NEUROPROTECTION OF *ABELMOSCHUS ESCULENTUS* L. AGAINST DIABETIC NEUROPATHY

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ABSTRACT

Objective: The present study was designed to determine the neuroprotective effect of *Abelmoschus esculentus* L. on alloxan-induced diabetic neuropathy in rats.

Methods: Diabetes was induced in rats with a single intraperitoneal injection of alloxan monohydrate (130 mg/kg b.w.). The ethanol extract of *A. esculentus* L. at a dose of 100 and 200 mg/kg of body weight was administered at single dose per day to alloxan-induced diabetic rats for 21 days. The fasting blood glucose was screened in the intermittent on day 0, day 14, and day 21. Behavioral tests such as thermal hyperalgesia test and rotorod performance test were performed to assess the thermal sensitivity and muscle grip strength. At the end of the study period, experimental animals were sacrificed and sciatic nerve tissues were obtained for histopathological investigation.

Results: Animals treated with *A. esculentus* L. extract at a dose of 200 mg/kg of body weight significantly reduced (p<0.05) in rotarod performance. The sciatic nerve fiber of diabetic rats receiving 200 mg/kg of body weight of *A. esculentus* L. extract also showed no swelling of nerve fibers, and lesser demyelination was observed.

Conclusion: These findings demonstrate that *A. esculentus* L. exhibits significant antidiabetic and neuroprotective effect against alloxan-induced diabetic neuropathy in rats.

Keywords: *Abelmoschus esculentus* L, Neuroprotective, Antidiabetic, Histopathological investigation.

INTRODUCTION

Diabetes mellitus is a condition where body either does not produce enough insulin or response to insulin. According to the International Diabetes Foundation, in 2014, there were 3.3 million cases of diabetes in Malaysia. The prevalence of diabetes in adults between 20 and 79 years was 16.61% in 2015. The cost per person with diabetes was 565.35 USD [1].

Diabetes mellitus is best known as a multifactorial metabolic disorder characterized by chronic hyperglycemia with abnormal carbohydrate, protein, and fat metabolism due to deficiency of insulin or failure of body response to insulin or both [2]. Long-term exposure of organ to hyperglycemia could lead to chronic complications such as microvascular and macrovascular complications. Examples of microvascular diseases of diabetes are diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy while macrovascular disease is atherosclerosis. In short term, acute complications cause clinical manifestation such as polydipsia, polyuria, glycosuria, and polyphagia [3].

Diabetic neuropathy is defined as the presence of signs and symptoms of nerve fiber dysfunction in people with chronic hyperglycemia [4]. Approximately 50% of patients with long-standing type 1 and type 2 diabetes mellitus have developed diabetic neuropathy. In fact, diabetic neuropathy is likely to affect 23 million of 472 million patients with diabetes by 2030 [5]. It may be classified as polyneuropathy, mononeuropathy, and autonomic neuropathy. The risk factors are long duration hyperglycemia, poor control of glucose in blood, smoking, heavy alcohol intake, hypertension, and elevated triglycerides [6].

The diabetic neuropathy is due to the background of hyperglycemia, so investigation on the pathophysiology of diabetic neuropathy is mainly concerned [7]. The pathogenesis of diabetic neuropathy is not fully understood, but a number of theories can be described [8]. One of the important factors associated with diabetes mellitus is oxidative stress [7]. The oxidative stress is due to free radical production responded from activation of polyol pathway, advanced glycation end products, hexosamine, and diacylglycerol/protein kinase C [9,10].

