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EVALUATION OF THE HYPOGLYCEMIC EFFECT OF BALI-BALI PLANT (*EUPHORBIA TIRUCALLI LINN.* FAMILY: EUPHORBIACEAE) STREPTOZOCIN-INDUCED DIABETES MELLITUS IN SPRAGUE DAWLEY RATS

Anthony R. Marin[1], Dr. Irene M. Roco[2], Ihongo Barnabas K[3], Guiapar Lady Shalma I[3] and Julian Ma. Cristine Michelle A[3]

[1]College of Pharmacy, Program Chair, St. Dominic College of Asia, Philippines
[2]College of Nursing, Faculty of Nursing, King Saud University, Riyadh, K.S.A.
[3]College of Pharmacy, School of Health Science Professions, St. Dominic College of Asia, Philippines

ABSTRACT: *To extract bali-bali plant using ethanol extract, to evaluate short-term general toxicity of the extracts, to determine the hypoglycemic effects of bali-bali extract and to evaluate the effects of ethanolic extract of Euphorbia tirucalli on fasting blood glucose. A total of 84 Sprague-dawley rats of both sexes were employed and were divided into 7 groups. Group 1 served as the normal control, group 2 served as the negative control (NSS), group 3 served as a positive control (Metformin 200mg/kg), group 4 served as diabetic control (STZ only), groups 5-7 served as the treatment groups which were treated with the Bali-bali extract orally for two weeks. The FBG level of each group was measured using one touch glucometer on days 0, 7, and 15. Result in the toxicity analysis, the plant extract was shown to have little to no significant toxicity shown in the various dosages of the extract administered Kto the rats. Bali-bali was shown to have a significant effect on blood sugar causing hypoglycaemia with significant decrease in blood glucose level at the various dosages with the 75mg/kg dose seen to be more effective. The Fasting Blood Glucose evaluation revealed no statistically significant difference in the various dosages of bali-bali extract 50mg (p = 0.550), 75mg (p = 0.181) and 100 mg (p = 0.679) administered to the rats.*

KEYWORDS: Bali-bali on hypoglycemic1, *Euphorbia tirucalli* effect in Diabetes2, Euphorbiaceae on diabetes3, Natural plants for diabetes4

INTRODUCTION

Diabetes mellitus is a heterogeneous group of disorders characterized by persistent hyperglycemia. The two most common forms of diabetes are type 1 diabetes (T1D, previously known as insulin dependent diabetes or IDDM) and type 2 diabetes (T2D, previously known as non-insulin-dependent diabetes or NIDDM). Both are caused by a combination of genetic and environmental risk factors. However, there are other rare forms of diabetes that are directly inherited. These include maturity onset diabetes in the young (MODY), and diabetes due to mutations in mitochondrial DNA.

All forms of diabetes have very serious effects on health. In addition to the consequences of abnormal metabolism of glucose (e.g., hyperlipidemia, glycosylation of proteins, etc.), there are a number of long-term complications associated with the disease. These include

cardiovascular, peripheral vascular, ocular, neurologic and renal abnormalities, which are responsible for morbidity, disability and premature death in young adults.

Autoimmune destruction of beta cells is the major cause of T1D the pancreas represents approximately 10% of all cases with diabetes. Currently, the only treatment therapy for this disease is lifelong insulin therapy. Without exogenous insulin injections, individuals with T1D will not survive. In the world population today, people with T1D are <1%, the geographic variation in incidence is enormous, ranging from <1/100,000 per year in China, to approximately 40/100,000 per year in Finland. It has been in the world population today, people with T1D are <1%, the geographic variation in incidence is enormous, ranging from <1/100,000 per year in China, to approximately 40/100,000 per year in Finland.

Asthma is the most prevent childhood disorder more than T1D. It has been estimated that approximately 20 million people worldwide, mostly children and young adults, have T1D (Holt, 2004). The most common form of disease is T2D; almost 90% of individuals are affected with this disease. When the fasting plasma glucose concentration is >7.0 mmoL (>126 mg/day), or plasma glucose 2 hours after a standard glucose challenge is > 11.1 mmol/L (> 200 mg/dl), a diagnosis of T2D is made.

