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RESEARCH ARTICLE

Antidiabetic effects of *Cycas edentata* aqueous leaf extract on the blood glucose levels of alloxan-induced diabetic ICR mice (*Mus musculus* L.)

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ABSTRACT

Background: Diabetes mellitus is ranked among one of the most prevalent diseases in the world conveniently described with increased blood glucose levels. Several drugs have been in the market to control diabetes; however, there are certain side effects that may be more of harm than beneficial. With this, alternative therapeutic options have been sought for the control of the disease. **Aims and Objectives:** The aim of this study is to evaluate the effects of aqueous extracts of *Cycas edentata* on the blood glucose levels of alloxan-induced diabetic mice. **Materials and Methods:** Aqueous leaf extract from *C. edentata* was administered to imprinting control regions (ICR) mice to determine its effect on blood glucose level. Thirty ICR mice were designated into six groups and were administered with double-distilled water, glimepiride, or the *C. edentata* aqueous extract. **Results:** After 28 days of treatment, statistical analysis indicated that the *C. edentata* extract had antihyperglycemic effect. There was a significant difference between the negative control and each experimental group (positive, low, mid, and high). There was also no significant difference observed between the sham control and the other experimental group (positive, low, mid, and high). Furthermore, ICR mice treated with the *C. edentata* extract had a significantly lowered cholesterol level compared to the sham group. With the use of hematologic analysis, the hemoglobin level, packed cell volume, total red blood cell count, total white blood cell count, neutrophils, eosinophils, lymphocytes, monocytes, and eosinophil were found to be normal which indicates that *C. edentata* has no effect in this parameter. **Conclusion:** These results indicate that *C. edentata* leaves have antidiabetic property at doses between 250 and 1000 mg/kg bodyweight.

KEY WORDS: Anticholesteremic; Blood Glucose; *Cycas edentata*; Hematology; Hypoglycemic Agents; *Mus musculus*

INTRODUCTION

Diabetes mellitus is one of the most prevalent metabolic disorders in the world.[1] It is a group of metabolic disorders characterized by a chronic hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. It is often classified into two main types based on dependence on exogenous insulin. Insulin-dependent diabetes mellitus (type I) is an autoimmune disease, wherein there is an insufficient supply of insulin caused by destruction of pancreatic beta-cells in the islets of Langerhans.[2,3] On the other hand, non-insulin diabetes mellitus (type II) resulted from the lack of insulin secretion by beta-cells of the pancreas and greater insulin resistance.[3] The latter represents about 90% of diabetic population, which suffers from complications

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that include negative effects on the eye, kidney, and nervous system.[4] Treatment of diabetes mellitus is geared toward management and control of hyperglycemia. Oral medications and insulin intake have been most frequently prescribed in managing type II diabetes.[5] On the other hand, control of type I diabetes involves a lifelong insulin therapy, wherein injections of insulin daily are required.[6]

Sulfonylurea and biguanides are the most commonly prescribed antidiabetic drugs for non-insulin diabetes mellitus. This drug has an antihyperglycemic activity by stimulating insulin secretion from pancreatic beta cells.[7] However, the recent pharmacological studies illustrated that most drugs used in diabetes control do not have an activity that can suppress the progression of the disease.[4] Furthermore, the use of oral antidiabetics has adverse side effects, which includes hypoglycemic coma, hematological, cutaneous and gastrointestinal reactions, and disturbances of the liver and kidney functions, and they are not suitable for use during pregnancy.[8]

