Activity of ethanolic extract from leaves of *Lagerstroemia speciosa* (L.) Pers. (Banaba) on pancreatic insulin secretion and its protective effect in alloxan-induced diabetic rats

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Abstract: Diabetes is a growing health problem in the Philippines, which is characterized by hyperglycemia resulting from defects in pancreatic insulin secretion. The leaves of *Lagerstroemia speciosa* (L.) Pers., a Southeast Asian tree commonly known as banaba, have been traditionally consumed by Filipinos as medication. Its leaves are known to contain an active compound called corosolic acid, to which its glucose-lowering activity is attributed. Thus, the study was therefore intended to investigate the effect of ethanolic leaf extract of banaba on blood glucose and insulin. Likewise, it was designed to assess its protective activity by measuring insulin secretion of pancreatic β cells at different doses. Diabetes was induced intraperitoneally with alloxan (40 mg/kg) and banaba crude leaf extract was given orally once a day for 10 days. Results of the present study indicated significant decrease ($p<0.05$) in glucose levels and increase in insulin levels after treatment. On the other hand, protective activity to pancreas was evaluated by giving three doses of banaba crude leaf extract prior to treatment with alloxan, specifically 500, 1000 and 2000 mg/kg being Groups II, III, and IV, respectively with Group I being the normal control group. Significant rise in insulin levels was observed in Group II (1000 mg/kg) with a greater increase in Group IV (2000 mg/kg). Multiple comparisons by post-hoc analysis were conducted using Tukey method which revealed significant differences among the normal group, Group III and Group IV. All results were supplemented by histopathological analysis which indicated that both Group III and Group IV have favorable effects, though the latter showed a closer tonormal structure of pancreatic islet cells. Based on these findings, *Lagerstroemia speciosa* (L.) Pers. crude leaf extract exhibited hypoglycemic and

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protective activity at 1000 mg/kg and 2000 mg/kg concentrations, respectively.

Keywords: *Lagerstroemia speciosa (L.), hypoglycemia, corosolic acid, alloxan, insulin, diabetes*

INTRODUCTION

Diabetes is a disease that is known worldwide and has been recognized as one of the leading causes of mortality (Park & Lee, 2011).

According to the World Health Organization (WHO) in 2013, 347 million people worldwide have diabetes. In 2004, an estimated 3.4 million people died from consequences of fasting high blood sugar.Asimilar number of deaths have been estimated for 2010. More than 80% of diabetes deaths occur in low- and middle-income countries (Danaei, Finucane, Lu, Singh, Cowanet al., 2011). Moreover,in the Philippines, diabetes ranked 6th among the leading causes of death according to the annual update of the International Diabetes Federation (IDF) Diabetes Atlas in 2011.

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces (Lenzen, 2008).

The two types of diabetes are referred to as type 1 and type 2. Former names for these conditions were insulin-dependent and non-insulin-dependent diabetes, or juvenile onset and adult onset diabetes, respectively.In the more prevalent type 2 diabetes mellitus, the cause is normally a combination of resistance to insulin and an inadequate compensatory insulin-secretory response (Amin, Lotfy, Mahmoud-Ghoneim, Adeghate, Al-Akhraset al., 2011). In people with type 2 diabetes, insulin is produced but it is not utilized by the body due to muscle, fat, and liver cells that were compromised and do not respond to insulin efficiently; thus, a condition known as insulin resistance. Individuals with insulin resistance require more insulin to aid glucose in entering cells. Owing to this increased demand, pancreas attempts to sustain but soon fails, causing excess glucose to build up in the circulation. Eventually, the effects of recurring high blood glucose levels impair beta cells, hence reducing the ability of the pancreas to produce insulin (Lenzen, 2008).

Type 2 diabetes mellitus accounts for about 90 percent of all cases of diabetes and rises from a pool of genetic and acquired factors that damage cell function on one hand, and insulin sensitivity on the other. However, there is increasing substantiation that β-cell impairment is the crucial defect for the development of this kind of

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diabetes, as revealed by certain studies (Marchetti, Bugliani, Boggi, Masini,& Marselli, 2008). One of which is in a study conducted with newly diagnosed type 2 diabetic patients followed up to ten years, secondary failure of plasma glucose control after initial successful response to diet therapy occurred progressively in those with greater β-cell dysfunction, and the ongoing decline in B-cell function closely mirrored the steady rise in fasting plasma glucose (Marchetti, Bugliani, Boggi & Marselli, 2012).

