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Antihyperglycemic Activity of the Crude Ethanolic Extract of *Artocarpus heterophyllus* Lam. (langka) Unripe Fruit against Alloxan-induced Hyperglycemia in *Mus musculus* (White Mice)

Ma. Socorro G. Leong-on

University of San Agustin, Gen. Luna St., Iloilo City, 5000, Philippines

Abstract: ***This study was conducted to determine the antihyperglycemic activity of the crude ethanolic extract of Artocarpus heterophyllus Lam.(langka) unripe fruit using the method alloxan induced hyperglycemia in Mus musculus (white mice). The different extract concentrations of A. heterophyllus were orally administered to the fasted hyperglycemic white mice. On the first, second and third hour after extract administration, fasting blood sugar (FBS) was measured and percent reduction in FBS were compared. The study showed that the crude ethanolic extract of A. heterophyllus unripe fruit exhibited anti-hyperglycemic activity at 5mg/20g (250mg/kg), 10mg/20g (500mg/kg), 15mg/20g (750mg/kg) on the third hour after the extract administration. Their results differ significantly to the negative control - dist. water (7.27+0.52) (P<0.05). The concentration of the extract at 15mg/20g body weight (bw) of white mice (lowered FBS by 37.19+0.65%) exhibited comparable antihyperglycemic activity to the positive control (Glibenclamide) (40.57+1.13) (P>0.05). On the first and second hour, both 10mg and 15mg/20g bw of white mice exhibited anthyperglycemic activity but were not comparable to the positive control. The antihyperglycemic activity was concentration and time dependent. The best concentration that exhibited antihyperglycemic activity was at 15mg/20g bw of white mice and best time was on the third hour. The study demonstrated the antihyperglycemic activity of the crude ethanolic extract of A. heterophyllus unripe fruit at 5mg/20g, 10mg/20g, 15mg/20g against alloxan-induced diabetes in white mice. The study provided the scientific basis of the folkloric claim of the antihyperglycemic effect of A. heterophyllus. This study could also serve as basis for future formulation of effective, low cost and locally available antihyperglycemic agent from A. heterophyllus unripe fruit subject for further study.***

Keywords: anti-hyperglycemic activity, Artocarpus heterophyllus, alloxan-induced hyperglycemia, crude ethanolic extract

1. Introduction

Hyperglycemia or high blood sugar, is associated with diabetes mellitus (DM). Hyperglycemia could have resulted due to the lack of insulin secretion, failure of the insulin to act or could be their combination [1]. The current available treatments for diabetes includes sulphonylurea, glinides and biguanides. Although there are drugs in the market for diabetes, some rural folks were using the unripe fruit of *A. heterophyllus* fruit to lower their blood sugar. This practice has been persistent through decades. However, no scientific studies have been done to validate their folkloric claim.

Diabetes mellitus is the leading cause of kidney failure, heart attack, blindness and lower limb amputation. It is the fourth main cause of death in most developed countries. According to International Diabetes Federation, the prevalence of diabetes is estimated to reach 330 million by the year 2025 especially in Africa and Asia. It is also estimated that by the year 2025, over 75% of people with diabetes are residing in developing countries, as compared to 62% in 1995 [2].

Various plant extracts have been proven to exhibit anti-diabetic or anti-hyperglycemic activity scientifically. Therefore, they are considered as promising substitute for anti-diabetic drugs [3]. Thus, screening of more medicinal plants with anti-diabetic or anti-hyperglycemic effects are encouraged since diabetes mellitus is a major global health problem.

One of the plants that has folkloric claim as antihyperglycemic agent is *A. heterophyllus* Lam. It belongs to Moraceae family. It is an evergreen fruit tree cultivated in many tropical regions. The phenolic compound from its wood exhibited anti-proliferative effect on cancer cell line [4]. The butanol fractions of the root bark and fruits of *A. heterophyllus* was found to be active against bacteria [5]. A study also, confirmed that *A. heterophyllus* leaf extracts has antioxidant, anti-hyperglycemic and hyperlipidemic activities [6]. The flavonoid fraction of its leaf exhibited hypoglycemic effect [7] and wound healing activity [8]. The polyphenolic compound from its tegmen showed antitumor activity [9]. However, the antihyperglycemic activity of the unripe fruit of *A. heterophyllus* has not yet been established.

This study generally aimed to evaluate the antihyperglycemic activity of the crude ethanolic extract of *A. heterophyllus* unripe fruit against alloxan-induced hyperglycemia in *Mus musculus* (white mice). The study specifically aimed to determine the concentration and the time (first, second, and third hour) when the crude ethanolic extract of *A. heterophyllus* unripe fruit showed the best anti-hyperglycemic activity.

