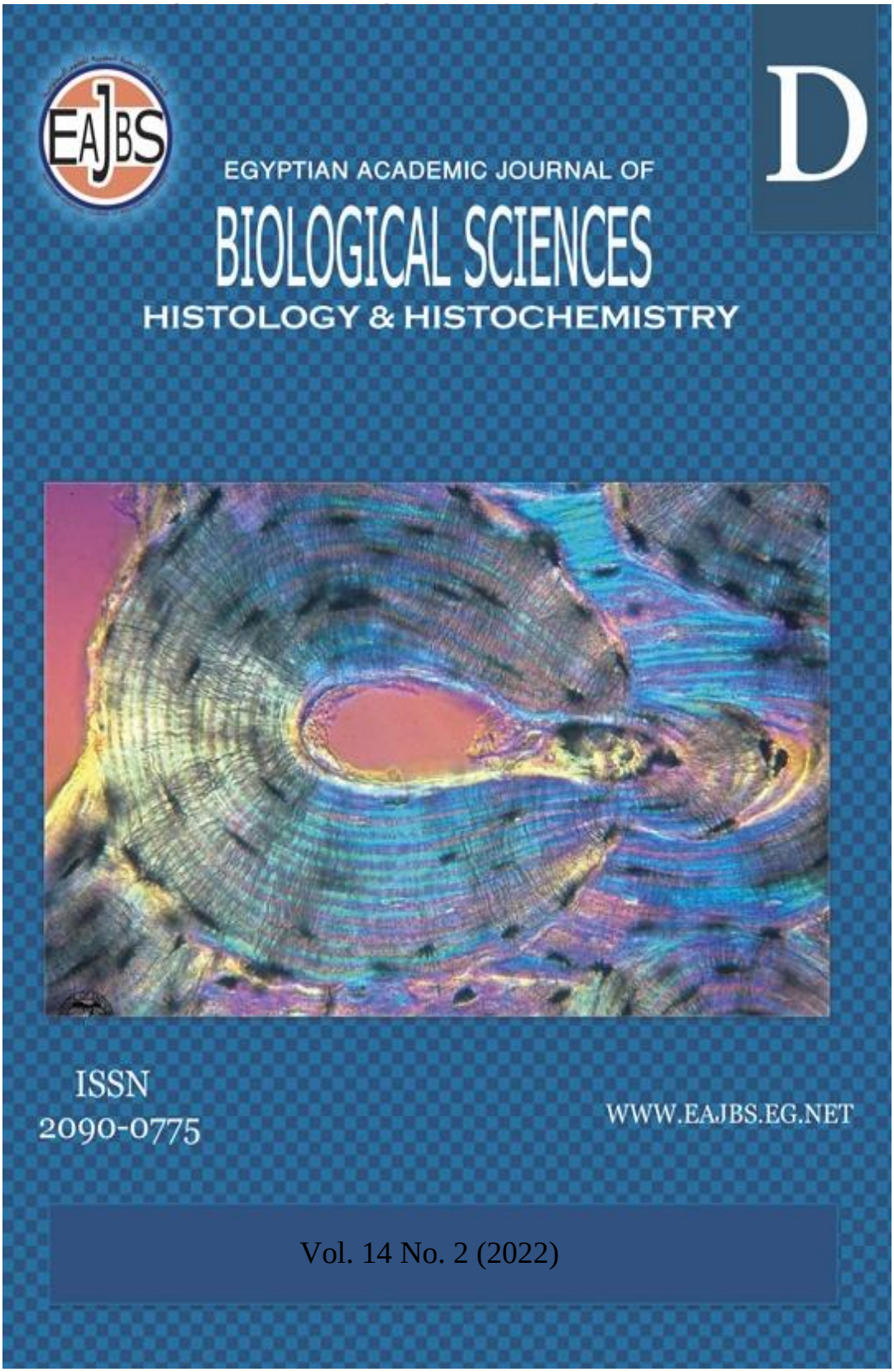

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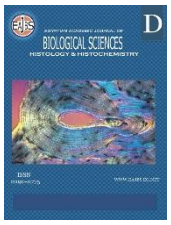

Sciatic Histological, Ultrastructural and Biochemical Investigations on Improving the Effect of Pregabalin by Flaxseed Oil on Diabetic Peripheral Neuropathy Induced in Albino Rats

Samah M. Arisha
Zoology Department, Faculty of Science, Menoufia University, Menoufia, Egypt
E-mail*: arishasamah@yahoo.com

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Diabetes, Sciatic nerve, Pregabalin, Oxidative stress markers, Antioxidants, Histology, Ultrastructure.

ABSTRACT

Background: The most common complication of diabetes mellitus is peripheral diabetic neuropathy associated with neurodegenerative effects. Food and Drug Association approved pregabalin as an anticonvulsant drug, for the treatment of diabetic neuropathy. There were many studies that reported no interest in the effect of this drug because it did not achieve complete recovery of diabetic neuropathy. So, this **study aimed** to improve its effect by combining its therapeutic dose with some medicinal plants such as flaxseeds. **Materials and Methods:** the experimental animals were divided into five groups. Group I, the control group, was kept without any treatment. Group II administered flaxseed oil (FXO) (1ml/kg body weight/day) for six weeks. A single intraperitoneal dose of alloxan (150 mg/kg b.w.) was used to induce diabetes, then the diabetic animals were divided into the following groups; Group III served as diabetic control. In Group IV the diabetic rats were supplemented with pregabalin (30mg/kg body weight /day) for six weeks. Group V diabetic rats were orally given pregabalin, and after2hours they were given the FXO for six weeks. **Results:** Diabetes significantly decreased the body weight, superoxide dismutase and catalase activities, and increased the malondialdehyde levels. Microscopic examination of the sciatic nerve of rats showed severe pathological and ultrastructural changes. When diabetic animals were treated with pregabalin only revealed mild improvement that recorded in the biochemical investigation only. However, the sciatic nerve examination of diabetic animals treated with pregabalin and FXO restored the normal histological and ultrastructural pictures and recorded a marked improvement in the biochemical parameters. **Conclusion:** Flaxseed oil improved the neuroprotective effect of pregabalin against diabetic peripheral neuropathy due to its anti-hyperglycemic, antioxidant and anti-inflammatory properties.

INTRODUCTION

Diabetes mellitus (DM) is a disease usually associated with long-term complications affecting metabolic disorders especially hyperglycemia that results from insulin deficiency (type 1) and/or insulin resistance (type 2) that is combined with neuropathy (American Diabetes Association, 2017). Abnormal function of the central or peripheral nervous system is associated with neuropathic pain that is driven by signaling from damaged or pathological neurons (Abdi *et al.*, 2004).