Figure 1. ***Lagerstroemia speciosa*** **(L.) Pers.(Banaba)leaves**

Despite the ongoing introduction of new hypoglycemic drugs, diabetes, and its related complications, remains a major global Medical problem. Modern drugs including insulin and other oral hypoglycemic agents such as sulphonylureas control blood glucose level as long as they are regularly administered, but can also produce many undesirable side effects (Zhou, Zhou, Tang, Zhang, Guanget al., 2009). Substantial interests in alternative approaches to minimize the burden of complications associated with this disease were currently considered. Thus, several experimental studies have been conducted to evaluate either raw or purified active ingredients of an array of natural products (Frode & Medeiros, 2008).

Lagerstroemia speciosa (L.) Pers. (Banaba) is a deciduous tree widely distributed in different parts of the Philippines, India, and Southeast Asian countries. The tea from its leaves has traditionally been used in the Philippines as a folk medicine for the prevention and treatment of diabetes (Vijaykumar, Murthy, Kannababu, Syamasundar,& Subbaraju, 2006). *L. speciosa*accounts for various local names such as Parasa-bukung, Agaro, Bugarom, Nabulong, Pamalauagon, Pamarauagon, Tagangau, Tabangau, Tauagnau, Duguan, Kauilan, Makablos, and Mitla. Likewise, other scientific names include *Munchausia speciosa* Linn. and *Lagerstroemia reginae Roxb*. Its international names are Crape Myrtle, Crepe Myrtle, *Lagerstroemia flos-reginae*, Munchausia speciosa, Myrte de Crêpe, Pride-of-India, Queen of India, Pyinma, and Queen's Crape Myrtle (Orwa, Mutua, Kindt, Jamnadass & Simons, 2009).Over the years, its leaves have been widely studied for different biological activities, particularly the ability to lower blood glucose level. A study (Park & Lee, 2011) showed that banaba leaf containsa triterpenoid compound called corosolic acid which is thought to stimulate glucose uptake (Deocaris, Aguinaldo, dela Ysla, Asencion & Mojica, 2005). That same study found out that

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corosolic acid increased the uptake of glucose into the cells which then yields a decrease in blood glucose levels.

A number of studies proved corosolic acid from banaba leaves to produce hypoglycemic effect. What is not known yet is the effect of banaba extract with its active compound, corosolic acid on pancreatic insulin secretion. Alterations in blood glucose and in cell number reflect abnormalities in β-cells function and structure. Pancreatic β-cell failure plays a key role in the pathogenesis of type 2 diabetes; hence, the study was made to evaluate the protective effect of ethanolic leaf extract of *L. speciosa* on insulin secretion as well as its effect on the integrity of the pancreas, which serves as a potential alternative to hypoglycemic agents. Thus,identifying the optimum activity of the extract as to its protective activity will be possible along with the effects considered significant brought about by the extract to the pancreas.

MATERIALS AND METHOD

Plant Material

Leaves of *L. speciosa* were obtained from Shell Residential Compound, Tabangao-Ambulong, Batangas City and submitted toUniversity of the Philippines, Los Baños, Laguna, Institute of Biology, for authentication.

Preparation of Banaba Extract

Three kilograms of fresh green leaves of *L. speciosa* with sizes ranging from 3 to 12 inches were processed in batches, with 250 leaves each batch. These were boiled for one hour to remove the water-soluble substances followed by three cycles of extraction with 25% aqueous ethanol for 30 minutes and then by 95% aqueous ethanol for one hour at 80°C. After cooling at room temperature for ten minutes and subsequent filtration, the extract was treated with activated carbon to remove chlorophyll, with a gram of activated carbon per 500 mL extract. The solution was filtered with Whatman filter paper no. 1. Every 9 mL solution was added with one mL 38% w/w hydrochloric acid to allow precipitation. The sediments were washed with distilled water to obtain total crude triterpene acid, which were dissolved with ethanol (He, Liu, Bai, Huang & Wang, 2010).

