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Evaluation of Anti-Hyperglycemic Effect *of Passiflora Edulis Sims* (Yellow Passion Fruit) Juice on Alloxan Monohydrate Induced Diabetes Mellitus in Male Sprague Dawley Rats

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ABSTRACT

Nowadays, the incidence of Diabetes Mellitus (DM) is growing around the world. In fact, it is one of the most serious diseases among adults in the age of 45 and a life - threatening disease over the age of 50, resulting to be the leading causes of death in which Filipinos are also affected by the disease. One of the most known way to treat DM is using insulin. However, side effect, toxicity, and organ damage may happen due to continuous usage of synthetic anti-hyperglycemic agent. Therefore, finding non-toxic and affordable treatment for Diabetes such as medicinal plants is presented. Present investigation was undertaken to investigate the ability of *Passiflora edulis sims* (yellow passion fruit) pulp juice as anti-hyperglycemic agent on male Sprague-dawley rats, and to compare and evaluate which concentration used in the study of *Passiflora edulis sims* pulp juice is significantly better as anti-hyperglycemic agent. Thirty (30) individual rats were used in this experiment, 10 of them are used for toxicity testing and no toxic effect has been observed after 24 hours. The remaining 20 rats were randomly divided into five (5) groups, DM was induced by using Alloxan monohydrate dissolved in 0.5 M buffered citrate at a pH of 4.5 and was injected intraperitoneally. Treatment was done by oral administration of yellow passion fruit pulp juice, 1ml, 1.5ml, 2ml, and 2.5ml respectively. The effectiveness of fruit juice was compared with a control group this include diabetic rats treated with water. Results of the study revealed that 1.5 mL, 2mL and 2.5mL concentration of the fruit juice significantly decrease the blood glucose of the diabetic rats while pre and post blood glucose only shows a significant decrease on group 4 and group 5, the highly evidence of anti-hyperglycemic factor occur. Therefore, the *Passiflora edulis sims* pulp juice fruit juice can be used as alternative treatment for diabetes mellitus

Keywords
Anti-hyperglycemict; diabetes mellitus; passion fruit; *Passiflora edulis sims*; alloxan.

1. INTRODUCTION

According to American Diabetes Association (ADA), hyperglycemia (high blood glucose) is a condition in which the body has too little insulin or the body cannot use the insulin properly [1]. It is also a hallmark sign of diabetes (both type 1 diabetes and type 2 diabetes) and pre-diabetes. Several factors can contribute to hyperglycemia in people with diabetes, including food and physical activity choices, illness, non-diabetes medications, or skipping or not taking enough glucose- lowering medication [2].

Diabetes mellitus is a disease of public health concern not only because of the costs involved in the care of individuals with this disease but also because of the vascular complications resulting from poor blood sugar control which impact on quality of life and shorten life span [3]. In 2016, diabetes was the direct cause of 1.5 million deaths and high blood glucose was the cause of another 2.2 million deaths [4].

Biguanides and sulphonylurea are oral hypoglycemic agents which are available along with insulin, were used as a treatment for diabetes mellitus but side effects associated with their uses are reported.Adhikari et al. [5] in their study reported that Biguanide (Metformin) was responsible for 87.5% of the adverse drug events whereas, Sulfonylureas accounted for 15.63% of the cases. Long-term usage of these drugs may ultimately lead to cardiovascular problems, liver disease, kidney disease and weight gain too[6].This has led to the use of herbal plants as an alternative agent. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown. Even the World Health Organization (WHO) approved the use of plant drugs for different diseases, including diabetes mellitus [7].

Currently, studies on new hypoglycemic drugs have been performed especially using medicinal plants. Of the several medicinal plants used for the treatment of diabetes only, some have been scientifically validated and recommended by the World Health Organization (WHO).

Passiflora edulis Sims, which is known as yellow passion fruit, is a popular remedy for treating anxiety, epilepsy, headache, and abdominal pain. The dry extract of *Passiflora* helps control glycemia and lipid levels. There are about 500 species distributed in warm temperatures and tropical regions.