The rates of diagnosed diabetes in adults by race/ethnic background are:7.4% of non-Hispanic whites, 8.0% of Asian Americans, 12.1% of Hispanics, 12.7% of non-Hispanic blacks 15.1% of American Indians/Alaskan Natives. Diabetes was the seventh leading cause of death in the United States in 2015 based on the 79,535 death certificates in which diabetes was listed as the underlying cause of death (Diabetes.org, 2018).

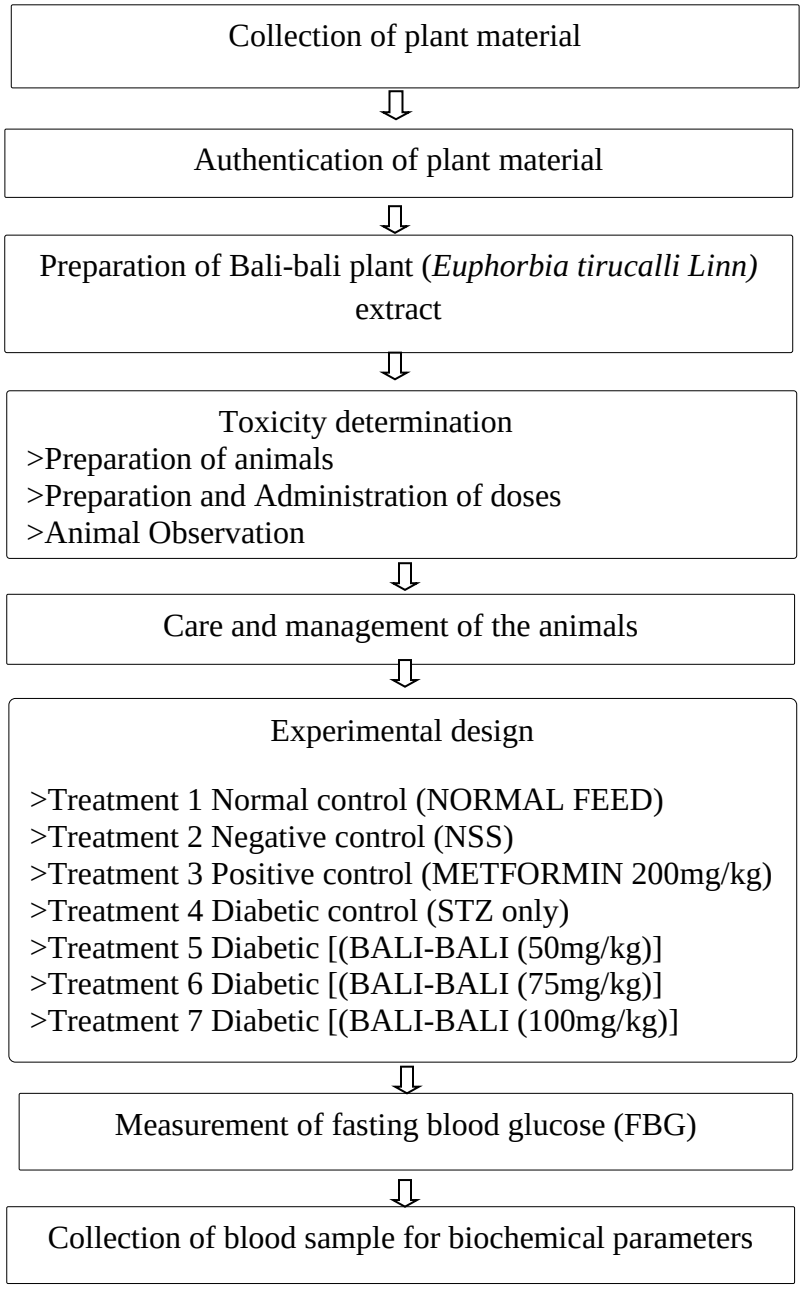
LITERATURE/THEORETICAL UNDERPINNING

Statement of the problem

The primary purpose of this study is to evaluate the Hypoglycemic Effect of Bali-bali (*Euphorbia tirucalli Linn*) in Sprague Dawley Rat. Specifically, the study answered the following questions:

