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THE NEUROPROTECTIVE EFFECT OF BEE VENOM AGAINST DIABETIC NEUROPATHY IN A RAT MODEL: ULTRASTRUCTURE AND MORPHOMETRIC STUDY

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ABSTRACT

Context: Diabetes mellitus is a metabolic disorder characterized by hyperglycemia that accompanied by complications such as polyneuropathy. Bee venom (BV) therapy is an old traditional remedy that has been used to treat various diseases safely. ***Objectives:*** To evaluate the effect of BV on neuropathy of sciatic nerve in streptozotocin (STZ)-induced diabetes in rats using ultrastructure and morphometric methods. ***Materials and Methods:*** Thirty adult male Wister rats were divided randomly into (group I) the negative control group, (group II) the BV control group; rats received intraperitoneal injection (IP) of 0.5 mg/kg BV twice weekly for four consecutive weeks; (group III) the STZ-induced diabetic group and (group IV) was the BV treated group. Blood glucose levels were assessed for all groups twice weekly. At the end of fourth week, animals in all groups were sacrificed and the sciatic nerves of all groups were harvested and processed for semithin sections and ultrastructure study. Total fiber diameter, axon diameter, myelin thickness and the g ratio were measured and were statistically analyzed. ***Results:*** Blood glucose level was decreased to normal in BV treated group. Statistical analysis showed that treatment with BV significantly increased ($P<0.05$) sciatic nerve diameter in normal and diabetic rats and the g ratio was significantly decreased in diabetic rats. The ultrastructural examination revealed that BV reversed the axonal and myelin damage and degenerative changes of axonal mitochondria and Schwann cells that occurred in diabetic rats. ***Conclusion:*** BV therapy could protect against peripheral neuropathy resulting from STZ-induced hyperglycemia.

KEYWORDS: Diabetic neuropathy, Bee venom, axon diameter, g ratio.

INTRODUCTION

Peripheral neuropathy is one of the most common chronic complications which affects approximately 70-90% of people suffering from diabetes mellitus type 1 and type 2.[1] All types of peripheral nerves including sensory, motor and autonomic nerves are affected by diabetes which subsequently cause neuropathic pain, diabetic foot and may result in amputation.[2] The exact mechanism of pathogenesis of diabetic neuropathy is not fully understood till present. The two main hypotheses include microvascular damage that lead to nerve ischemia and metabolic disorders that are caused by hyperglycemic state leading to activation of the polyol pathway and production of large amount of Reactive Oxygen Species (ROS).[3,4] Other probable mechanisms include interactions between neuronal and immune systems and activation of glial cells.[5,6]

Since this serious complication affect the quality of life, its treatment or even prevention of its accompanying symptoms has been considered as a major goal. Thus, several drug categories have been currently investigated in prevention and treatment of diabetic neuropathic pain such as antioxidants like vitamin E[7] and melatonin,[8] antidepressant like duloxetine,[6] analgesics as tapentadol[9] and fatty acid contained diets like Omega 3.[10] However, no definite treatment has been approved for this complication.

Bee venom (BV) therapy has been utilized for treatment of various diseases in traditional medicine.[11] It is formed of a complex mixture of many components including enzymes (phospholipase A_2, hyaluronidase, phosphatase), polypeptides (melittin, apamin, secapin) and low molecular compounds (histamine, dopamine, norepinephrine).[12]

The most active ingredient in BV is melittin that has powerful anti-inflammatory and anti-nociceptive effects, therefore BV was used to treat many inflammatory diseases such as rheumatoid arthritis, osteoarthritis, tendinitis, dermatitis and psoriasis.[11,13,14] Furthermore, apamin and phospholipase A_2 present in BV have strong immunoregulatory effect, thus several reports suggested

that treatment with BV may be helpful in neurodegenerative diseases such as multiple sclerosis, Alzheimer`s and Parkinson`s diseases.[15]

The present study was designed to investigate the possible role of bee venom in reestablishment of sciatic nerve structure in Streptozotocin induced diabetes in a rat model as well as to evaluate its effect on blood glucose level.

MATERIAL AND METHODS

Animals

Thirty male Wistar rats weighing 150-200 gm were used in this study. All procedures for animal care and experiments were done according to ethics committee recommendations of Ain Shams University. They were housed in standard conditions of illumination and ventilation and allowed free access to standard laboratory chow and water. The experiment was performed in the Medical Research Center (MRC), Ain Shams University hospitals.

Experimental design

The rats were divided randomly into four groups: ***group I*** (control group, n=5), animals in this group received a single injection of 0.1 M citrate buffer pH 4.5, ***group II*** (BV control group, n=5), animals in this group were injected intraperitoneally (IP) with 0.5 mg/kg BV (purchased from Department of Allergy and Clinical Immunology, Faculty of Medicine, Ain Shams University) twice weekly at fasting condition for four consecutive weeks, ***group III*** (diabetic group, n=10), animals were injected with single IP injection of 45mg/kg STZ (Sigma, USA) in 0.1 M citrate buffer pH 4.5 and ***group IV*** (BV treated group, n=10),rats in this group were induced to be diabetic by STZ as done in group III and were injected IP with 0.5 mg/kg BV twice weekly at fasting condition for four consecutive weeks.[16]

Animals in all groups were sacrificed after four weeks from the beginning of the experiment.

To confirm induction of diabetes in groups III and IV, blood glucose level was measured three days after STZ injection. Animals were considered diabetic when blood glucose level was above 250 mg/dl.[17]

Blood glucose level analysis

A drop of fresh blood was collected from the animals' tail vein using a lancet at fasting conditions. Blood glucose levels were measured in all groups twice weekly using glucometer instrument (Accua-check, ROCHE, Germany).

