



Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (14):104-108
(<http://scholarsresearchlibrary.com/archive.html>)



Hypoglycemic effects of *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell (Miracle Berry) fruit and leaf extracts on the blood glucose level of albino rats

Ma. Klarisse M. Dioso¹, Diane Cate A. Satsatin¹ and Johnny A. Ching^{1,2}

¹Biological Sciences Department, College of Science and Computer Studies, De La Salle University-Dasmariñas, City of Dasmariñas, Cavite, Philippines

²Graduate Studies Department, College of Science and Computer Studies, De La Salle University-Dasmariñas, City of Dasmariñas, Cavite, Philippines

ABSTRACT

Synsepalumdulcificum (Schumach. & Thonn.) Daniell(Sapotaceae), also known as miracle berry because of its ability to make sour foods taste sweet was the focus of the present study. The hypoglycemic effects of its fruit and leaf extracts on the blood glucose level of alloxan-induced diabetic albino rats were determined and compared with the positive control using metformin. Twenty-five albino rats were induced with type 2 diabetes mellitus using galloxan and were grouped and treated into five treatments (positive control using metformin, 50% fruit extract, 50% leaf extract, 100% fruit extract, 100% leaf extract).Blood glucose level were analyzed and compared. Results revealed that the two concentrations, i.e. 50% and 100%, of both fruit and leaf extracts induce the same significant lowering effects on the blood glucose level of the test rats. This may be due to the natural compound miraculin in the fruit and phytochemicals, such as flavonoids and saponins in its leaves.

Key terms: *Synsepalum dulcificum* (miracle berry), miraculin, diabetes, flavonoids, saponins

INTRODUCTION

Diabetes is one of the most known diseases worldwide. This disease is caused by the low production of insulin or insufficient levels of the hormone in the pancreas. The basic defect in diabetes is an absolute or relative lack of insulin which leads to abnormalities in metabolism[1]. According to the Global Report on Diabetes in 2016 by World Health Organization (WHO), the number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014. In 2012, an estimated 1.5 million deaths were directly caused by diabetes and another 2.2 million deaths were attributed to high blood glucose [2].

Diabetes can be treated and its consequences can be avoided or delayed with proper diet, physical activity, medications and regular screening and treatment for complications. One of the medications is the use of herbal medicines. Many plant species have confirmed that they are remarkably effective based on their plant components that could help control the blood glucose level which are resistant to insulin to prevent diabetes related problems [3]. Some examples of these popularly known herbal medicines are *Momordica charantia*[4-5], *Syzygium cumini*[6-7], and *Lyciumbarbarum*[8].

Another plant species with the potential to control blood glucose level is *Synsepalum dulcificum* or miracle berry under family Sapotaceae. The plant has been discovered due to its remarkable property of glycoprotein known as

miraculin which could help modify a sour taste into sweet taste[9]. In the Philippines, the miracle berry is known in Davao and some parts of Mindanao [10]. The value of the fruit as a taste modifier has a possible benefits for human health. It is discovered that it could help as a dietary supplement and an artificial sweetener among cancer patients. There are claims that miracle berry are indeed effective [9].

The present study determined and compared the effects of fruit and leaf extracts of miracle berry in reducing the blood glucose level of diabetic (alloxan-induced) albino rats.

MATERIALS AND METHODS

Five different treatments, including a positive control, were used in this experimental study. These are T₊ (treated with metformin), T₁ (50% concentration of miracle berry fruit extract), T₂ (50% concentration of miracle berry leaf extract), T₃ (100% concentration of miracle berry fruit extract) and T₄ (100% concentration of miracle berry leaf extract).

Procurement and Acclimatization of Test Rats

Twenty-five (25) male albino rats of same ages and body weights (ranging from 150 g to 200 g) were obtained at the Bureau of Food and Drugs in Muntinlupa City. The test rats were caged individually in wooden wire cages and acclimatized with normal pellet diet and ad libitum water for one week [11]. After acclimatization, the test rats were grouped into five with five test rats each for the different treatments.

Collection and Preparation of Fruit and Leaf Extract

Approximately 200 g of fruits and 150 g of leaves of miracle berry plant were collected and washed with clean tap water to remove the dirt. The fruit and leaves were cut into smaller pieces and were macerated in 800 mL 95% aqueous ethanol with occasional shaking for four days, followed by maceration with the same solvent for 2 days before filtration. After filtration, ethanol was evaporated through rotary evaporator at 37°C [12].

