Original Article

The Role of Hesperidin in Ameliorating the Histological Changes of Sciatic Nerve in Streptozotocin-Induced Diabetic Rats: Light and Electron Microscopic Study

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ABSTRACT

Introduction: Diabetes mellitus (DM) is a metabolic disorder with a well-known neurotoxic effect on peripheral nerves. Hesperidin is a natural flavanone that has an anti-oxidative, anti-inflammatory and neuroprotective properties.

Aim of the Study: To evaluate the role of hesperidin in ameliorating DM-induced sciatic nerve histological changes in adult male albino rats.

Material and Methods: Forty adult male albino rats were divided into three groups; group I as a control group, group II received hesperidin (100 mg/kg/day) orally for 4 weeks and group III was injected once intraperitoneally by streptozotocin (60 mg/kg) to induce DM. Then after 4 weeks, diabetic rats were divided into 2 subgroups: subgroup IIIa was left untreated and subgroup IIIb received hesperidin at dose similar to group II for another 4 weeks. Specimens from sciatic nerves were processed for histological, and immunohistochemical studies.

Results: The untreated diabetic group showed severe disorganization and loss of the regular architecture of the nerve fibers inside the sciatic nerve fascicles. A significant decrease in the expression of S100 protein in Schwann cells with a significant elevation in their apoptosis was recorded in comparison to control group. Electron microscopic examination revealed splitting of the myelin sheaths lamellae with some attenuated myelin sheaths. Schwann cells cytoplasm showed numerous vacuoles, dilated rER and swollen disrupted mitochondria. Administration of hesperidin evidently ameliorated all the pervious structural changes.

Conclusion: Hesperidin ameliorated the histological structure alterations that occurred in the sciatic nerve due to DM.

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Key Words: Diabetes mellitus, hesperidin, S100, sciatic nerve, ultrastructure.

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INTRODUCTION

Diabetes mellitus (DM) is a common worldwide metabolic disorder[1]. There are two known types of diabetes, Type-I diabetes caused by decrease in insulin synthesis and type-II diabetes caused primarily by insulin resistance, followed by the apoptosis of pancreatic β cells[2].

Peripheral neuropathy is a serious complication encountered with DM and named diabetic neuropathy (DN). Diabetic neuropathy is defined as symmetrical sensorimotor polyneuropathy that occurs due to metabolic and microvessel alterations, resulting from chronic hyperglycemia and causing different disabilities[3,4].

All peripheral nerves including pain fibers are affected by DN[5].The resulting neuropathic pain is considered a severe condition of chronic pain[6]. It was revealed that DN occurs due to the apoptosis of peripheral nerve cells with an imbalance between the process of nerve damage and repair[7,8]. This imbalance results mainly from oxidative stress causing DNA damage, followed by activation of the apoptotic process[9].

Hesperidin a natural flavanone glycoside extracted from citrus species, particularly in their peel and membranous parts including lemons and sweet oranges. Hesperidin was found to possess several beneficial effects including anti-oxidative, anti-inflammatory and anti-diabetic properties[10,11].

Hesperidin has recently gained great interest owing to maintaining various neuroprotective properties. Its role in modulation of neuroinflammation and oxidative stress at cortical, striatal and hippocampal levels was previously studied[12]. Many researchers supported the neuroprotective effect of hesperidin in experimental model of Parkinson's disease[13], and Alzheimer's disease[14]. Moreover, hesperidin was found to possess a vital role in controlling and alleviating neuropathic pain caused by various neurotoxins[15,16].

Streptozotocin (STZ), as a diabetogenic DNA alkylating or methylating agent, is usually used to make an experimental model for DM in rats[17]. Streptozotocin induces DM by causing irreversible damage to β-cell of

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pancreas[18]. Thus, the current work aimed to investigate the possible role of hesperidin in ameliorating DM-induced sciatic nerve histological changes in adult male albino rats.

MATERIAL AND METHODS

Chemicals

Streptozotocin (STZ) was purchased from Cornell lab-chemistry company, Cairo, Egypt.

Hesperidin was purchased in powder form from Sigma Chemical Co. (St Louis, MO, USA, CAS# 520-26-3).

Animals

Forty adult male albino rats, weighting 200 grams each, were used in this research. The rats were kept in plastic cages under standard housing conditions with free approach to water and food. They were allowed 2 weeks for adaptation before the beginning of the experiment. The study was permitted by the Ethical Committee of Medical Research, Faculty of Medicine, Tanta University (Approval Code: 35490).

The experimental design

The rats were divided into three main groups as the following:

Group I (Control group): included10 rats and was further subdivided into two equal subgroups:

- Subgroup Ia: The animals were left without any treatment.
- Subgroup Ib: Each animal received a single intraperitoneal injection of 1ml of 0.1M citrate buffer (PH = 4.5) (diluting vehicle of streptozotocin).

Group II (Hesperidin-treated group): included 10 rats that were left without any treatment for 4 weeks then received hesperidin dissolved into 1ml distilled water at a dose of 100 mg/kg/ day by oral gavage for another 4 weeks[19].

Group III (Streptozotocin-induced diabetic group): included 20 rats. After fasting overnight, each animal received 60 mg/kg of STZ dissolved into 0.1M citrate buffer (PH = 4.5) as single intraperitoneal injection[20,21]. Three days from STZ injection, blood samples were collected from the rat tail vein, used to measure blood glucose levels by one touch glucometer. Rats with blood glucose levels more than 250 mg/dl[22] for 2 successive days were considered to be diabetic. All experimental rats received STZ injection became diabetic. After 4 weeks from DM induction, the diabetic rats were subdivided into two equal subgroups;

- Subgroup IIIa (Induced diabetic group): included 10 rats and left without further treatment after induction of diabetes.
- Subgroup IIIb (Induced diabetic+Hesperidin group): included 10 rats that received hesperidin at the same dose and route as group II for 4 weeks following induction of diabetes.

Sample collection and tissue preparation

At the end of the experiment, after 8 weeks, blood samples were collected for blood glucose and plasma insulin levels calculation. Then all animals were euthanized with pentobarbital (40mg/kg)[23]. Both sciatic nerves were extracted for light and electron microscopic examination.

Biochemical assay

The blood glucose levels were evaluated using one touch glucometer and expressed as mg/dl. Quantification of plasma insulin levels was performed according to the producer`s instructions using a MyBioSource rat insulin ELISA kits and expressed as mU/ml.

Light microscopic study

Both sciatic nerves of each rat were dissected, cut into transverse sections and processed for:

Histological staining

Pieces of sciatic nerve were kept in 10% of formalin then, dehydrated and embedded in paraffin, cut and stained with hematoxylin and eosin (H&E) stain[24].

Immunohistochemical staining

Sciatic nerve sections (4μm thick) were handled for immunohistochemical staining as method described previously[25]. The sections were deparaffinized and rehydrated then incubated with primary antibodies, S100 (protein of Schwann cells) (Dako North America, Inc., CA, USA) and cleaved caspase-3 (an apoptosis marker) (ab4051 Abcam, Inc., Cambridge, UK). Biotinylated secondary antibodies were added. Positive reaction was visualized using 3,3-diaminobenzoicacid (DAB) and counterstained by hematoxylin[26]. The negative control sections were processed without increment of the primary antibodies. The positive control for S100 was melanoma and for cleaved caspase-3 was lung tissue .

Transmission electron microscopic study

Sciatic nerve specimens were sliced, fixed in phosphate buffered glutaraldehyde, post-fixed in 1% phosphate-buffered osmium tetroxide, dehydrated and embedded in epoxy resin. Ultrathin sections (80 nm) stained with uranyl acetate and lead citrate to be examined by JEOL electron microscope at 80 KV at Electron Microscope Unit of Faculty of Medicine, Tanta University[27].

Morphometric analysis

The acquisition of microphotographs was done by a light microscope (DM500, Leica, Switzerland) with a digital camera (ICC50, Leica, Switzerland). The software “ImageJ” (version 1.48v National Institute of Health, USA) was used for image analysis. Randomly selected ten non-overlapping fields from each sciatic nerve specimen in each rat group were examined at a magnification of 400 to quantify:

1. Mean area percentage of S100 protein -positive Schwann cells.
2. Mean area percentage of caspase-3 immunopositive expression.

Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's test was used for data analysis (IBM SPSS Statistics for Windows, IBM Corp, USA). Differences were considered significant if the probability value $p < 0.05$.

RESULTS

Effect of hesperidin on blood glucose and plasma insulin levels

The administration hesperidin to diabetic rats (subgroup IIIb) for 4 weeks decreased significantly ($P < 0.05$) the rise of blood glucose levels and also increased the levels of plasma insulin compared with untreated diabetic rats (subgroup IIIa). Although hesperidin intake could not lower the blood glucose to normal values nor return plasma insulin to its normal levels and showed a significant difference ($P < 0.05$) in comparison to control group (Table 1, Histogram 1).