Electron microscopic study

Sciatic nerves were isolated, cut into 2 mm segments and fixed in glutaraldehyde solution 2% in 0.1 M PBS for 24 h. The specimens were washed 3 times with PBS, post-fixed in 1% osmium tetroxide for 2 h., dehydrated in ascending grades of ethanol and finally embedded in Epoxy resin. Semithin sections (350 nm) were stained with 1% toluidin blue.

For ultrastructural study, ultrathin sections (60-80nm) were stained with 1% uranyl acetate and 2% lead citrate, examined and photographed with Jeol, JEM- 1200 EX II Electron Microscope, Tokyo, Japan.

Morphometric analysis

For morphometric study, semithin sections of sciatic nerves were used to measure myelinated fiber diameter, axon diameter, myelin thickness and the g ratio (The ratio of the inner axonal diameter to the total outer diameter).

For each parameter, five slides from five different rats of each group were examined. For each slide, five different non-overlapping fields were taken. Five different readings from every captured photo were counted and the mean was calculated for each slide.

Digital images (20× objective lens, 3900× 3090 pixels) were captured by a digital camera (Leica Microsystems, GmbH, Wetzlar, Germany) fixed to a light microscope (Leica DM2500, Germany) installed on a computer in the Image analysis unit, Histology and Cell Biology Department, Faculty of Medicine, Ain Shams University. The sections were analyzed morphometrically by using image analysis software (Leica Q win V.3 program).

Statistical analysis

Data were presented as mean + SD. One-way ANOVA was used with the Tukey multiple comparisons test to determine whether values differed. A *P* value less than 0.05 was considered statistically significant. The measured parameters in the different groups were compared with each other using the statistical package for the social sciences (SPSS) computer program analysis (version 20.0. IBM Corporation, New York-USA).

RESULTS

- Histological results

Semithin sections: In BV control group, the sciatic nerves almost appeared histologically similar to those of the control. Toluidine blue stained sections showed that each bundle of sciatic nerve was surrounded by connective tissue; the perineurium. The myelinated nerve fibers were of variable diameters, each was formed of central axon with clear axoplasm surrounded by regular dark compact rings of myelin sheaths. The myelin of some fibers appeared consisted of two separate rings as the sections were passed through Schmidt-Lanterman clefts. Unmyelinated nerve fibers were occasionally seen. The spaces in between the nerve fibers were filled with loose connective tissue the endoneurium "Fig. 1".

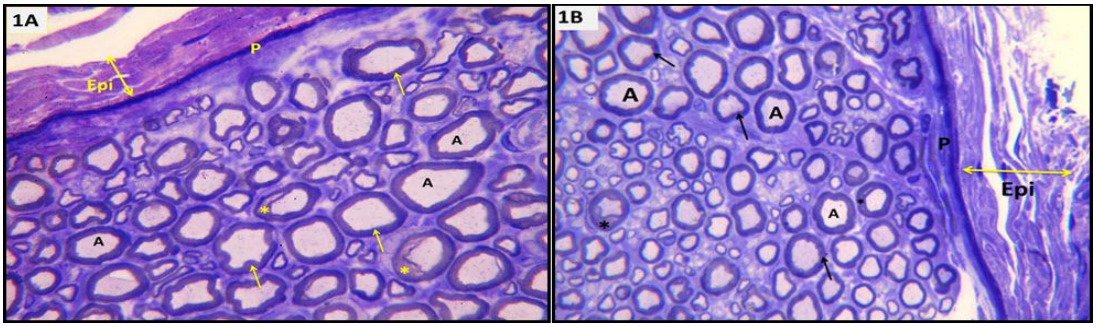

Figure 1: Semithin sections of rat sciatic nerves. (A) Group I; Control. (B) Group II; BV Control. In both groups, sciatic nerve is surrounded by the dense connective tissue epineurium (Epi). Each nerve fascicle is enclosed in the specialized connective tissue perineurium (P). The axons appear clear with variable diameters (A). The myelin is represented by dark ring surrounding the axons (↑). Some axons are surrounded by two separate rings of myelin (*). (Toluidine blue stain, × 1000).

In STZ-induced diabetic group, the perineurium appeared thickened as compared with the control groups. The axons of large myelinated fibers were severely degenerated and atrophied. Signs of myelin affection were obviously observed with variable degrees ranging from myelin infoldings into axoplasm, presence of myelin detachments or even marked myelin thickening with complete loss of their axons. Moreover, marked edema was observed in the endoneurium "Fig. 2".

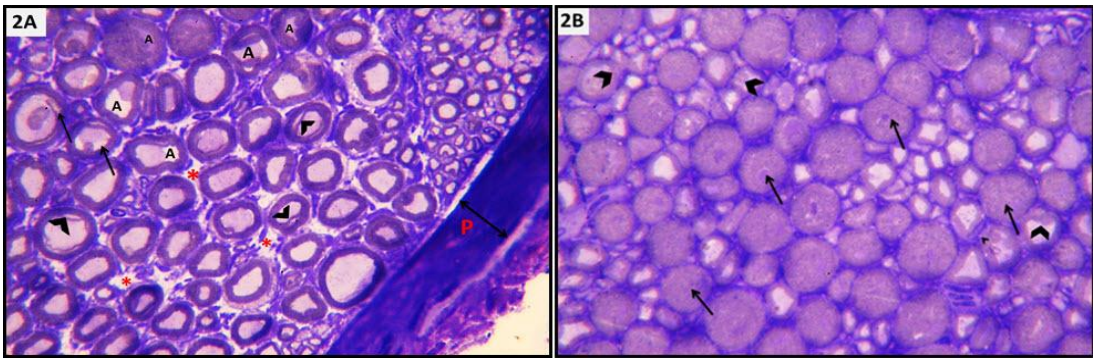

Figure 2: Semithin sections of sciatic nerves from STZ-induced diabetic rats (group III). (A) The nerve fascicle is surrounded by relatively thick perineurium (P). The nerve axons are shrunken and atrophied (A). Myelin sheath appears fragmented and separated in some fibers (↑). Edema is also noticed in between the nerve fibers (*). (B) Other field shows marked thickening of less dense myelin sheath in large myelinated fibers with loss of their axons (↑) and myelin infoldings in smaller ones (arrow heads). (Toluidine blue stain, × 1000).

