

Antidiabetic Activity of *Azadirachta excelsa* Extract on Alloxan Induced Diabetic Rats

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Abstract: Plant-based remedies have been used to treat numerous diseases for thousands of years in various parts of the world. *Azadirachta excelsa* or sentang traditionally used by Malay as an agent for lowering blood glucose. The study was designed to evaluate the antidiabetic properties of this plant by measuring the plasma blood glucose level, the percentage of HbA1c and the plasma insulin level. Thirty two *Sprague dawley* rats were divided into four groups which received different treatments. The first and second groups served as positive and negative control were treated with saline respectively while the rats in the third group were received metformin. The fourth group was treated with ethanolic extract of *A. excelsa*. After 14 days of treatment, the plant extract was significantly reduced the fasting blood glucose and the concentration of HbA1c in diabetic rats by 72.89% and 6.58% respectively. The plasma insulin in diabetic rats was also significantly increased after treated with plant extract. These results suggest that the ethanolic extract of *A. excelsa* possess antidiabetic activity by improving the insulin secretion, with consequent decrease in the level of plasma blood glucose and HbA1c.

Keywords: *Azadirachta excelsa*, blood glucose, HbA1c, insulin.

INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder that has affected substantial population regardless of sex, age and socio economic status [1]. According to World Health Organization, the number of diabetes cases in Southern Asia and Western Pacific currently at 171 million and it was expected to reach 336 million by the end of 2030 [2]. Specific diabetic risk factor such as glycation of protein including haemoglobin and coagulation abnormalities, and subsequent endothelial dysfunction, may also contribute to cardiovascular complications. Glycosylated peptides are elevated several folds in diabetics. The use of the glycosylated hemoglobin (HbA1c) assay for long-term diabetic monitoring of diabetic control is gaining much wider use and acceptance [3].

There were many reviews on plants remedies that possess experimental and clinical antidiabetic activity [4]. In Malaysia, medicinal plants are used according to the folk traditions of natives or people who came from India, China or Arab countries. Such plants are used in formulations of home remedies such as teas, decoctions or tinctures. *Azadirachta excelsa*, a typical wild plant found in Malaysia. *Azadirachta* is a genus from two species of trees in the flowering plant of family Meliaceae. To our best knowledge, the antidiabetic activity of this plant has not been investigated scientifically. However, previous work was reported that the chloroform extracts of *A. excelsa* stem demonstrated anticancer activity [5]. Young shoots can be

eaten in salad and are believed to be good for lowering blood pressure.

MATERIALS AND METHODS

Preparation of *Azadirachta excelsa* Leaves Extract

The powdered leaves of *A. excelsa* were soaked in 70% ethanol for two days at room temperature (37°C). The suspension was then filtered to obtain the extract. The extract was then evaporated using the rotary evaporator at 40°C until it was completely dried [6]. A dark semi-solid material was obtained and stored at 4°C to be used later on.

Induction of Diabetes

Rats weighing between 160 to 300g were fasted overnight and injected by intraperitoneal administration of freshly prepared alloxan monohydrate (130 mg/kg) in saline solution of pH 7.4. On day seventh, the rats showing stable glycemic values above 130 mg/dl or 7.0 mmol/L were considered diabetic and selected for further experimentation. Blood glucose levels of the rats were estimated by collecting the blood samples from the tail vein by using Glucometer Accu-check Active.

Experimental Design

The rats were divided into four groups with six rats in each group and treated orally twice a day as follows:

Group A: normal rats received saline.

Group B: diabetic rats received saline.

Group C: diabetic rats received metformin.

Group D: diabetic rats received ethanolic extract of *A. excelsa* at dose of 500 mg/kg.

