Research Article

# Antioxidant Potential of Ethanolic Extract of *Azadirachta excelsa* in Streptozotocin Induced Diabetic Rats

**Nur Syimal'ain A.*, Nooraain H., Nurdiana S., Noor Syaffinaz N.M.Z**
School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, Selangor, 40450. Shah Alam, Malaysia.
***Corresponding author's E-mail:** syimalainazmi@gmail.com

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**ABSTRACT**

Free radicals and lipid peroxide, easily formed in the diabetic state, play an important part in the development of diabetic complications. Plant flavonoid has emerged as the popular therapeutic nature drugs effective against a broad range of diseases including diabetes. Antidiabetic properties of extracted *A. excelsa* have been proven in animal models. The present study designed to investigate the antioxidant activity of ethanolic extract of *A. excelsa*. The ethanolic extract of *A. excelsa* was administered orally (250 mg/kg, for 56 days) to streptozotocin-induced diabetic rats (STZ, 60 mg/kg b.wt). Antioxidant profile in diabetic rats treated with *A. excels* extract were assessed and compared to control (normal and diabetic rats). *A. excels* has higher potential to increase pancreatic antioxidant enzymes. Moreover, *A. excelsa* was also found to improve pancreatic cells replenishment through increase in pancreas weight as compared to diabetic control. Therefore, ethanolic extract of *A. excelsa* depicted a potential to ameliorate pancreas in diabetic rats better than the common drugs metformin. These results emphasize that *A. excelsa* could be a potential agent to attenuate pancreatic oxidative damage and advocate their therapeutic potential for treating DM.

**Keywords:** Diabetes Mellitus (DM); *A. excelsa*, quercetin, streptozotocin (STZ); antioxidant.

## INTRODUCTION

Diabetes mellitus (DM) is a silent pandemic with substantial morbidity and death rate, characterized by the presence of high blood glucose.[1,2] It is now considered an epidemic by considered previously as a disease of the affluent. The world prevalence of DM in 2010 was estimated at 6.4% or 285 million adults and it is expected this number will rise to 439 million adults in 2030.[3] Therefore, currently in 2011 it is involve about 347 million people worldwide, out of which 1.2 million have been diagnosed in Malaysia.[4] DM is a heterogeneous group of metabolic disorders characterized by hyperglycemia with impaired metabolism of carbohydrate, fat, and proteins as a result of defects in insulin secretion, insulin action, or both.[5]

There have been recent reports describing the potential of antioxidants to be developed as new therapies of diabetes. The leaves extract of *Azadirachta excelsa* which mainly contains azadirachtin has been proven to give promising effect as an antioxidant, antimicrobial, antimalarial, antiseptic agent and antidiabetic properties in animal models.[6] A study by Nurul`Izzati *et al.* showed *A. excelsa* leaves extract was also able to improve the liver catalase activity in rat models and help to improve plasma insulin secretion and decreasing the levels of fasting blood glucose and glycated haemoglobin (HbA1c).[7,8] It is expected that *A. excelsa* leaves extract might protect destruction of pancreas caused by diabetes. *A. excelsa* might help in regeneration of islet of Langerhans of pancreas and ameliorate the pancreas structure. Successful investigations, precise insights on the mechanism underlying the protective effect of *A. excelsa* on diabetes may contribute to a finding of the new alternative therapy for diabetic patients.

## MATERIALS AND METHODS

### Experimental Animal and Animal Management

Males Sprague Dawley rats with body weight range from 150 g to 200 g aged three months old were used in these studies. The animals were acclimatized for a week before being used for the experiments. The rats were kept in cages and maintained under standard room temperature between 24 °C to 27 °C.

Inductions of diabetes were conducted through intraperitoneal injection of 0.5 ml Streptozotoxin (STZ) at 60 mg/kg bwt. STZ was freshly prepared in saline (9% sodium chloride) at 4ºC. During the induction period, food and drink were provided *ad libitum*. In order to prevent from the drug-induced hypoglycemic shock, 5% glucose water was given to the rats orally by using oral gavage for two days soon after the injection of STZ.[9] After 1 week of induction, the rats were divided into four groups six rats each (Table 1).