Treatment of the diabetic rats with BV caused prevention of these abnormal histological changes in the sciatic nerves. Remyelination of the large fibers had occurred by the hypertrophied Schwann cells. Their axons appeared intact as well as those of thin myelinated fibers. However, some endoneurial edema was still noticed in between the nerve fibers "Fig. 3".

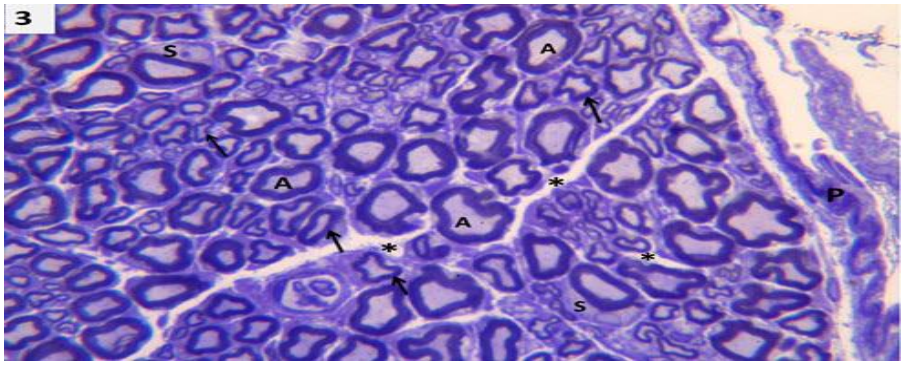

Figure 3: A semithin section of sciatic nerve from the BV treated rats (group IV), showing thick myelinated nerve fibers with intact axons (A) surrounded by hypertrophied Schwann cells (S). The thin myelinated nerve fibers have normal appearance (↑). Note the presence of edema in the endoneurium (*) and beneath the perineurium (P). (Toluidine blue stain, × 1000).

Ultrastructure results: Myelinated nerve fibers of the control groups were of small, medium and large sized diameters with homogenous axoplasm that contained intact mitochondria, microtubules and neurofilaments. The myelin sheaths appeared regular and compact with Schmidt-Lanterman clefts. Schwann cells surrounded the myelinated nerve fibers with euchromatic nuclei and few cytoplasmic organelles. Collagen fibrils of the endoneurium filled the spaces between the nerve fibers "Figs. 4,5".

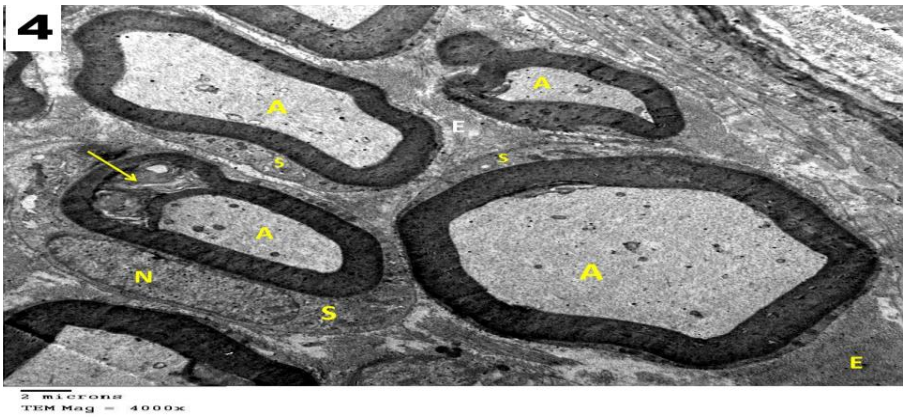

Figure 4: An electron micrograph of sciatic nerve of group I; (control) showing myelinated nerve fibers of variable diameters. The axoplasm appear homogenous (A) surrounded by compact regular myelin sheath with Schmidt-Lanterman clefts (↑). Schwann cells surround myelinated nerve fibers (S), having euchromatic nuclei (N). Endoneurium present in between nerve fibers is formed of collagen fibrils (E). (TEM, × 4000).

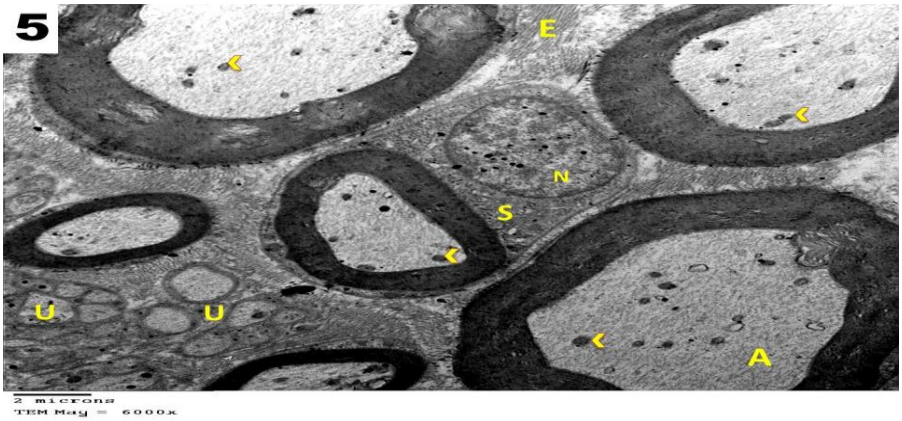

Figure 5: An electron micrograph of sciatic nerve of group group II; (BV control) showing axoplasm of myelinated fibers contain intact mitochondria (arrow heads). Schwann cell (S) is seen surrounding a myelinated fiber and having euchromatic nucleus (N). Unmyelinated nerve fibers can be seen (U). (TEM, × 6000).