With this, researchers steered to medicinal plants to manage diabetes mellitus since these plants contain active compounds that showed efficacy in most chronic disease such as diabetes mellitus.[9] Medicinal plants contain high contents of antinutrients and phytochemicals[10], and one potential medicinal plant is *Cycas* sp. It is commonly found in tropical and subtropical areas of the world, mainly in central and southern Africa, Australia, South and Central America, Caribbean, Asia, and Pacific Islands.[11] These species resemble ferns and palm trees with their leaf and stem forms. In the Philippines, multiple species of *Cycas* were identified to be present such as *Cycas edentata*. There have been different studies on the various medicinal use of *Cycas* sp. such as antimicrobial,[12] anticancer,[13] and antidiabetic activities[14] but thoroughly studied in *C. edentata*. Phytochemical analysis of compounds in *Cycas pectinata* showed that flavonoids, alkaloids, saponins, tannins, polyphenols, terpenoids, and steroids were present in the aqueous leaf extract.[15]

The plant kingdom has been utilized as medicines for thousands of years,[16] and medicinal plants have known to be an abundant source of phytochemicals.[17] These chemical compounds have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation, and modulation of hormone metabolism and anticancer property.[18] Since then, researchers have been studying different plant species to find out their exact effects within the body. Despite that no record on the efficacy of some medicinal plants exists in literature, majority of the Philippine population still resort to utilize these medicinal plants as an alternative treatment for common diseases and illnesses, such as colds and cough. The World Health Organization reported that most Filipinos still rely on medicinal plants for their psychological and physical health requirements, since they cannot afford the products of Western pharmaceutical industries together with the side effects and lack of health-care facilities.[19] With this, the use of *C. edentata* as a control of diabetes mellitus can serve as an alternative and accessible treatment to this chronic disorder. Furthermore, *C. edentata* could be a source of many potent and powerful drugs, which can be used, in the medical sector. This could be used as a future reference for the scientists and researchers who would like to conduct researches and future studies about *C. edentata*.

MATERIALS AND METHODS

Procurement of Plant Sample

7 kg of *C. edentata* stems was acquired from Kimaya, Consuelo, Misamis Oriental, Mindanao Island, Philippines. It was identified and authenticated by Dr. Esperanza Maribel G. Agoo at the De La Salle University Herbarium with the identification number of DLSUH 3150.

Preparation of Plant Extracts

The leaves were washed with tap water. They were then air dried for 3 days. Using a mechanical blender, the leaves were powdered. 500 g (dry weight) of the powdered *C. edentata* leaves were then soaked in 2.5 liters of water. After 3 days, through a muslin cloth, the solution was filtered. The filtrate was filtered again using a Whatman No. 1 filter paper. After this, a brown semisolid extract was obtained once it was allowed to freeze-dry and be lyophilized. This was weighed and then stored in an airtight and waterproof container at 4°C. The *C. edentata* extract was reconstituted by adding distilled water for the administration to the mice, by means of oral gavage.

Phytochemical Analysis

The collected *C. edentata* leaf extract was then sent to the Institute of Pharmaceutical Sciences, National Institutes of Health, and University of the Philippines Manila for phytochemical screening to determine the present compounds in the extract.

Procurement of Animals

A total of 36, 8-week-old male, mice weighing around 25-30 g imprinting control regions (ICR) mouse were obtained from the Food and Drug Administration, Alabang, Muntinlupa city. Six animals will be used for the acute oral toxicity test while the 30 animals will be used for the antidiabetic tests. Sample size was determined based on the methods of Charan and Kantharia.[20]

Animals were then placed in the Animal House at De La Salle University and were placed in individual standard-sized

cages. Standard commercial rodent food (pellet form) and drinking water were provided. The cages were lined with autoclaved paddy husk and cleaned twice a week. Before the experiment, all mice were acclimatized for 1 week to adapt to an environment with the temperature of 23°C and 55% humidity at a 12 h light: 12 h dark cycle. All succeeding experiments in animals were approved by the Institutional Animal Care and Use Committee of De La Salle University (reference #2015-002). The experiment from extraction to administration of samples and extrapolation of results were performed from September 2015 to April 2016.