Light microscopic results

H&E results

Examination of sections from control subgroups (group I) and group II exhibited normal morphology of sciatic nerves. The sciatic nerve was formed of fascicles of nerve fibers; each fascicle was surrounded by thin layer of specialized connective tissue called perineurium. The myelinated nerve fibers formed of axons surrounded by unstained area of dissolved myelin, nuclei and neurilemmal sheath of Schwann cells. Unmyelinated nerve fibers were also seen (Figure 1).

In induced diabetic group (subgroup IIIa), loss of the normal architecture of a sciatic nerve fascicles was observed. Most of the nerve fibers were widely discrete from each other. Dilated blood vessels and inflammatory cellular infiltration were prominent (Figure 2). Many groups of severely disorganized nerve fibers were seen. In addition, some nerve fibers appeared with discontinuous neurilemmal sheath (Figure 3).

Induced diabetic+Hesperidin group (subgroup IIIb) showed improvement of the architecture of nerve fascicles with closely related and intact nerve fibers. However, focal areas of mildly disorganized nerve fibers inside the fascicles were still present (Figure 4).

S100 immunohistochemical results

Control group showed positive immunoreaction for S100 protein in nearly all of Schwann cells (Figure 5). However, induced diabetic group showed an apparent decrease in positive distribution of S100 protein in Schwann cells (Figure 6). Concerning induced diabetic+hesperidin group, an apparent increase in the distribution of the immunoreaction of S100 protein in Schwann cells were noticed (Figure 7).

The mean area percentage of S100 protein positive immunoreaction in induced diabetic group (0.903±0.151) revealed a significant decrease ($p<0.05$) in comparison with the control group (3.516±0.2397), whereas induced diabetic+Hesperidin group (3.247±0.109) recorded a non-significant difference ($p>0.05$) from the control group (Table 2, Histogram 2)

Caspase-3 protein immunohistochemical results

The control group revealed positive caspase-3 immunohistochemical reactivity in the cytoplasm of few Schwann cells (Figure 8). However, induced diabetic group showed positive caspase-3 immunoreaction in the nuclei/cytoplasm of numerous Schwann cells (fFigure 9). Contrary, induced diabetic+Hesperidin group revealed positive caspase-3 immunoreaction in the nuclei/cytoplasm of some Schwann cells (Figure 10).

The mean area percentage of caspase-3 immunoreaction in induced diabetic group (1.758±0.0649) revealed a significant increase ($p<0.05$) compared to the control group (0.254±0.0747), whereas induced diabetic+Hesperidin group (0.379±0.1234) recorded a non-significant difference ($p>0.05$) in comparison with the control group (Table 2, Histogram 2).

Electron microscopic results

The control group showed myelinated axons containing axoplasm and surrounded by compact regular myelin sheaths. The axoplasm contained mitochondria, microtubules and microfilaments. Moreover, the cytoplasm of Schwann cells wrapped around the myelinated axons forming the multilayers of myelin sheaths (Figures 11,12).

In induced diabetic group, many axons with extensive splitting of their myelin sheaths lamellae were observed. In addition, other myelinated axons showed multiple focal splitting in their sheaths in the form of whorls. While, some attenuated myelin sheaths with deep invagination and decreased densities of their axoplasmic contents were also noticed (Figures 13,14,15). Additionally, the cytoplasm of many Schwann cells contained numerous vacuoles, dilated rER and swollen mitochondria with disrupted cristae (Figures 15,16). Some nerve fibers appeared as one fiber engulfing another (Figure 17). Other sections showed myelinated axons with rounded myelin fragments in their axoplasms and had irregular outlines (Figure 18).

Regarding induced diabetic+Hesperidin group, the myelinated nerve fibers appeared more or less normal with intact and regular myelin sheaths. Despite few areas of minimal focal splitting in the myelin sheaths lamellae and few vacuoles in the cytoplasm of some Schwann cells were still observed (Figures 19,20).

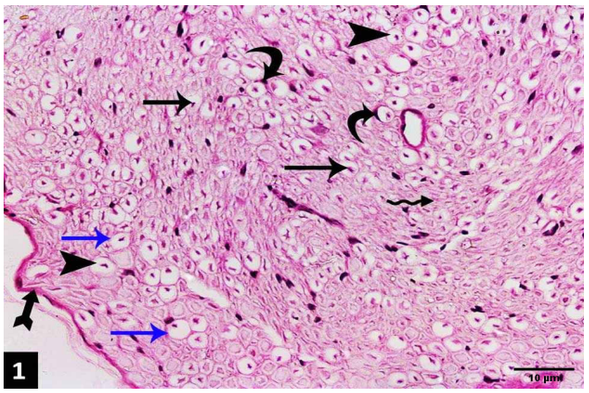


Fig. 1: photomicrograph of a transverse section of sciatic nerve of control group (group I) showing part of nerve fascicle surrounded by perineurium (bifid arrow). Myelinated nerve fibers are formed of axon (arrows) surrounded by unstained area of dissolved myelin (arrowheads), nuclei (curved arrows) and neurilemmal sheath (blue arrows) of Schwann cells. Notice: group of unmylinated nerve fibers (wavy arrow). (H&E X400, scale bar =10µm).

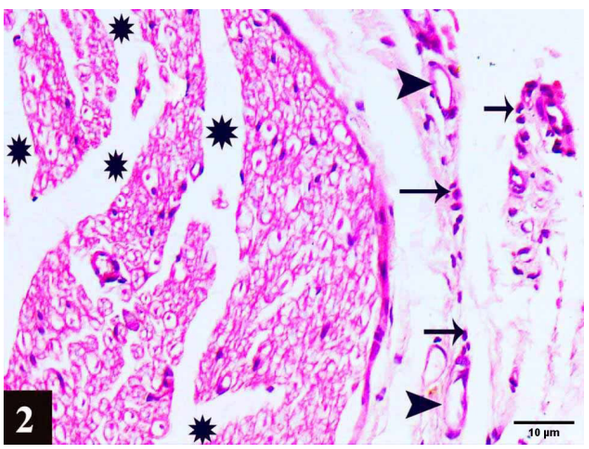


Fig. 2: A photomicrograph of a transverse section of sciatic nerve of induced diabetic group (subgroup IIIa) showing loss of the normal architecture of a sciatic nerve fascicle. Nerve fibers are widely separated from each other (asterisks). Notice dilated blood vessels (arrowheads) and inflammatory cellular infiltrate (arrows). (H&E X400, scale bar =10µm)

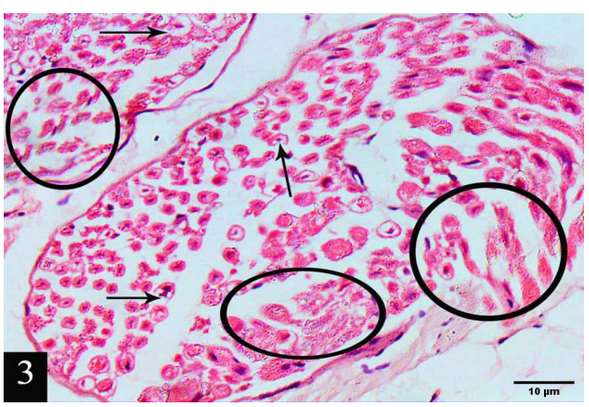


Fig. 3: A photomicrograph of a transverse section of sciatic nerve of induced diabetic group showing severely disorganized of nerve fibers (circles). Notice: some nerve fibers have discontinuous neurilemmal sheath (arrows). (H&E X400, scale bar =10µm)

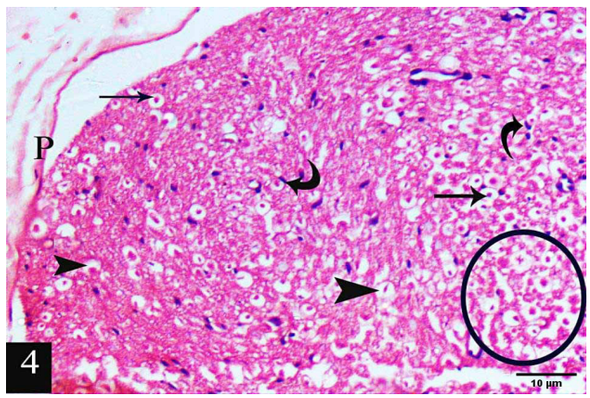


Fig. 4: A photomicrograph of a transverse section of sciatic nerve of induced diabetic+Hesperidin group (subgroup IIIb) showing nearly normal architecture of sciatic nerve fascicle with closely related nerve fibers surrounded by perineurium (P). Nerve fibers appear with axons (arrows) surrounded by unstained myelin (arrow heads) and nuclei of Schwann cells (curved arrows). Notice focal area of mildly disorganized nerve fibers (circle) (H&E X400, scale bar =10µm)

