The Potential of Psidium guajava in Lowering Blood Glucose Levels of Diet-induced Hyperglycemic Female White Sprague Dawley Rats

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Diabetes mellitus is a chronic metabolic disease that affects a lot of people worldwide. In line with this, aqueous leaf extracts from different varieties of Psidium guajava were evaluated for their potential in lowering elevated total blood glucose levels (hyperglycemia) by using diet-induced female Sprague Dawley rats. Both young and old leaves of the varieties of P. guajava were also examined. Sixteen healthy female Sprague-Dawley rats weighing about 140-150 grams, bought from Java Pet Shop in Cebu, were used in the study. Diabetes in the rats was induced by feeding the animals with food of high glycemic index (white bread and sugar solution) for at least four weeks. After Diabetes induction, the rats were treated by the specific variety of P. guajava leaf aqueous extracts at a dose of 500 mg/kg body weight, and the synthetic drug, Metformin (500 mg) at a dose of 3 mg/kg daily for three days, once a day. After the three days of treatment, the total blood glucose level of the rats decreased significantly ($P<0.05$). Furthermore, this was comparable to the antidiabetic drug, Metformin. Paired t-test revealed that based on one-tailed and two tailed test, blood glucose levels of rats treated with Native Guava (Young Leaves), Native Guava (Old Leaves), Pink Guava (Young Leaves), Pink Guava (Old Leaves), Apple Guava (Young Leaves), and Apple Guava (Old Leaves) all showed significant difference ($P<0.05$) compared with the hyperglycemic control. Pink Guava (Young Leaves) was found to be the most effective in treating hyperglycemia in the rats at a highest significant difference ($p= 0.0001<0.05$) compared with the Hyperglycemic control. There was no significant difference ($P>0.05$) compared with the synthetic drug, Metformin, which suggests that the effect of P. guajava aqueous extracts is as effective as the established drug for Diabetes mellitus in the market.

Keywords: Psidium guajava, anti-hyperglycemic, Diabetes, blood glucose, ethno-medicinal

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease, which can be classified into type 1 diabetes (insulin-dependent diabetes mellitus or IDDM) and type 2 diabetes (non-insulin dependent diabetes mellitus or NIDDM) (Oh *et al.,* 2005). It results from shortage or lack of insulin or the reduced ability of the body's tissues to recognize insulin (Prasad *et al.,* 2009). The prevalence of diabetes is rapidly increasing in industrialized countries, and type 2 diabetes accounts for 90% of the disease (Oh *et al., 2005*). It is also noteworthy that diabetes mellitus is also increasing in developing countries. In type 2 diabetes, insulin resistance is a characteristic feature and several drugs to increase the insulin sensitivity are currently being used in clinic. However, currently available drugs in the market for type 2 diabetes have a number of limitations, such as adverse effects and high rates of secondary failure (Oh *et al.,* 2005). This is why having treatments for diabetes mellitus that have no side effects is still a big challenge for the medical community (Prasad *et al.,* 2009).

Psidium guajava is an important food crop and medicinal plant in tropical and subtropical countries. It is widely used as a food and in folk medicine around the world (Gutierrez, Mitchell, & Solis*, 2008*). More recent ethnopharmacological studies reveal that *Psidium guajava* is used in many parts of the world for the treatment of a number of diseases, such as anti-inflammatory, for diabetes, hypertension, caries, wounds, pain relief and reducing fever (Gutierrez *et al.,* 2008). Because there are a lot of accounts that put *Psidium guajava* as a cure for many diseases including *Diabetes mellitus*, then there is a need to establish a clear scientific or clinical data about the ethno-medicinal use of the plant.

MATERIALS AND METHODS

Plant Material

Fresh leaves of the different varieties of Guava plants (Fig. 1, 2, 3) were collected from *Psidium guajava* trees of Tanjay City, Negros Oriental. The authenticity of the plant varieties was confirmed by Renee B. Paalan from the Biology Department of Silliman University, Dumaguete City.

Figure 1. Photo of leaves and young fruits of Native Guava also locally called "Bayabas Bisaya."

Figure 2. Photo of leaves and young fruits of Apple Guava.