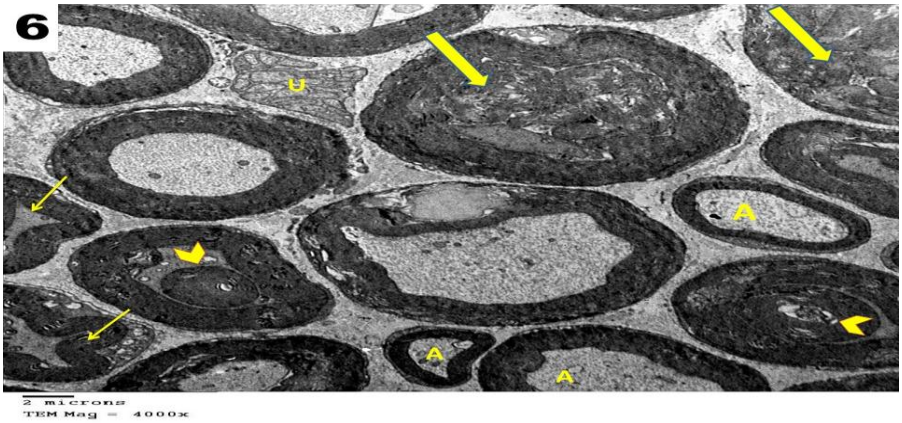

Figure 6: An electron micrograph of sciatic nerve from STZ-induced diabetic rats (group III) showing that the myelin sheaths of large myelinated fibers are markedly thickened with no apparent axoplasm (thick arrow). Other fibers show infolding and outfolding of the myelin sheath (↑) or even presence of myelin detachment (arrow heads), but unmyelinated (U) and thin myelinated fibers (A) are unaffected. (TEM, × 4000).

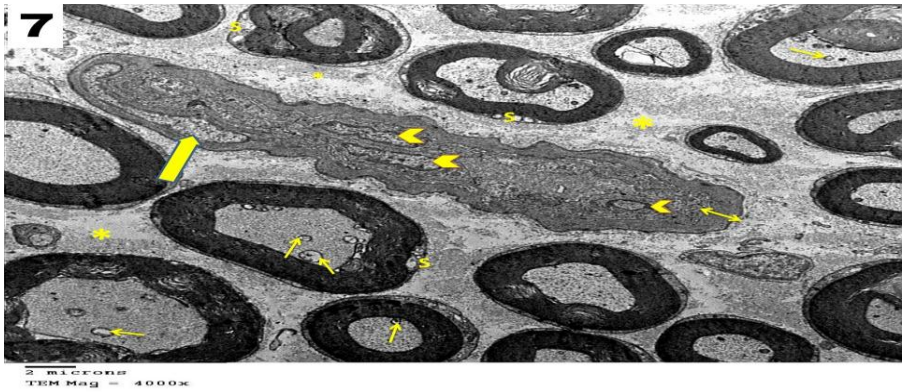

Figure 7: An electron micrograph of sciatic nerve from STZ-induced diabetic rats (group III) showing a collapsed endoneural blood vessel with proliferation of endothelial cells (arrowhead) and marked increase in its basement membrane thickness (double headed arrow) enclosing the pericyte (thick arrow). The axoplasm of some fibers contain vacuolated mitochondria (↑). Schwann cells of large myelinated fibers contain cytoplasmic vacuoles (S). Edema can be seen around the blood vessel and in between the nerve fibers (*). (TEM, × 4000).

In diabetic rats, the myelin sheaths of medium and large myelinated fibers were at different stages of degeneration. Some fibers showed several infoldings and outfoldings of their myelin assumed a star-shaped appearance and compressed the axoplasm. Other fibers showed marked thickening of the myelin with complete axonal atrophy. Multiple vacuoles could be seen within myelin sheaths and myelin fragments compressing the axoplasm of other fibers. Meanwhile, the small myelinated and unmyelinated nerve fibers appeared of normal structure "Fig. 6". An endoneural blood capillary appeared collapsed with proliferation of its endothelial lining that resting on markedly thickened basement membrane. The axoplasm of some fibers near this blood capillary contained vacuolated mitochondria and their Schwann cells were degenerated as well. Edema was noticed especially around the blood capillary and in between the nerve fibers "Fig. 7".

BV administration resulted in marked improvement of histopathological changes at ultrastructure level of the sciatic nerve except for the persistence of endoneurial edema. The myelinated nerve fibers appeared well organized with intact axoplasm contained normal mitochondria. The myelin sheaths were of normal appearance with narrow Schmidt-Lanterman clefts "Fig. 8". An endoneurial blood capillary appeared patent lined with flattened endothelial cells that rested on thin basement membrane enclosing pericyte "Fig. 9".