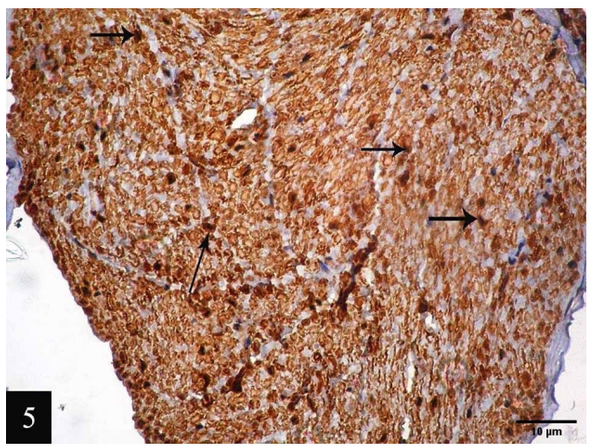


Fig. 5: A photomicrograph of a transverse section of sciatic nerve from the control group showing the immunopositive distribution of S100 protein in Schwann cells (arrows). (S100 X400, scale bar =10µm)

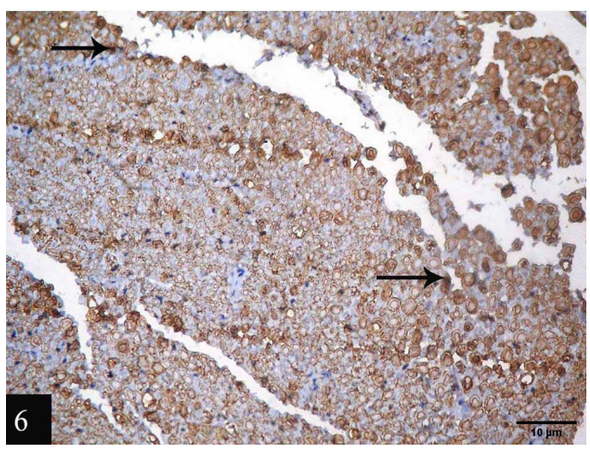


Fig. 6: A photomicrograph of a transverse section of sciatic nerve from induced diabetic group showing the apparent decrease in the immunopositive distribution of S100 protein in Schwann cells (arrows). (S100 X400, scale bar =10µm)

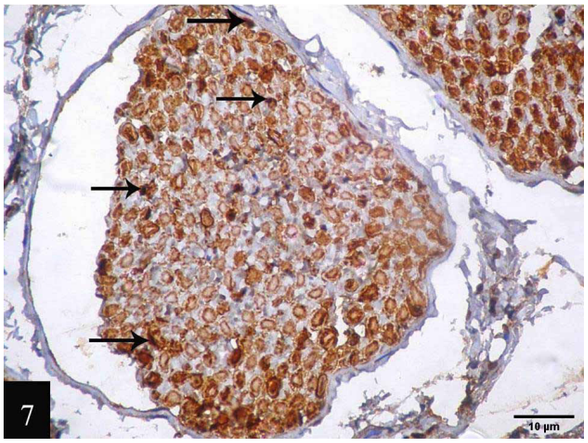


Fig. 7: A photomicrograph of a transverse section of sciatic nerve from induced diabetic+Hesperidin group showing apparent increase in the immunopositive distribution of S100 protein in Schwann cells (arrows). (S100 X400, scale bar =10µm)

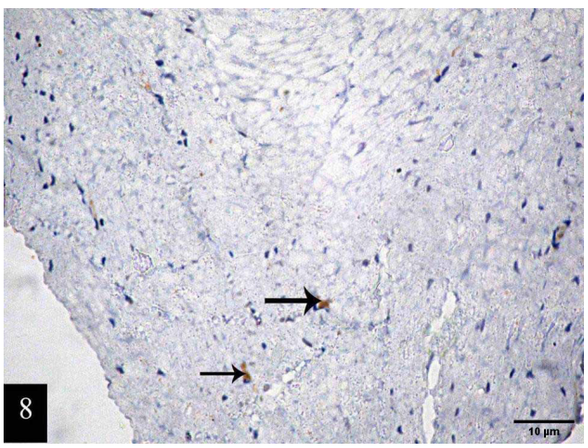


Fig. 8: A photomicrograph of a transverse section of sciatic nerve from the control group showing positive caspase-3 immunoreaction in the cytoplasm of few Schwann cells (arrows). (Caspase-3 X400, scale bar =10µm)

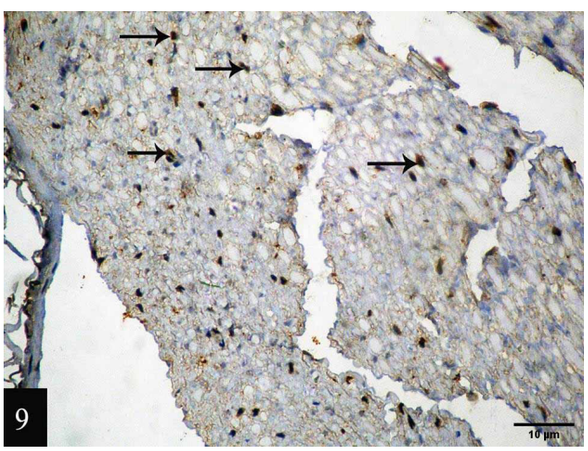


Fig. 9: A photomicrograph of transverse section of sciatic nerve from induced diabetic group showing positive caspase-3 immunoreaction in the nuclei/cytoplasm of numerous Schwann cells (arrows). (Caspase-3 X400, scale bar =10µm)

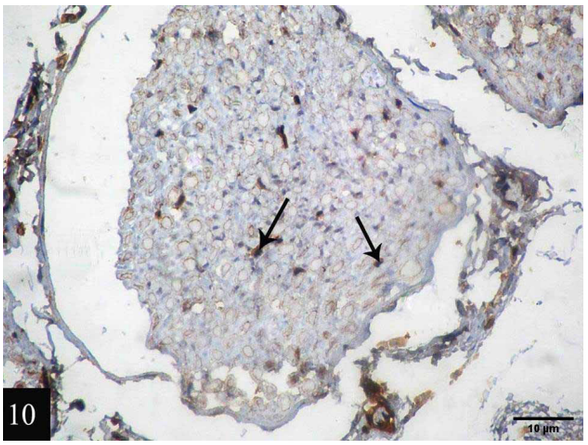


Fig. 10: A photomicrograph of transverse section of sciatic nerve from induced diabetic+Hesperidin group showing positive caspase-3 immunoreaction in the nuclei/cytoplasm of some Schwann cells (arrows). (Caspase-3 X400, scale bar =10µm)

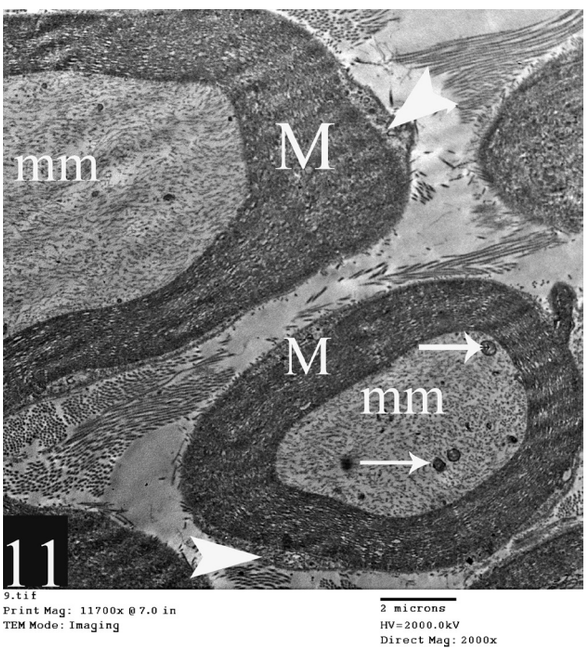


Fig. 11: A photomicrograph of an ultrathin section of sciatic nerve of control group showing compact regular myelin sheaths (M) of myelinated nerve fibers. The myelinated axons are surrounded by parts of Schwann cells cytoplasm (arrow heads). The axoplasm contained mitochondria (arrows), microtubules and microfilaments (mm) (X 2000).

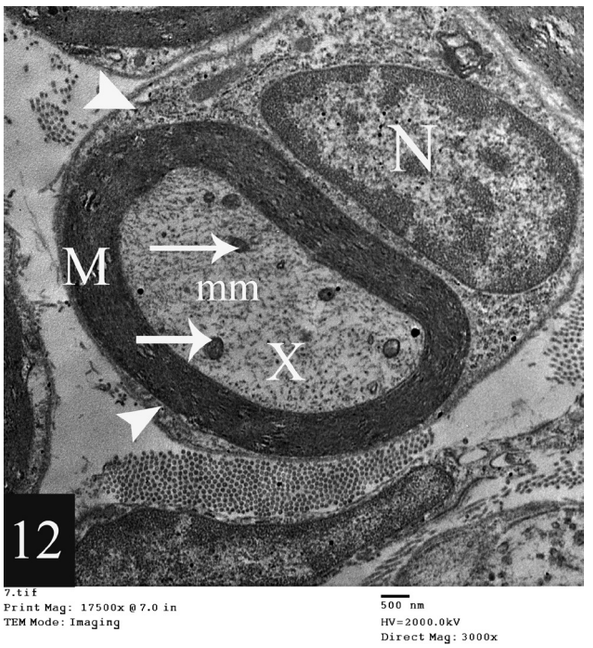


Fig. 12: A photomicrograph of an ultrathin section of sciatic nerve of control group showing an axoplasm (X) ensheathed with compact myelin sheath (M) with uniform thickness. Schwann cell (arrowheads) with large nuclei (N) is wrapping around the myelinated axon. Notice: The axoplasm (X) contains mitochondria (arrows), microtubules and microfilaments (mm) (X 3000).