Preparation of Plant Extract (Biswas et al., 2011; Reddy et al., 2012)

The collected leaves were air dried for thirty minutes. After air drying, the leaves (1 kg) of the different varieties of *Psidium guajava* were boiled in H2O (5 l), and the liquid portion of the strained decoction were orally-administered to the animal models.

Figure 3. Photo of Pink Guava or locally called "Supiro"

Test Animals

Sixteen healthy female Sprague-Dawley rats (Fig. 4) weighing about 140-150 grams, bought from Java Pet Shop in Cebu, were used in the study. These rats were chosen for they were best suitable for this type of study.

Sprague-Dawley rats are fast growing rats; they are docile and easy to handle (Janvier Labs, 2015). They are albino outbred with elongated heads and tails that are longer than their body (Janvier Labs, 2015). Their breed/strain was created by R.W. Dawley in 1925 from a hooded male hybrid of unknown origin and an albino female, which is probably Wistar, and was then crossed with the female's progeny for seven generations.

The animals were housed individually in appropriate cages containing sterile sawdust as bedding, maintained under standard conditions (12 hrs light and 12 hrs dark cycle, 25 +5°C and 40-60% humidity) (Prasad *et al.*, 2009). All the rats were given a period of acclimatization for two weeks before starting the experiment. They were given pigeon and general developer pellets and tap water *ad libitum* at room temperature (Sobrevilla *et al*., 2011). The mice considered to be "fasting" were deprived of food for at least 16 hours but were allowed free access to drinking water (Prasad *et al*., 2009).

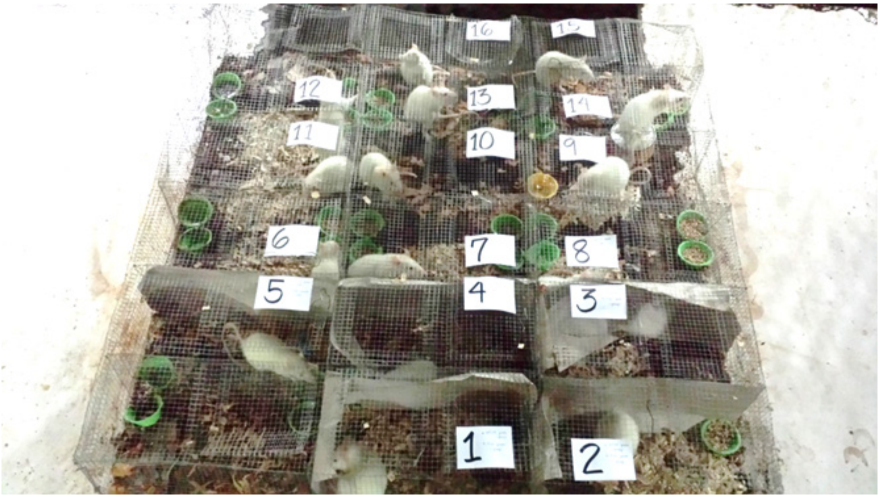

Figure 4. Photo of rats in individual cages that prevented them from interacting with one another.

Induction of Diabetes (Adeyi et al., 2012)

Diabetes in the rats was induced by feeding the animals with food of high glycemic index for at least four weeks. White bread, which has glycemic index value of 70, was fed to the rats while granulated sugar with glycemic index value of more than 100 was dissolved in drinking water at a concentration of 1g/ml. Surviving rats after four weeks with blood glucose concentration of approximately 180-200mg/ml were considered as food-induced hyperglycemic rats.

Metformin

Metformin is a hypoglycemic drug, which is effective in the treatment of non-insulin-dependent *Diabetes mellitus*. It is increasingly used in Canada and Europe (Klip & Leiter, 1990). The compound is often called insulin sensitizer as it increases the effects of insulin (Mestrovic, 2015). The major effect of the drug is on glucose utilization; it acts on the insulin receptors and glucose transporters (Klip & Leiter, 1990). Metformin stimulates the insulin-induced component of glucose uptake into skeletal muscle and adipocytes in both diabetic individuals and animal models, with an enhanced action of the drug in the hyperglycemic state (Klip & Leiter, 1990). The increase in glucose uptake is also reflected in an increase in the insulin-dependent portion of glucose oxidation (Klip & Leiter, 1990). In human and rat muscle cells in culture, metformin increased glucose-analogue transport independent of and additive to insulin, suggesting an insulin-independent action. In human and rat muscle cells in culture, metformin increase glucose-analogue transport independent of and additive to insulin, suggesting that it had an insulin-independent action (Klip & Leiter, 1990). Results of studies suggest that the basis for the hypoglycemic effect of this biguanide is probably at the level of skeletal muscle by increasing glucose transport across the cell membrane (Klip & Leiter, 1990).