1. How will Bali-bali (*Euphorbia tirucalli Linn*) plant be extracted?
2. At what dosage will Bali-bali (*Euphorbia tirucalli Linn*) cause toxicity to the Sprague Dawley rats?
3. What is the hypoglycemic effect of Bali-bali (*Euphorbia tirucalli Linn*) extract?
4. Does Bali-bali (*Euphorbia tirucalli Linn*) have any significant effect on fast blood glucose?

Research Flowchart



Anti-diabetic Activity of Bali-bali Research Flow-Chat

METHODOLOGY

Research Method and Procedure

Research Design

Experimental study designs for testing the hypoglycemic activity of Bali-bali (*Euphorbia tirucalli* Linn) stem in eighty-four (84) sprague dawley rats of both sexes were employed.

Plant Material

Collection of the Sample

Bali-bali (*Euphorbia tirucalli* Linn) was collected from subdivision of Tierra Nevada, General Trias, Cavite, Philippines. Sample was washed thoroughly with distilled water to remove dirt and another adhering extraneous constituent. The plants were authenticated in Museum of Natural History in University of the Philippines Los Baños, Laguna,Philippines under the Botanical Section.

Plant Material Extraction and Sample Preparation

The plant was collected from subdivision of Tierra Nevada, General Trias, Cavite, Philippines.

The fresh plant material will be dried at room temperature and grinded using mechanical grinder to obtain a fine powder. The extraction was achieved by maceration with ethanol (100%) for 24 hours. Later on, the extract was filtered and the solvent removed using rotatory evaporator (40 °C) to get a crude extract. After that, the crude extract was then be fractionated by vacuum liquid column chromatography on silica gel at room temperature. The column was eluted with appropriate solvent to separate the different fraction. All fractions were evaporated to dryness under reduced pressure using rotatory evaporator and stored in a freezer at −20 °C prior to use.

Animals Grouping and Experimental Design

Adult Sprague Dawley rats of both sexes, weighing 210 ± 10 g, was purchased from Mots Animals House Laguna. The animals were acclimatized for 2 weeks before the experiments were performed. All animals were housed 1 per cage under environmentally controlled conditions of light (12-light/dark cycle, lights on at 8:00 a.m.) and temperature (24 ± 1 °C) with free access to food and tap water. The animals were randomly divided into seven groups of ten Sprague Dawley rat each as follow:

Group (1) was kept as normal control (G-1).

Group (2) serves as negative control (NSS).

Group (3) serves as a positive control (Metformin 200mg/kg).

Group (4) serves as diabetic control (STZ only).

Group (5) serves as diabetic group (was treated by given two *E. tirucalli* fractions, with orally of 50mg/kg body weight for two weeks).

Group (6) serves as diabetic group (was treated by given two *E. tirucalli* fractions, with orally of 75mg/kg body weight for two weeks).

Group (7) serves as diabetic group (was treated by given two *E. tirucalli* fractions, with orally of 100mg/kg body weight for two weeks).

The last remaining fourteen (14) Sprague dawley rats were used for toxicity test. All procedures involving the use of animals in this study comply with the guiding principles for research involving animals as recommended by the declaration of Helsinki and the guiding principle in the care and use of animals (World Medical Association, 2014).

Toxicity Test

The acute oral toxicity of Bali-bali (*Euphorbia tirucalli* Linn) plant extract was evaluated in fourteen (14) Sprague dawley rats according to the produced outlined by Organizational for Economic Cooperation and Development (OECD). The animals were kept fasting overnight and provide only with water after the extract administered orally in a single dose of 250mg/kg, 500mg/kg, 750mg/kg, 1000mg/kg, 1500mg/kg, 3000mg/kg and 5000mg/kg body weight by oral gavages.