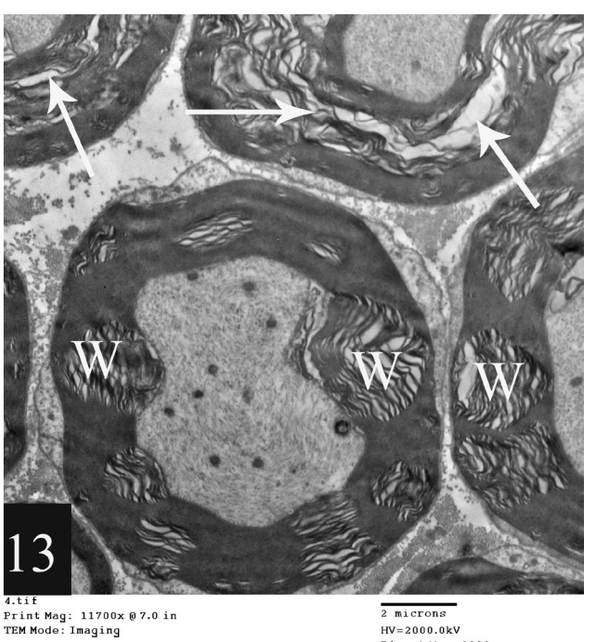


Fig. 13: A photomicrograph of an ultrathin section of sciatic nerve of induced diabetic group (subgroup IIIb) showing multiple axons with extensive splitting of the myelin sheaths lamellae (arrows). Other myelinated axons are showing multiple focal splitting in the form of whorls (W). (X 2000).

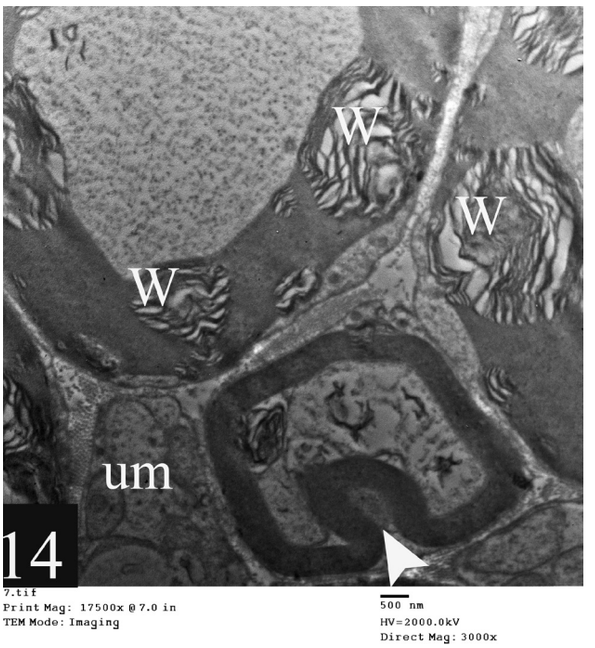


Fig. 14: A photomicrograph of an ultrathin section of sciatic nerve of induced diabetic group showing multiple focal splitting in the myelin sheaths lamellae in the form of whorls (W). An attenuated myelin sheath has deep invagination (infolding) (arrow head) with decrease in the density of the axoplasm contents. Notice: the unmyelinated nerve fibers (um) present inbetween the myelinated ones. (X 3000).

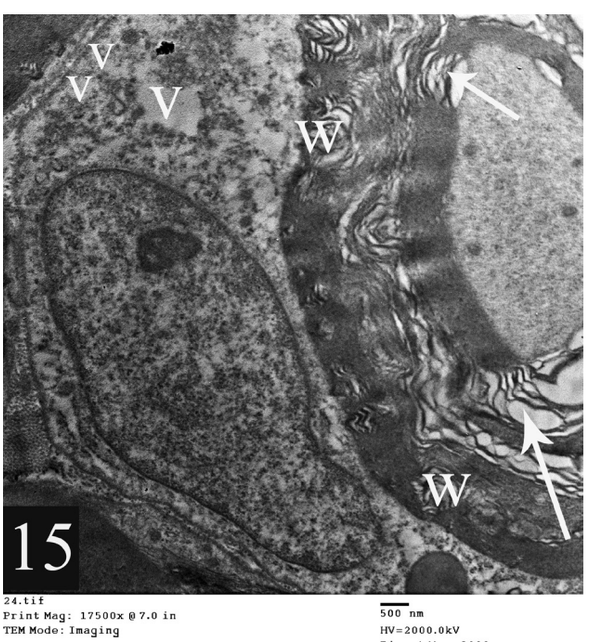


Fig. 15: A photomicrograph of an ultrathin section of sciatic nerve of induced diabetic group showing extensive splitting of the myelin sheath lamellae (arrows). Cytoplasm of Schwann cell contains cytoplasmic vacuolation (V) (X 3000). Note the myelin whorls (W). (X 3000)

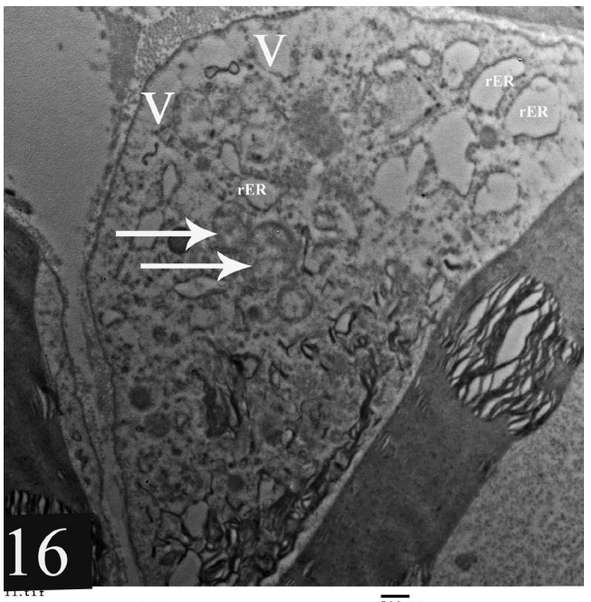


Fig. 16: A photomicrograph of an ultrathin section of sciatic nerve of induced diabetic group showing the cytoplasm of Schwann cell contains peripheral cytoplasmic vacuolation (V), dilated rER (rER) and swollen disrupted mitochondria (arrows). (X 3000).

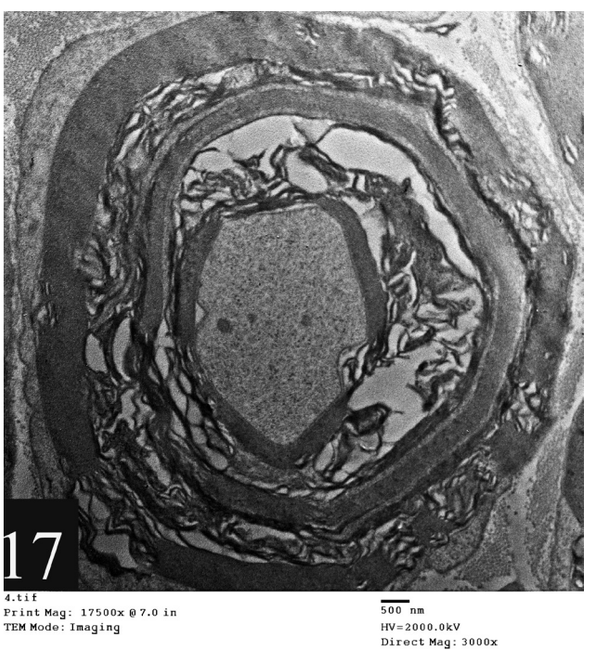


Fig. 17: A photomicrograph of an ultrathin section of sciatic nerve of induced diabetic group showing extensive splitting of myelin sheath appearing as one fiber engulfing another one (X 3000)