Result of this study established the scientific basis of the folkloric claim of use of *A. heterophyllus* unripe fruit as an alternative treatment for hyperglycemia.

2. Materials and Methods

2.1 Plant Identification and Collection

A. heterophyllus (fig. 1) was initially identified based on the book of Quisumbing (1978) [10] and Owa *et al.*, (2009) [11] and authenticated by a licensed agriculturist. The fruit was collected from Barangay Piapi, Hamtic, Antique, Philippines.

Figure 1: *Artocarpus heterophyllus* (langka) fruit

A. heterophyllus is locally called langka or jackfruit. It is described as an evergreen tree that reach the height of 8 to 25m. It forms canopy dense with rarely buttress trunk with greyish-brown and scaly trunk. Its leaves are glossy leaves with dark green color on the top and light green underside and alternately arranged. *A. heterophyllus* has racemoid inflorescence and has a multiple, spiky, yellow fruit and has waxy, oval to oblong shaped seeds [11].

2.2 Extraction of Plant Components

A. heterophyllus unripe fruit was washed with tap water and rinsed with distilled water. After the removal of the peelings, it was chopped and ground using a blender. The ground unripe fruit was homogenized in 90% ethanol (2g plant material/5ml ethanol) for 3 days with shaking every 8 hours. Homogenized ground unripe fruit was filtered. The filtrate was dried. The crude ethanol extract was used for the antihyperglycemic screening [12].

2.3. Raising and Acclimatization of Experimental animal (White Mice) and Ethical Considerations

White mice were bred in a clean cage and fed with pellets and water *ad libitum* at room temperature. Animals were treated under the required protocol of the Animal Welfare Act of 1998 and guidelines made by the Philippine Association of Laboratory Animal Science. A permit was obtained from the Bureau of Animal Industry for the use of white mice in the study. All mice were subjected to cervical dislocation after the experiment.

2.4 Induction of hyperglycemia

Fifty (50) white mice, male, weighing 20-30 grams, 2-3 month old, were utilized in this study. The white mice were fasted for 8 hours prior to the blood sample collection. A small drop of blood sample was obtained from the mouse's tail by pricking the major vein with needle. A drop of was blood placed on a disposable test strip. This was done to determine the fasting blood sugar level (mg/dl) using a glucometer. Alloxan monohydrate (Sigma) was dissolved in distilled water just prior to oral administration to the mice. Hyperglycemia was induced among mice by orally administering it at a dose of 150 mg/kg bodyweight (bw) after overnight fasting [13]. After 48 hours, the mice with fasting blood glucose levels of 112mg/dl (13 to 15 mmol/L) were separated and included in the study [14]. This procedure was followed from the work of Syiem *et al.*, (2002) with modifications.

2.5 Antihyperglycemic Test

Mice with 112mg/dl (13-15 mmol/L) fasting blood glucose were divided into five groups. The first three groups received different concentrations of *A. heterophyllus* unriped fruit crude ethanolic extract (5mg, 10mg, and 15mg per 20g body weight of white mice in 0.5ml distilled water). The fourth group received Glibenclamide (5mg/kg), the positive control for this study. The fifth group received the solvent of the plant extract, the negative control (distilled water). The fasting blood sugar (FBS) was measured on the first, second and third hour after administration of the treatments [15]. This procedure was patterned after Djomeni *et al.*, (2006) with modifications. Five replicates of mice were made in every group. This is in compliance with Animal Welfare Act of 1998. The percent reduction in FBS level was calculated using the formula below and subjected to statistical analysis:

$$\text{Percent reduction in FBS level} = \frac{\text{FBS level initial} - \text{FBS level final}}{\text{FBS level initial}} \times 100$$

2.6 Statistical Analysis

Analysis of Variance (ANOVA one-way) was used to compare the means of the percent reduction in FBS of the different treatments per hour as well in comparing the activity of the extracts at different hours. Least Significant Difference (LSD) was the post hoc test. *P value* equal to or less than 0.05 was considered statistically significant. The results were expressed as mean±sd.

