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Hypoglycemic Effect of *Musa acuminata* Aqueous Leaf Extract on Alloxan-induced Diabetic ICR Mice (*Mus musculus*)

John Eduard R Bascon[1], Tristan Josef E Galvez[1], Kay T Jabola[1], Rodel Jonathan S Vitor II[1], Paolo Robert P Bueno[2]

[1]*Biology Department, De La Salle University, Manila, Philippines*
[2]*Department of Biochemistry and Molecular Biology, University of the Philippines, Manila, Philippines*

ABSTRACT

Diabetes mellitus is a metabolic disease characterized by abnormally high glucose level in blood. Plant-based compounds are now being studied as alternatives for diabetes. Due to limited information on the pharmacologic activity of Musa acuminata, also known as "lakatan", this study was carried out to determine the effect of Musa acuminata aqueous leaf extract on blood glucose level of alloxan-induced diabetes mice and to assess its safety in vivo. Thirty male ICR mice (Mus musculus) were divided into six groups (n=5) and were administered intraperitoneally with alloxan monohydrate (75 mg/kg) (5 groups), or normal saline solution (sham control group). Treatment groups were given distilled water (both sham and negative control groups), glimepiride (17.6 µg/kg, positive control), or one of the three doses of M. acuminata leaf aqueous extract tested (250, 500 and 1000 mg/kg) concomitantly via oral gavage for 28 days. The blood glucose levels of the mice were measured at baseline and at 0^{th}, 14^{th} and 28^{th}. Phytochemicals present as well as signs of general toxicity were also determined. Results showed that M. acuminata (1000 mg/kg) significantly decreased blood glucose level ($P<0.05$) and has comparable activity with that of the positive control. Phytochemical analysis revealed the presence of tannins, cardiac glycosides, alkaloids, steroids and terpenoids. Also, there were no changes in behavior and activity observed in mice treated with M. acuminata. These observations strongly suggest that the M. acuminata leaf extracts has blood glucose lowering properties and is safe for consumption.

Keywords: Diabetes mellitus, *Musa acuminata*, Hypoglycemic effect, Phytochemicals, Alloxan-induced mice

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by abnormally high glucose level in blood-known as hyperglycemia. This may be brought about by defective insulin secretion or insulin action or may be both [1]. In fact, for every 10 s, there is at least a person who dies from diabetes-related diseases such as heart diseases, eye complications, and kidney diseases. In 2013, an estimated of four million deaths worldwide and it has been estimated to be 438 million deaths by 2030 [1].

The Philippines, as one of the emerging hotspots of diabetes, had been ranked to be in the Top 15 in the world for the prevalence of the disease. Currently, there is a ratio of one is to five having diabetes with at least four million as diagnosed and a large number of people who are still unaware of having the said disease [2].

A number of treatments for diabetic patients have been developed. Some of which are insulin injections and oral drugs like metformin, sulfonylureas, meglitinides and Sodium-glucose co-transporter-2 (SGLT-2) inhibitors. Unfortunately, adverse effects have been reported such as nausea, diarrhea, weight gain, yeast infections and urinary tract infections [3] and thus, new therapeutics are needed in the field.

The World Health Organization recommends the use of traditional and plant based medicines for the management of diabetes mellitus [4]. There is a high prevalence of utilization of alternative medicines due to their perceived effectiveness, safety, affordability, and acceptability, with minimal side effects in clinical experience, and relatively low cost [5]. Some of these medicinal plants are *Allium cepa*, *Brassica oleracea*, *Gongronema latifolium*, *Momordica charantia*, *Nauclea latifolia* and *Catharathus roseus* [6-11].

Musa acuminata belongs to family *Musaceae* which originated in Southeast Asia and other surrounding tropical and subtropical regions. There are nine subspecies known for this *Musa* species [12]. In the Philippines, *M. acuminata* is known as "lakatan" and has been reported that its cultivation began in the Philippines. This species flourished in the Philippines since it was able to thrive well in warm and humid tropic [13]. To date, there are no reports on the effect of *M. acuminata* leaves on hyperglycemia. Hence, this study was designed to investigate the effect of aqueous leaf extract on blood glucose levels of alloxan-induced diabetes mice and to evaluate its safety *in vivo*.

MATERIALS AND METHODS

Plant sample collection

About 7 kg of fresh *Musa acuminata* leaves were collected from Cavite, Philippines. Samples were brought to the Bureau of Plant and Industry (BPI) for the plant identification and authentication (PLT-ID-CRD-148-15).

Plant extract preparation

The collected leaves were then washed using distilled water and air-dried for three days. Dried leaves were powdered using a mechanical blender. About 500 g were submerged in distilled water (2.5 L) for 72 h. Then it was filtered using a muslin cloth. The filtrate was re-filtered using a Whatman No. 1 filter paper. The re-filtered extract was subjected to lyophilization and was stored at 4°C.

Phytochemical screening

Qualitative phytochemical tests of *M. acuminata* leaf extract were performed at the Institute of Pharmaceutical Sciences, National Institutes of Health, University of the Philippines, Manila in order to find out if alkaloids, cardiac glycosides, terpenoids, anthraquinones, flavonoids, saponins, tannins, polyphenols were present.

