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Research Article

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 rotarod 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. extarct at a dose of 200 mg/kg of body weight significantly reduced ($p<0.05$) in hyperglycemia and thermal hyperalgesia and significantly increased ($p<0.05$) in rotarod performance. The sciatic nerve fiber of diabetic rats receiving 200 mg/kg of body weight of *A. esculentus* L. extract also shows 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.

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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 complication 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. Approximate 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].

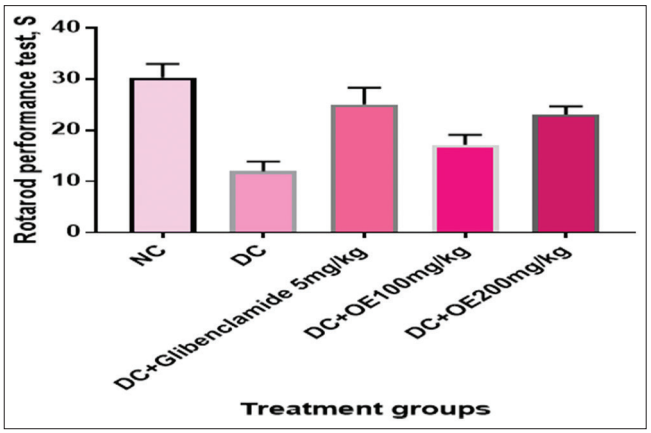
Preparation of A. esculentus L. extract

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.



Graph 1: Histological and morphological study of sciatic nerve

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].

Behavioral investigation

Thermal hyperalgesia test

Neuropathic pain is evaluated using Eddy's hot plate, which is an instrument designed by Eddy *et al*. to assess thermal sensitivity. The plate was preheated and each of the rats is put on the hot plate at fixed temperature of 55°C, and the rat was placed on the hot plate and nociceptive threshold, with respect to licking of the hind paw or jumping, was recorded in seconds. The cutoff time of 20 s was maintained [19].

Rotarod performance test

Rotarod performance test is used to evaluate balance and sensory-motor coordination of the subjects. The speed is accelerated to 40 rpm/min. The fallingtimeofeachratfromrotatingspindlewasrecordedduring5minperiod. Mean change in the locomotor activity was recorded for each group [20].

Histological and morphological study of *sciatic* nerve

The rat is anesthetized using ketamine, i.p. injection, 70 mg/kg. According to Suri *et al*., 2002, and Martins *et al*., 2005, rat is placed in prone position and sciatic nerve is exposed at the dorsocaudal region. An incision is made strating 0.5 cm laterally from the animal's midline and extending laterally for 3 cm toward the tibiofemoral articulation. The femoral biceps and gluteus muscles are separated using blunt dissection to allow access to provide exposure of the sciatic nerve. Then, the nerve is fixed *in situ* for 20 min with 4% glutaraldehyde in 0.1 M of phosphate-buffered saline at pH 7.4. The sciatic nerve is cut from the sciatic notch to the knee level and immersed in 2.5% glutaraldehyde in 0.1 M phosphate buffer solution at pH 7.4 for overnight. Then, approximately 3 mm lengths of sciatic nerve are cut and post-fixed for 2 h in 1% buffered osmium tetroxide and dehydrated in graded concentrations of acetone and embedded in epoxy resin. A thin layer of transverse section of sciatic nerve is cut using blade and stained with 1% toluidine blue and observe under a light microscope. The morphological changes of sciatic nerve between normal control group, diabetes- induced, and treatment rats are observed.