The most common complication of DM is diabetic neuropathy (DN) which occurs in almost 50 % of diabetic patients causing atrophy, demyelination of nerve axons and a high mortality rate. Therefore, controlling blood glucose levels improved these disabilities (Callaghan *et al*., 2012). Moreover, diabetes is associated with the increase in the generation of reactive oxygen species (ROS), which causes oxidative stress and is necessary for the onset of DN and other complications associated with diabetes (Premkumar and Pabiddi 2013).

Pregabalin is a structural homologue of the inhibitory transmitter gamma-aminobutyric acid that produced a disruption of calcium channels and inhibits the release of the excitatory neurotransmitter, leading to tonic inhibition of the over-excited neurons (Rajappa *et al.,* 2016). pregabalin was recommended by the United States Food and Drug Administration for the treatment of diabetic peripheral neuropathic pain, injury of the spinal cord, post-herpetic neuralgia, and fibromyalgia and as a complementary treatment for partial-onset seizures (Etemad, *et al.*, 2013; Morse, 2016). Recently, pregabalin is the first line of treatment for DN pain at a dose ranging from 150-600mg/day and its analgesic effect appears in the first few days of treatment and is sustained over time (Ungard *et al.*, 2020).

Linum usitatissimum, flaxseeds or linseeds, is an annual plant one of the family Linaceae, that grows in all countries as a dual plant for both fiber and seeds oil (Varghese et al., 2017). Flaxseed oil (FXO) contains high levels of omega-3 (α-linolenic) and omega-6 (linoleic) fatty acids that elevate their quality exert potential health benefits and have many pharmacological and therapeutic effects. Such properties were the antidiabetic, antioxidant, anti-inflammatory, anti-cancer, immunomodulatory, and neurological effects and improving the metabolic syndromes (Newairy and Abdou 2009; Hendawi *et al.,* 2016). One of FXO components is the α-linolenic acid, which has a significant impact on the integrity of cell membrane and protects s-pancreatic cells. It stimulates calcium levels and insulin release by influencing the functions of protein kinase and cyclic monophosphate adenosine. Secoisolariciresinaol, the major FXO-lignanes, naturalizes free radicals and controls DM incidence and improper future outcomes (Karaca and Eraslan, 2013; Maghsoudi, 2016). According to data from gas-liquid chromatography of fatty acid methyl esters, Hendawi *et al*. (2016) reported that FXO purchased from the company El-capten includes large concentrations of the unsaturated fatty acids C18:1 oleic (45.63%), C18:3 -linolenic (8.93%), and C18:2 linoleic (4.19%). Additionally, it has been stated that FXO is the most popular edible oil in the world and is highly recommended as a food supplement (Ye *et al.,* 2019).

Many studies have been made about pregabalin and approximately all of them focus on its anti-epileptic role, neuropathy and its relation with pain. Whereas, there were few studies done on how to improve its effects in diabetic patients. Thus, the purpose of this study was aimed to determine the effect of adding FXO to pregabalin in the treatment of diabetic neuropathy. This can be approved in the present work on the sciatic nerve of diabetic rats by histological, ultrastructural and biochemical studies.

MATERIAL AND METHODS

Chemicals:

Alloxan (2, 4, 5, 6-tetra oxypyrimidine; 2, 5, 6-pyrimidinetetrone) was purchased from Eldawlia Company, Tanta City, Egypt.

Pregabalin, [(S)-3-(aminomethyl)-5-methylhexanoic acid], under the trade name Lyrica, was obtained from Pfizer Company, Cairo, Egypt, as capsules, each one contains 75 mg of active constitutes. A fresh solution of pregabalin capsule contents was

prepared with distilled water and was administered every day for six weeks at a dose of 30 mg/kg body weight. (Chogtu *et al.*, 2011).

Flaxseed oil (FXO) *Linum usitatissimum* oil was bought from a local commercial company, El-Captain, El-Obour City, Cairo, Egypt. The FXO was orally administered to animals every day for six weeks at a dose of 1ml/kg body weight (Kaithwas and Majumdar, 2013).

Experimental Animals:

Forty healthy male albino rats (*Rattus norvegicus*), 120-140g, were purchased from Helwan farm, Ministry of Health, Cairo, Egypt. They were kept in typical rat cages and acclimatized, for one week before the beginning of experiments, at a controlled temperature (24 ± 2ºC) 12h dark/light cycle. A standard commercial pellets diet that the experimental animals fed was obtained from the Egyptian Company of Oils and Soap, Kafr-Elzayat, Egypt. The water was allowed *ad libitum.*

Induction of Diabetes:

A single intraperitoneal dose of alloxan (150 mg/kg body weight) dissolved in distilled water was used to induce diabetes (Ajibola *et al.*, 2014). Alloxan chemically established DM 72h after the treatment. Diabetes was defined using the determination of fasting blood glucose levels in rats by the glucometer. The animals became diabetic if the blood glucose levels were over 180mg/dl (Siddiqui *et al.*, 2011).

Experimental Design:

The normal (n = 16) and diabetic rats (n = 24) were separated into five experimental groups, eight animals each. The first group was kept as normal healthy control. The second one received the flaxseed oil (1ml/kg body weight/day) for six weeks. The third group contained diabetic control rats. The fourth group contained diabetic rats that were supplemented with pregabalin (30mg/kg body weight/day) for six weeks. The fifth group of diabetic rats was orally supplemented with pregabalin and after 2h they were given FXO for six weeks.

After six weeks, the final body weight of the control and treated groups were determined and then all rats fasted for 12h after the last dose and the levels of blood glucose were determined.

Investigated Parameters:

Histopathological and Ultrastructural Examinations:

For light and ultrastructural microscopic studies, perfusion fixative was done. Then the sciatic nerve from each leg was removed. Small sciatic nerve specimens (1.0 mm), obtained from each leg, were fixed in 2.5% gluteraldehyde solution for 24 hours, then the specimens were put in 1% osmium tetroxide in phosphate buffer (pH 7.4) for 2h as post-fixation treatment. Then samples were dehydrated in ascending series of ethanol (50% to 100%) and embedded in epoxy resin capsules. These capsules were put in an oven (60ºC) for 48h. Once hardened, the blocks were trimmed and the semi-thin sections (0.5µm) were cut and stained with toluidine blue and then examined with a light microscope, for histological study. Moreover, ultrathin sections were cut from the selected areas in the semi-thin sections and were stained with uranyl acetate and lead citrate**.** In the electron microscope unit, Faculty of Science, Alexandria University, Alexandria, Egypt, the ultrathin sections were examined with a 1400 plus-JSM transmission electron microscope (JEOL Ltd., Tokyo, Japan).

Biochemical Analysis:

A glucometer (Free Style Mini, manufactured by Abbott Diabetes Care Ltd. Range Road Witney, Oxon, Ox29OYL, UK) was used to measure the fasting blood glucose levels that were determined on day 0, initial and 72h after diabetes induction and at the end of the experiments.