The *Abelmoschus esculentus* L. aka Okra or lady’s finger is a flowering plant in the mallow family [11]. Okra immature fruits can be taken as vegetables or can be used with soup, salads, fresh or dried, fried, or boiled [12]. Okra is a powerhouse of valuable nutrients. Approximately half of it is soluble fibers in the form of gums and pectins while the rest are insoluble fibers, proteins, carbohydrates, minerals, and vitamins. Okra is also best known as antioxidant vegetable and has very good benefit on cardiovascular disease, Type 2 diabetes mellitus, digestive disease, and some cancer [13]. The medicinal values of Okra were revealed and reported to have properties on reducing blood glucose, lowering blood lipid, and neuroprotection [14,15]. This research is undertaken to investigate *in vivo* antioxidant and antidiabetic activity of Okra as well as its neuroprotection effects in alloxan-induced diabetic rats.

METHODS

Plant materials

7 kg of *A. esculentus* L. (Okra) was obtained from Pasar Besar Klang located in Klang which is in the state of Selangor, Malaysia. The plant material was identified by a resident botanist through comparison with specimen *A. esculentus* L. kept at the Forest Research Institute Malaysia.

Experimental animals

Male Sprague Dawley albino rats (150–200 g) were used to assess in this experiment. The animals were kept and maintained under
standard laboratory conditions (temperature [22°C±2°C] and humidity [45°C±5°C]) with 12:12 h day:night cycle. The animals were fed with standard laboratory diet and allowed to drink water. Studies were carried out in accordance with the Institutional Ethical Guidelines for the care of laboratory animals of Management and Science University, Malaysia.

Development of diabetes mellitus model in rats
The rats are fasted overnight. Diabetes is induced by intraperitoneal (i.p.) injection of alloxan monohydrate at a dose of 150 mg/kg body weight in 0.1 M cold citrate buffer (pH 4.5). To prevent alloxan-induced hypoglycemia, 10% dextrose solution is given to rats after 6 h of alloxan administration for next 24 h. Induction of diabetes is verified after 72 h by measuring blood glucose levels with strips using glucometer, and the animals are allowed 14 days for the stabilization of blood glucose level. If the blood glucose in rats has higher than 250 mg/L after day 14, animals are considered diabetic and used in experiment [16].

Preparation of A. esculentus L. extract
The A. esculentus L. is dried in the hot air oven at 60°C. The dried okra is made into fine powders using a blender. The fine powders will be extracted in 95% ethanol solvent using Soxhlet extraction method. Sequential extractions are performed. The extract (AeL extract) is evaporated at 60°C in a rotary evaporator. The remaining extract is dried in room temperature for several days to ensure the removal of any residual solvent [17].

Treatment of AeL extract to Alloxan-induced diabetic rat (positive vs. negative control)
Animals are divided into five groups, and each group consisted of 6 rats. The grouping details are follows:
- Group I - Normal/control animals received 1% tween 80, 3 ml/kg body weight per orally.
- Group II - Alloxan-induced diabetic rats received 1% tween 80, 3 ml/kg body weight per orally.
- Group III - Alloxan-induced diabetic rats received glibenclamide 5 mg/kg.
- Group V - Alloxan-induced diabetic rats received okra extract 100 mg/kg dissolved in 1% Tween-80.
- Group VI - Alloxan-induced diabetic rats received okra extract 200 mg/kg dissolved in 1% Tween-80.

Each group of rats is fed and observed for 30 days. After 30 days, the blood glucose level of each group of rats is determined with strips using glucometer and recorded [18].

Statistical analysis
Significance of differences between the mean values was determined by the analysis of variance (ANOVA), followed by Dunnett’s test. SPSS 24, USA, was used for statistical analysis. Graphs were prepared using GraphPad Prism 7. Results were considered statistically significant when the P < 0.05.

RESULTS
Induction of diabetes
Of total 45 rats that were induced, 10 of them dead due to severe high blood glucose and 5 of them were failed to become diabetes before the treatment was given.