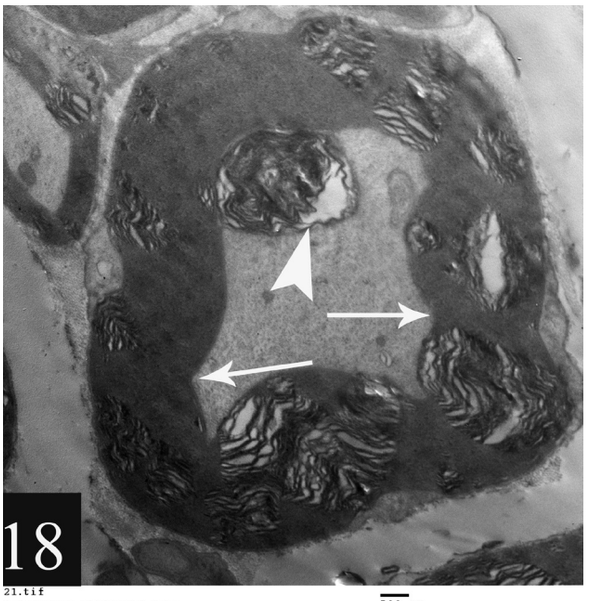


Fig. 18: A photomicrograph of an ultrathin section of sciatic nerve of induced diabetic group showing myelinated axon with rounded myelin fragment (arrowhead) in the axoplasm. Notice irregular outline of the axoplasm (arrows). (X 3000)

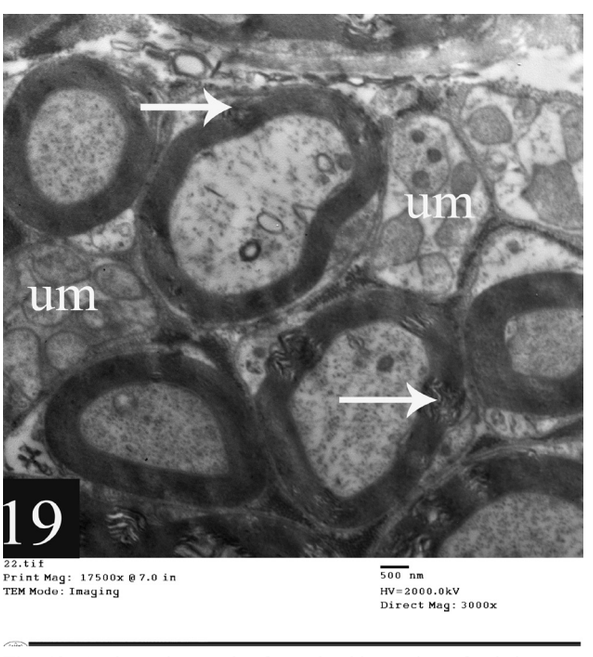


Fig. 19: A photomicrograph of an ultrathin section of sciatic nerve of induced diabetic+Hesperidin group showing more or less normal myelinated nerve fibers with few areas of minimal focal splitting of the myelin sheaths lamellae (arrows). Notice: the unmyelinated nerve fibers (um) present inbetween the myelinated ones. (X 3000).

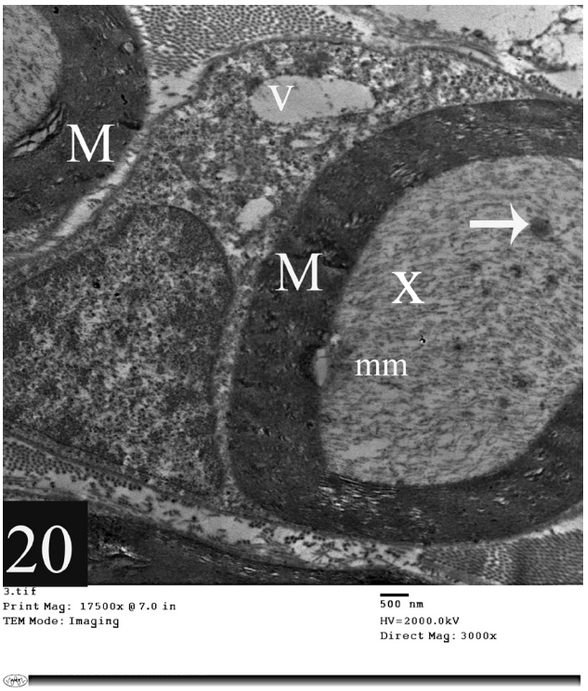


Fig. 20: A photomicrograph of an ultrathin section of sciatic nerve of induced diabetic+Hesperidin group showing myelinated nerve fiber with nearly intact and regular myelin (M) sheath. The axoplasm (X) contains intact mitochondria (arrow) and numerous microtubules and microfilaments (mm). The cytoplasm of Schwann cell is seen around the myelin sheath and contains few cytoplasmic vacuolation (V). (X 3000).

Table 1: Biochemical parameters of the studied groups

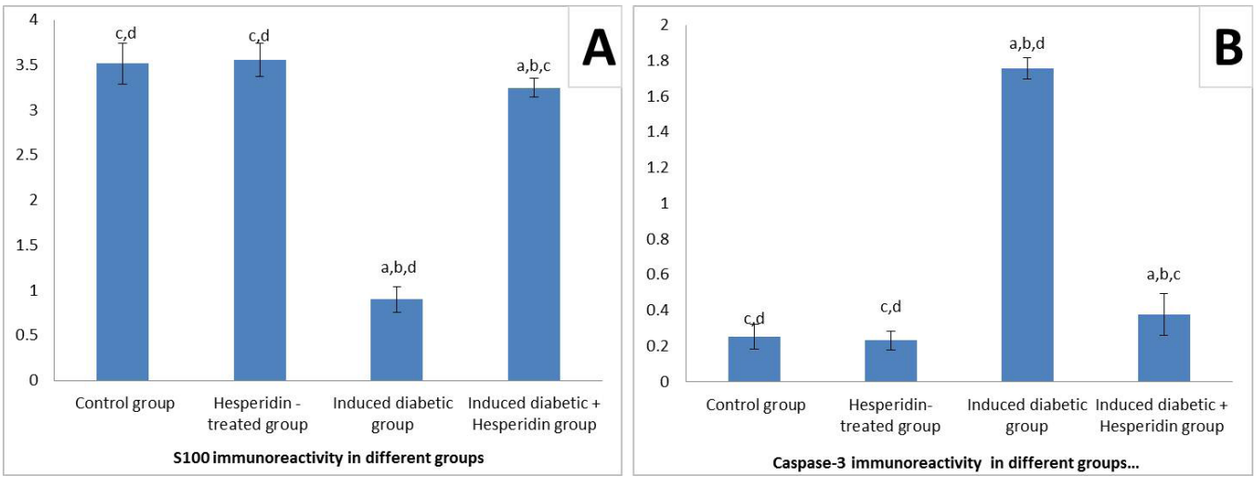
	Control group (Group I)	Hesperidin- treated group (Group II)	Induced diabetic- group (Subgroup IIIa)	Induced diabetic+ Hesperidin group (Subgroup IIIb)
Blood glucose level (mg/dl)	87.882 ± 2.587	91.082±3.678	271.994±4.615*	129.11±1.741*
Plasma insulin level (mU/ml)	17.415±0.725	16.91±1.055	7.542±0.801*	14.562±0.616*

Data are expressed as mean ± standard deviation. *$P < 0.05$ is significant value versus control group.

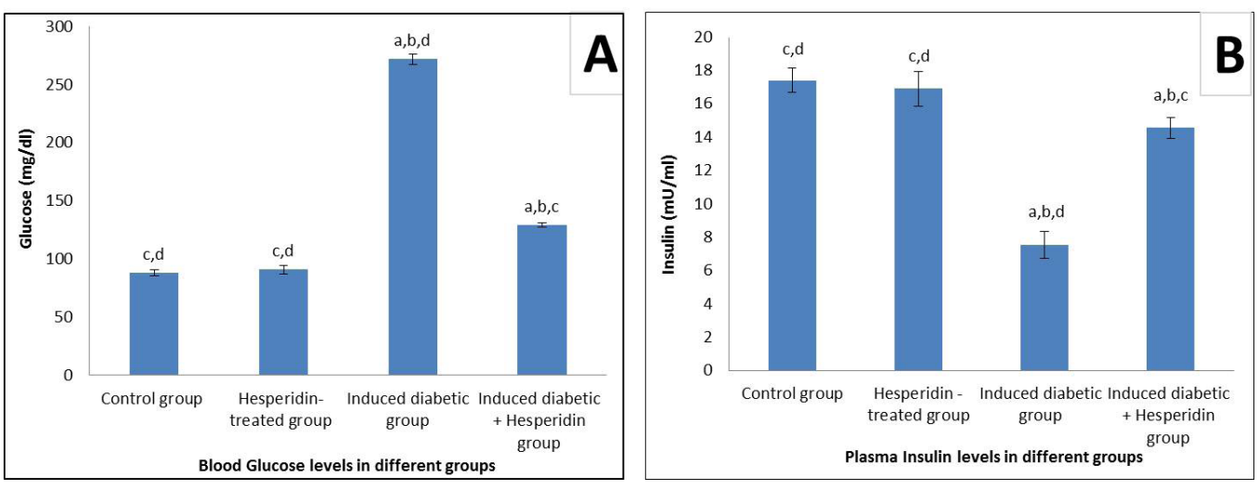
Table 2: Morphometric analysis of the studied groups

	Control group (Group I)	Hesperidin- treated group (Group II)	Induced diabetic- group (Subgroup IIIa)	Induced diabetic+ Hesperidin group (Subgroup IIIb)
Mean area percentage of S100 positive immunoreaction positive cells	3.516±0.2397	3.558±0.194	0.903±0.151*	3.247±0.109
Mean area percentage of caspase-3 immunoreaction positive cells	0.254±0.0747	0.232±0.0561	1.758±0.0649*	0.379±0.1234

Data are expressed as mean ± standard deviation *$P < 0.05$ is significant value versus control group



Histogram 2: Showing A) mean area percentage of S100 protein positive immunohistochemical staining , B) mean area percentage of caspase-3 positive immunohistochemical staining in different studied groups, results are expressed as mean ± SD (n=10) Values are considered statistically significant at $P < 0.05$. a ($P< 0.05$) as compared to control group. b ($P< 0.05$) as compared to hesperidin-treated group. c ($P< 0.05$) as compared to induced diabetic group. d ($P< 0.05$)as compared to induced diabetic + Hesperidin group.