Experimental Animals

Twenty-four (24) male Sprague-Dawley rats were divided into four groups (six rats in each group). They were acclimatized to standard laboratory conditions (temperature 24 ± 1°C, relative humidity

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55 ± 5% and a 12-hour photoperiod) in suspended wire-meshed galvanized cages for one week before the commencement of the experiment. During the entire period of study, the rats were supplied with a basal diet and water *ad libitum.* The first group was regarded as normal control which was kept without treatment under the same laboratory condition. The second group was treated with Banaba crude extract (BCE) at a dose of 2000 mg/kg b.wt. The third group was made diabetic with an intraperitoneal injection of Alloxan at a dose of 40 mg/kgb.wt and considered the diabetic control group. Lastly, the fourth group was firstmade diabetic with 40 mg/kg alloxan, followed by treatment with BCE at a dose of 2000 mg/kg b.wt. Treatments were given through oral gavage (Vijaykumar et al., 2006).

Group I : Normal control group.
Group II: BCE at a dose of 2000 mg/kg b.wt. for 10 days.
Group III: Alloxan 40 mg/kgb.wt for 10 days (diabetic control)
Group IV:Alloxan 40 mg/kgb.wt plus BCE at a dose of 2000 mg/kg b.wt.

Induction of Diabetes Mellitus

After one week stabilization period, blood glucose levels were tested prior to treatment. Then, rats fasted for 12 hours before diabetes was induced using Alloxan at a dose of 40 mg/kg that was freshly dissolved in saline. For intraperitoneal injection, the rat was held in onehand in dorsal position, the injection site was swabbed using povidone-iodine solution and the designated amount of alloxan was injected using sterile 25g needle (Abeeleh, Ismail, Alzaben, Abu-Halaweh, Al-Essaet al., 2009).

Blood Analysis

After 72 hours, blood was obtained from tail vein. Glucose was estimated using Blue Care Plus Glucometer with reagent strips by glucose oxidase method. Rats with blood glucose levels of 180 mg/dl or more were categorized as diabetic (Abeeleh at al., 2009). For the determination of insulin levels, blood samples were collected from retro-orbital vein. Two mL of blood were obtained to perform insulin testing by ECLIA method (Thuppia, Rabintossaporn, Saenthaweesuk, Ingkaninan & Sireeratawong, 2009).

Test for Protective Effect

Four groups were tested, each containing three rats. Group I served as normalcontrol. Group II was administered with Banaba crude extract (BCE) with a dose of 500 mg/kg.Group III was administered

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with a dose of 1,000mg/kg; and Group IV was administered with a dose of 2,000mg/kg. After the administration of treatment, diabetes was subsequently induced by administering alloxan (40 mg/kg) to BCE-treated rats. Protective effect of the extract to pancreas was evaluated after 15 days through measurement of insulin levels using ECLIA method (Abdul-Hamid & Moustafa,2013).

Group I : Normal control group.
Group II : Treated with 500 mg/kg BCE.
Group III: Treated with 1000 mg/kg BCE.
Group IV:Treated with 2000 mg/kg BCE.

Histopathological Examination of Pancreas

All rats were sacrificed under diethyl ether at the end of the study period. The pancreas of normal and diabetic animals was isolated for histopathological examination a day after insulin secretion test. These were fixed in 10% formalin and sent in the laboratory for hematoxylin and eosin staining (Jelodar, Mohsen & Shahram, 2007).

RESULTS AND DISCUSSION

Plant Extract

Upon extraction of three kilos of banaba leaves with six liters ethanol, a final volume of two and a half liters was obtained; resulting to a percent yield of 41.67%, and thecolorproduced upon subjecting the leaves to series of boiling and filtration was dark mossy green with watery consistency. However, when activated carbon was added to the solution to remove the chlorophyll, the color turned light green with similar consistency.

Figure 2. *Lagerstroemia speciosa* (L.) Pers. (Banaba)ethanolic leaf extract

Biochemical Analysis

To evaluate the hypoglycemic activity of *L. speciosa L.* leaf extract, glucose levels were determined using a glucometer. Correspondingly, insulin levels were quantified using ECLIA to assess further effect. For the protective activity of the extract, insulin levels were likewise tested using the same method.