Effects of okra extracts on blood glucose level in alloxan-induced diabetic rats
Thermal hyperalgesia test [Tables 1-3]

Graph 1: Histological and morphological study of sciatic nerve

Table 1: Effect of daily oral administration of extracts on blood glucose level of alloxan-induced diabetic rats

| Treatment groups | Fasting blood glucose (mmol/L) |
|------------------|--------------------------------|
|                  | Day 0 | Day 14 | Day 21 |
| Normal control   | 6.00±0.63 | 5.92±0.41 | 6.28±0.77 |
| Diabetic control | 14.07±1.89 | 12.95±2.25 | 14.75±1.76 |
| Diabetic control with glibenclamide (5 mg/kg) | 15.2±1.46 | 10.91±0.88 | 9.85±0.92* |
| Diabetic control with Okra extract (100 mg/kg) | 12.4±1.36 | 11.23±1.22 | 10.63±0.84 |
| Diabetic control with Okra extract (200 mg/kg) | 13.75±0.87 | 10.75±1.58 | 9.27±0.91* |

Values are given as mean±S.E.M for 6 rats in each group (n=6). *p<0.05=glibenclamide and 200mg/kg compared with diabetic control. S.E.M: Standard error of the mean.
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| Normal control   | 14.09±1.18  |
|------------------|-------------|
| Diabetic control | 8.29±0.83*  |
| Diabetic control with glibenclamide (5 mg/kg) | 12.24±0.70* |
| Diabetic control with okra extract (100 mg/kg) | 9.74±0.42  |
| Diabetic control with okra extract (200 mg/kg) | 12.46±0.71* |

Values are given as means±S.E.M for 6 rats in each group (n=6). *p<0.05=diabetic control compared with normal control. OE 100 mg/kg compared with diabetic control. S.E.M: Standard error of the mean.

Table 3: Effect of Abelmoschus esculentus L. extract on rats subjected to motor coordination test

| Treatment groups       | Reaction time (s) |
|------------------------|-------------------|
| Normal control         | 30.27±2.70        |
| Diabetic control       | 11.95±1.94*       |
| Diabetic control with glibenclamide (5 mg/kg) | 25.05±3.24*      |
| Diabetic control with okra extract (100 mg/kg) | 17.15±1.95       |
| Diabetic control with okra extract (200 mg/kg) | 23.08±1.58*      |

Values are given as means±S.E.M for 6 rats in each group (n=6). *p<0.05=diabetic control compared with normal control, whereas *p<0.05=glibenclamide and OE 100 mg/kg compared with diabetic control. S.E.M: Standard error of the mean.

DISCUSSION

In this study, A. esculentus L. extract was given as neuroprotection of sciatic nerve in alloxan-induced diabetic rats. Alloxan gain popularity because it is the most common complications of diabetes, diabetic neuropathy is characterized by the signs and symptoms of nerve fiber dysfunction in people with chronic hyperglycemia [4]. In this study, we further evaluated the role of A. esculentus L. extract on fasting blood glucose concentration of alloxan-induced diabetic rats.

As the most common complications of diabetes, diabetic neuropathy...
in rat, and our morphological study of sciatic nerve revealed that administration of *A. esculentus* L. extract, especially at dose of 200 mg/kg, shows no swelling in nerve fibers and lesser extent of demyelination to close to the control group. Therefore, dose dependence of 200 mg/kg body weight of *A. esculentus* L. extract was proposed its antidiabetic and neuroprotective effect whereby it exerts a substantial protective effect against alloxan-induced diabetic neuropathy in sciatic nerve of rats.

**CONCLUSION**

The present study has shown that dose dependence of 200mg/kg of the extract of *A. esculentus* L. has attenuated the alloxan-induced diabetic neuropathy in rats, whereas 100 mg/kg did not. These effects may be indirectly attributed to its potential anti-hyperglycemia properties. Which is causing a total 32.6% reduction in fasting blood glucose. These findings provide a therapeutic potential for future treatment of diabetic neuropathy. However, further studies are required to elucidate the mechanism of neuroprotection of *A. esculentus* L. on sciatic nerve. Besides, the treatment group of neuroprotective agent such as Vitamin B complex can be included as the standard treatment.

