medicinal properties of Serpentina whose intake have shown favorable effects to people with diabetes.

This effect of Serpentina plant may be attributed to andrographolide that has been reported to have a wide range of biological activities, such as those that are anti-inflammatory, anti-allergic, anti-platelet aggregation, hepatoprotective, and anti-HIV. Andrographolide enhances the surface uptake of glucose by adipose tissues and inhibit glucose absorption from intestine and glucose production from liver.

Review of literature also shows that some flavonoids and saponins isolated from medicinal plants significantly reduce blood sugar levels. Flavonoids stimulate the secretion of insulin in B-cells of islet of Langerhans of pancreas. It is possible that the presence of glycosides, flavonoids and tannins are responsible for their activity (Thanasekaran et. al, 2012).

# THANK YOU! ☺