Measurement of Fasting Blood Glucose (FBG) and Body Weight (BW)

The FBG level of each animal in normal and diabetic groups was monitored on several days, 0, 7 and 15. The FBG level was measured using One Touch Glucometer (company name and country) for drop of blood which collected from the tip of tail vein of each rat. On the other hand, initial and final body weights were recorded as well as the percentages of change (%) in FBG level and body weights was calculated.

Estimation of the Normal Range of Blood Glucose in Rats

The average FBG and PBG of normal Wistar rats was (3.95 +/- 1.31) mmol/L and (5.65 +/- 1.63) mmol/L, and the 95% upper limit was 6.2 mmol/L and 7.9 mmol/L respectively. The differences of fasting duration, sample source, time before separating serum and testing methods, excepting for rat species, were significant ($P < 0.05$). The results showed that the blood glucose tested by glucose monitor method was not comparable with GOD method while the range of blood glucose was out of 7-11 mmol/L. The difference of blood glucose between fasting for 5h and 11 h was 0.8 mmol/L. Blood glucose from abdominal aorta was 40% higher than that from tail. The reduction of blood glucose was 8% in 60 min and over 50% in 120 min after blood being sampled. After the test requirement being regulated, the upper limit for FBG and PBG was expanded to 7.5mmol/L and 10.4mmol/L, respectively.

Statistical Analysis

The results are expressed as Mean ± SEM. The data obtained is subjected to statistical analysis using t-test followed by Dunnett's post hoc test for comparison between control and test groups. A 'p' value < 0.05 are considered to be significant.

RESULTS/FINDINGS

Presentation, Interpretation and Analysis of Data

This chapter discusses the results and subsequent statistical analyses obtained from experimentation conducted.

How will Bali-bali plant be extracted?

Bali-bali (*Euphorbia tirucalli*) was collected from subdivision of Tierra Nevada, General Trias Cavite, Philippines Samples were thoroughly washed with distilled water to remove dirt and another adhering extraneous constituent. The fresh plant was dried at room temperature and rinsed using mechanical grinder to obtain a fine powder. The extraction was achieved by maceration with ethanol (100%) for 24 hours. The extract was filtered and solvent removed using rotatory evaporator (40°C) to get a crude extract. All fractions were evaporated to dryness under reduced pressure using rotatory evaporator and stored in a freezer -20°C prior to use.

At what dosage will Bali-bali (*Euphhorbia tirucalli Linn*) cause toxicity to the Sprague Dawley rat?

Forteen Sprague Dawley rats were used for toxicity texting, the 14 Sprague Dawley rats were treated with *E. tirucalli.* The animals were kept for 24 hours without food and provided only with water after the extract administered orally in a sningle dose of 250mg/kg, 500mg/kg, 750mg/kg, 1000mg/kg, 1500mg/kg,3000mg/kg and 5000mg/kgbody weight by oral gavages. There was no tyoxic effect caused by the plant, we concluded that it is effective.

What is the hypoglycemic effect of Bali-bali (*Euphhorbia tirucalli Linn*) extract?

Table 1: Effect of Bali-bali *(Euphorbia tirucalli Linn)* Group 5 Diabetic

Rat Group	Rat Weight (g)	Baseline (mg/dl)	Induction of STZ (ml)	Pre-Test (Check Glucose level)	Treatment	Post-Test (Check Glucose level)	Mean
Group 5 Diabetic (Bali-bali 50mg/kg)	155	80.67	0.902215	207.83	7	134.33	97.62
Total	**155**	**80.67**	**0.902215**			**134.33**	

