Protective Effects of *Peperomia pellucida* (L.) Crude Methanolic Extract Against Elevated Blood Glucose Levels and Alterations in Pancreas Histology of HFD/STZ-Induces Diabetic *Mus musculus*

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**ABSTRACT**

*Peperomia pellucida*, locally known in the Philippines as pansit-pansitan, is an annual herb with many ethnomedicinal properties. This study aims to determine the antihyperglycemic property of *P. pellucida* methanolic crude extract (PPE) through measurement of its effect on blood glucose levels and pancreas histology of High Fat Diet/Streptozotocin-induced diabetic mice. Thirty-five male mice were divided into seven groups: (1) Negative control group, (2) Positive control group, (3) Diabetic group treated with metformin, (4) Diabetic group treated with PPE at 200mg/kg, (5) Diabetic group treated with PPE at 400mg/kg, (6) Diabetic group treated with PPE at 800mg/kg, (7) Non-diabetic group given PPE at 400mg/kg. Blood glucose levels were monitored, pancreas histology was analyzed, and results were compared with the metformin-treated control. Administration of *P. pellucida* for two weeks had no significant effect on the blood glucose levels but prevented the necrosis, apoptosis, and inflammation of the Islets of Langerhans. These results suggest that *P. pellucida* has protective effects on the pancreas and may be an alternative to metformin in the treatment of type II diabetes.

Keywords: *Pepperomia pellucida*; pancreas; blood glucose; histology

**INTRODUCTION**

Type II diabetes is a metabolic disorder characterized by insulin resistance and beta cell dysfunction. Medications to control diabetes include synthetic insulin and metformin which can induce severe adverse effects such as lactic acidosis (Fitzgerald et al., 2009). Because of the rapidly increasing prevalence of type II diabetes over the past decade, there is a need for cost-effective alternative to expensive antihyperglycemic treatments.

*Peperomia pellucida*, an annual herb belonging to the pepper family or Piperaceae, is native to the American tropics but is widely cultivated and naturalized in different regions in Asia, as it grows in loose, humid soils of the tropical and subtropical regions (Staples and Kristiansen, 1999; Theresa-Ibibia, 2012). *P. pellucida* has been widely-used as an alternative to treat various diseases despite the lack of proper scientific validations to most of these claims. Among the scientifically-proven uses of *P. pellucida* are its analgesic, anti-inflammatory, antimicrobial, and antioxidant activities (Khan and Omoloso, 2002; De Fatima et al., 2004; Benjamin et al., 2013)

The widespread distribution of *Peperomia pellucida* and its popular use in traditional medicine for treating a wide range of diseases make it a target for further scientific explorations. Therefore, scientific evidences on the traditional medicinal uses of *P. pellucida* will be very helpful to developing countries, such as the Philippines. This study aims to investigate the antihyperglycemic property of *P. pellucida* in high fat diet (HFD)/streptozotocin (STZ)-induced diabetic mice through the measurement of blood glucose levels and analysis of pancreas histology.

**MATERIALS AND METHODS**

2.1. Preparation of Crude Methanolic Extract

*Peperomia pellucida* plants were collected from Barangay Bolbok, Taal, Batangas. The roots of the plant were removed while the aerial parts were cleaned with water and air-dried for three weeks. The dried plant materials were grounded after which, the powdered material was soaked in 6L 95% methanol for 72 hours. The solution was filtered using Whatman No.1 filter paper and evaporated using rotary evaporator to obtain the crude extract.
2.2. Animals
Forty (40) male ICR mice aged between 6 to 7 weeks were obtained from FDA. Procedures for housing, acclimatization and introduction of drugs and extracts were reviewed and approved by the UPM Institutional Animal Care and Use Committee.

2.3. Induction of Diabetes
Diabetes was induced by the high fat diet (HFD) and Streptozotocin (STZ). After two weeks of consumption of high fat diet or normal chow, mice was fasted for 20 hours and was given an intraperitoneal injection of STZ (40mg/kg) for 5 days. To confirm diabetes, blood glucose levels were measured one week after STZ treatment. Diabetic mice were then distributed into treatment groups.

2.4. Experimental Set-up
The mice were treated for two weeks. After treatment, they were subjected to overnight fasting then blood sample were collected from the periorbital sinus of the mice. To sacrifice the mice, they were anesthetized with 30 mg/kg Zoletil injected intraperitoneally. They were sacrificed via cervical dislocation and the pancreas was excised.