Table 1 presents the effect of ethanolic leaf extract of *L.speciosa* (L.) Pers. (banaba) on glucose levels.In Group I (normal

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control), pre-test yielded a value of 89.333 mg/dl whereas post-test was 89.833 mg/dl. Apparently, values were nearly identical with each other; neither alloxan nor BCE treatment was done. On the other hand, Group II which was administered with BCEat a dose of 2000 mg/kg yielded pre-test and post-test values of 88.500 mg/dl and 76.166 mg/dl, respectively. There was a drop in blood glucose level, demonstrating the glucose- lowering activity of the extract known to contain significant amount of corosolic acid. According to Miura, Ueda and Yamada (2006), corosolic acid had significantly decreased the blood glucose levels of mice but on the contrary, it had produced no effect on the blood glucose in normal mice.In Group III, alloxan was administered at a dose of 40 mg/kg, which produced a post-test value of 179 mg/dl with baseline level of 101 mg/dl. There was an evident increase in the glucose level brought about by repeated administration of alloxan.This was because one of the most potent methodsto induce experimental diabetes mellitus ischemical induction by alloxan (Etuk, 2010).

Table 1
Effect of ethanolic extract of *Lagerstroemia speciosa* (L.) Pers. (Banaba) leaves on glucose levels

	Pre-test (mg/dl)	Post-test(mg/dl)
Group I Normal control	89.333	89.833
Group II Extract treatment (2000 mg/kg BCE)	88.500	76.166
Group III Diabetic control (40 mg/kg alloxan)	101	179
Group IV (40 mg/kg Alloxan; 2000 mg/kg BCE)	183.500	140.333

**BCE- Banaba crude extract*

Similarly, it was noted in the findings of Rohilla & Ali (2012) that inducing alloxan was the most consistent and certainly reproducible method of developing diabetes among experimental mice. Group IV received two treatments; alloxan at a dose of 40 mg/kg which produced glucose level of 183.500 mg/dl, whereas when treated with BCE yielded a lower level of 140.333 mg/dl.A marked decrease in glucose level was observed which was comparable to group II, and was believed to be due to high amount of corosolic acid present in BCE. This correlates with the findings of Judy, Hari, Stogsdill,

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Judy,&Naguib et al., (2005), wherein *L. speciosa* leaves standardized to 1% corosolic acid, presented a significant decrease in the blood glucose level in type 2 diabetes. Likewise, results coincide with the study conducted by Miura et al., (2006), wherein they have stated that repetitive administration of corosolic acid yielded to hypoglycemia.

Table 2
Significance of BCE on glucose levels

	p-value	Significance
Group I Normal control	0.973	Not significant*
Group II Extract treatment (2000 mg/kg BCE)	0.000	Significant
Group III Diabetic control (40 mg/kg Alloxan)	0.042	Significant
Group IV (40 mg/kg alloxan; 2000 mg/kg BCE)	0.039	Significant

**Significant at p-value < 0.05; Tukey Method*

Table 2 presents the significance of BCE based on results of pre-test and post-test.Since Group I was regarded as the normal control, no significance was observed with a p-value of 0.973. On the other hand, in Group II, p-value was 0.000 which was considered significant, since as seen from the post-test result in Table 1, BCE produced a remarkable decrease in glucose level. Also, Group III which was the diabetic control gave a value of0.042 which is lower than 0.05 which is significant; thus, proving the action of alloxan justified by the extensive increase in glucose levels. A p-value of 0.039 was observed in Group IV which was considered significant, attesting that the extract which was thought to contain a substantial amount of corosolic acid can actually cause a decrease in blood glucose.

These results agree with the observations of Kakuda, Sakane, Takihara, Ozaki, Takeuchi et al., (2006) who showed that an extract of Banaba leaves exerted hypoglycemic effects through multiple mechanisms.

Insulin levels for these groups were also tested. Table 3 presents the effect of banaba crude extracton insulin levels. This shows pre-test values of all groups to be 0.200 uIU/ml. Group I retained its baseline value as this group was the normal control. Group II which received BCE had an increase in insulin level to 0.218 uIU/ml. Correspondingly, group IV got an increase in its insulin level to 0.312 uIU/ml upon administration of the same dose of the extract. Since both

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continued treatment, the results were believed to be the effect of BCE which agreed with the reported findings in the study of Deocaris et al., (2005) who mentioned thathigher levels of insulin present greater effectiveness of lowering the blood glucose level in alloxan-induced mice. However, Group III retained its baseline value (0.200). This indicates that BCE did not cause any decrease or increase in insulin levels and is supported by the study of Miura, Takagis, & Ishida (2012) who worked on the management of diabetes and its complication with banaba and the active constituent corosolic acid that the extract may or may not have any effect in the insulin levels or have it retained.