**Table 1:** Treatment groups

| Group | | Treatment | Dosage |
|---|---|---|---|
| I | Normal control (NC) | Saline | - |
| II | Diabetic control (DC) | Saline | - |
| III | Diabetic rats + Metformin (DMET) | Metformin | 1000 mg/kg b. wt |
| IV | Diabetic rat + *A. excelsa* (DAE) | *A. excelsa* extract | 250 mg/kg b. wt |

Metformin and ethanolic extract of *A. excelsa* were dissolved in saline. Those treatments were given once daily for a duration of 8 weeks (56 days). General health and behavior of the animals were monitored during the entire study. At the end of the treatment, rats were fasted overnight and under slight anesthesia using diethyl ether, then the blood samples collected through cardiac puncture into plain sterilized centrifuge tubes. Serum were suppurated and stored at -80ºC until analysis.

**Animals Use and Ethics in Research**

The experimental protocols involving the use of rats as well as internationally accepted practices for the usage and maintenance of laboratory animals as contained in guidelines, reference number: UiTM CARE, 112/2015.

**Preparation of *Azadirachta excelsa* Extract**

The leaves of *A. excelsa* plant (UKMB40314) were obtained from Forest Research Institute of Malaysia (FRIM), Kepong, Kuala Lumpur. The leaves of *A. excelsa* plant were cut, dried and ground to form powder. It was then soaked in 70% ethanol at 1:10 ratio (100 g powder: 1000 ml ethanol) for three days at room temperature (27ºC). The ethanolic extract was collect in a conical flask. The mixture was filtered by using a vacuum pump, Buchner funnel and filter papers. The filtrate obtained was evaporated by using a rotary evaporator at 40°C.[10] A dark semi-solid paste obtained was stored at 4ºC for further use.

**Preparation of Tissue Homogenates**

Tissue samples were homogenized in 5–10 ml/g of homogenizing buffer (50 mM Tris-HCl, 5 mM EDTA, 1 mM DTT pH 7.5) using a Teflon pestle (Glass-Col, USA) at 900 rpm as described previously by Saxena et al. (2015).[11] The homogenates were centrifuged at 9,000 ×g in a refrigerated centrifuge (4°C) for 10 minutes to remove nuclei and debris. The supernatant obtained was used for biochemical assays and FT-IR analysis. Protein concentration was estimated by the method of Lowry (1951), using bovine serum albumin as the standard.[12]

**Estimation of MDA Levels**

The levels of MDA equivalents were determined in tissue samples by TBARS assay kit (Cayman, MI, USA) as describe by Hardwick *et al.* 2010.[13] The absorbance was determined spectrophotometrically at a wavelength of 540 nm using a spectrophotometer.

**Assessment of Antioxidant Enzymatic Activities**

Glutathione peroxidase (GPx) activity was measured using an assay kit (Cayman, MI, USA). The experimental procedures were carried out according to the manufacturer's instructions.[14] The measurement of GPx activity is based on the principle of a coupled reaction with glutathione reductase (GR). The oxidized glutathione (GSSG) formed after reduction of hydro peroxide by GPx is recycled to its reduced state by GR in the presence of NADPH. The oxidation of NADPH is accompanied by a decrease in absorbance at 340 nm. One unit of GPx was defined as the amount of enzyme that catalyzes the oxidation of 1 nmol of NADPH per minute at 25°C.

Superoxide dismutase (SOD) activity was determined using an assay kit (Cayman, MI, USA). This kit utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD was defined as the amount of enzyme needed to produce 50% dismutation of superoxide radical.

**Statistical Analysis**

All data was analyzed with the Statistical Package for the Social Sciences (SPSS) 21.0 software. Data are expressed as means ± standard error of means (SEM) for each group of treatment. Statistical analysis using One way Analysis of Variance (ANOVA) was performed to determine the difference of the means of between the treatments at $p<0.05$.

## RESULTS

**Oxidative Stress Marker and Antioxidant Enzymes**

The oxidant and antioxidant status in pancreas was monitored to support the pancreas-protective effect of *A. excelsa*. Table 2 summarizes the results for the effect of *A. excelsa* on pancreas lipid peroxidation and the activities of antioxidant enzymes. There was a significant decrease in the activity of SOD and GPx of DC rats as compared to NC rats, reflecting the depletion of endogenous antioxidant enzymes activities in serum. However, administration of *A. excelsa* to diabetic rats caused significant increase in the SOD and GPx values relative to DC. Surprisingly, DAE indicate the lowest TBARS level eventually compared with NC group. Unbelievably, DMET remarked the highest TBARS level; consequently with reduce in both SOD and GPx values.