Once the perfusion process was finished small pieces of each sciatic nerve were carefully removed and cleaned in ice-cold saline. Using an Ultra Turrax T25 homogenizer (Omni International, Kennesaw, GA, USA) the

sciatic specimens were homogenized in a cold 50 mM phosphate-buffered saline (pH 7.4). The homogenates of the sciatic nerves were then centrifuged at 20000 rpm for 15 minutes. For further investigation, the supernatants were collected and kept at -20°C. Malondialdehyde (MDA) levels were measured spectrophotometrically in sciatic tissue homogenates to determine lipid peroxidase using a method described by Ohkawa *et al.* (1979). Using diagnostic kits and a Shimadzu UV-1601 spectrophotometer, tissue Superoxide dismutase (SOD) activity was determined according to Sun *et al*. (1988) using the Ransod kit (Randox Laboratories GmbH, Deutschland). Tissue catalase (CAT) activity was determined according to Sigma-Aldrich kit (Spruce Street, St. Louis, MO 63103 USA) according to Aebi (1984).

Statistical Analysis:

The data were presented as mean ± standard deviation (mean ±SD). The one-way analysis of variance (ANOVA) test with post-hoc analysis was used to assess the significance of differences between the means. It was done with the help of the SPSS program, version 20 (Inc., Chicago, USA). At $P \leq 0.0001$ and $P \leq 0.05$, the results were considered highly significant and significant, respectively.

RESULTS

The Impact of Various Treatments on The Body Weight:

Data in Table 1 showed a significant decrease in the final body weights in rats treated with FXO compared with control ones ($P \leq 0.05$). However, the diabetic rats recorded a highly significant decrease ($P \leq 0.0001$) in the final body weight compared to the control group. On the other hand, the diabetic animals treated with pregabalin recorded non-significant changes in the final body weight compared to the diabetic ones. While animals were given pregabalin and FXO a highly significant increase ($P \leq 0.0001$) in the final body weight was recorded when compared with the diabetic ones.

Histological Observations:

The semi-thin sections examination of control animals and animals given FXO (Figs. 1a and b) showed the normal histological structure of the sciatic nerve. In these animals, the sciatic nerve appeared surrounded by epineurium which consists of dense connective tissue filling in between the nerve bundles. Each bundle is invested by a thin layer of dense connective tissue called perineurium and contains transverse sections of various-sized myelinated nerve fibers. Moreover, the endoneurium appeared in between nerve fibers formed of fine connective tissue. The nerve fibers presented as unmyelinated or myelinated ones. Axons of these fibers are shielded by a membrane known as axolemma while the axoplasm is referring to its cytoplasm. Schwann cells, a type of glial cell, appeared in between the nerve fibers and produce the fatty insulating material known as the myelin layer. Schwann cells are surrounded by a basal lamina and have euchromatic nuclei. Also, the blood vessels appeared.

Light microscopic examination of the sciatic nerve of diabetic rats (Fig. 2a-d) showed dramatic pathological features. Most of the nerve fibers appeared widely separated from each other and from perineurium with degenerative changes of myelin structure, including disruption of myelin density giving them a distorted appearance. Myelin debris, enfolding and out folding of the myelin sheath, irregular thickening of the myelin sheath, and a mild increase in inter-fiber space suggesting edema were observed. Moreover, prominent vacuolar degeneration in the cytoplasm of Schwann cells occasionally appeared. A relative increase in the fibrous component in the interstitial tissue and dilated blood vessels were also observed.

In diabetic animals treated with pregabalin (Fig. 3a), some fibers appeared with irregular myelin sheath with disruption of myelin density and

others appeared thick or swollen. Moreover, clusters of normal nerve fibers with thin myelin coats were observed. Diabetic animals treated with pregabalin and flaxseed oil showed an advanced degree of improvement including normal dark well compact myelinated nerve fibers inside endoneurium with normal pale stained Schwann cells. Few degenerated features still appeared as shrinkage and loss of myelin sheath (Fig. 3b).

Ultrastructure Observations:

Ultrathin sections examination of the specimens of the control and FXO groups revealed the normal ultrastructure of the sciatic nerve including myelin sheath, Schwann cells and interstitial tissue. Nerve fibers with normal myelin sheath consisting of compacted and regular myelin lamellae were observed. Outside the myelin sheath of these nerve fibers, Schwann cell appeared and its cytoplasm contains lipid droplets, small spherical mitochondria, numerous granular endoplasmic reticula, prominent nuclei, and neurotubules. The nerve fibers are separated from each other by collagen and other constituents of the endoneurium. The axoplasm of nerve fibers appeared homogenous with microtubules and few mitochondria. On the other hand, the non-myelinated axons appeared invaginated into the surface of Schwann cells (Figs. 4a-d).

The major lesion of ultrastructural alternations and severe degenerative features appeared in sections obtained from diabetic animals. In some areas, the nerve fibers appeared separated from each other by widening endoneurium exhibiting edema, in addition to collagen fibers. Moreover, shrunken and swollen axons were common; the myelin sheath breaks down with vacuolization and lamellar separation exhibiting extensive demyelination or focal lysis of the myelin sheath. Splitting of myelin lamellae, detachment or fragmentation of parts of the myelin sheath and invasion towards axoplasm were evident in this group. Loss of neurotubules and neurofilaments, swollen mitochondria, and altered granularity were all observed in the axoplasm. There were many vacuoles that contained the myelin debris which was engulfed by macrophages or within Schwann cells. The cytoplasm of these cells appeared vacuolated and contained degenerative organelles. Swelling of Schwann cells at certain areas resulting in dispersion of organelles was detected. Few collagen fibers among the myelinated fibers also appeared (Figs. 5a-e).

In the diabetic animals treated with pregabalin, a few of the ultrastructural changes were relatively improved when compared with the diabetic rats. Some fibers appeared with variable thickness of myelin sheath in spite of the presence of normal nerve fibers with regular compact myelin sheath and new thin myelinated ones and their axoplasm contained sparse small vesicles. Moreover, Schwann cells not associated with nerve fibers were found. Also, the fibrous components of interstitial tissue and unmyelinated nerve fibers were relatively increased (Figs. 6a and b).

On the other hand, most of the previously mentioned alterations in nerve fibers were markedly attenuated in diabetic animals treated with pregabalin and FXO. The myelin coat of some fibers was relatively thinner which may represent a newly formed one. Also, rich-organelle Schwann cells appeared except for some containing few remnants of phagocytic activity (Figs. 6c and d).