3. Results

The study showed that the crude ethanolic extract of *A. heterophyllus* unripe fruit exhibited anti-hyperglycemic activity at 5mg/20g (250mg/kg), 10mg/20g (500mg/kg), 15mg/20g (750mg/kg) against alloxan-induced hyperglycemic in white mice on the third hour after the extract administration. Their results differ significantly to the negative control ($P<0.05$). The concentration of the extract at 15mg/20g body weight of white mice had comparable effect to the positive control ($P>0.05$). On the first and second hour, both concentrations of the extract at 10mg and 15mg/20g bw of white mice exhibited antihyperglycemic activity but were not comparable to the positive control. The anti-hyperglycemic activity was concentration and time dependent. The best concentration that exhibited anti-

hyperglycemic effect was at 15mg/20g bw of white mice. The time when the extract exhibited the best activity was on the third hour after extract administration (table 1).

Table 1: Antihyperglycemic activity of the crud ethanolic extract of *A. heterophyllus* (langka) unripe fruit against alloxan-induced hyperglycemia in *Mus musculus* (white mice)

Treatments (extracts)	% reduction in FBS (mean±SD)		
	1hr	2hr	3h
5mg	^{a}04.84±0.14^{a}	^{b}07.60±0.20^{a}	^{c}11.68±0.41^{b}
10mg	^{a}11.83±0.63^{b}	^{b}18.13±0.82^{b}	^{c}24.86±0.86^{c}
15mg	^{a}19.97±0.61^{c}	^{b}27.44±0.82^{c}	^{c}37.19±0.65^{d}
- control (dist. water)	^{a}3.62±1.30^{a}	^{a}5.37±0.82^{a}	^{a}7.27±0.52^{a}
+ control (Glibenclamide)	^{a}24.10+0.95^{d}	^{b}35.43±1.29^{d}	^{c}40.57±1.13^{d}

Note: Letters after the values denote comparison among treatments, while letters after the values denote comparison among hours. a>b>c>. The same letter means comparable.

4. Discussion

The crude ethanolic extract of *A. heterophylus* unripe fruit exhibited anti-hyperglycemic activity at 5mg/20g (250mg/kg), 10mg/20g (500mg/kg), 15mg/20g (750mg/kg) against alloxan-induced hyperglycemic in white mice. *A. heterophyllus* may have corrected the hyperglycemia by increasing insulin secretion, enhancing glucose uptake by adipose and muscle tissues, inhibiting glucose absorption from intestine and inhibiting glucose production from hepatocytes. These are the ways by which the efficacies of hypoglycemic herbs are achieved [16]. *A. heterophyllus* may have the same effect as the *Tragia involucrate* that could stimulate the residual pancreatic insulin or by increasing peripheral utilization of glucose, resulting to its antihyperglycemic effect. Glycosides, flavonoids, tannins, organic sulphur compounds, cathecol and alkaloids are active ingredients of hypoglycemic plants. *T. involucrata* contains tannins, flavanoids, alkaloids and saponins which could have antihyperglycemic activity [17]. The crude ethanolic extract of *A. heterophyllus* unripe fruit has the same activity with *A. heterophyllus* leaf which exhibited a non-toxic and significant hypoglycemic activity in male Wistar rats [7]. It has the same activity also to the aqueous extract of *M. charantia* which induced also significant hypoglycemic activity in hyperglycemic and normal mice [18]. The crude ethanolic extract of *A. heterophyllus* unriped fruit at the concentration of 10mg/20g (500mg/kg) was effective as the *Musa paradisiaca* at the dose of 500mg/kg bw of mice [19]. Other plants that showed anti-hyperglymemic activities like the crude ethanolic extract of *A. heterophyllus* unripe fruit were *C.auriculata*, *A.marmelos* [20], *Catharanthus roseus* [21] and *Musa sapientum* root extracts [22].

These findings were true only when the crude ethanolic extract of *A. heterophyllus* unripe fruit at the concentrations of 5, 10, 15mg/20g bw of white mice and the alloxan induced hyperglycemia method were used. Different results can be obtained when different concentrations, plant part, method and extractant will be utilized.

5. Conclusion

The study demonstrated the antihyperglycemic activity of the crude ethanolic extract of *A. heterophyllus* unripe fruit at 5mg/20g (250mg/kg), 10mg/20g (500mg/kg), 15mg/20g (750mg/kg) against alloxan-induced hyperglycemia in white mice. The best concentration that exhibited anti-hyperglycemic effect was at 15mg/20g bw of white mice. The time when the extract exhibited the best activity was on the third hour after its administration. The study provided the scientific basis of the folkloric claim of the antihyperglycemic effect of *A. heterophyllus*. This study could also serve as basis for future formulation of effective, locally available, natural and low cost anti-hyperglycemic agent from *A. heterophyllus* unripe fruit subject for further study.

6. Recommendations

Studies on the concentrations that will give higher than 37% reduction in fasting blood sugar level is recommended. Genotoxic effect, cytotoxicity level and the use of other extractants for unripe fruit of *A. heterophyllus* are also recommended for further study.