Acute Oral Toxicity Test

Six ICR mice underwent the acute oral toxicity test. A 5000 mg/kg dose was administered orally, once to the subjects. Alertness, grooming, touch response, pain response, tremors, convulsion, righting reflex, gripping strength, pinna reflex, corneal reflex, writhing, pupillary reflex, urination, salivation, skin color, lacrimation, and hyperactivity are the responses that were observed in the ICR mice. After giving the test substance, both food and water were withheld for 2 h. The number of survivors during the first 24 h cycle was recorded, and later, daily for 2 weeks. Any death that occurred among the subjects were recorded.

Administration of Samples

The ICR mice were fasted for 8 h before the experiment. Hyperglycemia was then induced through administration of alloxan monohydrate (150 mg/kg) intraperitoneally in normal saline. The control group was only given normal saline intraperitoneally. After 7 days, EasyMate glucose, cholesterol, and uric acid (GCU) meter was used to measure blood glucose (Bioptik Technology, Inc., Taiwan). Only mice with blood glucose levels greater than 300 mg/dL which characterizes hyperglycemia were included in the study.

Experimental Design

Thirty ICR mice were randomly designated to six groups ($n = 5$). For 28 days, through the use of oral gavage, different treatments were administered to the subjects (Table 1).

Antidiabetic Test

Blood was collected through the tail-nick method on the 1st, 4th, 7th, 14th, and 28th day. In these days, blood glucose was collected using the EasyMate GCU meter (Bioptik Technology, Inc.). On the 28th day, blood cholesterol levels were measured using the same GCU meter.

Hematology

On the last day of the experiment, all mice were anesthetized using zolazepam-tiletamine (40 mg/kg) overdose intraperitoneally, and blood was then collected through intracardiac venipuncture. Hematocrit, total red blood cell (RBC) count, hemoglobin, total white blood cell (WBC) count, differential WBC count, and thrombocyte count were measured using Mythic 18 Vet (Orphee SA, Switzerland).

Data Analysis

The differences in blood glucose levels (mean ± standard deviation) were analyzed using repeated measures analysis of variance (ANOVA) for differences within groups. The differences between the groups were also checked through one-way ANOVA. Tukey's test was used to compare the means to determine significant difference among the treatment groups at $P<0.05$. All statistical analysis was performed using STATA version 12.

RESULTS

Phytochemical Analysis

The phytochemical analysis showed that the *C. edentata* extract contains carbohydrates, reducing sugars, alkaloids, and saponins (Table 2).

Acute Oral Toxicity Test

According to the OECD Guideline for testing of chemicals, a limit test at a dose level of 5000 mg/kg is carried out with

Table 1: Experimental design of the study

Groups	Treatment
Group 1 sham control	Distilled water
Group 2 negative control	Distilled water
Group 3 positive control	Glimepiride
Group 4 low dose	250 mg/kg
Group 5 mid dose	500 mg/kg
Group 6 high dose	1000 mg/kg

Table 2: Phytochemical Analysis of *C. edentata* by the Institute of Pharmaceutical Sciences, National Institutes of Health, and University of the Philippines Manila

Phytochemical	Indication
Carbohydrates	(+)
Reducing sugars	(+)
Flavonoids	(-)
Tannins	(-)
Glycosides	(-)
Alkaloids	(+)
Steroids and terpenoids	(-)
Saponins	(+)
Resins	(-)

C. edentata: Cycas edentata

at least three animals. If mortality is observed, a lower level is carried out. The significance of the said test is performed if the extract is most likely to be non-toxic. With regard to the treatments, the highest starting dosage level that can be administered is 2000 mg/kg. However, when there is no information on the substance, it is recommended to have a starting dosage of 300 mg/kg.

Alertness, touch response, grooming, and hyperactivity were among the responses that were observed during the allotted period of observation. On the other hand, the other half showed tremors, convulsion, and lack of both alertness and touch response before their death. With this, the sample can be safely administered because of the normal state of the subjects that survived throughout the test.

Antidiabetic Test

The results of the antidiabetic tests are presented in Table 3 and Figure 1.