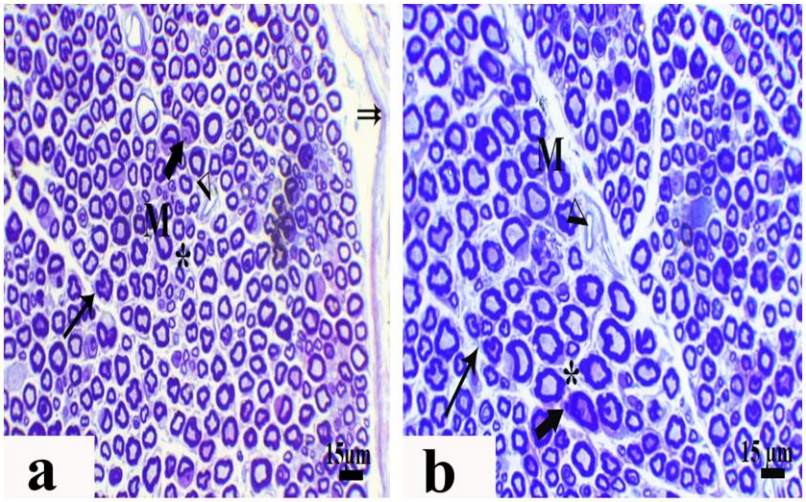

Fig.1: Photomicrographs of semi-thin sections in sciatic nerves from a control animal (a) and FXO - treated animal (b). Note, normal nerve surrounded by epineurium (double arrow), nerve bundles surrounded by perineurium (arrow), dark regular myelinated nerve fibers (M), Schwann cell (thick arrow), endoneurium (*) and blood vessel (arrowhead), (X 400).

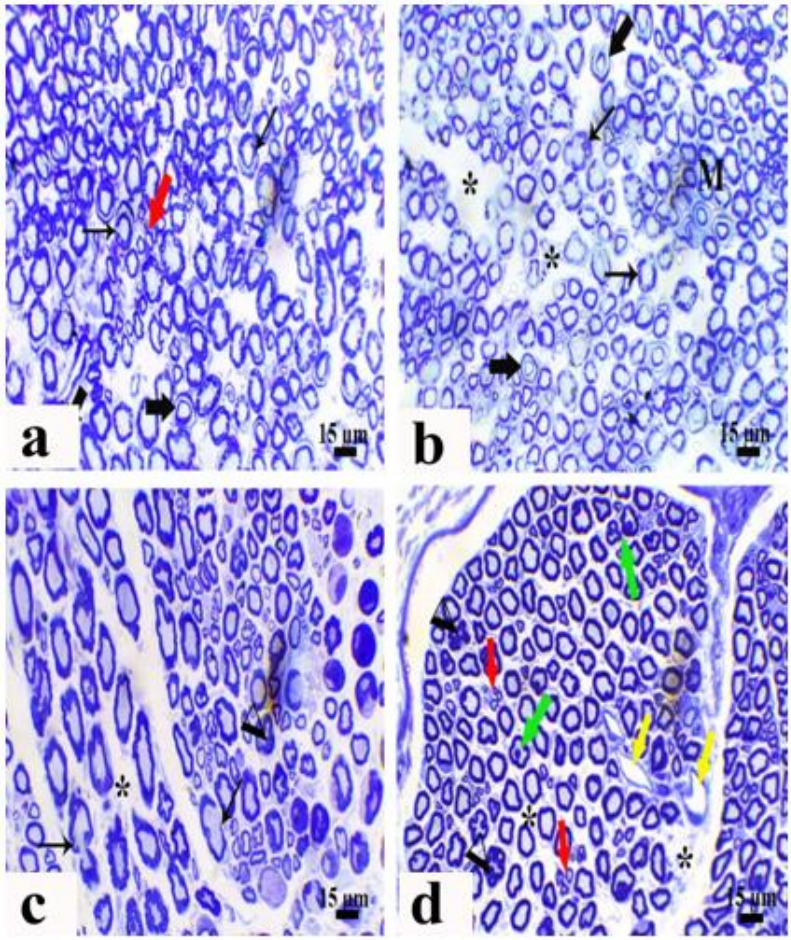

Fig. 2: Photomicrographs of semi-thin sections in sciatic nerve from diabetic animals (a - d) showing extensive myelin degeneration (M), variable thick of myelin sheath (thin arrows), complete demyelination of some nerve fibers (thick arrows), enfolding and out folding of myelin sheath (arrowheads), thin myelinated nerve fibers (red arrows), folding of myelin in axoplasm (green arrows), dilated blood vessels (yellow arrows) and increase of inter-fiber space, edema (*), (X 400).

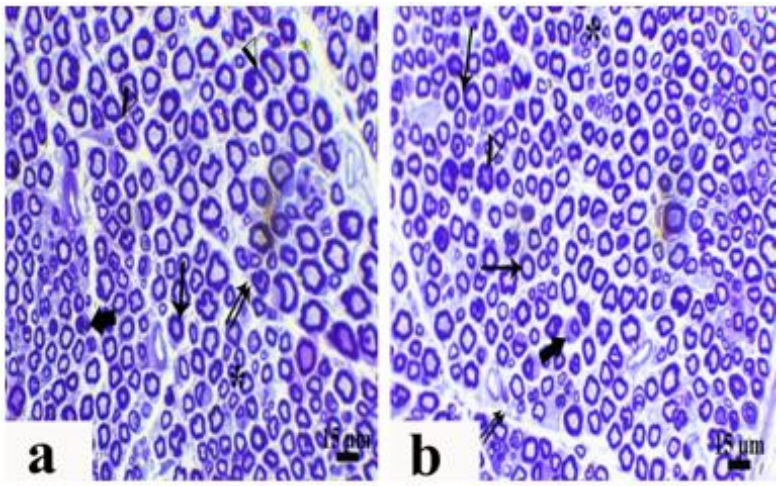

Fig. 3: Photomicrographs of semithin sections in sciatic nerve (a) from diabetic animals treated with pregabalin showing mild improvement with the presence of some degenerated mylemated nerve fibers (arrowhead), and (b) from a diabetic animal treated with pregabalin and FXO showing a marked improvement. The impact regular myelinated fibers (thin arrows) are found in large numbers, surrounded by perineurium (doubled arrows). New small and thin myelinated fibers (*) and normal Schwann cells (thick arrows) are present (X 400).