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Author Profile

MA.SOCORRO GONZAGA-LEONG-ON, PhD, College of Liberal Arts, Sciences and Education, University of San Agustin, Iloilo City, 5000 Philippines. Ma. Socorro G. Leong-on is a holder of Doctor of Philosophy in Science Education major in Biology from West Visayas State University. She graduated with highest distinction. She was a recipient of a scholarship grants from the Department of Science and Technology (DOST)-Science Education Institute (SEI), and University of San Agustin, Iloilo Faculty Development. She also holds a Master of Science in Biology from University of the Philippines in the Visayas as a scholar of St. Paul University, Iloilo and got a DOST fundings for her thesis. She obtained her BS Biology from the University of San Agustin, as cum laude, awardee in community service, and awardee in cooperation. She was a P.D. 451, Alumni-Chicago, Illinois Chapter, and University of San Agustin academic scholar.

Dr. Leong-on was the first placer during the search for best thesis and dissertation in Region 6, in 2005 with her study "Bioactivity Screening of Selected Ethnobotanicals for their Potential Uses in Reproduction and Fertility Management" a DOST funded study. She was also the 2012 Gregor Mendel Professorial Awardee on the study "Anti-arthritic Activity of the Ethanolic Extracts of Calamansi (*Citrus microcarpa*), Pomelo (*Citrus maxima*) and Pagatpat (*Sonneratia alba*) Leaves in White Mice (*Mus Musculus*). She is also a 2015 winner in the International Conference on Inquiry-Based Science and NOSTE Biennial Convention with her study "Antihyperglycemic Activity of the Ethanolic Extracts of *Artocarpus heterophyllus* (langka) unripe fruit in White Mice (*Mus Musculus*). She was also a 2017 winner, International Conference on Inquiry-Based Science and NOSTE Biennial Convention with her study "Genotoxicty Screening of the Crude Ethanolic Extracts of *Artocarpus heterophyllus* (langka) unripe fruit." Her dissertation "Competencies of Junior High School Science Teachers on Selected Biology Laboratory Procedures: Inputs to Instructional Material Development" (DOST and University of San Agustin funded study) won first place – Best Oral Presenter Award during the National Organization of Science Teachers' Educators (NOSTE) 2018 National Research Conference & Training Workshop. She has published her researches in national and international journals. She is a research presenter in regional, national and international research fora. She is also a consultant in various biological researches, and since then produced many winners in science investigatory projects and researches among college students. She serves as judge in regional and national research presentations. She also serves as resource person in many speaking engagement like in DOST 6- Health Research Consortium on Screening of Plant Extracts for Medicinal Uses. A speaker also on Instructional Material Development Research and Topics on Science Investigatory Project. She was also a research reviewer, National Medical Admission Test and Licensure Examination for Teachers' reviewer of West Visayas State University. She is a committee member of the Human Resource and Development, Western Visayas Health Research Development Consortium –

Department of Science and Technology, Region 6. She is the former Chairman – University of San Agustin Institutional Animal Care and Use Committee of the University of San Agustin. She served as the board of director, auditor and recording secretary, treasurer of the Philippine Society for Microbiology. She is also a member of the Philippine Association of Laboratory Animal Science and Biology Teachers' Association. She has attended various seminars and trainings. Her area of research is on bioactivity screening of plant extracts. She acquired skills on procedures in testing plant extracts using mice in the following studies: precoitos or anti-ovulatory (antifertility), postcoital antifertility, abortifacient, and teratotogenicity testing. She has also skill on, mutagenicity, analgesic, antihyperglycemic, anti-arthritis, anti-diarrheal, anti-inflammatory, and anti-depressant testing. She has been performing studies on cytotoxicity testing using brine shrimp, antimicrobial testing, genotoxicity, larvicidal assay, anti-pediculocidal assay, molluscicidal testing phytochemical analysis and instructional material development. She has a training on animal handling by the Philippine Association of Laboratory Animal Science, communicating researches to stakeholders by DOST, writing scientific papers for publication by DOST, and completing the 3rd Course on Transforming Philippine Plants into Quality Herbal Medicines for a Healthier Nation by University of the Philippines - Manila and Institute of Herbal Medicine. Dr. Leong-on was a former faculty member and the team leader of the Science Department of St. Paul University Iloilo. Currently, she is a faculty member of the University of San Agustin. She is married to Rey N. Leong-on, and blessed with three children, Rae Marie, Rae Angelique and Rae Danielle.