Attenuation of Pancreatic Histology, Hematology and Biochemical Parameters in Type 2 Diabetic Rats Treated with *Azadirachta excelsa*

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Abstract—*Azadirachta excelsa* or locally known as sentang are frequently used as a traditional medicine by diabetes patients in Malaysia. However, less attention has been given to their toxicity effect. Thus, the study is an attempt to examine the protective effect of *A. excelsa* on the pancreas and to determine possible toxicity mediated by the extract. Diabetes was induced experimentally in rats by high-fat-diet for 16 weeks followed by intraperitoneal injection of streptozotocin at dosage of 35 mg/kg of body weight. Declination of the fasting blood glucose level was observed after continuous administration of *A. excelsa* for 14 days twice daily. This is due to the refining structure of the pancreas. However, surprisingly, the plant extract reduced the leukocytes, erythrocytes, hemoglobin, MCHC and lymphocytes. In addition, the rat treated with the plant extract exhibited increment in AST and eosinocytes level. Overall, the finding shows that *A. excelsa* possesses antidiabetic activity by improving the structure of pancreatic islet of Langerhans but involved in ameliorating of hematology and biochemical parameters.

Keywords—*Azadirachta excelsa,* diabetes, pancreas, hemato-biochemical parameters.

I. INTRODUCTION

DIABETES mellitus is a chronic progressive metabolic disorder linked by abnormalities in carbohydrate, fat and protein metabolism. It currently affects about 347 million people worldwide [1], out of which 1.2 million have been diagnosed with diabetes in Malaysia [2]. Despite these alarming statistics, there is no specific and definite therapy currently for diabetes. Researchers have revealed many potential plant species that posses good bioactivities to alleviate hyperglycemia problem. Progressive scientific research works have been done on various Malaysian plants; however, there is paucity of reports regarding their toxicity effect.

Azadirachta excelsa is a species under the family of Meliaceae. It is also known by several common names such as marrango tree, the Philippine neem tree, (Jack) Jacobs. and 'sentang'. It is being used traditionally by Malay as an agent for reducing blood glucose level. Our findings have justified the traditional use of *A. excelsa* as antidiabetic agent. *A. excelsa* was found able to improve plasma insulin secretion consequent with reduction in fasting blood glucose and glycated haemoglobin (HbA$_{1c}$) levels in alloxan induced-

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diabetic rats [3]. However, the toxicity of *A. excelsa* in ameliorating hematology and biochemical parameters has yet to be justified by any researches. Hence, the aim for this research is to evaluate whether the potential ability of *Azadirachta excelsa* in treating hyperglycemia without having any toxicity effect or not in type 2 diabetic rat models.

II. METHODOLOGY

A. Plant Extract Preparation

The leaves of *A. excelsa* were dried, grounded, and mixed with the ethanol for at least 48 hours. The extractions were filtered by using a filter paper. The excess ethanol in the extract was eliminated by using the rotary evaporator and crudes were kept in the fridge until it further used.

B. Induction of Diabetes

Diabetes was induced experimentally in rats by high-fat-diet for 16 weeks and will be followed by intraperitoneal injection of streptozotocin (STZ) at dosage of 35 mg/kg of the body weight. The control group was injected with the same volume of normal saline. The rats with the blood glucose concentration ≥ 11 mmol/L were considered as diabetic.

C. Experimental Design

The rats were divided into four groups with a number of four rats in each group. Treatment then had been given twice a day as mentioned in Table I by force feeding for 14 days.

TABLE I
EXPERIMENTAL DESIGN

Groups	Treatment
1	Normal rats (Negative control)
2	Untreated diabetic rats
3	Diabetic rats had been treated with metformin (Positive control)
4	Diabetic rats had been treated with *Azadirachta excelsa*

D. Histological Study

The pancreas was harvested from the sacrificed rats after dissection, and were weighed and washed with saline. The specimens were stretched on filter paper and fixed in 10% buffered formalin (pH 7.4). The fixed specimens were sliced, processed, and embedded into paraffin blocks. The blocks were cut into 4 μm paraffin sections by a rotator microtome. The sections were stained with Hematoxylin and Eosin (H&E).