Experimental animal models are used to test the pathophysiology of any disease. The emerging results are used to design and develop the drugs for its treatment. Test organisms are experimentally induced with a chemical to control the pre conditions of the animal. One of the most potent methods to induce experimental diabetes mellitus is chemical induction by Alloxan, a well- known diabetogenic agent used to induce Type I diabetes in experimental animals. As it has been widely accepted that alloxan selectively destroys the insulin-producing beta-cells found in the pancreas, hence, it is used to induce diabetes in laboratory animals [8].Alloxan induces irreversible diabetes mellitus after 24 hours following its administration and the condition proves to be chronic by laboratory tests after seven days [9].

Passiflora edulis sims was used in the study because it was hypothesized to possess anti-hyperglycemic effect. Other studies had utilized different parts of *Passifloraedulis* in determining its anti-glycemic effect. Aqueousleaf extract of *P.edulis*(200mg/kg) orally consumed, contained novel bioactive principles with antihyperglycaemic properties and is capable of reversing the altered carbohydrate and lipid profile in diabetes [7]. Apart from reducing aggregation and oxidizing species production in platelet of diabetic animals, the extract of passion fruit leaves was able to improve glycaemic control, renal function, as well as lipid profile due to the presence of flavonoid C-glycoside [10].de Queiroz et al.[11] used passion fruit peel flour to test type 2 diabetes. Hypoglycemic properties in diabetic rats were tested using passion fruit pectin [12], passion fruit seed [13], mesocarp fiber of passion fruit [14]. Likewise, the present study utilized fruit juice to test alloxan induced in Sprague-dawley rats and to find an alternative solution in treating diabetes mellitus using a cost effective natural treatment.

2. METHODOLOGY

2.1 Research Design

The study utilized the experimental research design. The fruit juice was tested on four different groups of rats and included a water treated alloxan-induced diabetic group as a control, 1 mL *Passiflora edulis sims* pulp juice treated diabetic rats group, 1.5 mL *Passiflora edulis sims* pulp juice treated diabetic rats group, 2 mL Passiflora edulis sims pulp juice treated diabetic rats group, and 2.5 mL *Passiflora edulis sims* pulp juice treated diabetic rats group. Methods of other related researches were adopted, modified, and used in this study.

The study included the evaluation of the anti-hyperglycemic activity of *Passiflora edulis sims* (yellow passion fruit) pulp juice among alloxan-induced Diabetic Sprague–dawley rats. The study also determined the blood glucose level of rats from the start of the study, after induction of alloxan and after the treatment of yellow passion fruit pulp juice using a point of care testing device a glucometer (ACCU-CHEK). The study also covered the induction of diabetes mellitus using an aqueous solution of alloxan monohydrate combined with citrate buffer.

The study was conducted at a private animal house under the assistance of a licensed veterinarian for handling and collection of blood samples of the rats.

2.2 Plant Material and Extraction

The fruit of *Passiflora edulis sims* was obtained in a locality in the southern part of Luzon in the Philippines and was authenticated in a research university. **T**he fruit was stored 7 to 10°C (45 to 50°F) and has a potential storage-life of two (2) weeks [15]. The fruits were washed, cut and the fruit pulp was triturated in the blender for 1 minute. The fruit pulp was filtered using a strainer and was filtered three more times using a plain-woven cotton fabric to completely filter the crushed seeds, five kilograms of yellow passion fruit produced 1300 mL or 1.3 L of yellow passion fruit juice.

2.3 Experimental Animal

Sprague-dawley rats were obtained from the Department of Laboratory Animal Resources and were acclimatized prior to experiment. This ensured that the animal was not under stress. Some period of acclimation following transportation is generally suggested to restore homeostasis [16].