**Pancreas weight**

The pancreas weight and relative pancreas weight were measured in order to evaluate the treatment-related effects. The pancreas weight was significantly decreased in all diabetic groups as compared to NC group. Thus, it was observed that the mean values of pancreas weight in DAE increased approaching NC weight as compared to other diabetic treated rats. Therefore, relative pancreas weight was significantly decreased in DC rats. These data indicate that STZ administration already caused a significant loss in pancreas physical measurement. Nevertheless, treatment of diabetic rats with Metformin, *A. excelsa* and quercetin does not significantly improve pancreas physicals measurement.

## DISCUSSION

STZ used for the induction of diabetes is known to mediate the destruction of β-cells by establishing redox-cycles resulting in the formation of ROS which constitute the major inducer agents for cell damage and hence diabetes in the rats.[15]

**Table 2:** Oxidative stress marker and antioxidant enzymes of various experimental groups.

| Groups | Oxidative stress marker | Antioxidant enzymes | |
|---|---|---|---|
| | TBARS (nmol MDA/mg protein) | GPx (U/mg protein) | SOD (mU/mg protein) |
| NC | 1.33 ± 0.096 | 22.38 ± 0.300$^{d}$ | 10.01 ± 0.390$^{b}$ |
| DC | 2.15 ± 0.147 | 7.95 ± 0.545$^{b}$ | 0.64 ± 0.499$^{a}$ |
| DMET | 2.25 ± 0.128$^{a}$ | 3.24 ± 0.240$^{a}$ | 1.22 ± 0.204$^{a}$ |
| DAE | 0.28 ± 0.064 | 18.30 ± 0.187$^{c}$ | 7.95 ± 0.600$^{ab}$ |

Supercripts $^{a, b, c, d}$ in a column differ significantly at $p<0.05$.

**Table 3:** Mean absolute, relative pancreatic weights of various experimental groups.

| Groups | Pancreas weight(g) | Relative pancreas weight (%) |
|---|---|---|
| NC | 4.10 ± 0.058$^{b}$ | 0.97 ± 0.101 |
| DC | 1.20 ± 0.100$^{a}$ | 0.40 ± 0.125$^{a}$ |
| DMET | 1.63 ± 0.186$^{ab}$ | 0.64 ± 0.014 |
| DAE | 3.27 ± 0.133$^{ab}$ | 1.08 ± 0.107$^{b}$ |

Supercripts$^{a, b}$ in a column differ significantly at $p<0.05$

On the other hand, this study concede that *A. excelsa* helps to prevents oxidative stress in diabetic rats through the increased of pancreatic SOD levels. As it well known, the physiological role of an antioxidant is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals.[16,17] Besides, diabetes is characterized by hyperglycemia and hyperlipidemia, two biochemical feature associated with inhibition of endothelial nitric oxide synthase (eNOS), leading to diminished NO production, increased formation of reactive oxygen species (ROS) and formation of free radicals, lower efficacy of antioxidant systems, that are important agents in the development of diabetic complication.[16,18]In this study, administration of *A. excelsa* under diabetic conditions induced an improvement in hyperglycemia and in β-cells function and these effects are associated with antioxidative properties of the extract. In that sense, *A. excelsa* extract possess potent activities that help to prevent oxidative stress restoring mass of β-cells and its structure by reducing damage of pancreatic islets in diabetic rats.[19,20] The plant can exert its action by increasing the proliferation or the renewal of the islet β-cells following destruction by STZ.

Interestingly, the major components found in the ethanolic extract of *A. excelsa* were polyunsaturated fatty acids (51.95%) while another 37.41% were saturated fatty acids and about 10.63% was made up of phytol.[20] Phytol is an acyclic diterpene alcohol which is a part of chlorophyll and also a precursor of vitamin E and K1.[21] DM is reported to elevate the synthesis of saturated fatty acids in the tissues and reduce the polyunsaturated fatty acid levels as polyunsaturated fatty acids were more prone to the reactive oxygen species (ROS) attack.[22] It has been proven that a dietary consisting of polyunsaturated fatty acids has significantly lowered the prevalence of steatosis in patients with type II diabetesmellitus.[23] As this ethanolic plant extract also contained higher polyunsaturated fatty acid levels, it could be a promising treatment for hyperlipidemia and tissue steatosis in type II diabetes mellitus. Thus, this present study suggests that ethanolic extract of *A. excelsa* possesses a great potential to treat diabetes mellitus based on the identified compounds that might work as a synergy and hence, enable this extract to exert an antidiabetic effect.

## CONCLUSION

In conclusion, this study demonstrated that *A. excelsa* promote pancreas cell replenishment by improving the activities of pancreatic antioxidant enzymes. This raises the possibility of using *A. excelsa* and quercetin as a valuable ancillary treatment that could add a novel layer of protection for the β-cell.

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