Experimental Design

The procedures of this study were conducted in compliance with the ethical guidelines for animal care of Silliman University, which are based on the Department of Agriculture Administrative Order No. 40 Series of 1999.

Sixteen female Sprague-Dawley rats were divided into four groups (Prasad *et al.*, 2009):

Group I: The group consisted of four rats which first served as Hyperglycemic Control. They were given a diet composed of white bread (Mrs. Bread-worth) and sugar solution (1g/ml). After hyperglycemia had been induced, blood glucose level measurements were obtained for three consecutive days. After this, the rats were given two weeks of rest and were given the standard pellet diet. After two weeks, they were given a diet composed of food with high glycemic index. After hyperglycemia had been induced, they were treated orally with aqueous extract of the young leaves of the first variety (Native guava) of *P. guajava* leaves at the dose of 500 mg/kg body weight daily for three days, once a day. After treatment, the rats were again given two weeks of rest. After two weeks of rest, they were again given a diet composed of food with high glycemic index. After hyperglycemia had been induced, they were treated orally with aqueous extract of the old leaves of the first variety (Native guava) of *P. guajava* leaves at the dose of 500 mg/kg body weight daily for three days, once a day.

Group II: The group consisted of four rats which first served as Normal Control and were given the standard pellet diet and water only. Blood glucose measurements were obtained for three consecutive days. After this, the rats were given two weeks to rest. After two weeks, they were given a diet composed of food with high glycemic index. After hyperglycemia had been induced, they were treated orally with aqueous extract of the young leaves of the second variety (Apple guava) of *P. guajava* leaves at the dose of 500 mg/kg body weight daily for three days, once a day. After treatment, the rats were again given two weeks of rest. After two weeks of rest, they were given a diet composed of food with high glycemic index. After hyperglycemia had been induced, they were treated orally with aqueous extract of the old leaves of the second variety (Apple guava) of *P. guajava* leaves at the dose of 500 mg/kg body weight daily for three days, once a day.

Group III: This group consisted of four rats which were given a diet composed of food with high glycemic index. After hyperglycemia had been induced, they were treated orally with aqueous extract of the young leaves of the third variety (Pink guava) of *P. guajava* leaves at the dose of 500 mg/kg body weight daily for three days, once a day. After treatment, the rats were given two weeks of rest. After two weeks of rest, they were again given a diet composed of food with high glycemic index. After hyperglycemia had been induced, they were treated orally with aqueous extract of the old leaves of the third variety (Pink guava) of *P. guajava* leaves at the dose of 500 mg/kg body weight daily for three days, once a day.

Group IV: This group consisted of four diet-induced hyperglycemic rats that were treated with Metformin at the dose of 3 mg/kg for three days, once day. Blood glucose levels were measured every day.

Blood glucose level was measured before the treatment on Day 1, Day 2, and Day 3 (which was the last day of experiment) with the help of a glucometer using a strip method (One-touch glucometer) (Fig. 5). Blood was sampled from the tip of the tail. The tail of each rat was slightly cut just enough to produce a round blood that can be measured by One-touch Glucometer, which uses a test strip that is inserted into it. The test strip sucked the blood from the rat's tail and the meter processed the total blood glucose level in the blood. Glucose in the blood sample was mixed with special chemicals in the test strip and a small electric current was produced (LifeScan, Inc., 2010). The strength of this current changed with the amount of glucose in the blood sample (LifeScan, Inc., 2010). The measured total blood glucose level served as the initial total blood glucose level and the baseline for the next measurement.