Table 3
Effect of BCEon insulin levels

	Pre-test(uIU/ml)	Post-test(uIU/ml)
Group I Normalcontrol	0.200	0.200
Group II Extract treatment (2000 mg/kg BCE)	0.200	0.218
Group III Diabetic control (40 mg/kg alloxan)	0.200	0.200
Group IV (40 mg/kg alloxan; 2000 mg/kg BCE)	0.200	0.312

**BCE- Banaba crude extract*

Table 4 discusses the significance of insulin levels for each group. No p-values were provided for Group I and Group III due to the fact that such computation cannot be done if results to be compared were similar; thus, interpreted as not significant, and that such interpretation poses no influence on the validity of the results for those groups. Therefore, outcomes for Group I and Group III were inconclusive with respect to p-values analyzed.On the other hand, Group II and Group IV were interpreted as significant, with p-values of 0.018 and 0.412, respectively. This strongly suggests that the increase in insulin levels generated were thought to be triggered by the ethanolic leaf extract. This theory coincides with the study of Rohila & Ali(2012) who conducted an experiment on diabetic rats and found that the extract from the leaves of *L. speciosa* can increase insulin level of alloxan-induced diabetic rats.

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Table 4

Significance of BCE on insulin levels

	p-value	Interpretation
Group I Normal control	-	Not significant
Group II Extract treatment (2000 mg/kg BCE)	0.018	Significant
Group III Diabetic control (40 mg/kg alloxan)	-	Not significant
Group IV (40 mg/kg alloxan; 2000 mg/kg BCE)	0.412	Significant

*BCE- Banaba crude extract

In the assessment of the protective activity, the leaf extract was administered preceding the induction of alloxan. Insulin levels were analyzed to discern whether the extract can protect the pancreas from high amounts of glucose through secretion, or by least retention of its insulin.

Table 5

Assessment of the protective activity of *Lagerstroemia speciosa L.* (Banaba) leaf extract through measurement of insulin levels

	Pre-test(uIU/mL)	Post-test(uIU/mL)
Group I (Normal group)	0.200	0.200
Group II (500 mg/kg BCE)	0.200	0.200
Group III (1000 mg/kg BCE)	0.200	0.290
Group IV (2000 mg/kg BCE)	0.200	0.360

*BCE- Banaba crude extract

Table 5 presents the baseline levels of insulin and levels after the treatment. Levels on all groups prior to administration of treatment were similar, with values of 0.200 uIU/mL. After the duration of

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treatment of the extract and alloxan, levels were retained for groups I and II. Group I was the normal control group so likely, no change was observed. On the other hand, group II which received 500 mg/kg BCE did not generate any rise or fall in insulin levels. Conversely, insulin in group III and group IV increased, with values of 0.290 and 0.360, respectively. Due to this, the researchers believed that this was a compensatory response of the pancreas to increased glucose levels brought about by the extract, which signifies its protective activity. This conclusion was supported by the study of Judy et al. (2005) which stated that *L. speciosa* leaf may also have some chemical components that exert regenerative effect on β cells, stimulate these cells to produce more insulin or it may have some insulin-like substances thatalong with other factors may cause hypoglycemic activity.

Table 6
Significance of BCE on its protective activity

	p-value	Interpretation
Group I (Normal control)	-	Not significant
Group II (500 mg/kg BCE)	-	Not significant
Group III (1000 mg/kg BCE)	0.017	Significant
Group IV (2000 mg/kg BCE)	0.004	Significant

*Significant at p-value < 0.05; Tukey Method

Significance was also evaluated individually as illustrated in Table 6. The p-values for Group I and Group II were not calculated since no variation occurred between pre-test and post-test results; thus, interpreted as not significant by statistical analysis, implying no substantial effect withrespect to insulin level.As mentioned earlier, banaba crude extract may exhibit its protective activity in a way that insulin levels may remain unchanged. However, Group III attained a p-value of 0.017. This was inferred as significant and theorized to be due to the leaf extract's protective activity that insulin levels increased as way to compensate for the increase in glucose. Likewise, Group IV which received the highest dose, 2000 mg/kgyielded a p-value of 0.004 and was therefore, significant.This dose was believed to exert the maximum protective activity. Such idea agrees with the study of Leng, Lu & Xu (2005) which stated that the protective effect of banaba may be mediated through insulin secretion from the remnant *β*-cells and increase insulin sensitivity.

