Hypoglycemic Effect of Scarlet Spiral Flag (Costus woodsonii) to Alloxan-Induced Diabetic Mice: An In Vivo Study

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Abstract

Objective: Diabetes Mellitus is a leading illness to the society and despite of a lot of researches, not a single cure has been discovered. Most of the time, people focused on fighting diabetes and medicinal plants were utilized. The purpose of this study was to investigate the hypoglycemic effect of Scarlet Spiral Flag (Costus woodsonii) and to be reviewed for preclinical trials.

Methods: 20 male Swiss mice were made diabetic by inducing Alloxan monohydrate. Then separated randomly into 4 groups with different treatments administered daily for 7 days; Control, Positive Control (600 µg/kg Glibenclamide), T1 (250mg/kg Scarlet Spiral Flag ethanolic extract) and T2 (500mg/kg Scarlet Spiral Flag ethanolic extract). Their blood sugar was monitored onset after 1st treatment (6th, 12th and 24th hours) and during the week within the 1st, 4th and 8th day.

Results: Both the onset and fasting blood sugar monitoring showed a significant decrease in the mean average blood glucose level of the mice.

Conclusion: The ethanolic extract of Scarlet Spiral Flag (Costus woodsonii) has a potent antidiabetic effect in Alloxan-induced diabetic mice. Since it is under the same Genus of the Insulin plant, it has a common effect in terms of lowering the blood glucose level.

Keywords: Acclimatization, Alloxan, Costus woodsonii, Ethanolic Extract, Oral Gavage

Introduction

One of the most common metabolic disorders is Diabetes Mellitus, wherein a person has a high blood sugar, either because cells do not respond to the insulin that is produced or the pancreas does not produce enough insulin. It is a struggle in the world of health and most especially to the diagnosed patients because it cannot be cured and it has never been reported that someone had totally recovered from Diabetes. It is considered as one the leading causes of death in the world. The number of people with Diabetes has risen from 108 million in 1980 to 422 million in 2014 worldwide. In the Philippines, there were over 3,721,900 cases of diabetes in 2017.

People in the community are thriving for wellness. Their resourcefulness in finding ways for the improvement of their health is a sign of a desperate move in looking into and prioritizing medicinal plants as a substitute for synthetic treatments. Medicinal plants are not primarily the mainstay for most people in the occidental countries in terms of the treatments. Synthetic or chemical-based drugs are constantly used in treating and curing diseases. But in the eastern countries, the utilization of herbal plants is getting popular until now either due to an out of stock of drugs in remote areas or because drugs are less affordable. They are widely used due to its numerous health benefits and are safe and less expensive than conventional drugs. They are known to cure serious illnesses.

Some people in the community, especially those diagnosed with Diabetes Mellitus, reported that they stopped taking insulin maintenance due to financial matters. Instead, they used Insulin plant (Costus igneus) leaves as a belief for an alternative for their treatment. In awe, some people reported that they had a decrease in blood sugar level after using the plant. Insulin Plant is under the genus Costus and it is an important medicinal plant belonging to the family Costaceae. Though these plants are not approved by the Department of Health (DOH) for herbal medication, various pharmacological activities are attributed under this genus such as hepatoprotective, antioxidant, antibacterial and antifungal activity and most common for its antidiabetic activity. Researches on hypoglycemic effectiveness usually used Costus such as Insulin Plant (C. igneus and C. pictus) and Crepe Ginger (C. speciosus) and really showed significant results worth for a preclinical trial.

The main purpose of this study was to investigate another species under genus Costus, Scarlet Spiral Flag (C. woodsonii), for its hypoglycemic effect to Alloxan-induced diabetic mice. The diabetic mice will be under monitoring and evaluation for fasting blood sugar in
days to determine the effectiveness of the plant. The result of the study will again be determined for a thorough scientific evaluation for a plant’s potential value in the future that will promote it as an alternative treatment for diabetes. This will give an impact to all diabetic clients for the improvement of their holistic health.

Methods

Chemicals and Materials
Alloxan monohydrate (Sigma cal# A7413, Chemline Scientific Corporation Pvt. Ltd. Quezon City), Glibenclamide (Mercury Drug, Malaybalay City), Normal Saline and 5% Dextrose Solution (Sanitarium, Valencia City), 95% Ethanol (Elmer’s Chemical Store, Iligan City), oral gavage (gage 20, straight), Glucometer (OneTouch), Blood glucose strips, tuberculin syringes, amber bottles, nitrile gloves, bandage scissors, cotton balls, alcohols, and cages.