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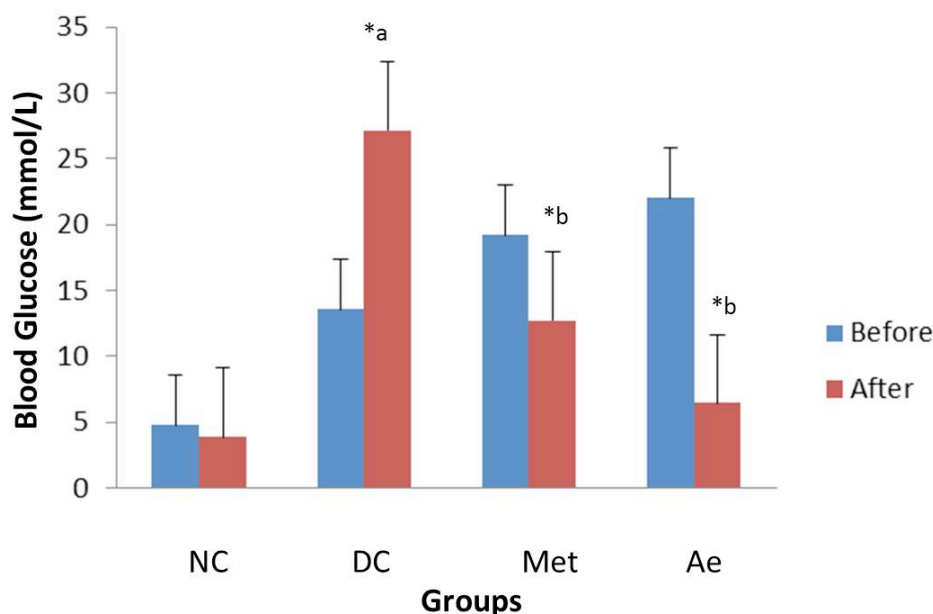


Fig. (1). Plasma blood glucose in experimental rats before and after treatment. Data are expressed as means \pm SEM ($n = 6$). *a- $P < 0.01$ compared with the corresponding value for normal control rats; *b- $P < 0.001$ compared with the corresponding value for diabetic control rats. Ae (*A. excelsa* ethanolic extracts treatment), Met (Metformin treatment), DC (Diabetic control), NC (Normal control).

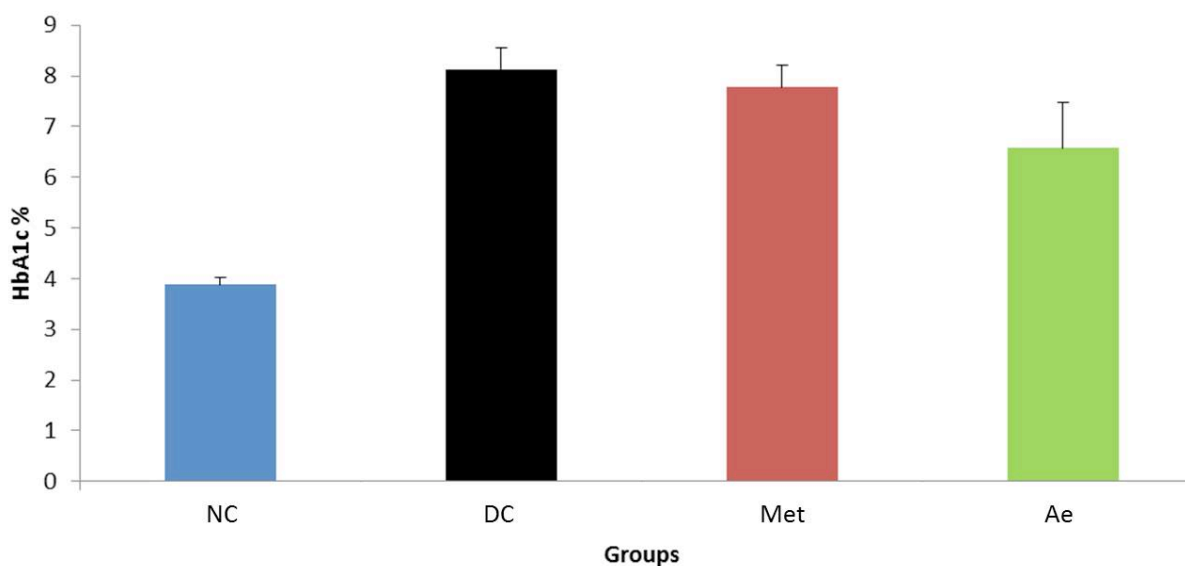


Fig. (2). The percentage of HbA_{1c} within 14 days of treatment. Data were expressed as means \pm SEM; $n=6$; Ae (*A. excelsa* ethanolic extracts treatment), Met (Metformin treatment), DC (Diabetic control), NC (Normal control).

On day 15 of treatment, after 8 hours of fast, all animals were sacrificed by decapitation. Blood were withdrawn for blood glucose, HbA_{1c} and insulin analysis while the uterine were harvested for histological study.

