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Anti-Diabetic Effect of *Leucaena Leucocephala* Linn (Ipil-Ipil) Seed Extract on Albino Mice

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Abstract

An experiment utilizing the Randomized Complete Block Design (RCBD) was done to prove the anti-diabetic effect of *Leucaena leucocephala* Linn. (Ipil-ipil) seed extract on albino mice as test animals. The study also aimed to detect the phytochemicals present in the extract, ascertain its physical properties, and compare its effect among three concentration levels and the positive control, a commercially prepared anti-hyperglycemic drug. Analysis of the extract's chemical properties showed the presence of alkaloid, flavonoid, saponin, tannin, and phenolic compounds; anthraquinone and steroid were not detected. The extract's color was orange, with a pleasant odor, a pH of 5.44, and was found to be miscible in both water and acetone, but was immiscible in chloroform. Among the three extract concentration levels used, 50% level was found to be most effective in reducing blood glucose levels (BGL) in the experimental animals, while the pure extract (100% concentration) was the least effective. However, reductions in BGL were not significantly different among the concentration levels and that of the commercial drug. Thus, it is clear that *Leucaena leucocephala* Linn has anti-diabetic potentials which are comparable to the commercial anti-hyperglycemic drug Metformin.

It is recommended that other parts of the plant be also tested for its anti-diabetic potentials taking into consideration also the detection of other secondary metabolites which may be present in the plant parts, and evaluating its other potential applications.

Keywords: Hyperglycemia; Anti-diabetic, BGL; *Leucaena Leucocephala*; Phytochemicals

Abbreviations: BGL: Blood Glucose Levels; RCBD: Randomized Complete Block Design.

Introduction

Hyperglycemia is a hallmark symptom of diabetes, a condition of a person experiencing high blood sugar levels because of complications in the production of insulin in the body or the body cells do not properly respond to the insulin produced. Diabetes is a chronic medical condition which can lead to blindness, kidney failure, and nerve damage. It is also an important factor in accelerating the hardening and narrowing of the arteries (atherosclerosis), leading to strokes, coronary heart disease, and other large blood vessel diseases, or what is referred to as macro vascular disease [1]. The disease is rapidly becoming a worldwide epidemic and the Philippines are not spared.

Humans are naturally resourceful; when they are ill, they never stop seeking and discovering alternative ways of cure. In fact, thousands of herbal medicines have already been identified in the Philippines, *Leucaena leucocephala* Linn. Included. The plant is known as 'miracle tree' in the 1970s and early 1980s because of its worldwide success as a long-lived and highly nutritious forage tree and a variety of other uses [2]. *L. leucocephala* is a small tree that originated in Mexico and escaped as a weed into tropical and warm temperate regions of other countries. The kernel of the seeds contains more than 20% oil. The plant is a bio-fuel and energy crop, and was used as concentrates for dairy animals [3]. The plant is also very well-known for its anthelmintic effect, but knowledge is limited in its anti-diabetic effect. Proving the anti-diabetic effect of this plant can be a big help for the poor, diabetic patients, since a less expensive alternative for commercial drugs would be readily available, hence, this study.

Methodology

This experimental research utilized 45 albino mice distributed accordingly following the Randomized Complete Block Design (RCBD), with 4 treatments replicated thrice, and was conducted at the Biology Laboratory Room, College of Science, University of Eastern Philippines, University Town, Northern Samar. Plant samples were collected from Zone 1 of the University campus and seed extract was subjected to phytochemical screening, using standard methods, alongside the determination of its physical properties. The experimental animals, ranging in weight from 10-25 grams, were acclimatized for 7 days prior to the experiment. Initial blood sugar levels were taken individually using a glucometer and was repeated after a 12-hour fast. Thirty (30) minutes after, 0.5ml of 70% glucose was administered to induce a rise in the BGL of the experimental animals. One (1) hour later, the treatments were administered. Data gathered were subjected to Analysis of Variance (ANOVA) and significant results were confirmed with the Scheffe's test.

Results and Discussion

This study was conducted to attain the following objectives:

a. Evaluate the phytochemical properties of Leucaena *leucocephala* in terms of alkaloids, anthraquinones, flavonoids, phenolic compounds, saponins, steroids, and tannins.
b. To ascertain the physical properties of *Leucaena leucocephala* seed extract in terms of pH, color, odor, miscibility, and density.
c. To evaluate the anti-diabetic effect of the different concentrations of ipil-ipil seed extract on albino mice.
d. To determine if a significant difference exist between the blood sugar levels of mice treated with different treatments.
e. To quantify the difference in the anti-diabetic effect of the most effective seed extract concentration and that of a commercial anti hyperglycemic drug (Metformin®).

Table 1 shows that *Leucaena leucocephala* seed extract possessed phytochemical substances like alkaloid, flavonoid, phenolic compounds, saponin, and tannin, but was negative in anthraquinones and steroids. This implies that the phytochemical constituents present in the extract are the chemicals responsible for the anti-diabetic effect of the plant extract. This was the same conclusion of Mukesh and Patil [4], while the phenolic compound present does not possess anti-diabetic effect, but as an anti-oxidant, as revealed in the study of Afza, *et al.*, (2007) who reported that the plant also possesses an anti-oxidant effect. The anthraquinone and steroids were found to have no contribution to the anti- diabetic effect of the plant [5,6].