One hundred percent (100%) of extract were collected from the evaporator and the desired 50% concentrations were prepared in graduated cylinder using the dilution formula: $C_1V_1 = C_2V_2$ where C_1V_1 are the initial concentration and volume of the extract and C_2V_2 are the desired final concentration and volume. The dilution factor was obtained by getting the difference between the final and initial volume [13].

Administration of Alloxan

The test rats undergo fasting for 8 hr but have an access with water. Body weights and blood glucose levels were recorded before and after the induction of diabetic condition with alloxan under the supervision of a veterinarian. 100 mg of alloxan dissolved in 200 mL distilled water was induced per kg body weight of the test rats through intraperitoneal injection [8]. The blood glucose level was monitored till it reached 126 mg/dl and above signifying a diabetic condition [14].

Administration of Fruit and Leaf Extracts

Immediately upon reaching diabetic condition, different treatments were administered using oral gavage method at a daily dose of 200 mg/kg body weight ratio after taking their 12 noon meal for four weeks [8]. Regular diet and proper hydration were observed during the whole period of experimentation.

Data Gathering and Statistical Analysis

Blood samples were extracted from the test rats using the tail vein method. Blood glucose levels were recorded through the use of glucometer before and after the induction of diabetic condition and after four weeks of treatment.

Paired T-test, analysis of variance and Tukey test were used to determine the significant differences on the blood glucose level among different treatments and to establish which of the plant parts and concentrations induced a significant effect in lowering blood glucose level. All statistical analyses were done at 0.05 significance level.

RESULTS AND DISCUSSION

The hypoglycemic effects of miracle berry were determined from the blood glucose levels of test rats induced with alloxan to attain diabetic conditions and treated with different concentrations of the fruit and leaf extracts. Table 1 shows the blood glucose levels before and after the induction of alloxan, and after three weeks of treatment.

Table 1. Blood glucose level of test rats before and after induction of alloxan and after three weeks of treatment

Treatment	Blood Glucose Level (mg/dL)			Mean difference*
	Before Induction of Alloxan	After Induction of Alloxan	After treatment	
T ₀ (positive control group)	100.2 ^a	132.8 ^b	105.4 ^a	-27.4 ^x
T ₁ (50% miracle berry fruit extract)	102.6 ^a	140.0 ^b	112.4 ^a	-27.6 ^x
T ₂ (50% miracle berry leaf extract)	98.2 ^a	137.4 ^b	103.4 ^a	-34.0 ^x
T ₃ (100% miracle berry fruit extract)	110.8 ^a	131.4 ^b	107.4 ^a	-24.0 ^x
T ₄ (100% miracle berry leaf extract)	98.2 ^a	136.2 ^b	108.2 ^a	-28.0 ^x

Letters a&b compare the blood glucose levels before and after induction of alloxan and after treatment (columns). Letter X compares and indicates no significant difference between the *mean difference(after treatment minus after alloxan induction) per treatment (rows).

The table shows that after one week induction of alloxan, the blood glucose levels of all the different treatments reached the target threshold of at least 126 mg/dl signifying a diabetic condition. The table also shows that after three weeks of treatments with different concentrations of miracle berry fruit and leaf extracts, the average blood glucose levels of the test rats in all of the treatments were significantly lower ($p \leq 0.05$) than the diabetic blood glucose levels. There was no significant difference ($p > 0.05$) between the blood glucose levels before the induction of alloxan and the blood glucose levels after three weeks of treatments. This only means that all the experimental treatments were able to significantly lower and return the blood glucose levels in its normal levels.

The table also shows the mean difference between the blood glucose level after the induction of alloxan and after three weeks of treatment. Statistically, results reveal that there is no significant difference between the different treatments in terms of their hypoglycemic effects. It only shows that the 50% concentration of fruit and leaf extracts induced the same hypoglycemic effects as that of the 100% concentrations. Likewise, all the concentrations effectively lower the blood glucose level in comparison with the positive control.