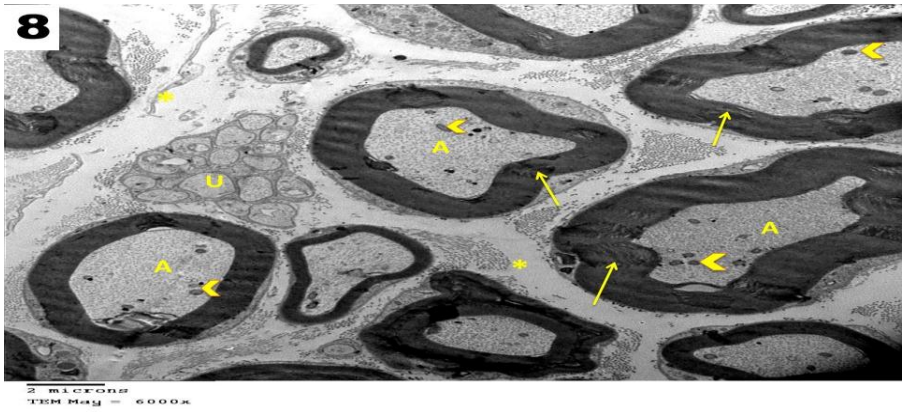

Figure 8: An electron micrograph of sciatic nerve from BV treated rats (group IV) showing normal myelinated nerve fibers with homogenous axoplasm (A) containing normal mitochondria (arrowheads). The myelin sheaths appear normal with narrow Schmidt- Lanterman clefts (↑). The unmyelinated nerve fibers appear unaffected (U). However, endoneurium still exhibit edema (*). (TEM, × 6000).

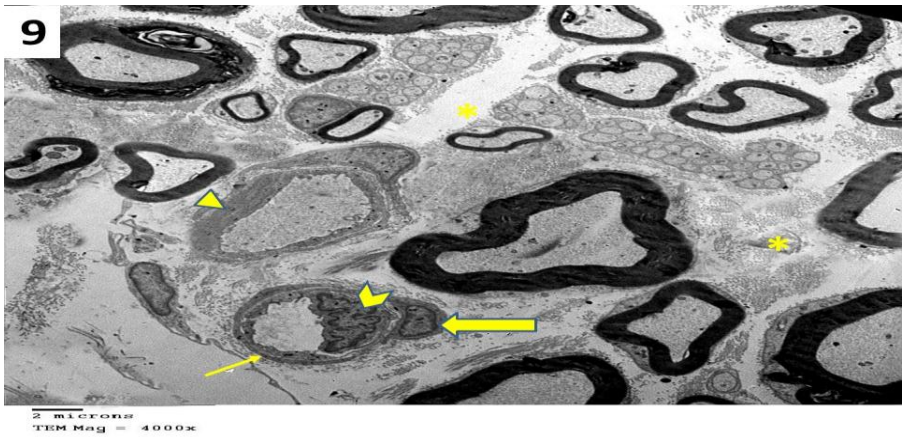

Figure 9: An electron micrograph of sciatic nerve from BV treated rats (group IV) showing patent endoneural blood capillary lined by endothelial cells (arrowhead) resting on basement membrane of normal thickness (↑), enclosing a pericyte in between (thick arrow). Most of the fibers are surrounded by thick electron dense myelin sheath, however, some fibers are surrounded by less dense non-uniform myelin sheath (Δ). Marked edema is still noticed in between the nerve fibers (*). (TEM, × 4000).

- ***Morphometric and statistical results: "Table 1"***

Effect of BV on blood glucose level in diabetic rats. Mean blood glucose level showed a considerable increase in STZ-induced diabetic rats (group III). On the other hand, the mean blood glucose level in BV control group (group II) and diabetic rats treated with BV (group IV) was almost similar to that of the control group.

The mean total fiber diameter. The mean total fiber diameter was significantly increased in the BV control (group II) in comparison to all other groups. In contrast, a non significant change was calculated between the mean values in the control group (group I), diabetic group (group III) and BV treated group (group IV).

The mean axon diameter. The mean axon diameter was significantly decreased (P<0.05) in the diabetic group in comparison to all other groups. On the other hand, BV injection resulted in significant increase (P<0.05) in the axon diameter in normal rats (group II) compared to the diabetic group, BV treated diabetic group and even the control group. A non- significant change was calculated between the mean values of the control group (group I) and the BV treated group (group IV).

The mean myelin thickness. The mean myelin thickness in the diabetic group (group III) and BV control group (group II) was significantly increased (P<0.05) in comparison to the control group (group I) and the diabetic group treated with BV (group IV). A non significant change (P>0.05) was calculated between the mean values of the BV control (group II) and the diabetic group (group III) as well as between the mean values of the control group (group I) and BV treated group (IV).

The mean g ratio. In the diabetic group, the mean g ratio was significantly decreased (P< 0.05) in comparison to the control group (group I) and the BV treated group (group IV). On the other hand, a non significant change (P>0.05) in the mean values was detected between the control group (group I), the BV control (group II) and the BV treated groups (group IV).

Table 1: Mean values and standard deviation of blood glucose level, total fiber diameter, axon diameter, myelin thickness and g ratio in different groups:

	Blood glucose level	Total fiber diameter	Axon diameter	Myelin thickness	G ratio
Group I	108±23.6	9.7 ± 2.33 (■)	6.94 ± 1.45 (■Δ)	1.59 ± 0.36 (■Δ)	0.72 ± 0.11 (Δ)
Group II	102 ± 10.58	12.71 ± 8.74 (∗Δ**O**)	8.57 ± 1.99 (∗Δ**O**)	1.87 ± 0.53 (∗**O**)	0.66 ± 0.17
Group III	323.4 ± 23.64	9.65 ± 2.8 (■)	5.58 ± 1.35 (∗■**O**)	1.84 ± 0.49 (∗**O**)	0.54 ± 0.22 (∗**O**)
Group IV	108.2±27.4	9.45 ± 1.96 (■)	7.18 ± 1.46 (■ Δ)	1.46 ± 0.36 (■ Δ)	0.73 ± 0.17 (Δ)

(*) Significant difference compared to group I, (■) Significant difference compared to group II, (Δ) Significant difference compared to group III, (O) Significant difference compared to group IV.