Histogram 1: Effect of hesperidin on A) blood glucose levels, B) plasma insulin levels in STZ-induced diabetic rats, results are expressed as mean ± SD (n=10). Values are considered statistically significant at $P < 0.05$. a ($P< 0.05$) as compared to control group. b ($P< 0.05$) as compared to hesperidin-treated group. c ($P< 0.05$) as compared to induced diabetic group. d ($P< 0.05$) as compared to induced diabetic + Hesperidin group.

DISCUSSION

In our work, the administration of hesperidin in the diabetic rats for a period of 4 weeks decreased significantly the elevation in the blood glucose levels and also increased the levels of plasma insulin compared with untreated diabetic rats. This finding could be related to the ability of hesperidin to exert an anti-diabetic effect through improving the function of pancreatic β-cells, inhibiting oxidative stress, as well as inflammatory cytokine-mediated pancreatic β-cell death[28].

In this research, H&E-stained sections of diabetic group showed abnormal architecture of sciatic nerve fascicles with severely disorganized nerve fibers. Most of the nerve fibers were widely discrete from each other with the presence of dilated blood vessels and inflammatory cellular infiltration. Moreover, some sections revealed areas of focal loss of neurilemmal sheath in some axons. These results come in agreement with many researchers[5,29,30].

Streptozotocin injection leads to glucose toxicity followed by induction of oxidative stress[3]. Moreover, the state of hyperglycemia after STZ injection leads to increase of advanced glycosylation end-products (AGE) in nervous tissue and results in generation of free radical and its subsequent damage[19]. The degeneration of the nerve fibers could be related also to the accumulated inflammatory cytokines as IL-1β and TNF-α[30]. Additionally, STZ induces the release of high levels of Nitric oxide (NO) being involved in neuronal damage via reacting with superoxide anions causing protein nitration, lipid peroxidation, DNA damage and neuronal apoptosis[31,32].

Schwann cells are the glial cells which provide support for axonal maturation and regeneration in the peripheral nervous system[33,34]. S100 protein is considered a specific indicator of mature Schwann cells; its expression indicates proliferation of Schwann cells and myelin sheath formation[35,36]. Many studies cleared that proliferating Schwann cells boost continuous regeneration and functional recovery of the sciatic nerve[37].

In our study, the expression of S100 protein in Schwann cells showed significant decrement in untreated diabetic group compared to the control group. The decrease of the distribution of S100 protein in Schwann cells could be related to hyperglycemia maintaining oxidative stress state particularly in Schwann cells[38].

Furthermore, induced diabetic group showed a significant increment in the mean area percentage of caspase-3 positive immunoreaction in Schwann cells in comparison to the control group. This was in accordance with the results of a previous work[5]. It is known that Schwann cells apoptosis with subsequent damage of myelin sheath is considered the main mechanism of diabetic induced peripheral neuropathy[39]. The hyperglycemic state induces apoptosis in different cells, through reactive oxygen species (ROS) production with subsequent increase in the activity of nuclear factor-kappa B (NF-KB). Additionally, elevated blood glucose levels causes depolarization of mitochondrial membrane with loss of uncoupling proteins (UCP), increasing ROS, with subsequent release of cytochrome c and activation of caspases[40,41].

Electron microscopic finding of this work confirmed the light microscopic results. Induced diabetic group revealed multiple axons with extensive splitting of the myelin sheaths lamellae, while other myelinated axons showed multiple focal splitting in the form of whorls. Similar changes were previously described[42]. These changes could be related to axonal degeneration, primary demyelination after dysfunction of Schwann cells and secondary segmental demyelination results from impairment of the control of myelination of axons with leanness of bands of Schwann cells[43]. Moreover, some fibers appeared as one fiber engulfing another. These results coincided with the results of some researchers[44,45]. The latter attributed these changes to different possible neurotoxic mechanisms including toxic metabolites, apoptosis, interference with DNA and metabolic function.

Furthermore, the diabetic group showed myelinated axons with round myelin fragment in the axoplasm and some fibers with attenuated myelin sheaths with deep invagination and decrease densities of their axoplasmic contents. Similar changes were previously described by some authors who attributed these changes to the course of cell activation in response to toxic injury of nerve tissue[46]. In addition, these findings were attributed to the disturbance of neuronal antioxidant balance, as a consequence of chronic hyperglycemia, generation of ROS, which lead to destruction of cellular components, especially proteins and lipids[47].

Additionally, the cytoplasm of many Schwann cells of the untreated diabetic rats contained numerous vacuolation, dilated rER and swollen disrupted mitochondria. These changes are a consequence of inflammation involved the endoplasmic reticulum together with metabolic alterations in Schwann cells. Therefore, activation of a demyelinating phenotype of Schwann cells and macrophages mediate the myelin sheath splitting and the myelin figures formation via their role in myelin clearance[48].

Administration of hesperidin after diabetic induction for 4 weeks, evidently improved the screened biological parameters and the architecture of sciatic nerve fascicles. Furthermore, hesperidin helped normal Schwann cells proliferation and myelin sheath formation as well as decreased significantly the Schwann cells apoptosis. These findings were similar to many previous studies[7,49]. These results could be related to the protective role of hesperidin in reduction of NO and TNF-α and IL-1β levels in sciatic nerve[40]. Furthermore, the anti-apoptotic effect of hesperidin is owing to its ability to modulate the expression of NF-KB and pro-inflammatory cytokines as well as apoptotic markers Bax, Bcl2 and caspase-3 in nervous tissue[50,51]. Also, it was reported that hesperidin induced direct neuroprotection through its antioxidant properties[52] and by decreasing the levels of HbA1c[19].

On the ultrastructure level, hesperidin ameliorated the process of myelination. The myelinated nerve fibers appeared apparently normal. Despite few areas of minimal focal splitting in the myelin sheaths lamellae and vacuolation in the cytoplasm of some Schwann cells were still present. These findings were supported by the results of researchers previously reported the role of hesperidin in reduction of demyelination[53]. Another mechanism of hesperidin is to decrease the demyelination process by inhibition of autoimmune T cell proliferation, regulatory T cells activation and prevent reduction in microglial cells[54].

CONCLUSION

Hesperidin is beneficial in ameliorating the histological structural changes that occurred in sciatic nerve fibers after STZ induced diabetes mellitus. Hesperidin neuroprotective action on sciatic nerve structure is mediated through its antioxidant, anti-apoptotic and anti-inflammatory effects.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Ghule AE, Jadhav SS, Bodhankar SL. Effect of ethanolic extract of seeds of Linum usitatissimum (Linn.) in hyperglycaemia associated ROS production in PBMNCs and pancreatic tissue of alloxan induced diabetic rats. Asian Pacific Journal of Tropical Disease. 2012; 1:2(5):405-10. DOI:10.1016/S2222-1808(12)60088-7

2. Moree SS, Kavishankar GB, Rajesha J. Antidiabetic effect of secoisolariciresinol diglucoside in streptozotocin-induced diabetic rats. Phytomedicine. 2013; 20(3-4):237-45. DOI: 10.1016/j.phymed.2012.11.011

3. Callaghan BC, Little AA, Feldman EL, Hughes RA. Enhanced glucose control for preventing and treating diabetic neuropathy. Cochrane database of systematic reviews. 2012; (6). DOI: 10.1002/14651858.CD007543.pub2

4. Tesfaye S, Selvarajah D. Advances in the epidemiology, pathogenesis and management of diabetic peripheral neuropathy. Diabetes/metabolism research and reviews. 2012; 28:8-14. DOI: 10.1002/dmrr.2239