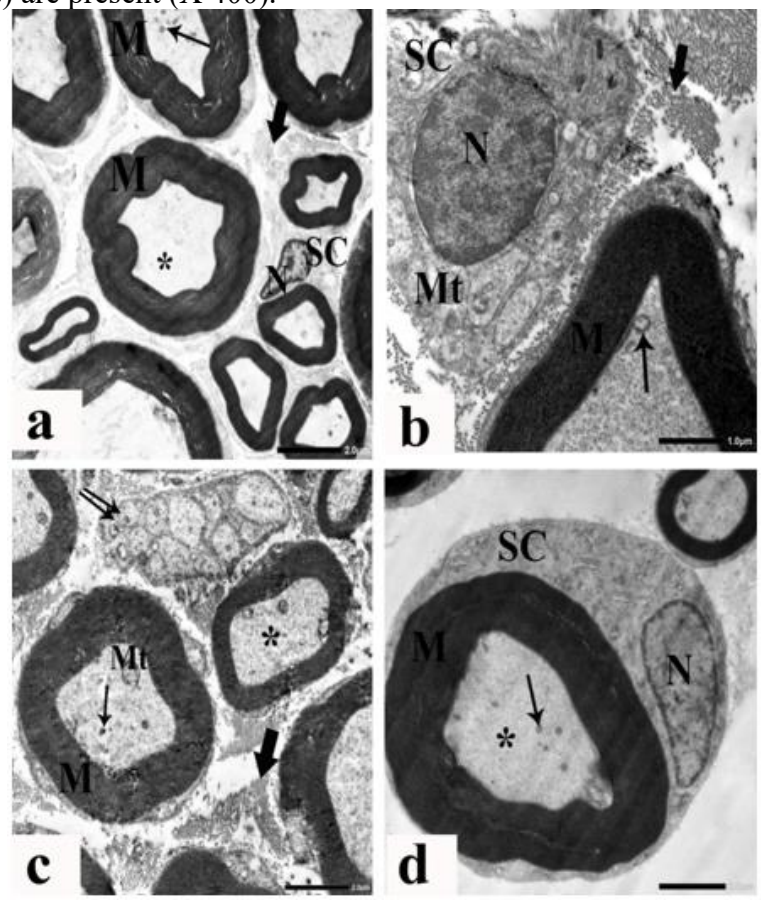

Fig. 4: Electron micrographs of sections in sciatic nerve from control animals (a & b) and FXO group (c & d) showing intact myelin sheath (M) of myelinated nerve fibers, homogenous axoplasm (*) contains microtubules (thin arrows) Schwann cell (SC) with vesicular nucleus (N), mitochondria (Mt), unmyelinated nerve fibers (doubled arrows) and collagen fibers (thick arrows), scale bar 2µm (X 3000), 1 µm (X 6000).

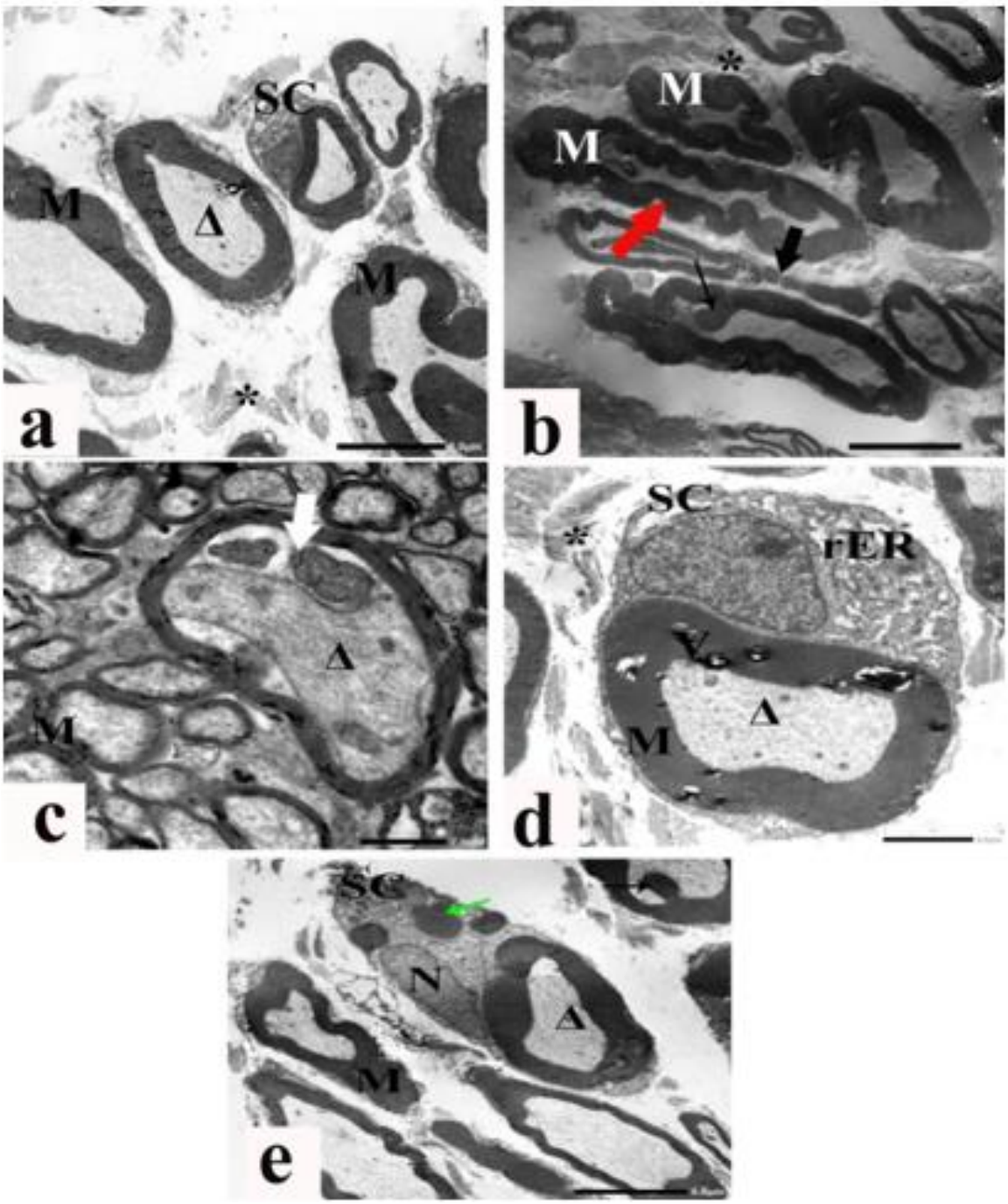

Fig. 5: Electron micrographs of the sciatic nerve from diabetic animals (a- e) showing variable thickness of myelinated nerve fibers (M), degenerated axolpasm (arrowhead), enfolding and out folding nerve fibers (red arrow), fragmentation of myelin sheath (thick arrow), expholiated within axolpasm (thin arrow), vacuolization of the myelin sheath (V), debris of myelin engulfed in Schwann cell (green arrow), few collagen fibers (*), degenerated Schawann cells (SC) contains dilated rough endoplasmic reticulum (rER). Scale bar 2µm (X 3000), 5µm (X 1500).

