Effect of *Coccinia indica* on Blood Glucose Levels in Alloxan-induced Diabetic Mice

Kathryn Niedzielski
Advisor: Dr. Linda Swift

ABSTRACT

Diabetes is a condition in the body where the pancreas does not produce enough insulin to process glucose or the insulin receptors are not working properly. In this study, the anti-diabetic effects of the medicinal plant *Coccinia indica* were tested on alloxan-induced diabetic mice and were compared to the reference drug, Tolbutamide. This study will try to determine how effective *Coccinia indica* is in lowering blood glucose levels over a short amount of time in moderately and severely diabetic mice. The mice were categorized as either moderately (100-300 mg/dl) or severely diabetic (over 300 mg/dl) according to their blood glucose levels. The drug dosage for the moderately diabetic mice was 200 mg/kg per body weight and 400 mg/kg for the severely diabetic group. Blood glucose levels were tested before the treatment and two and four hours after. *Coccinia indica* and Tolbutamide decreased the blood glucose levels for the moderately diabetic mice by 77.17 and 61.05 % and for the severely diabetic mice, 21.7 and 47.9 percent. The means of the percent change for the moderately and severely diabetic mice at 2 and 4 hrs were not significantly different ($p>0.05$), therefore *Coccinia indica* had the same ability to lower glucose levels as Tolbutamide.

INTRODUCTION

Diabetes mellitus is a disease that affects 17 million people in the Unites States, which is over six percent of the population. A growing number of people each day are diagnosed with diabetes mellitus, due to the rise in obesity and sedentary lifestyle (http://www.diabetes.org). In many places throughout the world, diabetes is kept under control by the use of medicinal plant treatments, although this type of treatment has not completely been taken seriously by the medical field . The World Health Organization says that the use of medicinal plants to treat diabetes mellitus is a topic that needs further research (Gray et al., 1999). It is known that diabetes is a condition within the body where the Beta cells of the Islets of Langerhans in the pancreas do not produce enough insulin or the insulin receptors are not working properly. Insulin is a hormone that helps move the glucose into the cell to produce energy. It results in an inadequate supply of insulin and therefore blood glucose levels are increased, which is known as hyperglycemia. When glucose builds up in the blood instead of going into the cells, it results in damage and stress on the body. Immediately, the cells are starved for energy, which leaves the person feeling lethargic and weak and over an extended period of time, high blood sugar levels may cause irreversible damage to eyes, kidneys, nerves and heart (http://www.diabetes.org). The symptoms of diabetes are increased blood sugar, increased appetite and thirst, unexplained weight loss, weakness, decreased blood pressure and blurred vision.

The traditional treatments of diabetes may include a low sugar and carbohydrate diet accompanied with exercise for mild cases. For more severe and harder to control glucose levels, diabetes can be treated by the administration of insulin and/or by sulfonylurea antidiabetic drugs that stimulate the production of insulin and therefore lower the blood glucose levels. The reference drug that will be used in this experiment is Tolbutamide, which starts to work within one hour and is effective for 6-12 hours. For sulfonylurea antidiabetic drugs to work there has to be some cells of the pancreas that are working and therefore are able to be stimulated to produce appropriate amounts of insulin (Silverman, 1986). Tolbutamide, along with all the other sulfonylureas drugs, increase the output of insulin by binding to the receptors of the Beta cells of the Islets of Langerhans located in the pancreas. Once they bind to the sulfonylureas receptors, the K+ -ATP channels are closed and therefore the membrane is depolarized and insulin production is stimulated (Rorsman, 1997).

Coccinia indica, is a member of the Cucurbitaceae family, which is natively grown in wastelands in Asia and Africa. The plant is a perennial herb that contains tuberous roots and will often form a dense covering over other flora. In Thailand, the roots are boiled to make a decoction that is taken two to three times a day orally to treat diabetes. Studies discussed in Thai Medicinal Plants, show that the plant has an antidiabetic effect on alloxan-induced diabetic rabbits, in which a 95% alcohol extract at doses of 2.5 g/kg

and 5.0 g/kg decreased blood glucose levels by approximately 50% after six hours. Also, when the leaves are administered twice a day orally to diabetics, a hypoglycemic effect was seen (Faculty of Pharmacy, 1992). In another study, the medicinal properties of this plant were tested and the results showed that the dried roots of *Coccinia indica* significantly lowered blood glucose levels in alloxan induced mice. In this study, the mice were induced with slow onset diabetes and the treatments were over a period of two and a half weeks (Sullivan, 1999).