Experimental animals

Thirty-four eight-week old male ICR mice, weighing from at least 20 to 30 g, were obtained from the Food and Drug Administration (FDA) in Alabang, Philippines and were housed in the De La Salle University (DLSU) Animal House under controlled conditions (23°C temperature, 55% humidity and 12 h light and dark cycle). The mice were acclimatized individually in standard sized cages for 7 days with free access to standard commercial rodent food (pellets) and water *ad libitum*. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) (Reference #2015-001).

Acute oral toxicity test

Acute oral toxicity was performed according to the Organization for Economic Cooperation and Development (OECD) [14]. A maximum dosage of 5,000 mg/kg was given orally to the mice. Animals were monitored for signs of general toxicity under the supervision of a veterinary doctor. The animals were monitored daily for 7 days. All signs of toxicity and mortality in the test were recorded. Behavioral and activity changes which includes: 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 were also noted.

Induction of diabetes

Thirty mice were fasted for 8 h overnight and their baseline blood glucose levels were measured. Twenty-five of these mice were administered intraperitoneally with alloxan monohydrate (75 mg/kg) to induce diabetes. On the other hand, the remaining 5 mice, which will serve as the non-diabetic or sham control group, were given Normal Saline Solution (NSS). After 3 days, blood glucose levels were measured using Easy Touch Glucose Cholesterol Uric acid (GCU) meter (Bioptik Technology, Inc., Taiwan). Mice with hyperglycemia (blood glucose>300 mg/dl) were the only ones used in the study.

Experimental design

Mice were randomly placed into six groups of five mice each using Saranomy Random Generator (USA). The treatments shown on Table 1 were given to the mice *via* oral gavage for 28 days.

Table 1: Experimental design for mice distribution

Group	Treatment
Group 1	Distilled water
Sham control	
Group 2	Distilled water
Negative control	
Group 3	Glimepiride (17.6 µg/kg)
Positive control	
Group 4	250 mg/kg
Low dose	
Group 5	500 mg/kg
Mid dose	
Group 6	1000 mg/kg
High dose	

Blood glucose collection and testing

Blood was collected using the tail-nick method. Blood glucose levels were measured at days 0^{th}, 14^{th} and 28^{th} day by using an EasyTouch® GCU meter (Bioptik Technology, Inc., Taiwan).

Data analysis

Data obtained from the different experimental groups were compared by one-way ANOVA followed by Tukey's test using STATA v.12 software. Differences with $P<0.05$ were considered significant. Data are presented as mean ± SD.

RESULTS AND DISCUSSION

Diabetes mellitus is a fast-growing, multi-factorial metabolic disease affecting millions of people worldwide [15]. It is considered to be one of the most common chronic diseases and is associated with hyperglycemia and co-morbidities such as obesity and hypertension [16]. In order to establish a scientific basis for the use of *Musa acuminata* in the treatment of diabetes, determination of the hypoglycemic activity of the aqueous extract on diabetic mice and assessment of its safety were carried out in the study.

Prior to determination of the hypoglycemic activity, acute oral toxicity of *M. acuminata* aqueous leaf extract was first conducted to evaluate its safety. The maximum dosage of 5,000 mg/kg of *M. acuminata* extract was administered to each of the ICR mice (*Mus musculus*) *via* oral gavage. In a report by OECD in 2001, 5,000 mg/kg is the dosage recommended to be used when the results of the study is directly relevant to the health of test subject [14]. After 7 days of observation for lethality and pathological change, the mice were noted to be in normal condition. There were no changes observed in behavior and activity. No deaths were observed during the conduction of the test in a span of 7 days. This suggests that *M. acuminata* is safe for consumption.

In animals, diabetes can be induced by partial pancreatectomy or by the administration of diabetogenic drugs such as alloxan, streptozotocin, ditizonx and anti-insulin serum [17]. Alloxan is a beta cytotoxin that induces diabetes through free radical production. Generated free radicals damage the insulin secreting pancreatic beta cells, resulting to a decrease of endogenous insulin release and to a dramatic increase in blood glucose levels [16-24]. In this study, alloxan was used as the diabetogenic agent in mice.

The hypoglycemic effect of the aqueous leaf extract of *M. acuminata* (250, 500 and 1000 mg/kg) and glimepiride (17.6 µg/kg) on mean blood glucose levels of diabetic and non-diabetic mice were shown in Table 2. After 14 days of administration with *M. acuminata* aqueous leaf extract, the 1000 mg/kg dose level showed a significant ($P<0.05$) decrease in the mean blood sugar level while the other doses (250 and 500 mg/kg) showed no significant effect ($P>0.05$). Moreover, mice treated with *M. acuminata* extract (1000 mg/kg) has higher reduction in mean blood glucose levels compared to that of the positive control group with 55.23% and 13.14%, respectively. Interestingly, it was observed that all groups except the sham control and high dose-treated group still exhibited diabetes on the 14th day of treatment.