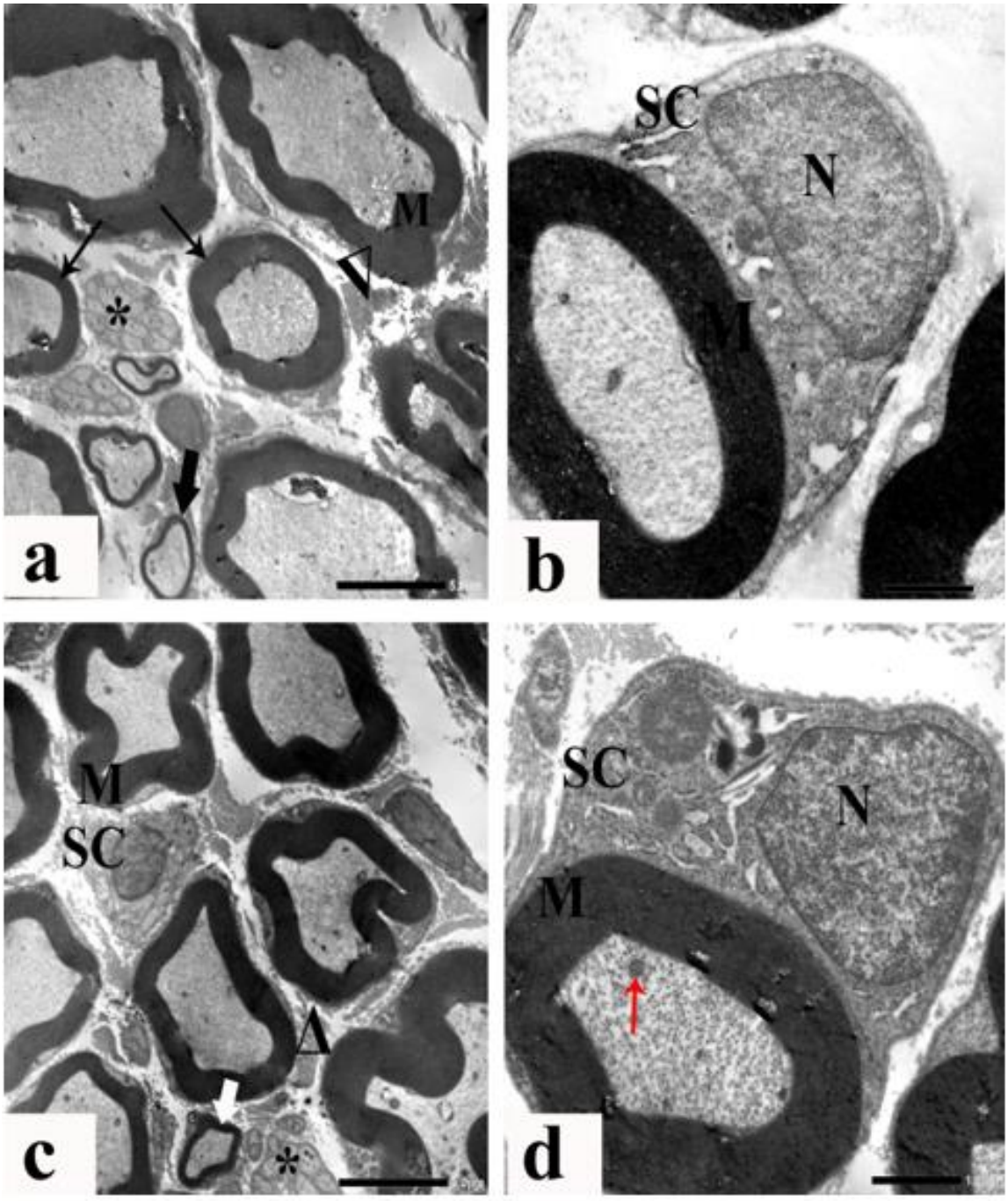

Fig. 6: Electron micrographs of the sciatic nerve of diabetic rats treated with pregabalin (a & b) and animals treated with pregabalin and FXO (c & d) showing, compact regular myelinated nerve fibers (thin arrows), new thin regular myelinated ones (thick arrows). The homogenous axoplasm contains microtubules (red arrow). Note, an increase in the thickness of myelin sheath (M), unmyelinated nerve fibers (*), collagen fibers (arrows heads) and Schwann cells (SC) with normal nucleus (N). Scale bar 5µm (X 1500), 1 µm (X 6000).

Biochemical Results:

Effects of Pregabalin and FXO on Blood Glucose Levels in Diabetic Male Rats:

Table 1 showed a non-significant difference in the blood glucose levels between all groups at baseline (initial glucose). Moreover, the diabetic animals recorded a highly significant increase in the glucose level compared to the control group ($P \leq 0.0001$). While diabetic animals given pregabalin showed a highly significant increase compared to the control group. While the treatment with pregabalin and FXO recorded highly significantly

reduce blood glucose levels compared to the untreated diabetic group ($P \leq 0.0001$).

Effects Of Pregabalin and FXO On the Sciatic MDA Level, SOD And CAT Activities in Diabetic Male Rats:

Data in Table 1 showed a non-significant difference in MDA level, SOD and CAT activities in the sciatic nerve of control and FXO- treated animals. While diabetic animals recorded a highly significant increase in MDA level and a highly significant decrease in SOD and CAT activities compared to the control group ($P \leq 0.0001$). The diabetic rats treated with pregabalin showed a significant increase in the MDA level and a significant decrease in SOD and CAT content compared with the control group ($P \leq 0.05$). Whereas, when diabetic animals were treated with pregabalin and FXO highly significant decrease in MDA level and a highly significant increase in SOD and CAT contents were recorded compared with the diabetic group ($P \leq 0.0001$).

Table 1: Effects of FXO and pregabalin and their combined usage on the body weight, level of blood glucose, malondialdehyde (MDA), and the activities of superoxide dismutase (SOD) and catalase (CAT) in the sciatic nerve of the different experimental groups.

Group	Initial b.w (g).	Final b.w. (g)	Initial blood glucose levels (mg/dL)	Final blood glucose levels (mg/dL)	MDA (µmol/g protein)	SOD (u/mg protein)	CAT (k/mg protein)
Control	129.2±3.64	145.8±8.29	136.2±19.16	135.6±12.02	15.0±2.02	78.0±10.65	84.4±9.017
FXO	130.2±4.03	120.3±7.07**	130.9±19.09	132.7±11.05	14.3±.2.05	80.1±10.54	86.5±9.098
Diabetes	125.6±7.83	99.2±6.38***	129.0±9.62	499.6±112.68***	31.9±4.95***	52.2±10.13***	28.4±6.731***
Diabetes+Pregabalin	128.4±6.47	101.0±10.34***a	130.2±23.89	233.0±55.63 ***a	22.9±3.17**a	63.4±7.43**a	49.0±12.550***a
Diabetes+Pregabalin +FXO	127.4±12.28	119.0±11.49**a	126.2±11.60	183.0±19.87***a	16.2±1.58a	72.0±11.00a	82.4±9.476a

The values are expressed as mean ± SD, n=8

(***): highly significant at $P \leq 0.0001$ compared to the control group.

(**): significant at $P \leq 0.05$ compared to the control group.

(a): highly significant at $P \leq 0.0001$ compared to the diabetic group.

DISCUSSION

The results of the present study recorded a highly significant decrease in the body weight of diabetic animals when compared to the control group which may be attributed to the metabolic disorders associated with diabetes. Balamurugan and gnacimuthu (2011) reported that diabetes-induced oxidative stress led to partial destruction of insulin β-cells and insulin deficiency that caused an extravagant breakdown of tissue proteins causing the observed loss in bodyweight that was associated with wasting muscle mass. Pregabalin treatment showed non-significant improvement in the body weight of diabetic rats. Similarly, Moustafa *et al.* (2018) found that treatment with pregabalin did not significantly affect the body weight of diabetic rats.