Figure 5. Photo of the One Touch ® Select Simple ™ Blood Glucose Monitoring System (OneTouch® Select ® Test Strips, Lancing Device, OneTouch ® SelectSimple™ Meter) that was used in measuring the blood glucose levels of the rats.

Body weight measurement (Prasad et al., 2009; Hemamalini et al., 2012)

Body weight was totally measured four times during the course of the study period [i.e., before induction of Hyperglycemia (initial values), and on the first, second, and the third day after the treatment period], using a weighing scale.

Computation of Dose for Positive Control

The dose of the drug Metformin solution that was administered on the rats that belonged to Group IV was computed using the formula below (Prasad *et al.*, 2009):

$$\frac{(\text{Dosage}) \text{ x } (\text{Weight of rat in kg})}{(\text{Concentration of solution})} = \text{volume to be administered on the rat}$$

Example:

$$\frac{(3 \text{ mg/kg}) \text{ x } (0.150 \text{ kg})}{(10 \text{ mg/ } 2 \text{ ml})} = 0.09 \text{ ml}$$

Statistical Analysis

The results are presented as mean + SEM. Statistical significant differences in the blood glucose levels on the initial and Day 3 of treatment for each group were analyzed using paired t-test, with the help of Microsoft Office Excel 2016. Before conducting a paired t-test, a Shapiro Wilk Normality Test was done in order to determine if the data were normally-distributed. One-way analysis of variance (ANOVA) was then employed followed by the post hoc Neuman-Keuls test with the help of Statistica®. A p value < 0.05 was considered as a significant difference in the analyses.

RESULTS

Effects of the Native Guava, Apple Guava, Pink Guava, and Metformin on elevated total blood glucose levels

After feeding the rats with high glycemic food specifically white bread and sugar solution for four weeks, the blood glucose levels of the rats increased and reached approximately 200 mg/dl. This blood glucose increase was about 2.2 fold when compared with the normal control rats. After the three days of treatment of leaf aqueous extracts from the different varieties of *Psidium guajava* at a dose of 500 mg/kg body weight on the experimental groups, the total blood glucose level of the rats decreased significantly (P<0.05) (Fig. 1 and Fig. 2). Meanwhile, the treatment of commercial antidiabetic drug, Metformin, at a dose of 3 mg/kg body weight, had also significantly decreased (P < 0.05) the total blood glucose level of rats belonging to Metformin group, based on a Paired t-test. Paired t-test also revealed that based on one-tailed and two tailed test, blood glucose levels of rats treated with Native Guava (Young Leaves), Native Guava (Old Leaves), Pink Guava (Young Leaves), Pink Guava (Old Leaves), Apple Guava (Young Leaves), and Apple Guava (Old Leaves) all showed significant difference (P<0.05) compared with the hyperglycemic control. On the other hand, the blood glucose levels of all the rats from all the treatments showed significant difference (P<0.05) compared with the normal control group.

