Antiglycemic effect of Bignay (*Antidesma bunius*) flavonoids in Sprague-Dawley rats

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ABSTRACT

Diabetes is increasing substantially in the global era. Several herbal medicines have been studied and some are proven to have a beneficial effect against it. Some from the group of organic acids, phenolic acids and flavonoids contribute to the decreasing effect of hyperglycemia. In previous studies, bignay (*Antidesma bunius*) showed an evidence of having the said contents. In the present study, the antiglycemic effect of bignay was determined after 14 days of treatment. The extracts of 200 mg/kg and 600 mg/kg of bignay proved their glucose lowering activity on fasted non-diabetic and Alloxan (ALX)-induced diabetic rats. Moreover, 600 mg/kg was more effectual and exhibited the same therapeutic outcome with glibenclamide. Therefore, the study shows that the extract of bignay (*Antidesma bunius*) and its flavonoid contents has a promising effect on decreasing blood glucose level in diabetes as well as the prevention of its occurrence.

Keywords: antihyperglycemic, hypoglycemic, diabetes, *Antidesma bunius*, flavonoids

INTRODUCTION

Diabetes has been highly increasing in proportions, affecting millions globally. Diabetes mellitus, a type of diabetes which exhibits hyperglycemia in nature, involves over 124 million individuals worldwide (Quinn, 2001; Quine and Raghu, 2005). The disease may be due to glucose tolerance impairment, insulin resistance, and unhealthy lifestyle. The type 2 diabetes mellitus epidemic in Asia is also rising (Chan et al., 2009) with 4.6% prevalence in the Philippine population (Morales et al., 2008). The metabolic changes are complex and the process of deregulation takes years (Tiwari and Madhusadana Rao, 2002). In fact, diabetes is associated with other risk factors for mortality like cardiovascular disease, especially in instances of long duration (Natajaran et al., 2005; Juutilanen, 2005) due to endothelial dysfunction that may occur (Shahab, 2006). In part of this is a socioeconomic burden, as well as in healthcare services. However, there are approaches in treating this kind of life threatening disease (Ono, 2006). Oral agents, advancement in technology and early intervention to the disease and its complications are some of the beneficial ways against it (Tripathi and Srivastarva, 2006). Still, the search for the prevention and

treatment of diabetes is given attention where mostly, plant extracts are developed through experimental researches.

The fruit of *Antidesma bunius*, commonly known as Bignay, is traditionally used by native Filipinos, boiled or eaten raw, for diabetes and hypertension. Methanolic extracts of bignay (*Antidesma bunius*) berries contains organic acids, phenolic acids, and flavonoids (Samappito and Butkhup, 2008), each of which encompasses their own beneficial actions on human health (Matsui et al., 2006). The organic acids found on bignay include tartaric acid, citric acid, benzoic acid, malic acid, lactic acid, oxalic acid, acetic acid and ascorbic acid. On the other hand, ferrulic acid, gallic acid, and caffeic acid were the phenolic acids present on the fruit. It also contains several flavonoids which comprise anthocyanin, luteolin, rutin, resveratrol, quercetin, procyanidin and catechins were the flavonoids found (Samappito and Butkhup, 2008).

Lactic acid (Ostman et al., 2002), acetic acid (Ogawa et al., 2000), caffeic acid (Jung et al., 2006) and ascorbic acid have also shown a positive effect on type 2 diabetes (Mullan et al., 2002).

Furthermore, flavonoids, the polyphenol compounds, (Lukacinova et al., 2008; Knekt et al., 2002), also anthocyanins (Ghosh and Konishi, 2007) have demonstrated significant effects on chronic diseases like diabetes by exhibiting a hypoglycemic action (Ahmad et al., 2000; Quine and Raghu, 2005) by inhibiting intestinal glucose transporter (Kwon et al., 2007). These phytochemicals decreased the risk of diabetes mellitus by glucose homeostasis (Kiec et al., 2008). Procyanidins exhibited antihyperglycemic effects (Pinent et al., 2004). Myricetin, epicatechin (Quine and Raghu, 2005) and quercetin also inhibit glucose uptake (Strobel et al., 2005; Sahu et al., 2001; Rizvi and Zaid, 2001). In addition, resveratrol had a hypoglycemic effect (Su et al., 2006). Lastly, catechins showed potential in the prevention of metabolic syndrome and glucose tolerance (Igarashi et al., 2007; Thielecke et al., 2007; Ostman et al., 2002).

In this study, the effect of ethanolic crude extract of bignay on the fasting blood sugar of diabetic and non-diabetic Sprague-Dawley rats was studied.

MATERIALS AND METHODS

Reagents and materials. Eight kilograms of ripe fruits was collected from Banay-Banay, San Jose, Batangas. Vouchers of the plant specimen were submitted to the Herbarium of University of Santo Tomas, Espana Manila for authentication (USTH-5540). Alloxan monohydrate was purchased from Sigma-Aldrich, MO, USA and the Glibenclamide from PT Aventis Pharma, Indonesia.