Before the mice were induced with diabetes, the blood glucose level of the samples was measured and showed no significant difference (Figure 1, base). This supported the fact that the samples were under the same condition before the experiment and that the there is no statistical difference in blood glucose level of the subjects. This means that there is no hyperglycemic activity present in the sample before the experiment was conducted.

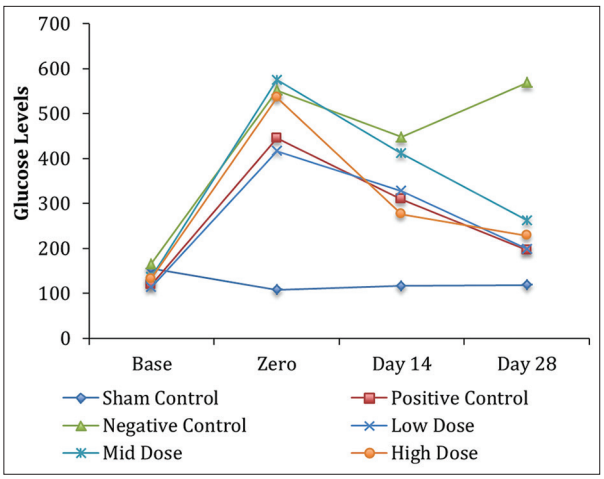

Figure 1: Mean glucose levels of mice groups from the baseline to the 28th day of treatment

After the samples from the five different experimental groups (positive, negative, low, mid, and high) have been induced with alloxan monohydrate (150 mg/kg), blood glucose levels were measured again and are shown under column zero. A significant difference exists between the sham and each of the treatment groups. Furthermore, data show no significant difference in the blood glucose level of the samples that were induced. This means that all experimental groups exhibited the desired hyperglycemic activity.

After 14 days of treatment, blood glucose levels were measured, shown under column 14 days. The mean blood glucose level of the negative control and mid-dosage treatment is significantly higher than the sham group, which is considered to be the normal level. Furthermore, no significant difference was observed among the sham, positive, low, and high dosage group. This implies that the blood glucose level of the samples under these groups was lowered. Although the said groups are significantly higher, no significant difference was observed among the experimental groups, which means that at 14 days, there is still insufficient evidence to conclude that one of the treatments is more effective than others.

28 days of treatment showed statistically significant effect on the blood glucose level of the samples. There is a significant difference existing between the mean of the negative control group and each of the other experimental group. Furthermore, no significant difference is observed between the sham group and the four treatment group (positive, low, mid, and high). This means that there is a significant decrease in the blood glucose of the samples since the treatments began. After 4 weeks of treatment, the hyperglycemic mice treated under the four treatment groups were found to be normal.

In addition, after 28 days of treatment, the subjects' cholesterol level was measured. All five experimental groups showed similar effect on the cholesterol level of the samples though only the subjects under the different *C. edentata* aqueous leaf extract treatments showed a significantly lowered cholesterol level than the sham group. This implies that *C. edentata* has a notable effect in lowering blood cholesterol level.

Table 3: Blood glucose level (mean±SD) of mice at different treatment levels

Group	Base	Zero	After 14 days	After 28 days	Chol
Sham (distilled water)	155.6±24.6	107.8±13.8	117.0±32.8^{b}	118.4±18.3^{a}	191.8±24.9^{b}
Positive (glimepiride)	119.8±53.8	445.2±112.3^{a}	309.4±158.9^{a,b}	196.8±228.0^{a}	146.2±42.2^{a,b}
Negative (distilled water)	167.0±68.3	552.0±57.7^{a}	448.8±139.1^{a}	568.4±48.5	138.2±51.1^{a,b}
Low (250 mg/kg)	114.0±28.7	416.0±156.6^{a}	328.0±182.7^{a,b}	198.8±67.2^{a}	125.0±29.5^{a}
Mid (500 mg/kg)	137.0±33.5	574.6±40.0^{a}	410.8±71.1^{a}	263.0±47.9^{a}	128.0±17.2^{a}
High (1000 mg/kg)	134.4±29.9	536.0±63.1^{a}	276.0±166.8^{a,b}	229.0±112.2^{a}	109.6±13.0^{a}