Glycated Haemoglobin Assay

A 1.5 μ L of blood sample were collected with the integrated sampling device by touching the surface of the blood drop until it filled the capillary end. Later, the sampling device was inserted into the Affinon Analyser system. The sample was automatically diluted and mixed with a buffer that lyses the erythrocytes and precipitates the haemoglobin. The analyser evaluated the precipitation on the membrane. The ratio between the glycated haemoglobin and the total haemoglobin intensities was proportional to the percentage HbA_{1c} displayed on the Analyzer screen.

RESULTS

The Plasma Blood Glucose Level

The effects of *A. excelsa* on blood glucose levels in diabetic rats are given in Fig. (1). The plant extract has produced a significant anti-hyperglycemic activity with 60.7% reduction in the blood glucose. Treatment of the diabetic rats with metformin (50 mg/kg b.w) has resulted in 31.3% reduction in their blood glucose.

The Percentage of HbA_{1c}

As shown in Fig. (2), the highest percentage of total HbA_{1c} was found in untreated diabetic rat which is 8.13%. However, administration of metformin and ethanolic extracts of *A. excelsa* for two weeks have successfully reduced the

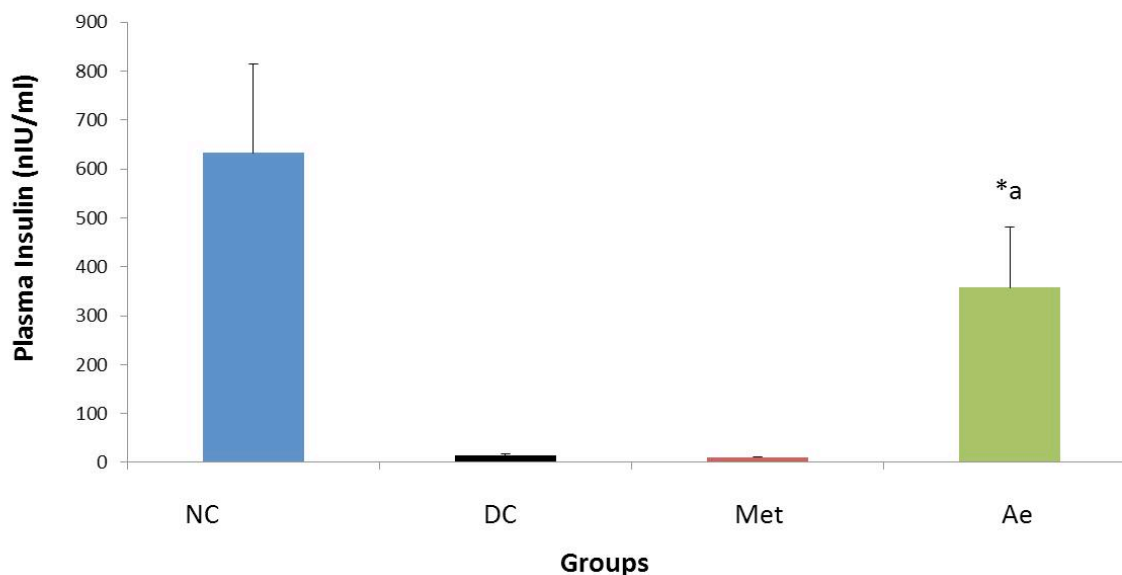


Fig. (3). The concentration of plasma insulin of each group after 14 days of treatment. Data were expressed as means \pm SEM; $n=3$; * statistical significant versus diabetic control ($p<0.05$); Ae (*A. excelsa* ethanolic extracts treatment), Met (Metformin treatment), DC (Diabetic control), NC (Normal control).

percentage of HbA1c which is 7.78% and 6.58% respectively.

Plasma insulin

As showed in Fig. (3), the secretion of insulin was suppressed effectively by a single intraperitoneal injection of alloxan monohydrate. Administration of *A. excelsa* leaves extract was significantly increases the plasma insulin level in the diabetic rats. The finding seems to suggest that the plant extracts work as antidiabetic agents via insulinogenic activity. *A. excelsa* might stimulate an immediate insulin secretion from the remnant β -cells or regenerated β -cells; as observed in other plant extracts [7]. However, administration of metformin during experimental period obviously failed to modify the insulin level of diabetic rats.