Preparation of Extract. The fruits were osterized and were filtered through Whatman filter paper. The sample was immersed into 80% (v/v) ethanol. The alcohol content was evaporated in ambient pressure at 40°C until the sample turned syrupy (Pineda, 2009).

Determination of Phytochemicals. The qualitative determination of phytochemicals was confirmed by the Industrial Technology Development Institute of the Department of Science and Technology, Taguig City, Metro Manila.

Test Animals. Sprague-Dawley rats were purchased from BioPhilippines, Manila. The rats of both sexes, weighing 151±29, were housed in cages, individually and in colony, for the diabetic and non-diabetic group respectively. A standard pellet diet and water were given *ad libitum.* They were acclimatized under controlled temperature with a 12-h light/12-h dark cycle for a period of one week. The animals were fasted for 12 hours with free access to water before glucose determination. The use of animals was approved by the Bureau of Animal Industry (BAI).

Induction of diabetes. Hyperglycemia was induced by a single intraperitoneal injection of ALX (150 mg/kg body weight) freshly suspended in 0.9 NaCl solution to the test animals after fasting. Following 48 hrs of induction, the fasted rats which exhibited hyperglycemia (>8 mmol/L) were selected for the study (Ahmed et al., 2010).

Experimental procedure.A total of 18 rats were used and received the following treatment for 14 days (Ahmad et al., 2000; Ahmed et al., 2010).

Diabetic rats (caged individually)

i.) Group I (four rats): 200 mg/kg B.W. bignay extract
ii.) Group II (four rats): 600 mg/kg B.W. bignay extract
iii.) Group III (two rats): 5 mg/kg B.W. glibenclamide
iv.) Group IV (one rat): 0.95% NSS

Non-diabetic rats(in colony cages)

i.) Group I (two rats): 200 mg/kg B.W. bignay extract
ii.) Group II (two rats): 600 mg/kg B.W. bignay extract
iii.) Group IV (three rats): 0.95% NSS

Glucose analysis. The fasting blood glucose levels collected through the tail vein (Hoff, 2000) were measured via glucometer (Optimum Xceed, Abbott, Berkshire, UK). Blood samples were measured on day 1, 7 and 14 of the study (Ahmed et al., 2010).

Statistical analysis. Each result was expressed as means ± Standard Error. The grouped data was evaluated statistically using one-way analysis of variance (ANOVA) and t-test for independent variables. *P*< 0.05 was considered significant (Zhou et al., 2009).

RESULTS

After the ALX induction, the blood glucose levels increased (**Figure 1**) due to the effect of the drug to the pancreatic cells of the rats (Szkudelski, 2001). The 200 mg/kg treatment of bignay showed an inconsistent antihyperglycemic effect on the first week. However, on the second week, a constant antihyperglycemic effect against increased fasted blood glucose levels was evident. On the other hand, 600 mg/kg of bignay showed a constant effect against rising blood glucose levels.

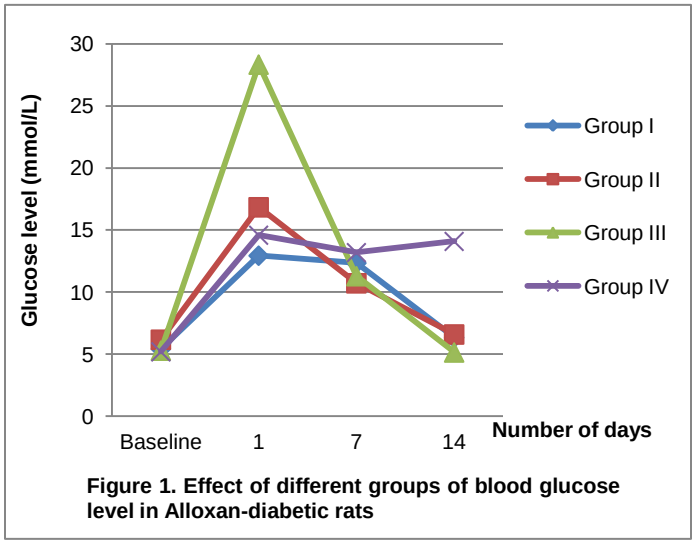

Figure 1. Effect of different groups of blood glucose level in Alloxan-diabetic rats

Both 200 mg/kg of bignay and 5 mg/kg of glibenclamide reduced FBG levels after two weeks of administration but only glibenclamide showed constant FBG levels decline proving its capability of stimulating insulin secretion (Jonsonn et al, 2000).Furthermore, it has been noted that there is no significant difference on the effect of 600 mg/kg of bignay and 5 mg/kg of glibenclamide at <0.05 level of significance. The normal control group (0.95% NSS) showed an inconsistent effect from a decreased FBG levels on the first week and increased FBG levels in the second week.