DISCUSSION

The most common cause of neuropathy in clinical practice is diabetes. Peripheral neuropathy develops in more than half of long term diabetics. To date no satisfactory treatment targeting the causes of neuropathy exists except for good metabolic control, which slows but does not prevent progression.

To our knowledge, there is no previous histological study demonstrated the preventive effect of BV against STZ-induced diabetic neuropathy. However, there are reports on the use of BV in different degenerative diseases of the central nervous system.[18,19] So, the objective of this study was to demonstrate the effect of BV on the

histological structure and morphometric measurements of sciatic nerve in STZ-induced diabetes in rats.

The present study demonstrated that BV had neuroprotective effects on sciatic nerves affected by diabetic neuropathy in STZ-induced diabetic rat model.

Based on histological and ultra structure examination, it was observed that endoneural blood vessels in the diabetic rats group were collapsed due to thickening of the basement membrane and endothelial cells hyperplasia. These alterations were enough to cause endoneural ischemia and edema.

These observations in STZ-model were in accordance to the findings observed by Malik et al., in diabetic patients.[20] They found increase in vessel wall thickness with and the basal lamina of arterioles and capillaries which led to nerve ischemia. As the blood flow decreases, shortage of oxygen and food supply follow, resulting in anaerobic metabolism and decrease in ATP production. This lack of energy hinders ATPase ion pump in nerve fibers and causes sodium, calcium and water accumulation and in turn degeneration of fibers and endoneural edema.[21]

On the other hand, BV treated diabetic group demonstrated patent endoneural blood vessels lined by flattened endothelial cells and resting on normal thickness basement membrane. Thus, it was concluded that, BV had prevented the diabetic vasculopathy, occurrence of ischemia and nerve fiber degeneration. However, the persistence of edema noticed in this group might be explained on basis of the presence of biogenic amine histamine within the composition of bee venom (0.7-1.5%) which increases the permeability of blood capillaries.[22]

The other main primary mechanism of diabetic neuropathy is metabolic disorders. Hyperglycemic state is responsible for activation of the polyol pathway, leading to increased production of sorbitol. Excess sorbitol accumulates intracellularly in nervous tissue causing osmotic stress, water influx, Schwann cell damage and nerve fiber degeneration.[23]

Ultrastructure findings of STZ-induced diabetic group in this study revealed degenerative changes in Schwann cells in the form of cytoplasmic vacuolations. Large and medium sized nerve fibers were the most affected fibers noticed in this study. They showed different stages of myelin disarrangements starting from splitting of myelin sheath, outfolding and infolding of myelin compressing the axoplasm, presence of myelin separated fragments inside the axon space and even marked thickening of the less dense myelin with no apparent axoplasm. On the other hand, the thin myelinated fibers and unmyelinated fibers appeared almost unaffected.

These results were in accordance with the results of a study in which a long-term experimental galactose neuropathy was induced; a model of increased polyol pathway activity. The study demonstrated that Schwann cells in myelinated fibers showed increased cytoplasmic volume, lipid droplets, periaxonal edema, enlarged mitochondria without recognizable cristae and lysis of Schwann cell cytoplasm.[24]

More recently, *Ma et al.,* found degeneration of Schwann cells, onion bulb type myelin destructions and lamellar separation in alloxan-induced diabetic rats.[25] They explained these results on basis that Schwann cells were sensitive to hyperglycemia, which in turn played an important role in myelin sheath degeneration.

In our study, treatment with 0.5 mg/kg bee venom ameliorated the degenerative changes occurred in Schwann cells and preserved the integrity of myelin of the nerve fibers as well as decreased blood glucose level to the normal range in STZ-induced diabetic rats.

The normal levels of blood glucose achieved in the current study after BV injection was supported by a previous study which reported that BV had anti-diabetic effect.[26] This could be due to the presence of melittin and phospholipase A_2 in BV which are the two-main components of the venom. These substances exert anti-inflammatory effect on Islets of Langerhans and hence increase insulin secretion with subsequent decrease in blood glucose concentration.[27,28] Furthermore, our results were in accordance with a recent study in which immunohistochemical staining of the pancreas with anti-insulin antibodies in a rat model of diabetes was performed. The results showed that BV caused significant increase in the area percentage of insulin positive cells in islets of Langerhans as compared with diabetic group.[29]

The ultra structure results of this study demonstarted swelling and vacuolation in the mitochondria present in the axoplasm of the nerve fibers in STZ-induced diabetic rats. On the contrary, apparently normal morphology of axoplasmic mitochondria was detected in BV treated diabetic group.

In diabetes, the increase in oxidative stresses and mitochondrial dysfunction due to the increase of free radicals in hyperglycemic conditions are considered among the main patho-physiologic causes of diabetic neuropathy.[30, 31]

In our study, the apparently normal morphology of axoplasmic mitochondria after BV injection suggested that BV restored the normal structure of the mitochondria through prevention of diabetic microvasculopathy and normalization the hyperglycemic conditions.

The statistical results obtained from the morphometric measurements in the current study revealed that the mean axon diameter was significantly decreased ($P<0.05$) in the diabetic rats in comparison to normal control rats, BV control rats and BV treated rats.