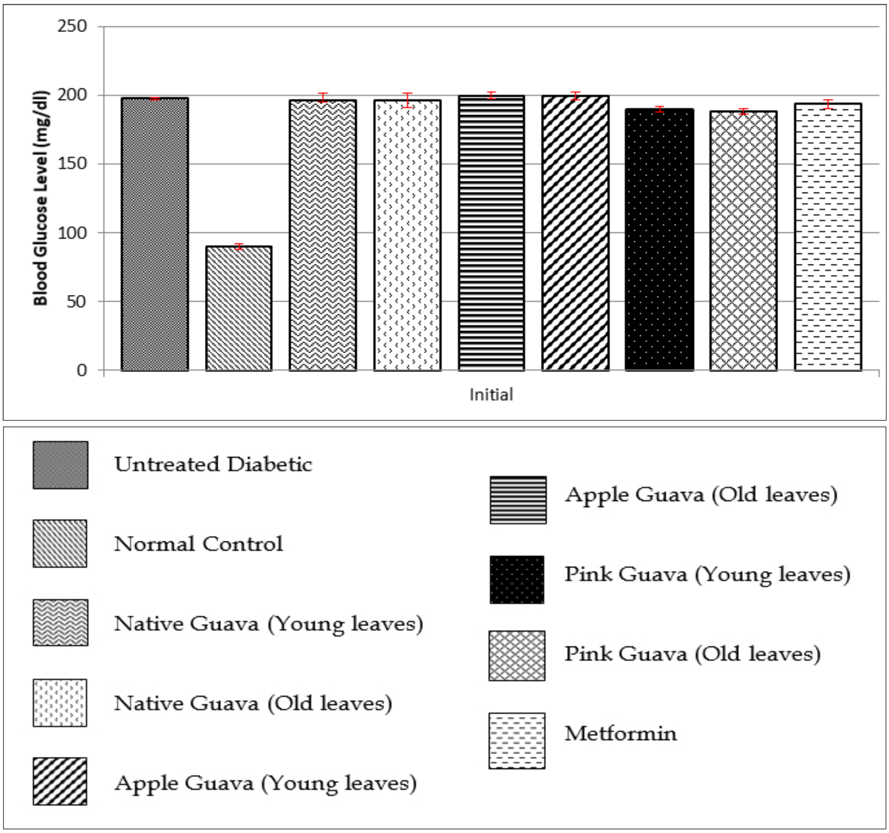

Figure 1. Mean of the total blood glucose levels before treatment (initial)

One-way Analysis of Variance (ANOVA) revealed that the p-value of the first variable (different varieties of *P. guajava*) and second variable (days of treatment) corresponding to the F-statistic were lower than the level of

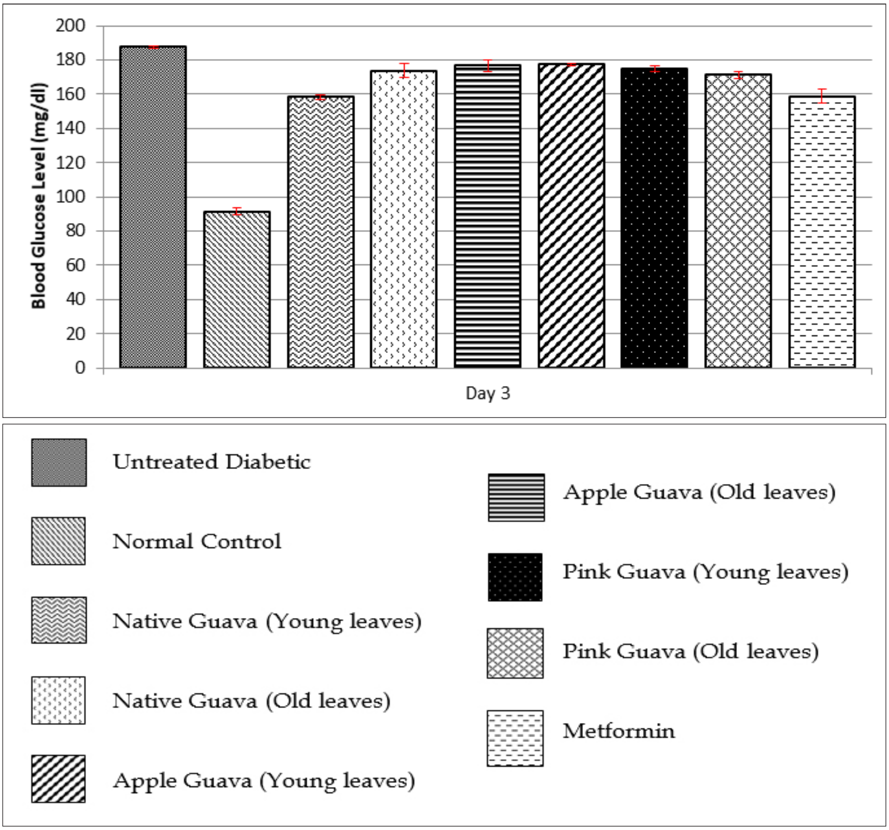

Figure 2. Mean of the total blood glucose levels on Day 3.