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]

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)	15.27±1.46	10.81±0.88	9.85±0.92*
Diabetic control with Okra extract (100 mg/kg)	12.40±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

Table 2: Effect of *Abelmoschus esculentus* L. extract on rats subjected to paw heat-hyperalgesia test

Treatment groups	Reaction time (s)
	Day 21 (end of treatment)
Normal control	14.09±1.18
Diabetic control	8.29±0.83[a]
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 mean±S.E.M for 6 rats in each group (n=6). [a]p<0.05=diabetic control compared with normal control *p<0.05=glibenclamide and 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 Day 21 (end of treatment)	Reaction time (s)
Normal control	30.27±2.70
Diabetic control	11.95±1.94[a]
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 mean±S.E.M for 6 rats in each group (n=6). [a]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

Rotarod performance test

Fig. 3a shows the normal control group of sciatic nerve. Fig. 3b shows sciatic nerve fiber of diabetic control group. There is obvious edema around the epineurium and leakage and infiltration of neutrophils around blood vessels, and swelling of nerve fibers (red arrow) was observed. Fig. 3c shows the sciatic nerve fiber of diabetic rats receiving glibenclamide (5 mg/kg). There are no obvious edema and infiltration of neutrophils into the nerve fiber bundle peaks, and no fiber swelling and demyelination were observed. Fig. 3d shows sciatic nerve fiber of diabetic rats receiving okra extract (100 mg/kg). Swelling of nerve fibers (red arrow) was observed, and partial demyelination was seen. Fig. 3e shows sciatic nerve fiber of diabetic rats receiving okra extract (200 mg/kg). No swelling of nerve fibers and lesser demyelination were observed.

DISCUSSION

In this study, *A. esculentus* L. extract was given as neuroprotection of sciatic nerve in alloxan-induced diabetic rats. Alloxan gain popularity to induce type-1 diabetes in rodents which resembles to human insulin dependent diabetes mellitus. Alloxan has its property of diabetogenicity by reacting with two-SH groups in the sugar binding site of glucokinase, resulting in the formation of the disulfide bond and inactivation of the enzyme. Glucokinase inhibition reduces glucose oxidation and ATP generation that further suppresses glucose-induced insulin secretion. In addition, alloxan also inhibits the biosynthesis of insulin through the same mechanism [21].

At day 0, a marked rise in fasting blood glucose level was observed in alloxan-treated groups as compared to normal group. At day 14, there are no significant differences between the treatment groups (glibenclamide 5 mg/kg and okra extract 100 mg/kg and 200 mg/kg) compared to diabetic control, whereas, at day 21, treatment with glibenclamide and okra extract 200mg/kg caused significant reduction (p<0.05) in the levels of fasting blood glucose compared with diabetic control. 32.6% reduction in fasting blood glucose from day 0 to day (Fig. 1). Thus, okra extract at dose of 200 mg/kg had demonstrated antiglycemic activity in alloxan-induced diabetic rats. The antiglycemic effect of *A. esculentus* L.

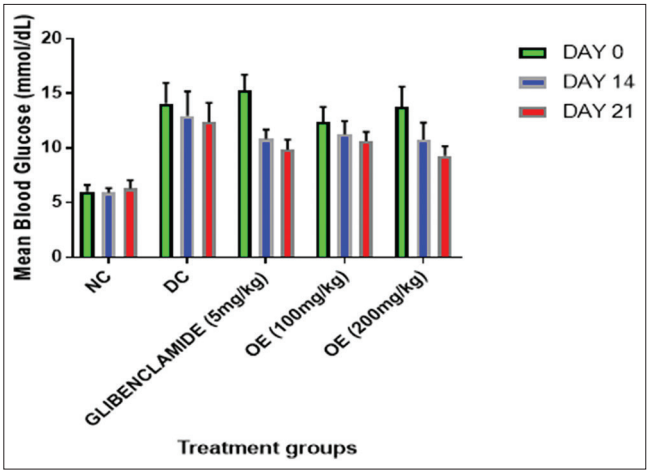


Fig. 1: Effect of *Abelmoschus esculentus* L. extracts on fasting blood glucose concentration of alloxan-induced diabetic rats