Secondary Metabolites	Results	Interpretation
Alkaloid	**Mayer's reagent:** Formed a white precipitate at the bottom of the test tube **Draggendorff's:** Formed an orange precipitate	**Positive** **Positive**
Anthraquinone	There was no presence of a pink, red, or violet color in the ammonia phase.	**Negative**
Flavonoid	Formed an orange coloration.	**Positive**
Phenolic Compound	There is a formation of bluish-green color.	**Positive**
Saponin	The height of the extract is not less than the half of the height of the water.	**Positive**
Steroid	Reddish brown color at the interface was not observed.	**Negative**
Tannin	There is a formation of jelly precipitate.	**Positive**

Table 1: Phytochemical Screening Results.

Table 2 shows that the *Leucaena leucocephala* seed extract has an orange color and a pleasant odor, and an average pH of 5.44, which means it is weakly acidic.

Physical Properties	Results
Color	Orange
Odor	Pleasant
pH	5.44
Miscibility in:	
Water	miscible
Acetone	miscible
Chloroform	immiscible
Density	1.1127 g/mL

Table 2: Physical Properties.

Miscibility test results for the extract show it to be miscible in water and acetone, but were immiscible in chloroform, which is interpreted that the extract is a polar compound. The density of the extract was calculated to be 1.1127 g/mL, which indicates that the extract is denser than water.

Table 3 shows the mean blood sugar levels (BSL) of the experimental animals before and after fasting, induction with glucose, and treatment of the extract. Mice treated with 50% concentration of the extract showed a mean difference in BSL after treatment of 167.78 mg/dL, for 75% concentration, it was 131.55 mg/dL, and for the 100%, it was 120.67 mg/dL. It shows that treatments utilizing the plant extracts are apparently not comparable in their effects, but they do manifest an anti-hyperglycemic effect that is comparable to the effect of the commercial drug. This implies that as the level of concentration increases, the effectiveness of the extract decreases. This is similar to the result reported by Kuppusamy, *et al*., [7]; however, this result is still inconclusive since the studies used different extraction methods.

Treatments	Initial BSL (mg/dl) (1)	BSL after fasting (mg/dl) (2)	BSL after induced Glucose (mg/dl) (3)	SL after extract treatmer (mg/dl) (4)	Difference (mg/dl) (3-4)
50% concentration	123.66	82.89	242.22	74.44	167.78
75% concentration	117.22	104.11	248.44	116.66	131.55
100% concentration	133.78	101.003	234.22	113.55	120.67

Table 3: Evaluation of the Three Concentrations of the Seed Extract.

The Analysis of Variance (ANOVA) was used to determine if there was a significant difference among the five treatments, whose decision rule says that if the F-computed value is greater than the F-tabular value, then significant differences exist among the five treatments, and such difference have to be sought utilizing the Scheffe's test. Calculations in Table 4 show that the F-computed value of 4.42 is greater than the F-tabular value of 2.61 at 0.05 level of significance, with 4 and 40 degrees of freedom, hence, it is concluded that there are significant differences in the extracts' anti-hyperglycemic activity. Therefore, the effectiveness of some of the treatments may not be comparable, and thus it needs to be verified through the Scheffe's test.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F = Value ∞ = 0.05	
				Computed	Tabular
Between Groups (K-1)	4	54, 043.24	13, 510.81	4.42	2.61
Within Groups (N-1) (K-1)	40	122, 307.56	3, 057.69		
Total	44	176, 350.80			

Table 4: Statistical Analysis of Results (One Way Anova).

Table 5 shows that the mean for T_1 (distilled water) is significantly different to the means of T2 (50% concentration of extract) and T5 (Metformin®) thus, the effectiveness of distilled water was obviously incomparable to Treatments 2 and 5. However, the mean of T_1 is not significantly different to T_3 (75% concentration) and T_4 (100% concentration), indicating comparable effects. On the other hand, the mean of T_2 (50% concentration) is not significantly different to treatments 3, 4, and 5 thus, the effectiveness of the 3 treatments are comparable with each other. Therefore, the treatments seed extracts and the commercial drug were comparable in terms of its effectiveness. This implies that treatments utilizing the plant extracts are apparently not comparable in their effects, but they do manifest an anti-hyperglycemic effect that is comparable to the effect of the commercial drug.

Between Treatments	F’	(F.05)(k-1)	Interpretation
X1 vs. X2	14.49	10.44	Significant
X1 vs. X3	5.84	10.44	Not significant
X1 vs. X4	3.99	10.44	Not significant
X1 vs. X5	11.45	10.44	Significant
X2 vs. X3	1.93	10.44	Not significant
X2 vs. X4	3.27	10.44	Not significant
X2 vs. X5	0.18	10.44	Not significant
X3 vs. X4	0.17	10.44	Not significant
X3 vs. X5	0.94	10.44	Not significant
X4 vs. X5	1.92	10.44	Not significant

Table 5: Scheffe’s Test Results.

Conclusions

Findings of the study seem to indicate that the presence of some phytochemical constituents in the seed extract together with the physical properties, account for the anti-diabetic effect of the plant extract. Among the three concentration levels tested, 50% concentration level was found to be the most effective as manifested by exhibiting the higher difference in BSL after treatment (167.78 mg/dL). Analysis of Variance (ANOVA), as confirmed by the Scheffe’s test, show insignificant differences in anti-diabetic effects between and among the concentration levels and the commercial drug, implying that the extract is a potential organic anti hyperglycemic agent.

Recommendations

The researcher recommends the testing of other plant parts for its potential anti-diabetic effects, and a more thorough phytochemical screening of the plant extract may be done to discover other potential medicinal applications. Further studies considering longer exposure times may also be considered in the future.

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