According to the Institutional Animal Care and Use Committee (IACUC) of the John Hopkins University, proper restraint and handling techniques are essential for reducing stress to laboratory animals. Although there are significant species differences when handling and restraining an animal, there are several important concepts that apply equally to all species. These include handling animals gently but firmly, approaching an animal slowly but purposefully, wearing personal protective equipment (PPEs) and the use of an appropriate method.

Thirty (30) adult male Sprague-dawley rats that were only four weeks of age were used in the study. The rats were acclimatized for one week and weighed at the beginning of the experiment. Twenty (20) rats were divided into five groups with four rats each.

Alloxan monohydrate and buffered citrate, the chemical used for inducing diabetes was of analytical grade. DM was induced by administration of an aqueous solution of alloxan monohydrate diluted to 0.05 M buffered citrate, pH 4.5 after 24 hours of fasting, injected intraperitoneally. The solution was prepared by combining 30 mg per rat weight (in kg) and 1 ml alloxan per 7.5 ml buffer.

2.4 Procedure

Rats were housed in individual cages. Initial blood glucose testing was conducted, after the initial blood glucose testing, DM was induced by administration of an aqueous solution of alloxan monohydrate diluted to 0.05 M buffered citrate, pH 4.5 after 24 hour of fasting, injected intraperitoneal.

The blood glucose was checked at the 5th day after induction to monitor the blood glucose activity. Rats were considered diabetic if they had blood glucose levels greater than or equal to 120 mg/dL. After the successful induction of diabetes, treatment of yellow passion fruit pulp juice was conducted. Group 1 – control group diabetic rats treated with water; group 2 – diabetic rats treated with fruit juice with 1.0 mL concentration; group 3 – diabetic rats treated with fruit juice with 1.5 mL concentration; group 4 – diabetic rats treated with fruit juice with 2.0 mL concentration; and group 5 – diabetic rats treated with fruit juice with 2.5 mL

concentration. The remaining 10 rats were used for toxicity testing of the yellow passion fruit juice. After the treatment of yellow passion fruit pulp juice, the blood glucose level was determined at the 7th day of treatment.

2.5 Euthanization of Rats after the Termination of the Study

According to the Institutional Animal Care and Use Committee of the University of Texas at Austin, euthanasia is generally performed at the termination of a project, or in some cases, at a point where animals experience severe or chronic pain or distress that cannot be relieved. Emphasis should be placed on making the animal's death distress-free and painless.Techniques should result in rapid loss of consciousness, followed by cardiac or respiratory arrest and ultimate loss of brain function. Personnel carrying out the euthanasia should be properly trained to perform the procedure in the most effective and humane manner.

2.6 Statistical Analysis

Analysis of variance (ANOVA) was used to compare between pre and post blood glucose level, values <0.5 were considered as statistically significant and among the treatment groups. Further, post hoc test was done to determine the groups that gave the significant result.

3. RESULTS AND DISCUSSION

Table 1 presented the results of the anti-hyperglycemic activity of the rats after the treatment with *Passiflora edulis sims* (Yellow passion fruit) pulp juice for seven days. The blood glucose levels of diabetic rats from all the groups increased after injection of alloxan. However, with the treatment of *Passiflora edulis sims*(Yellow passion fruit) pulp juice, there was a noticeable reduction of the blood glucose levels on all groups treated with yellow passion fruit juice, wherein the blood glucose levels normalized. The effect was more pronounced in case of the group 5 – 2.5 mL on rat number 1 (from 169.5mg/dL after alloxan injection to 62.00mg/dL on the 7th day). While in the case of the control group on rat number 2, values showed a slight decreased (from 138.25mg/dL, after alloxan injection to 74.00mg/dL). This implied that the juice was found to be effective against hyperglycemia. Besides, it was very remarkable that there were values that were not within the range of the normal value. Juice formulation at concentration 1.5 mL, 2 mL, and 2.5 mL showed a significant improvement or decreased in the blood glucose as compared to water treatment and 1mL of concentration suggesting that an increased concentration of the active ingredients was proportionally beneficial.