Plant Material
About 8 kilograms of healthy and mature leaves of Scarlet Spiral Flag (C. woodsonii) were collected from Salawagan, Quezon, Bukidnon. The collection was done 8:00-10:00 in the morning through hand-picking. The plant was then authenticated by Fulgent P. Coritico, a registered seller in Davao City. They were presented as Scarlet Spiral Flag (C. woodsonii) and then air-dried for a month at a room temperature to separate the extract from the ethanol. The pure extracts were then placed in a properly labeled and tightly sealed sterilized vial and then stored in a refrigerator under -20°C to separate the extract from the ethanol. The pure extracts were then placed in a properly labeled and tightly sealed sterilized vial and then stored in a refrigerator under -20°C for preservation until further use.

Ethanolic Extraction
In this procedure, extracts of the Scarlet Spiral Flag were prepared by soaking 150 grams of powdered leaves with 1,000 ml of 95% ethanol at room temperature for 48 hours which were then mixed by a stirring rod once daily at a same time. After extraction, the mixtures were filtered through a filter paper (Grade 1 Whatman) and the filtrate was subjected to a rotary evaporator (Stuart RE300, UK) with an initial speed of 50 rpm until 120 rpm at 40°C to separate the extract from the ethanol. The pure extracts were then placed in a properly labeled and tightly sealed sterilized vial and then stored in a refrigerator under -20°C for preservation until further use.

Experimental Unit: Mice
20 healthy male Swiss mice of 14-16 weeks old (25-30 grams) were bought from Philippine Institute of Traditional and Alternative Health Care (PITAHC), a registered seller in Davao City. They were presented as the experimental units in 4 different groups housed at the Rodent House of the Animal Experimental Station (AES), College of Veterinary Medicine, CMU, an approved experimental station in the Philippines by Institutional Animal Care and Use Committee (IACUC), with about 28°C and a relative humidity of 55%. Standard cages were provided with wood shavings and routinely cleaning of the cages were done. The experimental animals were provided with dog food (Pedigree) and distilled water. Only those with normal blood glucose concentration (60-130 mg/dl) 10 were considered as experimental animals. All the experimental animals were appropriately handled according to the guidelines of the IACUC.

Experimental Procedure

Acclimatization
A total of 20 male mice were used in this experiment. Acclimatization was done for 1 week. They were grouped by 4 and kept in 4 separate standard size cages. They were given with food and water in an ad libitum manner within 8:00am until 4:00pm. They were then restricted with any oral intake from 4:00pm until 8:00am. After 1 week of acclimatization, blood sampling was done through puncturing the tail vein and collected a drop (0.05ml) of blood to determine the initial blood glucose level of the male mice. A glucometer was used to determine the normal blood glucose level which is 60-130mg/dl.

Alloxan-Induced Diabetic Model
The experimental animals were made diabetic by injecting Alloxan monohydrate 1, 10-13 intraperitoneally with a dose of 150 mg/kg body weight based on the IACUC guidelines. After twenty-four hours from inducing diabetes mellitus, the blood glucose levels were determined using glucometer. One week after the induction, blood glucose levels were re-evaluated to make sure that the diabetes mellitus induced by Alloxan have not returned to normal levels before the treatment will be initiated. Blood collection was done before feeding (6:00am to 8:00am). Mice having blood glucose levels of equal to and/or more than 300 mg/dl were considered a criterion for diabetic mice.

In the 1st and 3rd phase after induction of Alloxan, there would be an expected transient hypoglycemia. They were given food in an ad libitum manner and a 5% dextrose solution in feeding bottle for a day as an appropriate intervention to overcome the early hypoglycemic effect 14.

Assignments of Mice into Treatments
The experimental animals were randomly assigned through blocking to one of the 4 treatment groups (Control, Positive Control, T1 and T2). The control, which was given only with food and water. The positive control, which was given with 600 µg/kg of Glibenclamide 6.15 orally once daily. T1 and T2 were
given a dose of 250mg/kg and 500mg/kg respectively once daily. The ethanolic extracts were administered orally with the use of a straight oral gavage needle gauge 20. 10 mice were treated with Scarlet Spiral Flag ethanolic extraction with concentrations of 250ppm (250mg/kg) for T1 and 500ppm (500mg/kg) for T2. The administration of the treatment was given in a homogenous manner every 10:00 am. The extract was given to treated groups of mice daily for 7 days. Then the other 10 mice will be under the other 2 groups. The positive control (5 mice), will be given daily with Glibenclamide of 600mcg/kg for a week as with the test groups. The control (5 mice) will neither be given Glibenclamide nor Scarlet Spiral Flag ethanolic extract. At the 6th, 12th and 24th hour after the first administration, blood collection for the mice were done to get its onset hypoglycemic effect. Then on the 1st, 4th and 8th day of the treatment period, another blood glucose monitoring was done every 6:00-8:00 am to get its fasting blood sugar.