2.5. Histopathology of Mice Pancreas
The excised pancreas was fixed in 10% buffered formaldehyde and brought to Hi-Precision Diagnostics for sectioning. The sections were stained with hematoxylin & eosin. The resulting slides were analyzed under the light microscope and the damages were measured using a histopathological index.

2.6. Blood Glucose Analysis
Blood glucose were determined using One Touch electronic glucometer. Blood samples were drawn from the peri-orbital sinus of the mice at weekly intervals.

2.7. Statistical Analysis
All body weight, islet of Langerhans diameter, glucose, and cholesterol values were analyzed by one-way ANOVA followed by Tukey’s HSD test. While the index scores were subjected to Kruskal-Wallis H test, followed by Mann-Whitney U test.

RESULTS
Mice that received HFD and STZ developed hyperglycemia, evidenced by the significant increase in blood glucose levels after the administration of HFD and injection of STZ (Fig 1). This goes to show that Type 2 diabetes was successfully induced across all treated groups. After induction of Type 2 diabetes, different groups received corresponding treatment as indicated in the experimental set-up. Results showed that there were no significant difference in the mean blood glucose values before and after treatment of metformin and in all doses of P. pellucida (Fig 2).

The islets of negative control exhibit normal islet architecture where the cells remain intact and have the same size. Histological abnormalities observed among treatment groups were immune cell infiltration, fibrosis, hemorrhage, fat deposits and parenchymal necrosis and hypertrophy of beta cells (Fig 3). With the use of a histological index, the tissues were scored and mean score were subjected to statistical analyses. Results revealed that P. pellucida exhibits protective effects on diabetic pancreas since there no significant differences in the histological appearance of the pancreas between the group fed with standard diet and the group treated with 200 mg/kg of PPE (Figure 4).
Figure 1. Mean blood glucose levels (mg/dL) of groups before and after administration of STZ. Different letters denote significant difference at p<0.05.

Figure 2. Mean blood glucose levels (mg/dL) of all groups before and after treatment of P. pellucida or metformin across groups (p<0.05). Different letters denote significant change within each group (p<0.05). Different numbers denote significant change across groups (p<0.05).

Figure 3. Representative islets for each group. Green arrow represents necrosis, black represents lymphocyte infiltration, blue represents hemorrhage, red represents fibrosis, and yellow represents fat deposits. A: Islet of negative control showing normal histological appearance (400x) B,C: Islets of positive control showing necrosis characterized by membrane breakdown and hypertrophy and leukocyte infiltration (1000x) D: Hemorrhage in Group 2 (400x). E: Islet of Group 3 (metformin) with lymphocyte infiltration and necrosis (1000x). F: Islet of Group 4 (PPE 200) showing normal islet architecture (1000x) G: Islet of Group 5 (PPE 400) with necrotic cell
CONCLUDING DISCUSSION

This study demonstrated the effect of *Peperomia pellucida* crude methanolic extract on HFD/STZ-induced diabetic mice through the measurement of blood glucose levels and evaluation of pancreatic histopathology using a modified scoring system.

Treatment of *P. pellucida* for two weeks did not result to any significant changes in the blood glucose levels in all groups. Furthermore, blood glucose continued to increase even after treatment. This may be attributed to the short duration of study since protective effects of *P. pellucida* on the pancreas were proven through histological analysis.

Histological examination via a scoring system showed that *P. pellucida* exhibits protective effects on diabetic pancreas since there no significant differences in the histological appearance of the pancreas between the group fed with standard and the group treated with 200 mg/kg of PPE. This finding suggests that *P. pellucida* is able to ameliorate damages in the pancreas caused by high fat diet and STZ. Most remarkable anomalies in the histology of the pancreas of HFD/STZ-induced diabetic mice include immune cell infiltration, beta-cell hypertrophy which is a characteristic of necrosis, and reduction of islet diameter via apoptosis or necrosis.

Lastly, 200 mg/kg of *P. pellucida* did not exhibit any significant difference with metformin in terms of effect on blood glucose levels and pancreas histology. This suggests that *P. pellucida* may be an alternative to metformin in the treatment of type 2 diabetes.

REFERENCES