The mean±SD values with the same letter superscripts within columns are not significantly different (P<0.05). SD: Standard deviation, ^{a,b}: $P < 0.05$

Table 4: Hematological values (mean±SD) of mice at different treatment levels

Group	Hemoglobin (g/dL)	PCV (%)	Total RBC (cells×10^{12}/uL)	Total WBC (cells×10^{3}/uL)	Platelet (cells×10^{9}/uL)
Sham (distilled water)	114.6±5.2	34.2±5.5	6.408±0.6	3460±336.2	758.2±86.0
Positive (glimepiride)	102.2±5.4	35±5.1	7.432±0.2	4160±684.1	764.4±68.7
Negative (distilled water)	123.6±14.1	40.2±2.4	7.416±0.2	3820±609.9	671.6±109.9
Low (250 mg/kg)	104.2±13.5	34.4±5.3	7.296±1.1	4300±905.5	780.6±123.4
Mid (500 mg/kg)	117±14.1	32.4±4.4	5.89±1.2	3760±403.7	824.6±140.1
High (1000 mg/kg)	107.2±16.1	34.2±5.6	6.674±1.4	3380±739.6	742.4±182.2

SD: Standard deviation, PCV: Packed cell volume, RBC: Red blood cell, WBC: White blood cell

Evaluation of Hematological Values

After 28 days of treatment, the hemoglobin, hematocrit or packed cell volume (PCV), total RBC, total WBC, and platelet count were measured (Table 4). In comparison with the sham control, the values for the other mice groups were lower except for the negative control since it has not been given treatment, but it still falls within the normal range. This implies that there is no significant difference with the observed results for hemoglobin.

Hematocrit or PCV also did not exhibit a significant difference among the mice groups. A notable difference is that the negative control exhibited higher values as compared to the other mice groups, specifically the sham control, since it was not given treatment.

Similarly, the total RBC count was also measured. All of the mice groups exhibited values within the normal range. However, in comparison with the sham control, the positive and negative control as well as the low-dose group has higher values. Still, there is no significant difference between the total RBC count of all mice groups.

Another blood group is the total WBC count, wherein all were also within the normal range. Furthermore, the WBC count in all the mice groups exhibited values within that of the sham control. This implies that there is no significant difference between the values of WBCs in the mice groups.

Finally, platelet count was also determined. It has been implied that the groups exhibited values within the normal range, with the sham control, and the positive control has the lowest values. Similar to the other blood groups, it is observed that there is no significant difference between the platelet count in all the mice groups.

Specifically, as seen on Table 5, the neutrophil, lymphocyte, monocyte, eosinophil, and basophil count were determined after treatment of *C. edentata* for 28 days. In comparison with the sham control, it was implied that the high-dose group has the lower values of neutrophil and monocytes as compared to the sham control. The mid-dose group also has the lowest value for lymphocytes, and also, the data for the positive control are the same for the sham control since the former is treated with glimepiride, which is a drug that lowers blood glucose. However, after statistical analysis, it was found that there are no significant differences between the different WBC subtypes and *C. edentata* aqueous leaf extracts tend to have no significant effects.

DISCUSSION

Phytochemical analysis showed that the *C. edentata* extract contains carbohydrates, reducing sugars, alkaloids, and saponins. Among these compounds, alkaloids and saponins have been studied that may be able to suppress and control blood glucose levels.