**Statistical Analysis**
The results were tabulated and compared using ANOVA for Repeated Measurements.

**After Care of the Experiment**
After the experimentation, the mice were anesthetized before euthanized as per IACUC Guidelines through cervical dislocation. Then they were disposed and buried in the soil.

**Results and Discussions**

Table 1: Onset hypoglycemic effectiveness of Scarlet Spiral Flag ethanolic extract on the subjects.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Initial Value</th>
<th>6th hour</th>
<th>12th hour</th>
<th>24th hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>386.20</td>
<td>384.60</td>
<td>387.60</td>
<td>380.00</td>
</tr>
<tr>
<td>Positive Control</td>
<td>385.60</td>
<td>375.80</td>
<td>360.40</td>
<td>350.20</td>
</tr>
<tr>
<td>T1</td>
<td>389.40</td>
<td>383.80</td>
<td>380.00</td>
<td>374.40</td>
</tr>
<tr>
<td>T2</td>
<td>388.60</td>
<td>380.80</td>
<td>367.20</td>
<td>359.20</td>
</tr>
</tbody>
</table>

Table 2 clearly showed that the average glucose level of the mice was within the range of 386.20mg/dl-397.45mg/dl initially which passed the method criterion for diabetes (≥300mg/dl). From the start of the treatments on the 1st day until the 8th day, different groups showed significant decrease in a mean average value of blood glucose levels of the mice in a different rate on different groups.

The between treatments test indicated that the variable treatment was significant, consequently in the graph (Figure 2) we see that the lines for the four treatments were rather far apart. The within subject test indicated that there was a significant day’s effect, in other words, the treatments did change over time. The four treatments were getting a decrease in fasting blood sugar level from the initial value until the 8th day. Moreover, the interaction of treatments and the days were significant.
which means that the treatments were changing over time but were changing in different ways, which depicted that in the graph the lines would not be parallel. The graph showed that the treatments have non-parallel lines that decreased and were getting progressively closer together over time, though the mice stayed diabetic during the treatments, meaning that it did not reach the normal blood glucose level (60-130 mg/dL). This might be caused by the effectiveness of the Alloxan induction, it still showed its hypoglycemic effect significantly.

Conclusion

The sole purpose of this study was to investigate the hypoglycemic effectiveness of Scarlet Spiral Flag (C. woodsonii) and, as a part of the healthcare team, to promote herbal plants as one of the potential medicinal treatments for diabetes. It was experimental in approach with the limitations of using 20 male Swiss mice as the experimental unit; Alloxan as the drug induced for diabetes; Scarlet Spiral Flag as the treatment and; a week of monitoring for the onset and the fasting blood sugar.

The study concluded that the ethanolic extract of Scarlet Spiral Flag (C. woodsonii) has a potent antidiabetic effect in Alloxan-induced diabetic mice. Since it is under the same Genus of the Insulin plant, they have a common effect in terms of lowering the blood glucose level. Though the mice stayed diabetic during the treatments, it might be because of the effectiveness of the Alloxan induction, it still showed its hypoglycemic effect significantly.

The present investigation would now be open as an avenue for further research especially with reference to the development of potent formulation for diabetes mellitus from C. woodsonii leaves. For the future researches, a longer duration of the study conducted is highly recommended to determine its chronic effect. After the experiment, there should be a histopathology study to visually examine the liver and/ or pancreas of the mice which are the main organs affected by the plant extract administered. A serum glucose analyzer can be recommended to determine exact amount of blood glucose level and a daily fasting blood sugar monitoring can be considered. Daily fasting blood sugar monitoring can be considered. Phytochemical analysis for the plant is highly recommended to determine the contents of the plant in the molecular level. Acute oral toxicity study must be followed to determine the toxicity level of the plant. The study presented its potential value for the treatment of diabetes with further review and comprehensive research in preclinical trials.

Acknowledgment

To the IACUC Committee, thanks for accepting the study despite the expertise of the researcher; modifying the methods into a better and standard way; and the guidance throughout the study.

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