**REFERENCES**

1. International Diabetes Federation. Diabetes in Malaysia-2014; 2014. Available from: https://www.idf.org/sites/default/files/Atlas-poster-2014_EN.pdf. [Last accessed on 2014 Jun].
2. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003;36 Suppl 2:1183-97.
3. Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiol Rev 2011;93:137-88.
4. Boulton AJ, Malik RA. Diabetic neuropathy. Med Clin North Am 1998;82:909-29.
5. Tesfaye S, Selvarajah D. Advances in the epidemiology, pathogenesis and management of diabetic peripheral neuropathy. Diabetes Metab Res Rev 2012;28:1-14.
6. Kasper D, Fauci A, Hauser S, Longo D, Jameson J, Loscalzo J. Harrison’s Principles of Internal Medicine. 19th ed. New York: McGraw-Hill, Medical Pub. Division; 2015.
7. Negi G, Kumar A, Joshi RP, Ruby PK, Sharma SS. Oxidative stress and diabetic neuropathy: Current status of antioxidants. Inst Integr Omics Appl Biotechnol 2011;2:71-8.
8. Pourmand R. Diabetic neuropathy. Neurol Clin 1997;3(3):569-76.
9. Edwards JL, Vincent AM, Cheng HT, Feldman EL. Diabetic neuropathy: Mechanisms to management. Pharmacol Ther 2008;120:1-34.
10. Yagihashi S, Mizukami H, Sugimoto K. -Mechanism of diabetic neuropathy: Where are we now and where to go? J Diabetes Invest 2011;2:18-32.
11. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal Plants. New Delhi: Council of Industrial and Scientific Research; 1956. p. 1-133.
12. Ndunguru J, Rajabu AC. Effect of okra mosaic virus disease on the above-ground morphological yield components of okra in Tanzania. Sci Horticult 2004;99:225-35.
13. Gemeni HF, Ratta N, Haki GD, Woldegiorgis AZ, Beyene F. Nutritional quality and health benefits of okra (*Abelmoschus esculentus*): A review. Food Sci Q Manage 2015. DOI: 10.4172/2157-7110.1000458.
14. Subrahmanyan GV, Sushma M, Alekya A, Neeraja CH, Harsha HS, Ravindra J. Antidiabetic activity of *Abelmoschus esculentus* fruit extract. Int J Res Pharm Chem 2011;1:17-20.
15. Ngoc TH, Ngo QN, Van AT, Phung NV. Hypolipidemic effect of extracts from *Abelmoschus esculentus* L. (Malvaceae) on tyloxapol-induced hyperlipidemia in mice. Warasan Phesatchasat 2008;35:42-6.
16. Sabitha V, Ramachandran S, Naveen KR, Panneerselvam K. Investigation of *in vivo* antioxidant property of *Abelmoschus esculentus* (L) Oench. fruit seed and peel powders in streptozotocin-induced diabetic rats. J Ayurveda Integr Med 2012;3:188-93.
17. Pendre NK, Nema PK, Sharma HP, Rathore SS, Kushwah SS. Effect of drying temperature and slice size on quality of dried okra (*Abelmoschus esculentus* (L.) Moench). J Food Sci Technol 2012;49:378-81.
18. Raju S, Hemamalini K. *In vivo* animal model for screening of antidiabetic activity. Asian J Pharm Clin Res 2012;5:118-24.
19. Austin PJ, Wu A, Moalem-Taylor G. Chronic constriction of the sciatic nerve and pain hypersensitivity testing in rats. J Vis Exp 2012;61:3393.
20. Deacon RM. Measuring motor coordination in mice. J Vis Exp 2013;75:2609.
21. Rohilla A, Ali S. Alloxan induced diabetes: Mechanisms and effects. Int J Res Pharm Biomed Sci 2012;3:819-23.