Table 2: Blood glucose level (mean ± SD) of mice at baseline, 0th, 14th and 28th days

Group	Baseline (mg/dl)	Day 0 (mg/dl)	Day 14 (mg/dl)	Day 28 (mg/dl)
Sham control (Normal mice control)	55.6 ± 24.56	107.8 ± 13.82	117 ± 32.79^{a}	118.4 ± 18.26^{a}
Positive control (Glimepiride 17.6 µg/kg)	119.8 ± 53.83	356.2 ± 30.82^{b}	309.4 ± 158.87^{a-c}	196.8 ± 228.00^{a-c}
Negative control	107 ± 105.18	552 ± 57.71^{a}	448.8 ± 139.10^{b,c}	568.4 ± 48.51^{d}
Low dose (250 mg/kg)	165.8 ± 28.53	547.4 ± 87.60^{a}	511.2 ± 118.28^{c}	348.8 ± 93.00^{b-d}
Mid dose (500 mg/kg)	148.7 ± 42.75	513.6 ± 119.52^{a,b}	358.8 ± 227.66^{a-c}	409.2 ± 103.28^{c,d}
High dose (1000 mg/kg)	115 ± 51.24	440.8 ± 145.40^{a}	197.8 ± 105.84^{a,b}	185.6 ± 58.72^{a,b}

Means ± SD with the same superscript within the same column are not significantly different at $\alpha<0.05$

On the 28th day of treatment, the mean blood glucose levels of mice were lowered significantly ($P<0.05$) to 185.6 ± 58.722 and 196.8 ± 227.99 mg/dl from 0th day values (440.8 ± 145.398 and 356.2 ± 30.817) by *M. acuminata* aqueous extract (1000 mg/kg) and Glimepiride (17.6 µg/kg), respectively. In addition, *M. acuminata* showed greater hypoglycemic effect of 57.89% than that of 44.75% of the positive control. On the other hand, there were no significant lowering ($P>0.05$) observed in mice treated with the 250 and 500 mg/kg. Thus, these findings revealed that high dose of *M. acuminata* (1000 mg/kg) in order to exhibit significant hypoglycemic activity.

The hypoglycemic activity observed from the aqueous *M. acuminata* leaf extract can be accounted on the presence of different phytochemicals which could act synergistically or independently in lowering the blood sugar level. *M. acuminata* contained various families of secondary metabolites with potential biological activity, mainly tannins, cardiac glycosides, alkaloids and steroids and terpenoids (Table 3).

Table 3: Results of phytochemical analysis of *Musa acuminata* leaf extract

Phytochemicals	Indication
Tannins	(+)
Flavonoids	(-)
Cardiac glycosides	(+)
Saponins	(-)
Anthraquinones	(-)
Alkaloids	(+)
Steroids and terpenoids	(+)
Polyphenols	(-)

Alkaloids, tannins, terpenoids and sterols have been associated with hypoglycemic activity [19]. *Nauclea latifolia*, a plant screened to contain alkaloids, showed significantly reduced the blood glucose levels in alloxan-induced diabetic rats [20]. Four types of alkaloids found in *Catharanthus roseus*, exhibited blood glucose level lowering by inhibition of glucose uptake in the liver [11].

Tannins found in banaba extract blocked TA-induced glucose transport and induced translocation of glucose transporter 4 in the signaling pathway of insulin-mediated glucose transport [21]. The tannin epigallo-catechin-3-gallate exhibited anti-diabetic activity as demonstrated by Broadhurst et al. [22]. In a review done by Arif et al. different types of terpenoids have shown mechanisms acting upon high glucose levels by protective or inhibitory action against insulin degradative processes [23]. Isolated terpenoids from *Paeonia suffruticosa*, increased glucose uptake and enhanced glycogen synthesis by activating AMPK in insulin-resistant human HepG2 Cells while isolated terpenoids in *Lagerstroemia speciosa* Linn. showed significant stimulation of glucose transport activity and significantly lowered blood glucose levels [23].

A study done by Jawla et al. revealed that *M. paradisiaca* flowers showed significant reduction in the mean blood glucose level after 30 min of administration in glucose-loaded rats while the extract strikingly reversed the permanent hyperglycemia induced by alloxan monohydrate. These were attributed to the phytochemicals found in it which includes tannins, cardiac glycosides, alkaloids and steroids and terpenoids [18].

These phytochemicals have been reported to possess antioxidant properties which may aid against hyperglycemia induced oxidative stress. They have also been reported to be associated with improvement in the symptoms of diabetes [24].

CONCLUSION

The results showed that the *M. acuminata* leaf extract is non-toxic since there are no signs of lethality and behavioral changes observed for the span of seven days in acute oral toxicity test. The aqueous leaf extract of *M. acuminata* lowered blood glucose levels in all of the treatment groups but the high dose group (1000 mg/kg) showed the significant hypoglycemic effect even lower than that of the positive control. The present investigation revealed that the aqueous leaf extract of *M. acuminata* has hypoglycemic activity. However, comprehensive chemical and pharmacological investigation should be carried out to isolate the active compound and appropriate elucidation of its mechanism of action.

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