The histological results showed that the diabetes-induced severe histopathological changes of the sciatic nerve include loss of its regular architecture with degenerated nerve fibers, disruption of myelin density, degenerated Schwann cells and endometrium edema. These changes may be due to oxidative stress associated with diabetes disease resulting in neuropathy. The present study's findings were in agreement with the findings of Moustafa *et al.* (2018) who detected similar pathological alternations in the sciatic nerve tissue of diabetic rats. They confirmed that the noticeable nerve degeneration during hyperglycemia may be due to pancreatic β-cells partial devastation, overproduction of blood glucose and decreased utilization of glucose by tissues. Similarly, Farshid and Tamaddonfard (2015) found the same

histopathological changes in the sciatic nerve including fiber degeneration, edema, partial separation of the myelin sheath and axons appeared atrophied in diabetic rats.

Diabetes-induced severe ultrastructural features in the sciatic nerve that mainly affects myelin sheath in the form of fragmentation or focal degeneration of myelin, splitting of myelin lamellae, from the axons at some sites and degenerated Schwann cells and these degenerative changes may be due to the direct oxidative effect of diabetes that may initiate inflammation of the sciatic nerve resulting in peripheral neuropathy. Similarly, Altaf (2012) observed severe degenerative changes in the sciatic nerve of diabetic rats including demyelination or disruption of myelin of the myelinated nerve fibers and the cytoplasm of Schwann cells contains different-sized electron lucent fat vacuoles that may be attributed to degeneration of myelin sheath.

The neuronal cells are particularly sensitive to oxidative stress and because of their high metabolic activity; low antioxidant capacity, and non-replicative nature, cells are susceptible to oxidative damage (Lee *et al*., 2012). Premkumar and Pabbidi (2013) reported that sustained hyperglycemia during diabetes is responsible for neuropathy and the production of reactive oxygen and nitrogen species that has cortical roles in the pathogenesis of peripheral neuropathy. Indeed, oxidative stress leads to the development of diabetic peripheral neuropathy via activating various inflammatory mediators which caused the destruction of the lipid membrane and tissue injury (Creager *et al.* 2003). Moreover, chronic hyperglycemia is usually associated with degenerative abnormalities in the peripheral nervous system due to the production of ROS (Tomlinson and Gardiner, 2008).

Data in this study recorded a highly significant increase in the blood glucose levels of the diabetic animals while the pregabalin-treated animals recorded a significant decrease that confirmed the anti-diabetic effect of pregabalin. The ability of pregabalin to reduce blood glucose levels could be explained either by the suppression of pro-inflammatory cytokines, that promote insulin resistance by interfering with the cross-linking between the insulin receptor and its substrate (Tanti *et al.,* 2013) and/or the increase in endogenous gamma-aminobutyric acid and subsequent activation of its receptors in the α cells of the pancreas which suppresses glucagon secretion (Xu *et al.,* 2004).

In the current study, the diabetic rats recorded a highly significant increase in MDA level and a highly significant decrease in SOD and CAT content of sciatic nerves and these effects may be attributed increasing in the ROS production associated with diabetes. In this context, Farshid and Tamaddonfard (2015) found the same results and attributed that to chronic hyperglycemia which is companied by mitochondrial damage, increase production of ROS, and nerve blood flow decrease.

In the current work, although the biochemical changes were improved in animals treated with pregabalin, the architecture of the sciatic nerves still appeared unlike the control ones showed a moderate degree of improvement that was attributed to its anti-inflammatory activity which decreased the diabetic oxidative damage. The previous studies reported that pregabalin was used in the treatment of peripheral neurodegenerative diseases due to its anti-inflammatory and antioxidant properties (Pandhare *et al*., 2012; Jorige and Annapurna 2015). Similarly, Abdel Rasheed *et al.* (2018) observed that

pregabalin ameliorated the histopathological changes and performed an anti-inflammatory role mediated by significantly decreasing pro-inflammatory gene expression levels, such as interleukin-1β and tumor necrosis factor, decreased the release of pro-inflammatory cytokines which play a key role in mediating its anti-nociceptive actions on peripheral neuropathic pain. Moreover, Liu *et al.* (2015) reported that the demyelination of the sciatic nerve was relatively improved after delivered pregabalin.

When pregabalin was administered to diabetic rats in the current study, the ultrastructural characteristics slightly improved; many nerve fibers appeared with enfolding and out folding of the myelin sheath, although there was a large number of thin myelinated nerve fiber which indicated proliferation of sciatic nerve in addition to the Schwann cell appeared normal. Similarly, Kazanci *et al.* (2017) found that most of the ultrastructural changes in the sciatic nerve were either prevented or alleviated by pregabalin which protected the myelinated axons from injury. Pregabalin promoted nerve regeneration and functional recovery after a nerve crush injury which may be due to its capacity to increase the anti-inflammatory cytokines that are required in pathological conditions as transforming growth factor-*β* in the pregabalin-treated group (Celik *et al.*, 2014).

The treatment of diabetic animals with pregabalin recorded a significant decrease in MDA level and a significant increase in SOD and CAT contents in the sciatic tissue. Similarly, Moustafa *et al*. (2018) found similar results in diabetic animals after pregabalin treatment. Previous studies reported that pregabalin protected the tissues against oxidative stress by increasing the activities of antioxidant enzymes and up-regulating the levels of MDA in a dose-dependent manner (Kazanci *et al*., 2017).

Recently scientists turned their attention toward the use of medicinal plants to improve the effects of some drugs. The body weight significantly decreases in the present study of the FXO group compared with the control ones. Also, a significant decrease in the body weights of diabetic animals treated with pregabalin and FXO was recorded when compared with the control group. Similarly, Zhang *et al.* (2008) found that FXO treatment (dose of 600 mg/day for 8 weeks) caused weight reduction due to it gives a feeling of fullness that lasts for a long time and it contains extremely high both soluble and insoluble fiber and caused improved the lipid profile in humans with hypercholesterolemia and hypertriglyceridemia. Qureshi *et al.* (2018) observed significant weight loss and waist circumference reduction in flaxseed-treated animals which is concordant with our results. The same authors attributed the observed weight loss to the richness of linseeds with omega-3 and α-linolenic acid. Couto and Wichmann (2011) found that treatment with flaxseed (10 and 20 g/d for 2 months) decrease body mass index, and waist circumference, and retrained the lipid profile to the normal range in overweight women over 19 years.