Sugimura et al., reported similar results and suggested that the shrinkage observed in STZ diabetes might be due to hyperosmolarity of the interstitial tissue as the morphometric changes were similar to those resulting from fixation in hyperosmolar fixative solutions.[32] However, this hypothesis was denied because there is no condensation of axoplasmic organelles which would be expected with osmotic shrinkage of the axon.[33]

Instead a progressive defect in axonal transport of neurofilaments, which are the main indicator of fiber diameter, was suggested based on the characteristic axonal atrophy in STZ diabetes which was present in distal nerves whereas the proximal nerve fibers were not affected. As the neuropathy progresses proximal axons also become atrophic.[34]

Another view suggested that Schwann cells might be responsible for controlling the caliber of the axon, matching it to the thickness of the myelin sheath through a mechanism that involves myelin- associated glycoprotein. This leads to an increased phosphorylation of neurofilament arms. The negatively charged phosphate groups are thought to repulse each other, increasing the spacing of the neurofilament chains, and the caliber of the axon.[35]

Since, Schwann cell was affected in diabetic group in the current study, this might explain the significant decrease in the axonal diameter as well as in the g-ratio which is widely utilized as a functional and structural index of optimal axonal myelination.

Surprisingly, statistical analysis in this study demonstrated a significant increase ($P<0.05$) in the mean total fiber diameter in the BV control group in comparison with the diabetic group, BV treated group and even the control group. However, the results of the g-ratio of the BV control group showed a non significant change ($P>0.05$) in comparison to other groups.

These results could obviously suggest that BV succeeded to increase the fiber diameter in normal rats and failed to induce the same results in diabetic rats. This hypothesis was confirmed by recording a significant increase ($P<0.05$) in the fiber diameter in BV control group in comparison to all other groups using statistical analysis.

In the view of these results we suggested that BV had stimulated the synthesis of neurofilaments in the normal nerve fibers via either a neurotrophic component in its structure or stimulation of neurotrophic factors synthesis.

The neurotrophins, which regulate synthesis of medium and low molecular weight neurofilaments, are synthesized by the neuronal target cells and delivered to the neuronal perikarya by retrograde axonal transport. Nerve growth factor (NGF), is one of the most researched neurotrophins which is synthesized in the periphery target organs; muscle and skin and then transported retrogradely to sensory neurons and sympathetic ganglia. Retrogradely transported NGF binds to two receptors; the high affinity trkA and the low affinity p75-receptor.[36]

In diabetic rats, there was decreased synthesis as well as decreased axonal transport of NGF. In addition the mRNA expression of trkA and p75 in sciatic nerve are down regulated in diabetic rats.[37] This could explain failure of BV to increase the axon diameter in diabetic rats to the values recorded in normal rats.

As neurofilaments are the major structural determinants for the maintenance of axonal size, this implies that deficient neurotrophic factors in diabetic neuropathy are in part responsible for the characteristic axonal atrophy which was significantly improved by injecting BV in diabetic rats.[38]

CONCLUSION

Bee venom administration in early stages of STZ-induced diabetes had a neuroprotective role against the progression of diabetic neuropathy in rats as well as it normalized the blood glucose level.

REFERENCES

1. Low PA. Pathogenesis of diabetic neuropathy. In: Kahn CR, Weir GC, King GL, Jakobson AM, Moses AC and Smith RJ, editors. Joslin's diabetes mellitus. 14th ed. Lipponcott Williams and Wilkins, 2005; 839–51.
2. Boucek P. Advanced diabetic neuropathy: a point of no return. Rev Diabet Stud, 2006; 3: 143-150.
3. Pavy-Le Traon A, Fontaine S, Tap G, Guidolin B, Senard JM, Hanaire H. Cardiovascular autonomic neuropathy and other complications in type 1 diabetes. ClinAuton Res, 2010; 20: 153–160.
4. Oates PJ. Polyol pathway and diabetic peripheral neuropathy. Int Rev Neurobiol, 2002; 50: 325–392.
5. Miranda-Massari JR, Gonzalez MJ, Jimenez FJ, Allende-Vigo MZ, Duconge J. Metabolic correction in the management of diabetic peripheral neuropathy: improving clinical results beyond symptom control. Curr Clin Pharmacol, 2011; 6: 260–273.
6. Drel VR, Lupachyk S, Shevalye H, Vareniuk I, Xu W, Zhang J, Delamere NA et al. New therapeutic and biomarker discovery for peripheral diabetic neuropathy: PARP inhibitor, nitrotyrosine, and tumor necrosis factor-a. Endocrinology, 2010; 151: 2547–2555.
7. Skalska S, Kyselova Z, Gajdosikova A, Karasu C, Stefek M, Stolc S. Protective effect of stobadine on