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Table 7 shows multiple comparison on the protective activity of banaba crude extract on pancreas by means of measuring insulin secreted by the betacells as to their post-test values presented in Table 5.The value of insulin level in Group Iwas compared to the insulin levels of Groups II, III, and IV having p-values of 0.964, 0.042, and 0.038, respectively. Among these, only when group I was compared with Group III and Group IV that significance was noted, and vice versa. This meant that the results obtained were truly significant when the groups with treatment starting from the dose 1000 mg/kg have produced significant changes when compared with the normal group. On the other hand, when Group III was compared with Group IV and vice versa,significance was perceived with p-value of 0.038. This means that a greater protective activity could have been dependent on the dose administered, since Group IV had been treated with a higher dose, 2000 mg/kg. This finding concurs with the recommendations in the study of Thuppia et al., (2009) which asserted that higher dose of the extract, which was greater than 1000 mg/kg must be administered to achieve a better outcome.

Table 7
Multiple comparison of insulin levels as to the protective effect of BCE

		p-value	Interpretation
Group I (Normal group)	Group II	0.964	Not significant
	Group III	0.042	Significant
	Group IV	0.038	Significant
Group II (500 mg/kg BCE)	Group I	0.964	Not significant
	Group III	0.964	Not significant
	Group IV	0.099	Not significant
Group III (1000 mg/kg BCE)	Group I	0.042	Significant
	Group II	0.964	Not significant
	Group IV	0.038	Significant
Group IV (2000 mg/kg BCE)	Group I	0.038	Significant
	Group II	0.099	Not significant
	Group III	0.038	Significant

Therefore, it can be noted that BCE exhibited protective effect

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with values of 0.200 uIU/ml that retained and even an increase in baseline levels, reaching a level of 0.360 uIU/ml. However, the optimum dose that can be considered protective is 1000 mg/kg. The results correlate with the study done by Adewole, Caxton-Martins & Ojewole (2007) wherein their findings demonstrated that corosolic acid has a powerful hepatoprotective effect in rats.

Histopathological examination

For a better understanding on the extract's protective effect, histopathological examination was performed. Pancreatic tissue of normal control (Figure 2A) showed normal distribution of islet of Langerhans and within the exocrine part, islets were regular with well-defined boundaries.

The section consists of numerous serous acini of the exocrine pancreas and islets of Langerhans that are within normal limits. Since this group did not undergo any treatment, there was no significant finding in the final pathological diagnosis.

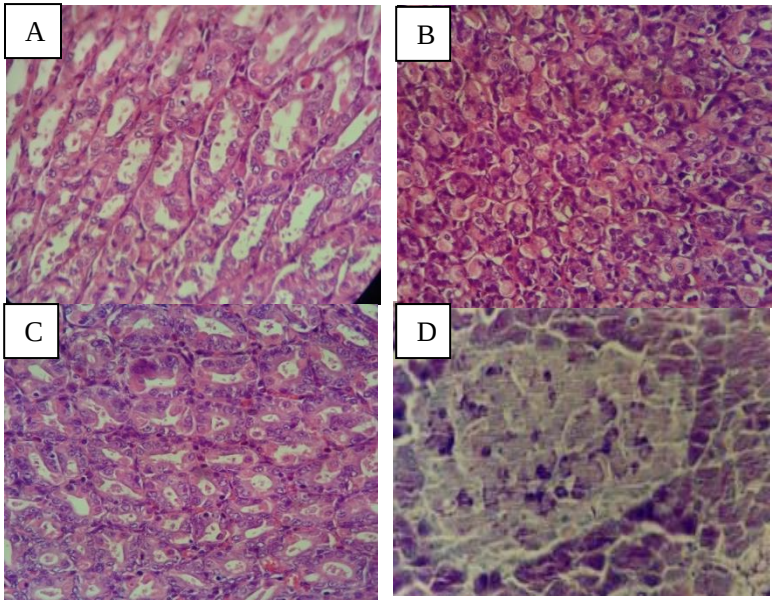

Figure 3.Histopathological view of pancreatic sections from different groups stained with hematoxylin andeosin. A. Normal group. B. 500 mg/kg BCE.C. 1000 mg/kg BCE.D. 2000 mg/kg BCE.