Results showed a strong evidence that administering 2.5 mL of *Passiflora edulis Sims* fruit juice provided antihyperglycemic activity while administering 1.5 mL to 2.0 mL had moderately strong evidence. Utilizing 1 mL of *Passiflora edulis Sims* fruit juice provided a very weak evidence of antihyperglycemic activity. Results of the study conformed to the study of Barbalho et al. [17], where diabetic wistar rat offspring were treated using passion fruit juice. Mesocarp fiber of passion fruit at concentrations of 15% and 30% were used on diabetic rats induced by alloxan (2%), and the result showed that the fiber had potential hypoglycemic effect [14].

Statistical analysis using post hoc - Duncan's Multiple Range test revealed that there was no statistically significant decreased in the blood glucose levels of the group 1 - control, and group 2 – 1 mL, while a statistically significance can be seen in the blood glucose of the group 3 – 1.5 mL, group 4 – 2 mL and group 5 – 2.5 mL

Table 1. Anti-hyperglycemic activity of *Passiflora edulis sims* in different concentration

Groups	**Mean**			**p-value**	**Interpretation**
	Initial glucose	**Glucose level, 5 days after**	**Glucose level, 7 days after**		

	testing (mg/dL)	**induction of alloxan (mg/dL)**	**treatment of passion fruit pulp juice (mg/dL)**		
Group 1- control group	76.25	178.25	91.50	0.08	Not significant
Group 2 – 1.0 mL	78.25	138.25	74.00	0.07	Not significant
Group 3 – 1.5 Ml	74.50	153.50	79.00	0.03	Significant
Group 4 – 2.0 mL	80.00	182.75	65.75	0.01	Significant
Group 5 – 2.5 mL	75.50	169.50	62.00	0.00	Significant

Note : Levels of significance ≤ 0.05

Table 2 tabulated the ANOVA results on the blood glucose level. Based on the table, there was no significant in the glucose level at day 5. However, there was a significant on the blood glucose level at day 7 after the treatment with passion fruit pulp juice. This statistic justified the findings that the group treated with *Passiflora edulis sims* (Yellow passion fruit) showed a decreased in blood glucose level. Wherein, those treated with high concentration of fruit juice decreased blood glucose levels to the extent that after treatment blood glucose level was lower than the initial blood glucose level. In addition, it was notable that blood glucose levels of the control group also decreased or normalized. The effect may be primarily due to the absorption and distribution processes that occurred in response to alloxan followed by elimination.

Table 2. Effect of *Passiflora edulis Sims* on the Blood Glucose Levels of Alloxan-Induced Diabetic Sprague-Dawley Rats

Groups	**F-value**	**Probability**	**Interpretation**
Glucose level, 5 days after induction of alloxan (mg/dL)	0.64	0.646	Not significant
Glucose level, 7 days after treatment of passion fruit pulp juice (mg/dL)	17.03	0.000	Significant

Note : Levels of significance ≤ 0.05

4. CONCLUSION

The ability of the *Passiflora edulis sims* (Yellow passion fruit) pulp juice to significantly decreased the blood glucose levels in diabetic rats supported its anti-diabetic activity in rats. Statistical analysis of the different concentrations revealed that 1.5 mL, 2 mL and 2.5 mL of *Passiflora edulis sims* (Yellow passion fruit) pulp juice significantly decreased the blood glucose level of the diabetic rats. Moreover, data revealed that with 2 mL and 2.5 mL concentration of yellow passion fruit pulp juice showed a significant decreased in blood glucose level 7 days after treatment. Therefore, the researchers conclude that *Passiflora edulis sims* (yellow passion fruit) pulp juice was an effective anti-hyperglycemic agent. Hence, it might help in preventing diabetic complications and may serve as a good alternative in the present selection of antidiabetic drugs. Likewise, *Passiflora edulis sims* can also be considered in pharmacological studies of plant based medications.

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