significance, which is 0.05, implying that one or more of the treatments in the study were significantly different. Thus, Student Newman Keuls Test (SNK) was employed in order to identify which among the treatments were or was significantly different. SNK revealed that treatment of Native Guava (Young Leaves) had a significant difference ($p=0.0001<0.05$) with the normal control group and the hyperglycemic control group ($p=0.0001<0.05$). Native Guava (Old Leaves) also showed a significant difference ($p=0.0009<0.05$) with the hyperglycemic control and normal control group ($p=0.0001<0.05$). Apple guava (Young Leaves) also had a significant difference ($p=0.016<0.05$) with the hyperglycemic control group and normal control group ($p= 0.0001<0.05$). Apple guava (Old Leaves) also showed significant difference with the hyperglycemic group ($p=0.011<0.05$) and normal control group ($p=0.0001<0.05$). Pink guava (Young Leaves) also showed significant difference with the hyperglycemic group ($p= 0.0001<0.05$) and normal control group (p=0.0001<0.05). Pink guava (Old Leaves) showed significant difference with the hyperglycemic group (p=0.0001<0.05) and normal control group (p=0.0001<0.05). Lastly, Metformin also showed a significant difference with the hyperglycemic group (p= 0.00011<0.05) and normal control group (p= 0.0001<0.05). A comparative account of the antihyperglycemic activity of the different varieties of *P. guajava* is well displayed in this study. Statistics revealed that Pink Guava (Young Leaves) has the highest statistical significance (p=0.000113<0.05) among the three varieties. Meanwhile, treatment of the rats with Metformin at the dosage of 3 mg/kg, had significantly decreased (p=0.00011< 0.05) the total blood glucose levels of rats belonging to the Metformin-treated group. Moreover, the reduction of blood glucose levels in Pink Guava (Young Leaves) did not show any significant difference (P>0.05) with the blood glucose levels of the Metformin-treated group, suggesting that both treatments have equal efficacy.

DISCUSSION

Knowledge when it comes to Diabetes can be traced back even to the Brahmic Period (Dhanukar & Thatte, 1989). Even during that time, they were already able to distinguish two types of diabetes: one that is attributed to the genes and the other to dietary factors (Dhanukar & Thatte, 1989). Several treatments have been formulated and the Indian ancient pharmacopoeia revealed treatments including dietary modifications and herbal treatments (Prasad *et al.,* 2009).

Since then, researches have revealed that there are a lot of possible plant and plant-based therapies that have a potential in controlling blood glucose levels and treating diabetes (Prasad *et al.,* 2009). Studies in the antihyperglycemic potential of plants usually use Streptozotocin(STZ)-induced hyperglycemic rats as animal models (Ivorra, Paya, & Villar, 1989). However, there are also studies that make use of diet-induced hyperglycemic rats just like the study of Adeyi *et al.*

Difference in the blood glucose levels can be attributed to the food intake of an individual. This is affected by the intake of foods with different glycemic index (GI). To determine the glycemic index of a food, volunteer individuals are typically given a test food that provides 50 grams of carbohydrate and a control food (white bread or pure glucose) that provides the same amount of carbohydrate on different days (oregonstate.edu). In the same way, rats can also be induced using a specific diet in order to obtain hyperglycemia. This study also made use of diet-induced hyperglycemic rats, following the methods of Adeyi *et al.* They were induced by feeding the animals with food of high glycemic index for approximately four weeks (Adeyi *et al.*, 2012). White bread which has glycemic index value of 70 was fed to the rats, while granulated sugar with glycemic index value of more than 100 was dissolved in the drinking water at a concentration of 1g/ml (Adeyi *et al.*, 2012).

Several phytochemicals have been speculated to account for the possible hypoglycemic activity of the leaves of *P. guajava.* Tannins, flavonoids, pentacyclic triterpenoids, guiajaverin, quercetin, and other chemical compounds present in the plant are the ones speculated to account for the observed hypoglycemic activity of the leaf extract (Ojewole, 2005). These phytochemicals have been speculated to have an action similar to insulin which is hypoglycemic (Ojewole, 2005). However, the specific chemical(s) that can be attributed for such hypoglycemic action, which was found in all the varieties of *P. guajava* have not yet been identified. A study by Oh *et al.* in 2005 suggested that the extract from *P. guajava* leaves possesses antidiabetic effect in type 2 diabetic mice model and these effect is, at least in part, mediated via the inhibition of Protein tyrosine phosphatase1B (PTP1B). The results of this study also correspond with the results of Ojewole in 2005, who used methanolic extracts of only variety of *P. guajava* and Streptozotocin-induced diabetic rats. Thus, Ojewole concluded that *P. guajava* had such hypoglycemic and even hypotensive properties.