DISCUSSION AND CONCLUSION

The use of plants for the treatment of patients with diabetes is common throughout the world. In the present study, alloxan-induced diabetic rats exhibit a significant decrease in plasma glucose level when treated with *A. excelsa* leaves extract. Glucose lowering effect of *A. excelsa* might be due to stimulation of surviving β -cells of islets of Langerhans leading to more insulin release. This was confirmed by the increased levels of plasma insulin in diabetic rats treated with *A. excelsa*. The plant was caused a large increase of plasma insulin in alloxan-treated animals but had no effect on insulin in control rats.

Determining the glycosylated haemoglobin (HbA1c) serves as an appropriate method for diabetic screening. Greater long term variability in HbA1c increases the risk of diabetic microangiopathies and macroangiopathies [8]. The risk of cardiovascular disease has been shown to be elevated in patients with types 2 diabetes mellitus with increase fluctuations in HbA1c [9]. Previous work was reported the increment of glycosylation proteins, including Hb found in uncontrolled or poorly controlled diabetes [10]. Aligned with other work [11], the high levels of Hb1Ac was observed in

alloxan-induced diabetic rats together with corresponding increased plasma glucose values. In our study, HbA1c increased in diabetic animals compared to normal rats. *A. excelsa* and metformin treatments reduced the HbA1c values to near normal levels due to improved glycemic control.

These findings indicated that *A. excelsa* acts primarily as antidiabetic agent by stimulating the secretion of insulin, consequent with reduction of plasma blood glucose and HbA1c values.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGMENTS

Declared none.

REFERENCES

- [1] Kannur DM, Hukkeri VI, Akki KS. Antidiabetic activity of *Caesalpinia bonducella* seed extracts in rats. *Fitoterapia* 2006; 77(8): 546-9.
- [2] Sunil C, Ignacimuthu S, Agastian P. Antidiabetic effect of *Symplocos cochinchinensis* (Lour.) S. Moore. in type 2 diabetic rats. *Journal of Ethnopharmacology* 2011; 134(2): 298-304.
- [3] Bhandari U, Jain N, Ansari MN, Pillai KK. Beneficial effect of *Embelia ribes* ethanolic extract on blood pressure and glycosylated hemoglobin in streptozotocin-induced diabetes in rats. *Fitoterapia* 2008; 79(5): 351.
- [4] Shokeen P, Anand P, Murali YK, Tandon V. Antidiabetic activity of 50% ethanolic extract of *Ricinus communis* and its purified fractions. *Food Chem Toxicol* 2008; 46(11): 3458-66.
- [5] Cui B, Chai H, Constant HL, et al. Limonoids from *Azadirachta excelsa*. *Phytochemistry* 1998; 47(7): 1283-87.
- [6] Mustafa MA, Al-Khazraji A. Effect of some plant extracts on the *Culex pipiens molestus* Forskal larvae. *Iraqi J Veterinary Sci* 2008; 22 (1): 9- 12.
- [7] Eliza J, Daisy P, Ignacimuthu S, Duraipandiyan V. Antidiabetic and antilipidemic effect of eremanthin from *Costus speciosus* (Koen.)Sm., in STZ-induced diabetic rats. *Chemico-Biol Interact* 2009; 1(182): 67- 72.

- [8] Koga M, Murai J, Morita S, Saito H, Kasayama S. Comparison of annual variability in HbA1c and glycated albumin in patients with type 1 vs. type 2 diabetes mellitus. *J Diab Complicat* 2013; 2: 122-5
- [9] Bouchi R, Babazono T, Mugishima, M, Yoshida, N, Nyumura, I, Toya, K. Fluctuations in HbA1c are associated with a higher incidence of cardiovascular disease in Japanese patients with type 2 diabetes. *J Diab Investig* 2012; 3: 148-55.
- [10] Carlsson J, Gullstrand C, Ludvigsson J, Lundström I, Winqvist F. Detection of global glycosylation changes of serum proteins in type 1 diabetes using a lectin panel and multivariate data analysis. *Talanta* 2008; 76 (2): 333-7.
- [11] Adeneye AA, Adeyemi OO. Further evaluation of antihyperglycaemic activity of *Hunteria umbellata* (K. Schum) Hallier f. seed extract in experimental diabetes. *J Ethnopharmacol* 2009; 126 (2): 238-43.

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