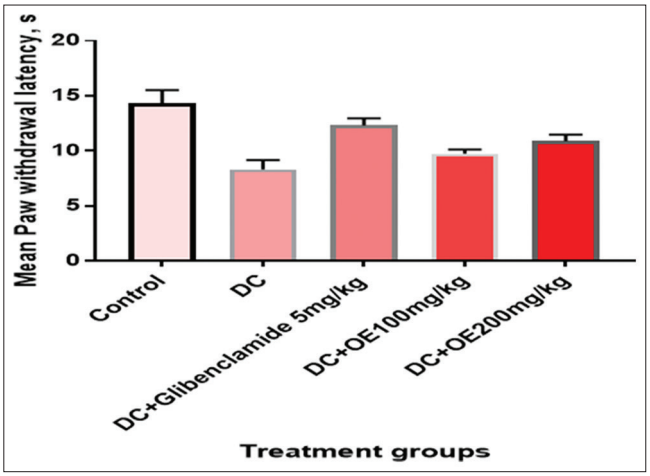


Fig. 2: Effect of *Abelmoschus esculentus* L. extracts on rats subjected to paw heat-hyperalgesia test

may due to isoquercetin and quercetin-3-O-beta-glucopyranosyl-(1"'--> 6")-glucoside isolated from the okra seed extract and was selectivity inhibited rat intestinal maltase and sucrose.

In first behavioral examination, diabetic rats were shown significant reduction in paw withdrawal latency compared with normal control rats, shown in Fig. 2, indicating that the diabetic rats decreased in nociceptive threshold to heat, resulting hyperalgesia. Other than that diabetic rats treated with *A. esculentus* L. extract in 200 mg/kg were shown significant reduction (p<0.05) in paw withdrawal latency compared with diabetic control rats. Thus, 200 mg/kg *A. esculentus* L. extract shows neuroprotection effect on alloxan-induced diabetic rats.

In second behavioral examination, diabetic rats were shown significant reduction in Rotarod performance compared with normal control rats (Graph 1), indicating a decrease in muscle grip strength of legs due to impairment of sciatic nerve. Other than that, diabetic rats treated with *A. esculentus* L. extract in 200 mg/kg were shown significant reduction (p<0.05) in Rotarod performance compared with diabetic control rats. Thus, 200 mg/kg *A. esculentus* L. extract shows neuroprotection effect on alloxan-induced diabetic rats.

As 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. extraction diabetic neuropathy

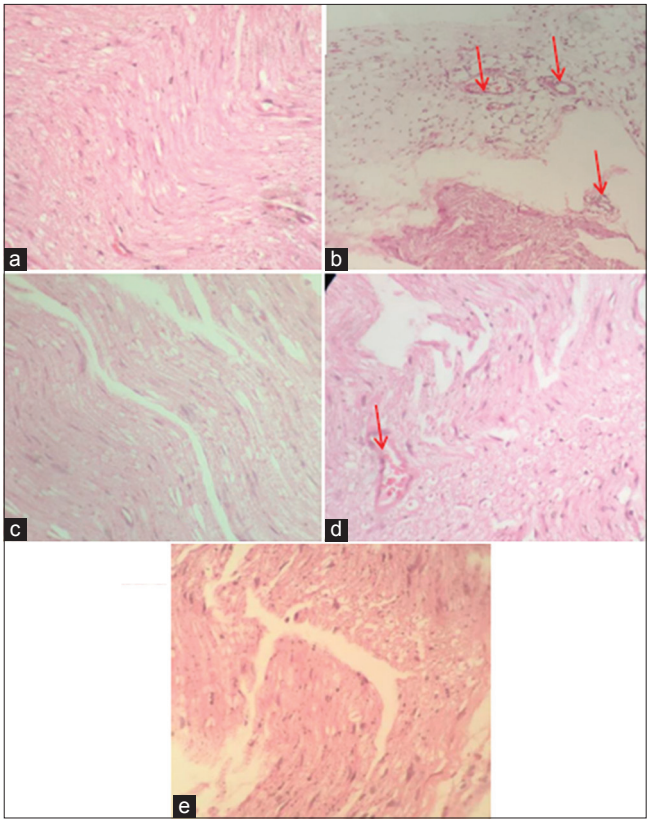


Fig. 3: Effect of *Abelmoschus esculentus* L. extracts on rats subjected Rotarod performance test

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.

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