Group II which received 500 mg/kg of banaba crude leaf

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extract illustrated by Figure 2Brevealed small bits and pieces upon microscopic examination which were not enough for a meaningful assessment. Intended parts to be studied might have been damaged upon removal from rats. Otherwise, no major diagnoses were found significant.

The group which received the dose of 1000 mg/kg of banaba crude leaf extract was shown in Figure 2Cdemonstrating moderate atrophied islets of Langerhans. Although there were some degenerative changes seen in the cells lining the duct under the microscope, still a lagre number of cells remain intact. These findings correlate with the study of Aleeva (2004) showing that corosolic acid produced a reliable increase in the population of insulin-producing cells.

Figure 2D, receiving the highest dose (2000 mg/kg) of banaba crude leaf extract showed near normal structure of pancreatic islet cells. It revealed section consisting of islets of Langerhans that are within normal limits. There were only mild degenerative changes in the cells lining the ducts and few lymphoid aggregates around blood vessels. However, the final pathologic diagnosis resulted to mild pancreatitis. This only showed that this dose delivered the maximum effect among the three groups with treatment but accompanying this was a negative effect which was mild pancreatitis. The protective effects of corosolic acid in experimental diabetes are mediated by decreasing oxidative stress, enhancing pro-liferation and cell survival and resulting in the preservation of pancreatic β-cell integrity(Amin et al., 2011).According to Leng et al., (2005), hypoglycemic effect of naturalproduct extracts is generally dependent upon the degree of β-celldestruction. Since the histopathological result of Group IV was nearly analogous to that of Group I,it was concludedthat Group IV produced the greatest positive effect. However, since upon the result of histopathological analysis, banaba crude leaf extract with the dose of 1000 mg/kg also yielded conclusive effects; thus, can be inferred as the optimum dose.

CONCLUSIONS

In conclusion, the study showed that *Lagerstroemia speciosa* (L.) Pers. ethanolic crudeleaf extract can lower glucose levels at 2000 mg/kg and exhibit a protective effectto the pancreas by retaining insulin level at 1000 mg/kg. Correspondingly, in some results, it can also hasten the production of insulin in response to increased amounts of glucose. The protective activity of the extract was supported by the results of histopathological examination.

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RECOMMENDATIONS

Based on the findings, the researchers highly recommend to test the effect of *L. speciosa* (banaba) crude leaf extract by giving doses with minimal interval to extensively monitor the effects on both glucose and insulin levels. If possible, pure corosolic acid should be obtained and tested to further specify its effect. Moreover, further investigation should evaluate the pancreatic B-cell mass as well as longer period of administration to better elucidate the mechanism for protecting the pancreas and assess the toxicity of the extract.

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REFERENCES

Abeeleh, M., Ismail, Z., Alzaben, K., Abu- Halaweh, S., Al-Essa, M., Abuabeeleh, J.& Alsmady, M. (2009). Induction of diabetes mellitus in rats using intraperitoneal streptozotocin: a comparison between 2 strains of rats. *European Journal of Scientific Research*,32(3), 398-402.

Abdul-Hamid, M.& Moustafa, N. (2013). Protective effect of curcumin on histopathology and ultrastructure of pancreas in the alloxan treated rats for induction of diabetes. *The Journal of Basic & Applied Zoology*. doi:10.1016/j.jobaz.2013.07.003

Adewole, S.O., Caxton-Martins, E.A.& Ojewole, J.A.O. (2007). Protective of Quercetin on the morphology of pancreatic β-cells of streptozotocin-treated diabetic rats. *African Journal of Traditional, Medicines, 4(1)*, 64-74.

Aleeva, G. N. (2004). Comparative assessment of cell mechanisms of the antidiabetic action of a new organic derivative (May–June (3),

ISSN: 2094-5906

42–44.

Amin, A., Lotfy, M., Mahmoud-Ghoneim, D., Adeghate, E., Al-Akhras, M.A., Al- Saadi, M. & Al-Rahmoun, S. (2011) *Banaba Uses, Health Benefits, Side Effects*. (n.d.). Retrieved 2011 from http://www.medicalhealthguide.com

Danaei G., Finucane M.M., Lu Y., Singh G.M., Cowan M.J. & Paciorek C.J. (2011). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet*, 378(9785):31–40.