NCV in streptozotocin-diabetic rats: augmentation by vitamin E. Gen Physiol Biophys, 2008; 27: 106-114.
8. Afifi NM. Neuroprotective effect of melatonin in a rat model of streptozotocin-induced diabetic neuropathy: Light and electron microscopic study. Egypt J Histol, 2013; 36: 321-335.
9. Schwartz S, Etropolski M, Shapiro DY, Okamoto A, Lange R, Haeussler J, Rauschkolb C. Safety and efficacy of tapentadol ER in patients with painful diabetic peripheral neuropathy: results of a randomized-withdrawal, placebo-controlled trial. Curr Med Res Opin, 2011; 27: 151–162.
10. Zangiabadi N, Ahrari MN, Nakhaee N. The effect of omega-3 fatty acids on nerve conduction velocity (NCV) and F-wave latency in patients with diabetic polyneuropathy. Am J Pharmacol Toxicol, 2007; 2: 1.
11. Abdela N, Jilo K. Bee venom and its therapeutic values: A review. Adv Life Sci Technol, 2016; 44: 18-22.
12. Son DJ, Lee JW, Lee YH, Song HS, Lee CK, Hong JT. Therapeutic applicationof anti-arthritis, pain-releasing, and anticancereffect of Bee Venom and its compounds. Pharmacol Ther, 2007; 115: 246–270.
13. Kwon YB, Lee JH, Han HJ, Mar WC, Beitz AJ, Lee HJ. Bee venom injection into an acupuncture point reduces arthritis associated edema and nociceptive responses. Pain, 2001; 90: 271-280.
14. Kang SS, Pak SC, Choi SH. The effect of whole bee venom on arthritis. Am J of Chin Med, 2002; 30: 73-80.
15. Castro HJ, Mendez-Lnocenio JI, Omidvar B, Omidvar J, Santilli J, Nielsen HS, et al. A phase I study of the safety of honeybee venom extract as a possible treatment for patients with progressive forms of multiple sclerosis. Allergy Asthma Proc, 2005; 26: 470-476.
16. Son DJ, Lee JW, Lee YH, Song HS, Lee, CK, Hong JT. Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. Pharmacol Ther, 2007; 115: 246-270.
17. Zangiabadi N, Sheibani V, Asadi-Shekaari M, Shabani M, Jafari M, Asadi AR et al.Effects of Melatonin in Prevention of Neuropathy in STZ-Induced Diabetic Rats. Am J Pharmacol Toxicol, 2011; 6: 59-67.
18. Doo AR, Kim ST, Kim SN, Moon W, Yin CS, Chae Y et al. Neuroprotective effects of bee venom pharmaceutical acupuncture in acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced mouse model of Parkinson's disease. Neurol Res, 2010; 32: 88-91.
19. Kim JI, Yang EJ, Lee MS, Kim YS, Huh Y, Cho IH et al. Bee venom reduces neuroinflammation in the MPTP-Induced model of Parkinson's disease. Inter J Neurosci, 2011; 121: 209-217.
20. Malik RA, Tesfaye S, Newrick PG, Walker D, Rajbhandari SM, Siddique I et al. Sural nerve pathology in diabetic patients with minimal but progressive neuropathy. Diabetologia, 2005; 48: 578-85.
21. Kihara M, Schmelzer JD, Kihara Y, Smithson IL, Low PA. Efficacy of limb cooling on the salvage of peripheral nerve from ischemic fiber degeneration. Muscle Nerve, 1996; 19: 203-209.
22. Hwang DS, Kim SK, Bae H. Therapeutic effects of bee venom on immunological and neurological diseases. Toxins, 2015; 7: 2413-2421.
23. Oates PJ: Polyol pathway and diabetic peripheral neuropathy. Int Rev Neurobiol, 2002; 50: 325–392.
24. Mizisin AP, Powell HC. Schwann cell changes induced as early as one week after galactose intoxication. Acta Neuropathol, 1997; 93: 611-618.
25. Ma ST, Liu DL, Deng JJ, Peng YJ. Protective effect of mulberry flavonoids on sciatic nerve in alloxan-induced diabetic rats. Brazilian Journal of Pharmaceutical Science, 2014; 50: 765-771.
26. Mousavi SM, Imani S, Haghighi S, Mousavi SE, Karimi A. Effect of iranian honey bee (Apismellifera) venom on blood glucose and insulin in diabetic rats. J Arthropod-Borne Dis, 2012: 6: 136–143.
27. Kim JY, cho SH, Kim YW, Jang EC, Park SY, Kim EJ et al. Effects of BCG, lymphotoxin and bee venom on insulitis and development of IDDM in non-obese diabetic mice. J Korean Med Sci, 1999; 14: 648-652.
28. Simonsson E, Karlsson S, Ahren B. Islet phospholipase A_2 activation is potentiated in insulin resistant mice. Biochem Biophys Res Commun, 2000; 272: 539-543.
29. Abdel Gawad S, Fikry H, Amin MM, Elmahdi AR, Abd Elaziz D. Effect of apitherapy on the pancreas and liver of streptozotocin induced diabetic rats. A biochemical and histological study. EJPMR, 2016; 3(7): 555-565.
30. Saini AK, Kumar HS, Sharma SS. Preventive and curative effect of edaravone on nerve functions and oxidative stress in experimental diabetic neuropathy. Eur J Pharmacol, 2007; 568: 164-172.
31. Kumar A, Kaundal RK, Iyer S, Sharma SS. Effects of resveratrol on nerve functions, oxidative stress and DNA fragmentation in experimental diabetic neuropathy. Life Sci, 2007; 80: 1236-1244.
32. Sugimura K, Windebank AJ, Natarajan V, Lambert EH, Schmid HH, Dyck PJ. Interstitial hyperosmolarity may cause axis cylinder shrinkage in streptozotocin diabetic nerve. J. Neuropathol. Exp Neurol, 1980; 39: 710-21.
33. Yagihashi S. Pathology and pathogenic mechanisms of diabetic neuropathy. Diab Metab Rev, 1995; 11: 193-225.
34. Sima AAF, Sugimoto K. Experimental diabetic neuropathy: an update. Diabetologia, 1999; 43: 774-788.
35. De Waegh SM, Lee VM, Brady ST. Local modulation of neurofilament phosphorylation,

axonal caliber, and slow axonal transport by myelinating Schwann cells. Cell, 1992; 68: 451-463.
36. DiStefano PS, Friedman B, Radziejewski C, Alexander C, Boland P, Schick CM et al.The neurotrophins BDNF, NT-3 and NGF display distinct patterns of retrograde axonal transport in peripheral and central neurons. Neuron, 1992; 8: 983-993.
37. Sima AA, Merry AC, Levitan I. Increased regeneration in ARI-treated diabetic nerve is associated with upregulation of IGF-1 and NGF receptors. Exp Clin Endocrinol Diabetes, 1997; 105: 60-62.
38. Yagihashi S, Kamijo M, Watanabe K. Reduced myelinated fiber size correlates with loss of axonal neurofilaments in peripheral nerve of chronically streptozotocin diabetic rats. Am J Pathol, 1990; 136: 1365-1373.