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E. Clinical Pathology Evaluation

Evaluations of whole blood were determined using an electronic cell counter (Baker 9000 Automated Cell Counter, Biochem Immunosystems, Inc., Allentown, PA). Differential leukocyte counts were done by visually classifying 100 cells on a thin blood film stained with a modified Wright's stain (Hema-Tek Slide Stainer, Miles Laboratories, Elkhart, IN; with Wright-Giemsa Hematology Pack, Volu-Sol, Salt Lake City, UT) under appropriate microscopic magnification. Levels of aspartate aminotransferase (AST), alanine transaminase (ALT) and total bilirubin in serum were determined via A Coulter Dacos Chemistry Analyzer (Coulter, Miami, FL)

F. Statistical Analysis

All the data were presented as mean ± SEM. The significance of the differences in the values had been performed by one-way analysis of variance (ANOVA) test and followed by Tukey post hoc test. The group means will be considered to be significantly different at the level of $p < 0.05$.

III. RESULTS

A. Fasting Blood Glucose

As seen in Fig. 1, both diabetic groups that received the metformin and *A. excelsa* respectively for 14 days possessed a significant reduction ($p < 0.05$) in fasting blood glucose level as compared to control group. Metformin has anti-hyperglycemic properties [4] to reduce the fasting blood glucose. Nevertheless, *A. excelsa* extract was found to be more effective in lowering blood glucose level compared to metformin which was most likely due to its high antioxidant actions [5].
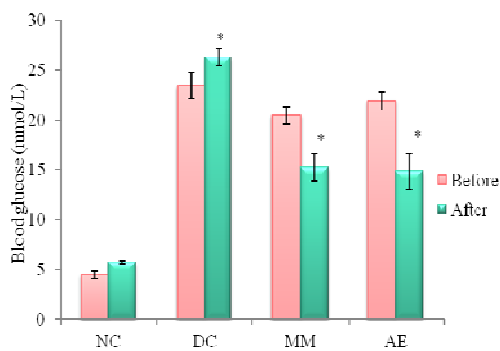
Fig. 1 Mean fasting blood glucose (mmol/L) in experimental groups of rats before and after treatment. The results are expressed as means ± SEM, n = 4. p values are shown as * p < 0.05 denoted the presence of statistically significant difference

B. Histological Appearance of the Pancreas

The normal rats (Fig. 2 (a)) showed normal architecture with normal acini and population of the islets of Langerhans similar to the study done by [6]. However, degenerated and necrotic cells were observed in the pancreatic islets of untreated diabetic rats (Fig. 2 (b)) which might be due to injection of streptozotocin. The streptozotocin causes the progressions of diabetes as a result of permanent pancreatic

cell destruction leading to degranulation and reduced insulin secretion [7]. In addition, the streptozotocin also causes the destruction of β-cells by necrosis [8].
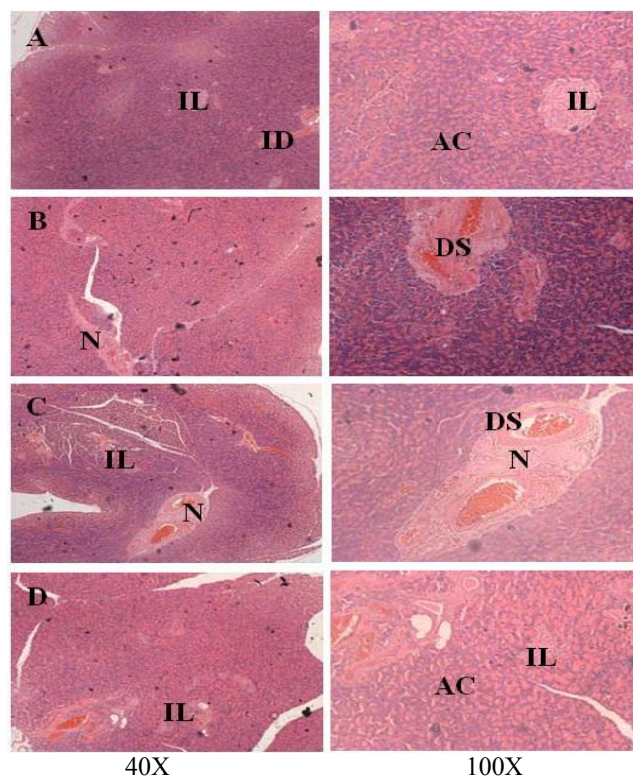
Fig. 2 Pancreas histology (a) Normal control, (b) Diabetic control, (c) Diabetic treated with metformin, (d) Diabetic treated with *A.excelsa*, stained with H&E (40X and 100X)

Chronic hyperglycemia was reported to be responsible in the destruction of pancreatic islets consequence with pancreatic failure [9]. Worryingly, administration of metformin (Fig. 2 (c)) showed a major alteration in islets of Langerhans, increased the atrophy of islet and cellular necrosis. However, interestingly, administration of *A. excelsa* (Fig. 2 (d)) among diabetic rats was able to against the damaged of pancreatic β- cells caused by the injection of STZ. Most probably it might be due to the presence of flavonoids and other phenolic compounds in the extract.