Deocaris, C.C., Aguinaldo, R.R., dela Ysla, J.L., Asencion, A.S. & Mojica, E. R.E. (2005). Hypoglycemic Activity of Irradiated Banaba (Lagerstroemia speciosa Linn.) leaves. *Journal of Applied Science Research, 1(1)*, 95-98.

Etuk E.U. (2010). Animals models for studying diabetes mellitus. 1:130-4 Frode, T.S. & Medeiros, Y.S. (2008) Animal models to test drugs with potential antidiabetic activity. *Journal of Ethnopharmacology*, 115, 173-183.

He, J., Liu, Q., Bai, Z., Huang, R. & Wang, Z. (2010). An optimized method for extracting corosolic acid from loquat leaves. International Diabetes Foundation (IDF) Diabetes Atlas, 5th edition (2011)

Jelodar, G., Mohsen, M., & Shahram, S. (2007). Effect of walnut leaf, coriander and pomegranate on blood glucose and histopathology of pancreas of alloxan induced diabetic rats. *Complementary and Alternative Medicines*, 4 (3): 299 – 305

Judy, W., Hari, S., Stogsdill W., Judy J., Naguib., Y. & Passwater, R. (2005). Antidiabetic activity of a standardized extract (GlucosolTM) from *Lagerstroemia speciosa* leaves in type II diabetics a dose-dependence study. Journal of Ethnopharmacology 87, 115–117

Kakuda, T., Sakane, I., Takihara, T., Ozaki, Y., Takeuchi, H. & Kuroyanagi, M. (2006). Hypoglycemic effect of extracts from *Lagerstroemia speciosa* L. Leaves in genetically diabetic KK-AY mice," *Bioscience, Biotechnology and Biochemistry*, 60(2), 204–208.

Leng, S.H., Lu, F.E. & Xu, L.J. (2005). Therapeutic effects of berberine in impaired glucose tolerance rats and its influence on insulin secretion. *Acta Pharma-cologica Sinica*, 25, 496-502.

Lenzen S. (2008). *Oxidative stress: the vulnerable beta-cell.* Biochem Soc Trans 2008; 36:343-347.

ISSN: 2094-5906

Marchetti P., Bugliani, M., Boggi, U., Masini, M.& Marselli, L. (2008) Pancreatic cells in human type 2 diabetes. *European Journal of Pharmacology*1(3),36-45, doi:10.4236/jdm.2011.13006

Marchetti P., Bugliani M., Boggi U. & Marselli L. (2012). The pancreatic β cells in human type 2 diabetes chapter 22. In S. I. Ahmad (ed.), *Diabetes: an old disease, a new insight* (pp. 288-309).

Miura T., Takagis S. & Ishida T. (2012). Evidence–Based Complementary and Alternative Medicine. 144, 1-8.

Miura, T., Ueda, N.& Yamada, K. (2006). Antidiabetic effects of corosolic acid in KK-Ay diabetic mice. *Biological and Pharmaceutical Board, 29(3)*, 585-587

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. & Simons A. (2009). Agroforestree Database: a tree reference and selection guide version 4.0

Park, C.& Lee, J.K. (2011). Banaba: The natural remedy as antidiabetic drug. *Biomedical Research 2011, 22 (2)*, 125-129.

Rohilla, A. & Ali, S. (2012). Alloxan Induced Diabetes: Mechanisms and Effects. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 3 (2), 819-823

Thuppia,A.,Rabintossaporn,P.,Saenthaweesuk, S., Ingkaninan, K. & Sireeratawong, S. (2009). The hypoglycemic effect of water extract from leaves of Lagerstroemia speciosaL. in streptozotocin-induced diabetic rats.

Vijaykumar, K., Murthy, P.B., Kannababu, S., Syamasundar, B.& Subbaraju, G.V. (2006). Quantitative Determination of Corosolic in *Applied Science and Engineering, 4(2)*, 103-114.

World Health Organization (2013)

Zhou, J., Zhou, S., Tang, J., Zhang, K., Guang, L., Huang, Y., Xu, Y., Ying, Y., Zhang, L. & Li, D. (2009) Protective effect of berberine on beta cells in streptozotocin- and high carbohydrate/ high-fat diet-induced diabetic rats. *European Journal of Pharmacology*,606, 262-268.

ISSN: 2094-5906