Table 2: Effect of Bali-bali *(Euphorbia tirucalli Linn)* Group 6 Diabetic

Rat Group	Rat Weight (g)	Baseline (mg/dl)	Induction of STZ (ml)	Pre-Test (Check Glucose level)	Treatment	Post-Test (Check Glucose level)	Mean
Group 6 Diabetic (Bali-bali 75mg/kg)	208	95.83	1.199068	199.17	7	145	109.37
Total	208	95.83	1.199068			145	

Table 3: Effect of Bali-bali *(Euphorbia tirucalli Linn) Group 7 Diabetic*

Rat Group	Rat Weight (g)	Baseline (mg/dl)	Induction of STZ (ml)	Pre-Test (Check Glucose level)	Treatment	Post-Test (Check Glucose level)	Mean
Group 7 Diabetic (Bali-bali 100mg/kg)	178	91.67	1.001162	214.83	7	142.5	105.83
Total	**178**	**91.67**	**1.001162**			**142.5**	

Based on the Bali-bali extract, the most effective is the 50mg which has the difference of 73.50, followed by the mean of 72.00 at 100mg and the last is the 75mg extract of Bali-bali which has the mean of 54.16. The 50mg has almost the same component as metformin which is a certified blood glucose therapeutically medication that lowers blood glucose.

Table 4: Does Bali-bali (*Euphorbia tirucalli Linn*) have any significant effect on fast blood glucose?

Positive Control	t Value	df	P value (Sig.)	Interpretation	Analysis
50 mg	.641	5	.550	There is no Significant Difference	Accept the Null Hypothesis
75 mg	1.554	5	.181	There is No Significant Difference	Accept the Null Hypothesis
100 mg	-.439	5	.679	There is No Significant Difference	Accept the Null Hypothesis

A paired sample t test was calculated comparing the positive control score of the Bali-bali extract using different concentrations prior to the implementation of the intervention. The analysis revealed that there was no significant difference found among the 3 concentrations (50mg: t = .641, p >.01; 75mg: t = 1.554, p >.01; 100mg: t = -.439, p >.01).

SUMMARY OF FINDINGS, CONCLUSIONS, AND RECOMMENDATIONS

Summary of Findings

From the research work, it was found that bali-bali could be successfully extracted using ethanol extract with a significant hypoglycemic effect with a short-term general toxicity observed in the rats.

Conclusions

Diabetes is possibly the world's fastest growing metabolic disease, and as knowledge of the heterogeneity of this disorder increases, so does the need for more appropriate therapies. Traditional plant medicines are used throughout the world for the management of diabetes because it is easily and cheaply available.The use of ethanol extract of Bali-bali compared to the control showed significant hypoglycemic effect with little short-term general toxicity. Thus, from the results of the present investigation, it may be concluded that the ethanolic extract of the bali-bali possess significant hypoglycemic activity against blood sugar. The hypoglycemic effect may be due to the presence of polyphenolic compounds. Studies have shown that a diet rich in fruits and vegetables contributes to the delay of the aging process and to the decrease of the inflammation and oxidative stress risk, related with chronic diseases. Acarbose is the current drug which works in your intestines to slow the breakdown and absorption of carbohydrates from foods that you eat. This effect helps lessen your blood sugar rise after a meal. Acarbose reversibly bind to pancreatic alpha-amylase and membrane-bound intestinal alpha-glycoside hydrolases. These enzymes inhibit hydrolysis of complex starches to oligosaccharides in the lumen of the small intestine and hydrolysis of oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharaides in the brush border of

the small intestine. Meformin as a current drug of choice fof lowering hypoglycemic has an antidiabetic effect like acabose. But does not have polyphenolic effect like acabose

Recommendations

1. Further chemical phytochemical work in the plant because the identification of new chemical compounds from the plant will be emergence of new potent anti-diabetic molecules.
2. The onset of diabetics in rat is judged as blood glucose being higher than the expanded normal upper level. The criteria for rats are close to that for human.
3. Further studies like isolation and characterization of the active principals responsible for hypoglycemic activity.

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