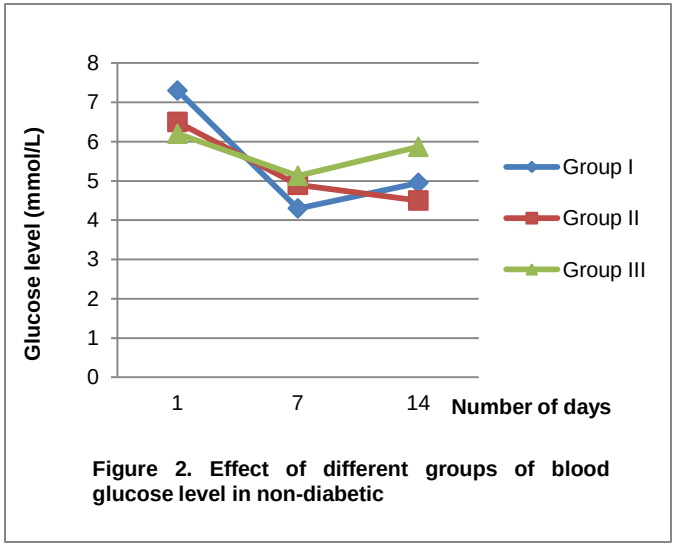

Figure 2. Effect of different groups of blood glucose level in non-diabetic

The results of the non-diabetic group (**Figure 2**) showed an inconsistent hypoglycemic effect using 200 mg/kg of bignay after 14 days of treatment. The 600 mg/kg bignay treatment in contrast, manifested a hypoglycemic effect which shows that bignay at 600 mg/kg can lower the blood glucose level of normoglycemic rats.

DISCUSSION

Diabetes mellitus has increased rapidly. The prevention and control of the disease is now a global concern (Chan et al., 2009). There are several therapeutic drugs available but still, the search for the prevention and treatment is given attention (Ono, 2006). However, some of the herbal medicines are being developed to help lessen the healthcare and socioeconomic burden diabetes brings.

The fruits of *Antidesma bunius* contains organic acids, phenolic acids, and flavonoids (Samappito and Butkhup, 2008) that in several studies were proved to have antihyperglycemic effect (Matsui et al., 2006; Mullan et al., 2002; Kwon et al., 2007; Lukacinova et al., 2008; Kiec et al., 2008).

The present study has proven that the ethanolic crude extract of *Antidesma bunius* has a good antihyperglycemic and hypoglycemic activity.

It is found out that the 600 mg/kg of ethanolic extract bignay is more effective than 200 mg/kg of bignay after 14 days of treatment in the diabetic group. This also means that a dose of 600 mg/kg of bignay shows similar curative effect as the standard drug, glibenclamide (5 mg/kg).

The first group had a 7.525 mmol/L increase in FBG levels after ALX induction. Upon the first week of treatment of 200 mg/kg of bignay, 0.6 mmol/L reduction in the FBG levels was observed. In the next 7 days of 200 mg/kg bignay treatment, a decrease of 6 mmol/L was seen. Moreover, from the 6.1750 mmol/L FBG baseline of the second group, an additional 10.675 mmol/L on FBG levels was detected after the induction of ALX. Following the first 7 days of treatment of 600 mg/kg bignay, FBG levels decreased by 6.125 mmol/L. On the next week of oral administration of bignay at 600 mg/kg, 4.125 mmol/L FBG decrease was expressed.

A demonstrable rise of 23.05 mmol/L of FBG was exhibited by the third group. After the first week, the fasted rats were administered orally with 5 mg/kg and produced 17.05 mmol/L FBG decrease. After the second week, a decrease of 6.15 mmol/L FBG levels was evident on 5 mg/kg glibenclamide oral administration. In the last group which is administered with 0.95% NSS, an increased 9.4 mmol/L was noted from the 5.20 mmol/L FBG levels. On the first and second week, a 1.4 mmol/L decreased FBG level and 0.9 mmol/L elevations were seen.

The non-diabetic group exhibited hypoglycemic effect with both doses of bignay but 600 mg/kg of bignay also has a greater activity than the 200 mg/kg of bignay. This was evaluated from the 3.0 mmol/L decline in FBG levels after the first week of oral administration administration of 200 mg/kg of bignay. The next week of 200 mg/kg of bignay treatment showed an increase of 0.6 mmol/L FBG levels. In the another treatment, 600 mg/kg of bignay, the first 7 days showed FBG level decrease by 1.6 mmol/L and the next 7 days with 0.4 mmol/L. The FBG levels of the last group which is orally treated with only 0.95% NSS showed a decrease of 1.07 mmol/L on the first week. On the second week of treatment an increase of 0.73 mmol/L was seen.

In conclusion, *A. bunius* ethanolic crude extract exhibited significant antihyperglycemic activities in normal and ALX-induced diabetic rats. However, a dose of 600 mg/kg exhibited significant effects than 200 mg/kg in the ALX-induced diabetic and normal rats. Thus, the activity of *A. bunius* ethanolic crude extract on both doses of 200 mg/kg and 600 mg/kg could be of value in the treatment of diabetes as well as the prevention of having the said disease. Furthermore, the authors conclude that a dose of 600 mg/kg of bignay can be used as a substitute for the standard drug, glibenclamide.

RECOMMENDATIONS

Acute toxicity study and the use of other extracts are highly recommended for further investigation of the effect of *Antidesma bunius* on hyperglycemia and hypoglycemia.

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