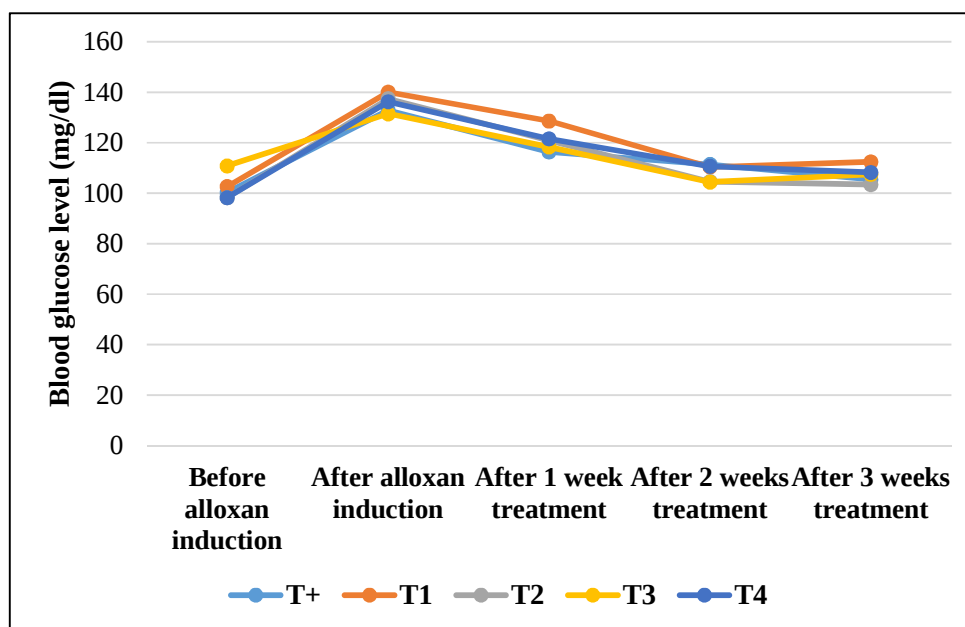


Figure 1. Trends in the blood glucose levels before and after alloxan induction and after weekly treatments

The blood glucose levels after weekly treatments for three weeks show a decreasing pattern (Figure 1). The different concentrations of miracle berry fruit and leaf extracts effectively induced a hypoglycemic effect to the test

rats. Both plant parts significantly lower the blood glucose levels as those treated with metformin in the positive control.

These results only showed that miracle berry fruit and leaf extracts were proven to have hypoglycemic effects on the blood glucose level of alloxan-induced albino rats. This effect can be attributed to the natural compounds that can be found in miracle berry fruits and leaves. Miracle berry fruit pulp is reported to contain antioxidant-rich phytochemicals, such as phenolics, flavonoids and the glycoprotein, miraculin[15] [16]. On the other hand, phytochemical screening of the leaves revealed that the leaves are rich in protein, fiber, polysaccharides, and moisture containing different phytochemicals such as tannin, cardiac glycosides, polyphenols, saponin and also flavonoids [16-17].

Many natural compounds, especially plants-derived medicines, have the potentials to treat diabetes mellitus in experimental animal and human trials via antioxidative mechanism[18]. Numerous studies has been reported that herbal constituents such as those found in miracle berry fruit and leaf extracts like saponins, tannins, flavonoids, phenolic compounds, glycosides alkaloids etc., shown to exhibit free radical scavenging role in vitro as well as in biological systems. However, among these phytochemicals, saponins and flavonoids are reported to possess the potential antidiabetic action [19-21].

Flavonoids is a potent antioxidant and free radical scavenger that has been shown to protect cell membranes from damage [22]. In vitro studies showed flavonoids have anti-diabetic, anti-inflammatory, anti-microbial and anti-cancer activities [23] [24]. Flavonoid compounds improve and stabilizes the secretion of insulin from pancreatic beta cells [25]. While saponins are known to reduce certain nutrients such as glucose and cholesterol at the gut. They may also aid in lessening the metabolic burden that are should be in the liver and are also known to inhibit the structure dependent biological activities [26]. On the other hand, the glycoprotein, miraculin, which is responsible for modifying sour taste as sweet in the mouth, has the ability to improve insulin sensitivity, thus it can be used as adjuvant for treating diabetic patients with insulin resistance [9].

CONCLUSION

Both extracts from the fruits and leaves of miracle berry plant exhibit hypoglycemic effects on the blood glucose level of alloxan-induced diabetic rats. Similarly, both extract concentrations, i.e. 50% and 100%, significantly induced the same lowering effect on the blood glucose level in comparison with the positive control treated with metformin. Thus, miracle berry fruit and leaf extracts can be used as an alternative supplement in reducing blood glucose level. These findings are attributed to the presence of natural compound miraculin in the fruit pulp and antioxidant-rich phytochemicals in its leaves, such as flavonoids and saponins

Acknowledgements

The suggestions and helpful critique of A/Prof. Myra N. Lagat, Dr. Jonathan S. Rubio, Dr. Mario S. Torres and Dr. Airill L. Mercurio, faculty members of the Biological Sciences Department, De La Salle University-Dasmariñas are duly acknowledged.