After the induction of diabetes using the special diet, blood glucose levels in the rats increased. When treated with the different varieties of *P. guajava* and the synthetic drug for diabetes, Metformin, blood glucose levels decreased within the span of the treatment which was three days. When it comes to efficacy in lowering blood glucose levels, Pink Guava (Young Leaves) showed the highest potential which is the same with the established synthetic drug, Metformin. Blood glucose levels of rats treated with Pink Guava (Young) Leaves did not show any significant difference ($P>0.05$) with the blood glucose levels of the rats treated with Metformin. This implies that treatment with the aqeous extract of young leaves of Pink guava is comparable with Metformin in lowering blood glucose levels ($P>0.05$). Blood Glucose levels of rats treated with Native Guava (Young Leaves and Old Leaves) also did not show significant difference ($P>0.05$) with the blood glucose levels of those treated with Metformin. Although the reduction of blood glucose level due to the native guava (young and old leaves) was numerically more than that by the Pink guava (Fig. 17), paired t-test value showed otherwise since the P value obtained was lower than that of the Native guava (Young and Old Leaves). The P-value was always compared with the positive control, Metformin. This hypoglycemic activity in the Guava plant can be attributed to the different phytochemicals present in the leaves such as Tannins, flavonoids, pentacyclic triterpenoids, guiajaverin, quercetin as proposed and investigated by Ojewole (2005). The molecular mechanism by which these phytochemicals in lowering blood glucose levels is still to be established.

CONCLUSION

The analysis of the data indicated Pink Guava (Young Leaves) to have the highest potential in treating hyperglycemia in diet-induced Sprague-Dawley rats. The other varieties of *P. guajava* also have significant hypoglycemic properties. The difference between Pink Guava (Young Leaves) and the synthetic drug for Diabetes, Metformin, which is not statistically-significant suggests that Pink Guava (Young Leaves) has a hypoglycemic activity which is comparable to the commercial drug, Metformin. Leaves of the Pink Guava are as effective as Metformin. Thus, among the three varieties of *Psidium guajava* namely Native Guava, Apple Guava, and Pink Guava, the Pink Guava (Young Leaves) has the highest efficacy in maintaining normal blood glucose level. This potential of a readily available and palatable food product is most significant that it deserves utmost attention.

REFERENCES

Adeyi, A., Idowu, B., Mafiana, C., Oluwalana, C., Ajayi, O., & Akinloye, O. (2012). Rat model of food-induced no-obese-type 2 diabetes mellitus: Comparative pathophysiology and histopathology. *International Journal of Physiology, Pathophysiology and Pharmacology, 4*(1), 51-58.

Dhanukar, S., & Thatte, U.. (1989). *Ayurveda revisited.* Bombay: Popular Prakashan.

Gutiérrez, R. M. P., Mitchell, S., & Solis, R. V. (2008). < i> Psidium guajava</i>: A review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology*, *117*(1), 1-27.

Ivorra, M.D., Paya, M., & Villar, A. (1989). A review of natural products and plants as potential antidiabetic drugs. *Journal of Ethnopharmacology, 27,* 243-275.

Janvier Labs. (2015). Retrieved from https://www.janvier-labs.com

Klip A., & L. Leiter. (1990). Cellular mechanism of action of Metformin. *Diabetes Care, 13*(6), 696-704.

Mestrovic, T. (2015). *Metformin mechanism.* Retrieved from http://www.news-medical.net/health/Metformin-Mechanism.aspx

Ojewole, J. A. O. (2005). Hypoglycemic and hypotensive effects of Psidium guajava Linn.(Myrtaceae) leaf aqueous extract. *Methods and findings in experimental and clinical pharmacology*, *27*(10), 689-696.

Oh, W. K., Lee, C. H., Lee, M. S., Bae, E. Y., Sohn, C. B., Oh, H., & Ahn, J. S. (2005). Antidiabetic effects of extracts from< i> Psidium guajava</i>. *Journal of ethnopharmacology*, *96*(3), 411- 415.