C. Clinical Pathology Evaluation

Ingestion of medicinal plant or drugs frequently associated with attenuation of haematological values [10]. Therefore, haematological it is becoming an important tool in the assessment of deleterious effect of drugs, as well as medicinal plant [11]. Administration of *A. excelsa* for 14 days at dosage of 500 mg/kg bw significantly reduced the RBC number, Hb and lymphocytes ($p < 0.05$) at the end of experiment period in relative to normal and diabetic control group. It also depicts a significant reduction in leukocytes count as compared to normal rats while eosinocytes rose significantly as compared to both normal and diabetic untreated rats. It is predicted that the continuous exposure of the body systems of animals to *A.*

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excelsa may cause neutropenia.

Similarly, the RBC count went down significantly after the injection of STZ. This finding agreed with the previous work done by [12] which reported that erythrocytes are more susceptible to lipid peroxidation among diabetic animal models.

In this study, metformin was revealed to have a better impact on hematology and biochemical parameters where it was able to increase the leukocytes and RBC count, Hb, MCHC, neutrophils and eosinocytes.

Serum ALT and AST levels were determined to evaluate the hepatic functions in order to examine the effect of *A. excelsa* supplementation on the regulation of biochemical parameters of the diabetic rats. STZ induced significant elevations in ALT as compared to respective control group. Surprisingly, administration of *A. excelsa* in diabetic rats significantly increased the level of AST almost two times higher than normal rats. Meanwhile, diabetes does not possess any significant changes in ALT level [13]. However, administration of *A .excelsa* in diabetic rats possesses higher level of ALT as compared to others. Based on this finding, it seems to suggest that *A. excelsa* may induce a high rate of liver failure among diabetic rats due to organelle membrane damage. ALT and AST is the enzyme released when injury involving organelles such as mitochondria occur [14].

TABLE II
SUMMARY OF HEMATOLOGY AND BIOCHEMISTRY VALUES

Parameters	Group 1	Group 2	Group 3	Group 4
Leukocytes (x10^9/L)	8.08±0.0323	6.70±0.1367	12.20±2.0839[*b]	2.6025±0.1126[*a]
Red blood cell (x10^{12}/L)	7.43±0.1750	8.06±0.01931[*a]	8.41±0.1382[*a]	5.33±0.1162[*ab]
Hemoglobin (g/L)	121.50±1.7559	146.50±13.1053	155.25±6.2899[*a]	86.83±0.2496[*ab]
Mean corpuscular volume (fL)	52.00±0.9129	51.75±0.8539	54.75±1.1087	51.75±0.8539
Mean corpuscular hemoglobin concentration (g/L)	315.25±1.93	328.50±0.6455	330.00±1.2910[*a]	332.25±1.1087[*a]
Neutrophils (x10^9/L)	2.59±0.2410	1.42±0.0132	5.14±1.2904[*b]	0.28±0.0091
Lymphocytes (x10^9/L)	4.62±0.1841	4.71±0.0836	5.39±0.5872	2.04±0.0129[*ab]
Monocytes (x10^9/L)	0.61±0.0609	0.58±0.0317	0.89±0.1984	0.32±0.0556
Eosinocytes (x10^9/L)	0.07±0.0048	0.07±0.0041	0.20±0.0104[*ab]	0.13±0.0111[*ab]
Aspartate aminotransferase (U/L)	70.03±0.2.337	244.57±0.3115[*a]	121.10±0.4874	395.97±0.9822[*ab]
Alanine transaminase (U/L)	154.43±0.5243	137.27±0.1287	187.57±2.1165	198.63±2.6874
Total bilirubin (umol/L)	0.7±0.0067	0.5±0.2541	1.2±0.5623	0.6±0.3549

IV. CONCLUSION

This investigation clearly showed that *A. excelsa* has favorable effects to lowering blood glucose effect probably by inhibiting the histopathological changes of the pancreas in type II diabetes. However, this study has recommended that *A. excelsa* leaves extract was not suitable for managing diabetes now by virtue of some liver functions may be compromised at long term administration of the extract. The degree of hepatic dysfunction was measured by using biochemical parameters like serum transaminases (ALT and AST). Thus, further study should be carried out to evaluate the time-dependent impact of *A. excelsa* on type I and type II diabetes management.

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