Alkaloids are found in aqueous extracts of *C. edentata* as seen in the phytochemical analysis. They have ammonia compounds[21] comprising of low molecular weight nitrogen-containing compounds[10] and are important in plant defense and survival against microorganisms, insects, pathogens, and herbivores.[18] It is studied that alkaloids can exhibit antidiabetic properties through various mechanisms.[22] In a study on *Cryptolepis sanguinolenta*, alkaloids have been shown to have antihyperglycemic effect in diabetic mouse models, which leads to a decline in blood glucose concentration.[23] A study on *Catharanthus roseus* (L.) G. Don yielded four alkaloids that were found to be able to ameliorate type 2 diabetes through protein tyrosine phosphatase-1B inhibition.[24] On the other hand, alkaloids isolated from the roots and stems of *Berberis* L. have antihyperglycemic activity due to its alpha-glucosidase inhibition and to decrease glucose transport in the intestinal epithelium.[22]

In addition to that, saponins are found also in aqueous extracts of *C. edentata*. They are considered to be secondary metabolites due to the presence of glycosylated steroids, triterpenoids, and steroid alkaloids[18] and are known to have antidiabetic properties. A study done by Singh, Farswan[25] used triterpenoid saponin from *Primula denticulata* in streptozotocin-induced diabetic rats. It was identified through the use of nuclear magnetic resonance, ultraviolet, and infrared

Table 5: Differential WBC values (mean±SD) of mice at different treatment levels

Group	Neutro	Lympho	Mono	Eosino
Sham (distilled water)	670.2±106.7	2767.8±280.4	22.0±32.4	0.0±0.0
Positive (glimepiride)	883±356.0	3249.4±349.4	27.6±61.7	0.0±0.0
Negative (distilled water)	688.2±427.7	3006.2±735.7	51.0±72.7	74.6 0.0±127.9
Low (250 mg/kg)	824.4±398.4	3322.6±612.3	133.4±195.9	19.6±27.8
Mid (500 mg/kg)	942.2±338.3	2769.0±346.5	32.8±44.9	16.0±35.8
High (1000 mg/kg)	505.8±307.3	2813.8±555.9	16.0±22.6	44.4±49.0

SD: Standard deviation, WBC: White blood cell

spectroscopic methods. The result found that triterpenoid saponin possesses an antidiabetic property since it reduces the blood-glucose level in the animal models and restores the insulin level of the mice. In another study, wherein triterpenoid saponin was isolated from the roots of *Momordica cymbalaria* and administered to an isolated diaphragm of both diabetic and non-diabetic animals, results suggested that the extract reduces the blood glucose, cholesterol, and triglyceride level of the samples, and at the same time, increases the serum insulin values.[26] A similar study conducted in our laboratory on effects of *Cajanus cajan* yielded similar results. The plant was studied to contain saponins and may also have the same effect on diabetic mice.[27] There are different attributes of how saponins in plants can exert its antidiabetic effects. Some of the most common is that it can release insulin from the pancreas, reduce activity of glucose-6-phosphatase in the liver, and also alpha-glycosidase inhibition.[28]

In this study, it can be inferred that there are phytochemicals, particularly, alkaloids and saponins that work synergistically, which may have antidiabetic activity. However, further characterization of the specific compounds needs to be performed to be able to further describe the mechanism of antihyperglycemic action of the plant. Furthermore, there are no conclusive data on the effects of the plant on hematological parameters.

CONCLUSION

C. edentata extract was able to decrease the blood glucose levels of the ICR mice. There is a significant difference observed between the four treatment groups (positive, low, mid, and high), wherein during the 28th day, the blood glucose level of the hyperglycemic mice was found to be normal. Compounds such as saponins and alkaloids could have contributed to the antihyperglycemic effect of *C. edentata. C. edentata* showed a significantly lowered cholesterol level effect on the ICR mice compared to that of the sham group. With this, it implies that the plant has a notable effect in lowering blood cholesterol level. For the hematology parameters of the samples, it was shown that there is no significant difference between the hemoglobin level, PCV, total RBC, total WBC, and platelet count and between the neutrophils, lymphocytes, monocytes, and eosinophils, respectively. Further studies, particularly, the isolation of the specific compounds should be performed to further elucidate the antidiabetic action of the plant.

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