5. Nasiry D, Ahmadvand H, Talebpour Amiri F, Akbari E. Protective effects of methanolic extract of Juglans regia L. leaf on streptozotocin-induced diabetic peripheral neuropathy in rats. BMC complementary and alternative medicine. 2017;17(1):1-1. DOI: 10.1186/s12906-017-1983-x

6. Pandhare RB, Sangameswaran B, Mohite PB, Khanage SG. Attenuating effect of seeds of Adenanthera pavonina aqueous extract in neuropathic pain in streptozotocin-induced diabetic rats: an evidence of neuroprotective effects. Revista Brasileira de Farmacognosia. 2012;22:428-35. https://doi.org/10.1590/S0102-695X2012005000008

7. Li K, Shi X, Luo M, Wu P, Zhang M, Zhang C, Li Q, Wang Y, Piao F. Taurine protects against myelin damage of sciatic nerve in diabetic peripheral neuropathy rats by controlling apoptosis of schwann cells via NGF/Akt/GSK3β pathway. Experimental cell research. 2019 ;383(2):111557. DOI: 10.1016/j.yexcr.2019.111557

8. Ismail CA, Long I. Models of Diabetes in Rats: A Focus on Diabetic Neuropathy and Biomarkers. InBiomarkers in Diabetes 2022: 1-23. Cham: Springer International Publishing. DOI:10.1007/978-3-031-08014-2_56

9. Yin DH, Liang XC, Zhao LI, Zhang H, Sun Q, Wang PY, Sun LQ. Jinmaitong decreases sciatic nerve DNA oxidative damage and apoptosis in a streptozotocin-induced diabetic rat model. Experimental and Therapeutic Medicine. 2015; 10(2):778-86. DOI: 10.3892/etm.2015.2543

10. Parhiz H, Roohbakhsh A, Soltani F, Rezaee R, Iranshahi M. Antioxidant and anti-inflammatory properties of the citrus flavonoids hesperidin and hesperetin: an updated review of their molecular mechanisms and experimental models. Phytotherapy Research. 2015;29(3):323-31. DOI: 10.1002/ptr.5256

11. Alu'Datt MH, Rababah T, Alhamad MN, Al-Mahasneh MA, Ereifej K, Al-Karaki G, Al-Duais M, Andrade JE, Tranchant CC, Kubow S, Ghozlan KA. Profiles of free and bound phenolics extracted from Citrus fruits and their roles in biological systems: content, and antioxidant, anti-diabetic and anti-hypertensive properties. Food & function. 2017;8(9):3187-97. doi.org/10.1039/C7FO00212B

12. Menze ET, Tadros MG, Abdel-Tawab AM, Khalifa AE. Potential neuroprotective effects of hesperidin on 3-nitropropionic acid induced neurotoxicity in rats. Neurotoxicology 2012;33:1265-75. DOI: 10.1016/j.neuro.2012.07.007

13. Antunes MS, Goes AT, Boeira SP, Prigol M, Jesse CR. Protective effect of hesperidin in a model of Parkinson's disease induced by 6-hydroxydopamine in aged mice. Nutrition 2014;30:1415-22. DOI: 10.1016/j.nut.2014.03.024

14. Sawikr Y, Yarla NS, Peluso I, Kamal MA, Aliev G, Bishayee A. Neuroinflammation in Alzheimer's disease: the preventive and therapeutic potential of polyphenolic nutraceuticals. Adv Protein Chem Struct Biol 2017;108:33-57. DOI: 10.1016/bs.apcsb.2017.02.001

15. Carballo-Villalobos AI, González-Trujano ME, Alvarado-Vázquez N, López-Muñoz FJ. Pro-inflammatory cytokines involvement in the hesperidin antihyperalgesic effects at peripheral and central levels in a neuropathic pain model. Inflammopharmacology. 2017;25(2):265-9. DOI: 10.1007/s10787-017-0326-3

16. Zhang Y, Wang B, Guo F, Li Z, Qin G. Involvement of the TGFβ1-ILK-Akt signaling pathway in the effects of hesperidin in type 2 diabetic nephropathy. Biomedicine & Pharmacotherapy. 2018;105:766-72. DOI: 10.1016/j.biopha.2018.06.036

17. Lenzen S: The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008; 51(2): 216–226. DOI: 10.1007/s00125-007-0886-7

18. Damasceno DC1, Netto AO2, Iessi IL2, Gallego FQ2, Corvino SB2, Dallaqua B2, Sinzato YK2, Bueno A2, Calderon IM2, Rudge MV2. 2014. Streptozotocin-induced diabetes models: pathophysiological mechanisms and fetal outcomes.Biomed Res Int. 2014;2014:819065. doi: 10.1155/2014/819065. Epub May 27. DOI: 10.1155/2014/819065

19. Visnagri A, Kandhare AD, Chakravarty S, Ghosh P, Bodhankar SL. Hesperidin, a flavanoglycone attenuates experimental diabetic neuropathy via modulation of cellular and biochemical marker to improve nerve functions. Pharmaceutical biology. 2014;52(7):814-28. DOI: 10.3109/13880209.2013.870584

20. Mohan C. A guide for the preparation and use of buffers in biological systems. Calbriochem R, EMD Biosciences INC. 2003.

21. Jin HY, Lee KA, Song SK, Liu WJ, Choi JH, Song CH, Baek HS, Park TS. Sulodexide prevents peripheral nerve damage in streptozotocin induced diabetic rats. European journal of pharmacology. 2012;674 (2-3):217-26. DOI: 10.1016/j.ejphar.2011.05.059

22. Solmaz V, Çınar BP, Yiğittürk G, Özlece HK, Eroglu HA, Tekatas A, Erbaş O, Taşkıran D. Neuroprotective effects of octreotide on diabetic neuropathy in rats. Biomedicine & pharmacotherapy. 2017;89:468-72. DOI: 10.1016/j.biopha.2017.02.027

23. Gaertner DJ, Hallman TM, Hankenson FC, Batchelder MA. Anesthesia and analgesia for laboratory rodents. In: Fish, R.E., Danneman, P.J., Brown, M., Karas AZ, editor. Anesthesia and Analgesia in Laboratory Animals. 2nd ed. Elsevier Academic Press, London (UK); 2008:239–97.

24. Bancroft JD and Gamble M. Theory and practice of histological techniques. 6th edition, Elsevier health science. 2008; 126- 127.

25. Ramos-Vara J. A., Kiupel M., Baszler T., Bliven L., Brodersen B., Chelack B., West K., Czub S., Del Piero F., Dial S., Ehrhart E. J., Graham T., Manning L., Paulsen D. and Valli V.E.: Suggested guidelines for immunohistochemical techniques in veterinary diagnostic laboratories. J Vet Diagn Invest. 2008; 20: 393–413. doi.org/10.1177/10406387080200040

26. Kabiraj, A., Gupta, J., Khaitan, T., Bhattacharya, P. Principle and techniques of immunohistochemistry- A review. Int. J. Biol. Med. Res. 2015; 6 (3), 5204–5210. CorpusID:3893346

27. Bozzola J and Russe L. Electron microscopy principles and techniques for biologists. 2nd ed. London: Toronto, 1999; 16–39. DOI: 10.4236/msa.2015.611094

28. Hanchang W, Khamchan A, Wongmanee N, Seedadee C. Hesperidin ameliorates pancreatic β-cell dysfunction and apoptosis in streptozotocin-induced diabetic rat model. Life sciences. 2019;235:116858. DOI: 10.1016/j.lfs.2019.116858

29. Kiasalari Z, Rahmani T, Mahmoudi N, Baluchnejadmojarad T, Roghani M. Diosgenin ameliorates development of neuropathic pain in diabetic rats: involvement of oxidative stress and inflammation. Biomedicine & Pharmacotherapy. 2017;86:654-61. DOI: 10.1016/j.biopha.2016.12.068

30. Dhaliwal J, Dhaliwal N, Akhtar A, Kuhad A, Chopra K. Beneficial effects of ferulic acid alone and in combination with insulin in streptozotocin induced diabetic neuropathy in Sprague Dawley rats. Life Sciences. 2020;255:117856. DOI: 10.1016/j.lfs.2020.117856

31. Kajal A, Singh R. Coriandrum sativum improve neuronal function via inhibition of oxidative/nitrosative stress and TNF-α in diabetic neuropathic rats. Journal of ethnopharmacology. 2020;263:112959. DOI: 10.1016/j.jep.2020.112959

32. Singh R, Rao HK, Singh TG. Neuropathic pain in diabetes mellitus: challenges and future trends. Obesity Medicine. 2020;18:100215. DOI:10.1016/j.obmed.2020.100215

33. Campana WM. Schwann cells: activated peripheral glia and their role in neuropathic pain. Brain Behav Immun. 2007; 21:522–527. doi: 10.1016/j.bbi.2006.12.008

34. Griffin JW, Thompson WJ. Biology and pathology of nonmyelinating Schwann cells. Glia 2008; 56:1518-1531. doi: 10.1002/glia.20778.