This study is going to look at the effects of *Coccinia indica* on the blood glucose levels of mice that were induced with acute diabetes. Also, it will look at how effective these treatments are in lowering glucose levels over a short period of time (2-4 hrs). The ability to decrease hyperglycemia in the mice for the medicinal plant treatment will be compared to Tolbutamide, a reference drug. I hypothesize that *Coccinia indica* will lower the blood glucose levels similarly to Tolbutamide. *Coccinia indica* is taken two to three times a day and Tolbutamide starts to work within one hour, so they both get into the body and start working very quickly and therefore this should be able to be seen in a four hour time period.

METHODS AND MATERIALS

To obtain the plant extract, dried *Coccinia indica* roots were ground in an electric grinder. The powder (20 g) was then mixed with 150 ml of water and boiled for 10 minutes. The mixture was cooled at room temperature and then filtered through fluted filter paper. The liquid was placed in a freeze drying tube and placed in the freezer until it was frozen. The tube was then placed on a lypholizer until it was dry. The freeze-dried extract was dissolved in isotonic saline solution (Alarcon-Aguilar et al, 1999).

Induction of diabetes was accomplished by three intraperitoneal injections of alloxan at 48-hour intervals. The mice were fasted for 18 hours before the first injection. The dosage of alloxan that was administered was 150-mg/kg body weight. One week after the third injection, the mice were fasted again and the blood glucose levels were recorded. The blood sample was obtained by using the tail clip method (Alarcon-Aguilar et al, 1999). A drop of blood was placed on a blood glucose test strip and was inserted into a glucosometer, ExacTech RSG Blood Glucose Testing System. Mice were categorized as either mildly or severely diabetic according to their blood glucose levels. Mice with basal blood glucose levels between 100 and 300 mg/dl were considered moderately diabetic and mice with basal glucose levels over 300 mg/dl were labeled as severely diabetic.

The dosage of the *Coccinia indica* and the Tolbutamide were determined according to the severity of diabetes. The treatments were administered orally by using an intubation tube. The moderately diabetic mice received 200-mg/kg per body weight of *Coccinia indica* or Tolbutamide and the severely diabetic mice received 400-mg/kg of either of the two treatments. Blood glucose levels were tested before the treatments were administered and then they were checked 2 hrs and 4 hrs after the treatment.

The percent change of the glucose levels were measured for the two and four hour time period for each treatment (before-after/before x 100). A T-test for the means of percent change for both time periods and the two treatments were compared according to this data.

RESULTS

The data in Table 1 and Figure 1, show the blood glucose levels before treatment, two hours after and four hours after for the treatments , Tolbutamide and *Coccinia indica*, and between severity of diabetes.

Table 1. Comparison of Average Blood Glucose Levels in Moderately and Severely Induced Diabetic Mice

Treatment	Initial (mean) Blood glucose	2 hours after treatment	4 hours after treatment
Coccinia indica (moderate)	450	358	352
Tolbutamide (moderate)	450	328	234
Coccinia indica (severe)	254	152	59
Tolbutamide (severe)	191.5	99	68.5

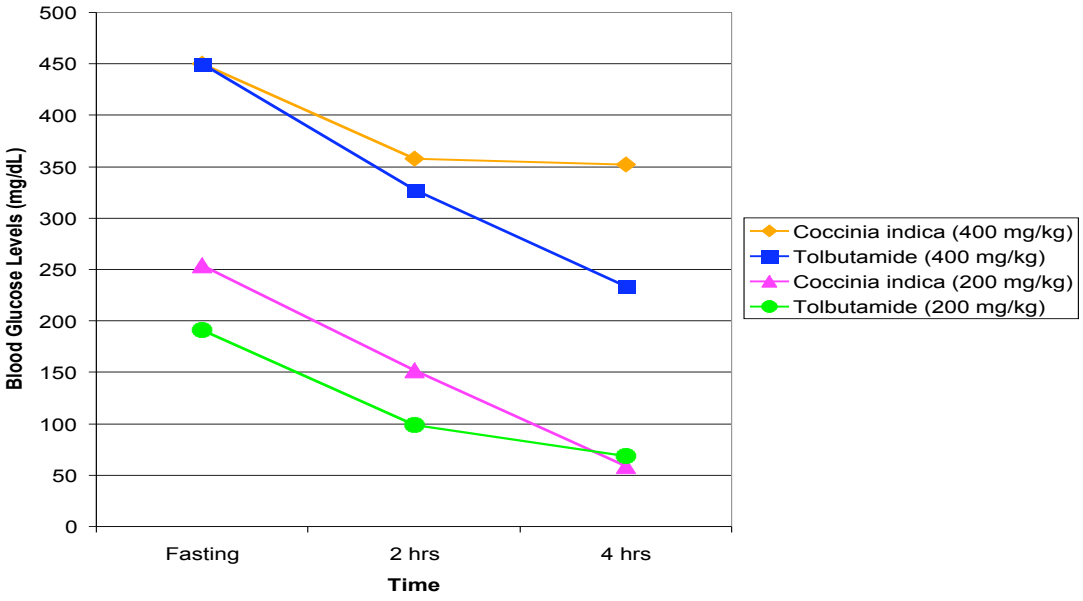

Figure 1. Comparison of Average Blood Glucose in Moderately and Severely Induced Diabetic Mice