REFERENCES

- [1] American Diabetes Association. *Diabetes Care*, **2009**, Jan. 32 (Suppl 1): S62 – S67. doi 10.2337/dc09-S062
- [2]World Health Organization. *Diabetes Fact Sheet*.Reviewed June **2016**, Available at <http://www.who.int/mediacentre/factsheets/fs312/en/>
- [3] DK Patel; SK Prasad; R Kumar; S Hemalatha. *Asian Pac J Trop Biomed*, **2012**, 2 (4): 320-330.
- [4] P Chaturvedi; S George. *J of Med Food*, **2010**, 13 (3), 520-527.
- [5] X Xu; B Shan; CH Liao; JH Xie; PW Wen; JY Shi. *IntJ of BioMacromol*, **2015**,81, 538-544.
- [6] M Ayyanar; P Subash-Babu; S Ignacimuthu. *Compl Therapies in Med*,**2013**,1: 3, 232-243.
- [7] F Raffaelli; F Borroni; A Alidori; G Tirabassi; E Faloia; RA Rabini; A Giulietti; L Mazzanti; L Nanetti; A Vignini. *Platelets*, **2015**, 26:8, 1369-1635.
- [8] F Muselin; D Brezovan; J Savici; R Cristina; E Dumitrescu; AO Doma; D Morar; A Trif. *Animal Sci& Biotech*, **2015**, 48:1, 373-376.
- [9] CY Chen; PY Wu; TS Huang; CW Lin; YC Li; RH Chou; HW Chang; HM Wang. *Cur Nutr & Food Sci*, **2009**, 5:2, 1-8.
- [10] LB De Jesus. *Madrid Agribusiness Digest*, **2012**, 23, 45-46.

-
- [11] J Li; F Gong; F Li. *Biomed Research*, **2016**, 27: 1, 132-137.
- [12] M Zihlif; F Afifi; A Abu-Dahab; AMSA Majid; H Somrain; MM Saleh; ZD Nassar; R Naffa. *BMC Compl&Alt Med*, **2013**, 13:358. doi: 10.1186/1472-6882-13-358.
- [13] HEH Biscocho; JA Ching. *J of Expl Bio & Agric Sci*, **2016**, 4:121-127. doi: <http://dx.doi.org/10.18006/2016>.
- [14] H Staff. *WebMD [Internet]*. Health Wise Inc. **c2005-2015**, Available from: <http://www.m.webmd.com/diabetes/blood-glucose>
- [15] L Du; Y Shen; X Zhang; W Prinyawiwalkul; Z Xu. *Food Chem*, **2014**, 153: 279-284.
- [16] CY Ragasa; K Cornelio; T Bauca; S Chua; C Shen. *Chem of Nat Products*, **2015**, 51:3, 588-589.
- [17] V Osabor; R Etiuma; M Ntinya. *American ChemSci Jour*, **2015**, 12:1, 1 – 8.
- [18] LW Qi; EH Liu; C Chu; YB Peng; HX Cai; P Li. *Current Top Med Chem*, **2010**, 10:434-457.
- [19] C Han; Q Hui; Y Wang. *Nat Products Res*, **2008**, 22:11, 12-19.
- [20] M Misra. *J Med Plants Res*, **2009**, 3:1-9.
- [21] PS Perumal; PV Answara; AMuthuraman; S Krishan. *Pharmacological Study*, **2014**, 35:2, 211-217.
- [22] Y Noda; T Kneyuki; K Igarashi; AMori A; L Packer. *Toxicology*, **2000**, 148: (2-3), 119-123.
- [23] TP Cushnie; AJ Lamb. *Int J of Antimicrobial Agents*, **2005**, 26 (5): 343-356.
- [24] S Granados; N Balcazar; AGuillen; F Echeverri. *Molecules*, **2015**, 20 (4): 6181-6193.
- [25] S Mohan; L Nandhakumar. *J of Med Hypothesis and Ideas*, **2014**, 8: 1, 1-6.
- [26] S Patel; D Santani; M Shah; V Patel. *Jof Young Pharmacists*, **2012**, 4 (3): 171-176.