35. Liu Y, Liu Y, Cao J, Zhu X, Nie X, Yao L, Chen M, Cheng X, Wang Y. Upregulated expression of Ebp1 contributes to schwann cell differentiation and migration after sciatic nerve crush. Journal of Molecular Neuroscience. 2014; 54(4):602-13. doi: 10.1007/s12031-014-0331-6

36. Zhao Z, Li X, Li Q. Curcumin accelerates the repair of sciatic nerve injury in rats through reducing Schwann cells apoptosis and promoting myelinization. Biomedicine & Pharmacotherapy. 2017; 92:1103-10. doi: 10.1016/j.biopha.2017.05.099.

37. Adiwardhana IS, Suyasa IK, Astawa P, Ridia IM. Administration of mesenchymal stem cells after rat sciatic nerve defect reconstruction with amnion tube expresses higher s100 protein. Jurnal Orthopaedi dan Traumatologi Indonesia. 2018;1(3):26-31.

38. Yang X, Yao W, Shi H, Liu H, Li Y, Gao Y, Liu R, Xu L. Paeoniflorin protects Schwann cells against high glucose induced oxidative injury by activating Nrf2/ARE pathway and inhibiting apoptosis. Journal of ethnopharmacology. 2016; 185:361-9. doi: 10.1016/j.jep.2016.03.031

39. Pan H, Ding Y, Yan N, Nie Y, Li M, Tong L. Trehalose prevents sciatic nerve damage to and apoptosis of Schwann cells of streptozotocin-induced diabetic C57BL/6J mice. Biomedicine & Pharmacotherapy. 2018; 105:907-14. doi: 10.1016/j.biopha.2018.06.069

40. Vincent AM, Olzmann JA, Brownlee M, Sivitz WI, Russell JW. Uncoupling proteins prevent glucose-induced neuronal oxidative stress and programmed cell death. Diabetes. 2004; 53(3):726-34. doi: 10.2337/diabetes.53.3.726.

41. Allen DA, Yaqoob MM, Harwood SM. Mechanisms of high glucose-induced apoptosis and its relationship to diabetic complications. The Journal of nutritional biochemistry. 2005; 16(12):705-13. doi: 10.1016/j.jnutbio.2005.06.007

42. Zangiabadi N, Mohtashami H, Hojatipour M, Jafari M, Asadi-Shekaari M, Shabani M. The effect of Angipars on diabetic neuropathy in STZ-induced diabetic male rats: a study on behavioral, electrophysiological, sciatic histological and ultrastructural indices. The Scientific World Journal. 2014; 2014. doi: 10.1155/2014/721547

43. Said G. Diabetic neuropathy—a review. Nature clinical practice Neurology. 2007; 3(6):331-40. doi: 10.1038/ncpneuro0504.

44. Okasha EF. Effect of long term-administration of aspartame on the ultrastructure of sciatic nerve. Journal of microscopy and ultrastructure. 2016 Dec 1;4(4):175-83. doi: 10.1016/j.jmau.2016.02.001

45. Abd-El-Hafez AA. Effect of leflunomide on sciatic nerve of adult albino rats: a histological and immunohistochemical study. Egyptian Journal of Histology. 2014 Jun 1;37(2):258-68. DOI:10.1097/01.EHX.0000446588.04196.df

46. Balaha M, Kandeel S, Kabel A. Phloretin either alone or in combination with duloxetine alleviates the STZ-induced diabetic neuropathy in rats. Biomedicine & Pharmacotherapy. 2018; 101:821-32. doi: 10.1016/j.biopha.2018.02.135

47. Bagher Z, Ehterami A, Nasrolahi M, Azimi M, Salehi M. Hesperidin promotes peripheral nerve regeneration based on tissue engineering strategy using alginate/chitosan hydrogel: in *vitro* and in *vivo* study. International Journal of Polymeric Materials and Polymeric Biomaterials. 2021; 70(5):299-308. doi.org/10.1177/0883911522108

48. Park HT, Kim JK, Tricaud N. The conceptual introduction of the “demyelinating Schwann cell” in peripheral demyelinating neuropathies. Glia. 2019; 67(4):571-81. doi.org/10.1007/s40120-021-00315-7

49. Kassab AA, Elkaliny HH. The possible role of propolis in ameliorating paclitaxel-induced peripheral neuropathy in sciatic nerve of adult male albino rats. Egyptian Journal of Histology. 2017; 40(2):141-55. DOI: 10.21608/EJH.2017.4073

50. Sato K, Sato T, Ohno-Oishi M, Ozawa M, Maekawa S, Shiga Y, Yabana T, Yasuda M, Himori N, Omodaka K, Fujita K. CHOP deletion and anti-neuroinflammation treatment with hesperidin synergistically attenuate NMDA retinal injury in mice. Experimental Eye Research. 2021; 213:108826. doi.org/10.1016/j.exer.2021.108826

51. Yıldız MO, Çelik H, Caglayan C, Kandemir FM, Gür C, Bayav İ, Genç A, Kandemir Ö. Neuromodulatory effects of hesperidin against sodium fluoride-induced neurotoxicity in rats: Involvement of neuroinflammation, endoplasmic reticulum stress, apoptosis and autophagy. NeuroToxicology. 2022; 90:197-204. doi: 10.1016/j.neuro.2022.04.002

52. Nones J, Gomes FC. Hesperidin, a flavone glycoside, as mediator of neuronal survival. Neurochemical research. 2011;36(10):1776-84. doi: 10.1007/s11064-011-0493-3

53. Baradaran S, Moghaddam AH, Ghasemi-Kasman M. Hesperetin reduces myelin damage and ameliorates glial activation in lysolecithin-induced focal demyelination model of rat optic chiasm. Life sciences. 2018; 207:471-9. doi: 10.1016/j.lfs.2018.07.001.

54. Haghmorad D, Mahmoudi MB, Salehipour Z, Jalayer Z, Momtazi Brojeni AA, Rastin M, Kokhaei P, Mahmoudi M. Hesperidin ameliorates immunological outcome and reduces neuroinflammation in the mouse model of multiple sclerosis. J Neuroimmunol 2017;302:23-33. doi: 10.1016/j.jneuroim.2016.11.009.

الملخص العربى

دور الهسبريدين في تحسين التغييرات النسيجية للعصب الوركى فى الجرذان المصابة بداء السكرى المستحدث بمادة الستربتوزوتوسين : دراسة بالمجهر الضوئي والإلكتروني

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المقدمة: داء السكري هو اضطراب أيضي له سمية معروفة على الأعصاب الطرفية. مادة الهسبريدين هى فلافانون طبيعى لها خصائص مضادة للأكسدة و للالتهابات وواقية للأعصاب.

الهدف من الدراسة: تقييم دور الهسبريدين في تحسين التغيرات النسيجية للعصب الوركي الناتجة عن داء السكري المستحدث في ذكور الجرذان البيضاء البالغة.

مواد وطرق البحث: تم تقسيم أربعين من ذكور الجرذان البالغة إلى ثلاث مجموعات. المجموعة الأولى كمجموعة ضابطة ،وتلقت المجموعة الثانية هيسبيريدين (١٠٠ مجم / كجم / يوميا) عن طريق الفم لمدة ٤ أسابيع وتم إعطاء المجموعة الثالثة حقنة واحدة من الستربتوزوتوسين (٦٠ مجم / كجم) داخل الصفاق للحث على إحداث داء السكرى. ثم بعد ٤ أسابيع ، تم تقسيم الجرذان المصابة بداء السكري إلى مجموعتين فرعيتين: المجموعة الفرعية الأولى تركت دون علاج والمجموعة الفرعية الثانية تلقت الهسبريدين بجرعة مماثلة للمجموعة الثانية لمدة ٤ أسابيع أخرى. ثم تمت معالجة عينات العصب الوركى للدراسات النسيجية والهستوكيميائية المناعية.

النتائج: أظهرت الجرذان المصابة بداء السكري وغير المعالجة بالهيسبيريدين عدم أنتظام حاد فى الحزم العصبية للعصب الوركى مع فقدان البنية المنتظمة للألياف العصبية. وانخفض بشكل ملحوظ ظهور بروتين S١٠٠ في خلايا شوان مع تسجيل ارتفاع كبير في نسبة الموت المبرمج بها مقارنة بالمجموعة الضابطة. كشف الفحص المجهري الإلكتروني عن انفصال في طبقات المايلين مع وجود بعض أغشية المايلين الواهنة. كما لوحظ ظهور فجوات سيتوبلازمية واتساع الشبكة الاندوبلازمية الخشنة وتورم الميتوكوندريا لخلايا شوان. وفى المقابل أتضح أن إعطاء الهسبريدين خفف من جميع المتغيرات النسيجية السابقة، كما ساعد على انقسام خلايا شوان بصورة طبيعية وقلل من الموت المبرمج لها.

الأستنتاج: استطاع الهيسبيريدين تحسين التغييرات النسيجية للعصب الوركي الناتجة عن مرض السكري.