Table 2 and Figure 2 show the average percent changes of blood glucose levels that were seen at the 2 hour and 4 hour time period for *Coccinia indica* and Tolbutamide in both the moderately and severely diabetic mice. In Table 2, the p-value from the T-test that compared the mean percent change of the two different treatments is also represented. The p-values for moderately diabetic mice after two and four hours showed that the means were not significantly different ($p>0.05$) for *Coccinia indica* and Tolbutamide. The same result was seen in the severely diabetic mice when comparing the mean percent change after 2 hours and 4 hours, the p-value (>0.05) and so the means were not significantly different.

Table 2. Mean Percent Change in Blood Glucose Levels in Moderately Severely Induced Diabetic Mice

Treatment	Mean % change After 2 hours	Mean % change After 4 hours	p-value
Coccinia indica (moderate)	39.75	77.2	p>0.05
Tolbutamide (moderate)	40.4	61.1	p>0.05
Coccinia indica (severe)	20.4	21.7	p>0.05
Tolbutamide (severe)	27.1	47.9	P>0.05

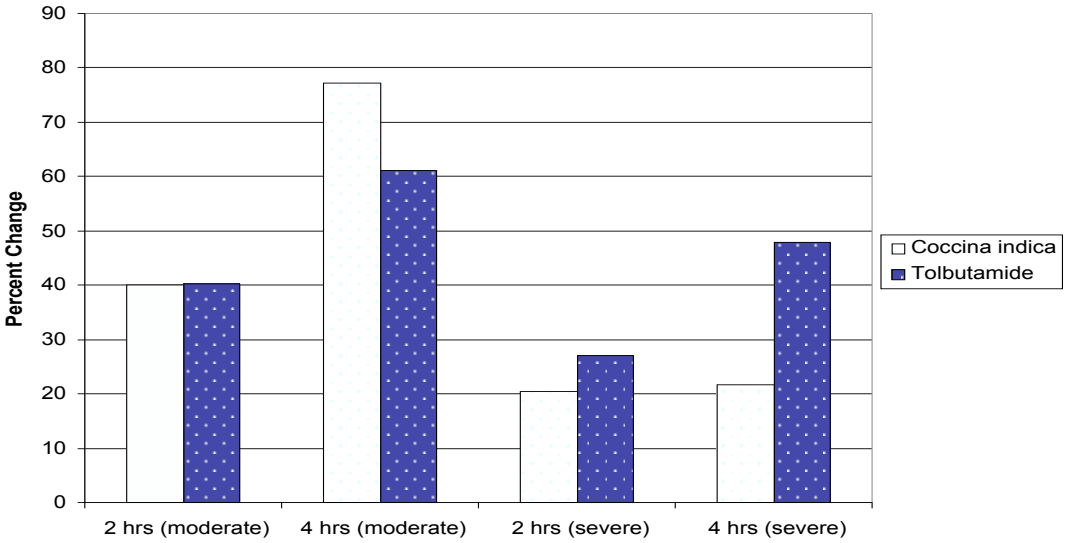

Figure 2. Mean Percent Change in Blood Glucose Levels in Moderately And Severely Induced Diabetic Mice

DISCUSSION

For moderately diabetic mice, *Coccinia indica* caused a 77.2 % change over 4 hours and Tolbutamide caused a 60.1 % change over this same time period. Since the p-value ($p>0.05$) showed there was no significant difference between the mean percent change for the two treatments, the conclusion can be made that *Coccinia indica* has a very similar ability to lower glucose levels in moderately diabetic mice. When the mice were considered to be severely diabetic, the *Coccinia indica* decreased the blood glucose levels by 21.7 % change over four hours and the mean percent change over 4 hours for Tolbutamide was 47.97 %. These values were not considered significant ($p>0.05$). So the same conclusion can be made about the efficacy of *Coccinia indica*, when compared to the reference drug, Tolbutamide. The blood glucose levels achieved after the four hour time period for the severely diabetic mice are still considered to be in the diabetic range, so further research needs to be done to find the optimal dose for severely diabetic people.

The results from this study, where mice were induced with acute diabetics and tested over a short period of time can be potentially useful for the Thai's who use *Coccinia indica* to treat diabetes. From these results that show that *Coccinia indica* has a very similar ability to Tolbutamide, a reference drug, to lower blood glucose levels, one can conclude that it can be considered as an alternative treatment for people who are suffering from diabetes. Further research needs to be done to fully understand how *Coccinia indica* is working within the pancreas and by what mechanism it is able to lower glucose levels in alloxan-induced diabetic mice.

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