The present study revealed that the sciatic nerve of the diabetic rats treated with pregabalin and FXO showed an obvious improvement. This treatment retained the normal histological structure of the sciatic nerve except for little degenerative features. These current observations may be due to the pharmacological constituents of FXO that performed the anti-inflammatory and antioxidant effects. Similarly, Yang *et al.* (2012) demonstrated that FXO has protective activity against hepatorenal toxicity and attributed that to its high content of

omega-3 and alpha-linolenic acid which have the ability to protect cell membranes against oxidative damage by its free radical scavenging ability. In addition, Ekebas *et al.* (2019) reported that flaxseeds extract improved hepatic necrosis, inflammatory cell infiltration and decrease fat vacuoles. Singh *et al.* (2008) reported that the FXO has an inhibition effect against the acute inflammatory reaction phases including protein exudation, vascular permeability as well as leukocyte migration and attributed that to the presence of alpha linolenic acid responsible for its anti-inflammatory activity. Moreover, Kaithwas and Majumdar (2013) reported that fixed oil found in the FXO affects an acute phase of local vasodilatation and enhanced capillary permeability resulting in exudation, followed by leucocytes migration. The previous results revealed that stearic, oleic, linoleic, linolenic, and palmitic are the main fatty acids in FXO, as well as its own high content of vitamin E and phenolic compounds all give it the pharmaceutical effects (El Makawy*et al.*, 2018). Additionally, the presence of polyunsaturated fatty acids, essential amino acids, vitamin E, and lignans may be responsible for FXO's antioxidant function. Additionally, the presence of dietary fibers makes flaxseed available to provide essential needs for maintaining human health and diet (Parikh *et al.*, 2019).

On the other hand, FXO and pregabalin treatment significantly decreased the blood glucose level and MDA level in the sciatic nerve tissue while the content of SOD and CAT significantly increase when compared with diabetic-animal treated with pregabalin only. The present data attributed that to the active constituents of FXO as the hypoglycemic and antioxidant ones. Similarly, Djaber *et al.* (2020) indicate that oral treatment with FXO attenuates the extensive changes in the high levels of glucose. In support, Goncalves *et al*. (2018) found that alpha-linolenic acid in FXO is responsible for improving glucose tolerance and reducing insulin resistance. Flaxseeds have different types of phytoestrogens, such as isoflavones (ginstein, didzein), and lignin (meta-ieisinol, secoisolarisinol) which protect pancreatic cells through naturalized free radicals, control DM, via blocking glucose uptake in the cell membrane of brush borders of renal cells. The insulin secretion from pancreatic cells was controlled by thyrosin kinase enzymes. Ginstein blocks thyrosin kinase enzyme function and can increase insulin secretion (Ranich *et al.,* 2001; Maghsoudi, 2016).

The mechanism of FXO in reducing hyperglycemia may be due to its high content of lignan containing omega-3 and secoisolariciresionol diglycoside that suppressed the expression of the phosphoenolpyruvate carboxykinase that involve in the process of glucose synthesis in the liver (Kaur *et al*., 2017). Increased polyunsaturated fatty acids in flaxseed provide higher membrane fluidity, increase the number of insulin receptors and insulin action finally maintains glucose homeostasis (Gorjao *et al*., 2009). Furthermore, FXO performed a critical role against DNA damage due to the presence of polyphenols and vitamin E. the protection of DNA from lesion-induced by ROS owing to polyphenols that ameliorate cell injury and have the capacity to scavenge free radicals as well as vitamin E which prevent DNA damage due to its antioxidant and enzymatic activities (Urquiaga and Leighton 2000; Songthaveesin *et al.* 2004).

It was demonstrated that dietary flaxseed supplementation increases antioxidant defenses via reducing ROS generation and increasing ROS detoxification (Lee *et al.*, 2009) and

resulted in a significant decrease in protein and lipid peroxidation caused by the oxidative damage (Goncalves *et al*., 2018). Moreover, the antioxidant effect of FXO might exert a hypoglycemic effect by significantly normalized CAT and SOD activities; the latter enzyme scavenges the superoxide radicals via the formation of H_2O_2 and molecular oxygen then protects the tissue against highly reactive hydroxyl radicals Karuna *et al.,* 2011). The main components of FXO that is responsible for its antioxidant activity are the phenolic compound (Zanwar *et al*. 2010). Also, flax contained other biological components such as tocopherols oils, fats enriched in omega-3 fatty acids, crude fiber, and proteins that are responsible for its antioxidant activity (Zou *et al*., 2017). In addition, FXO's antioxidant properties lowered oxidative stress in the liver and kidneys and preserved the integrity of tissue structure (Djaber *et al.,* 2020).

In conclusion, FXO is recommended as a dietary supplement to improve the level of blood glucose during diabetes, decrease peripheral neuropathic pain and improve the neuroprotective effect of pregabalin through its anti-hyperglycemic, antioxidant and anti-inflammatory activities due to its active constituents.

Ethics Approval and Consent to Participate:

The Ethics of Scientific Research and Laboratory Animal Care Committee, Faculty of Science, Menoufia University, Egypt, approved (Approval No. MUFS/F/HI/1/21), the guidelines for the care and use of laboratory animals, and all experiments were performed in accordance with it.

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ARABIC SUMMARY

الفحوصات النسيجية والتركيبية الدقيقة والكيميائية الحيوية لدور زيت بذور الكتان فى تحسين تأثير البريجابالين على الاعتلال العصبي السكري في العصب الوركى للجرذان

سماح محمد عريشه
قسم علم الحيوان - كلية العلوم - جامعة المنوفية – جمهورية مصر العربيه

يعتبر اعتلال الأعصاب الطرفى المرتبط بالتأثيرات العصبية التحللية من المضاعفات الأكثر شيوعًا لداء السكري. وقد وافقت منظمة الغذاء والدواء على البريجابالين كعقار مضاد للتشنجات لعلاج الاعتلال العصبي السكري. توجد العديد من الدراسات على تأثير هذا العقار فى علاج اعتلال الأعصاب السكري حيث أنه لم يحقق الشفاء التام. لذلك هدفت هذه الدراسة إلى تحسين هذا التأثير عن طريق اتحاد جرعته العلاجية مع بعض النباتات الطبية مثل بذور الكتان. إستخدم فى هذه الدراسة عدد أربعون ذكر من الجرذان البيضاء البالغة وقد تم تقسيمهم إلى خمس مجموعات تجريبية. المجموعة الأولى أُعتبرت بمثابة المجموعه الضابطة. المجموعة الثانية أُعطيت الجرذان زيت بذور الكتان (1 مل/ كجم من وزن الجسم/ يومًا) لمدة ستة أسابيع. تم حقن الألوكسان (150مجم/ كغ من وزن الجسم) كجرعة واحدة عن طريق الغشاء البريتوني للأربعة و عشررون جرذ الباقين لاحداث مرض السكرى. ثم تم تقسيم هذه الحيوانات عشوائيا إلى الثلاث مجموعات المتبقية. المجموعة الثالثة كانت ضابطة لداء السكري. المجموعة الرابعة بعد إصابة الجرذان بداء السكري تم معاملتها بالبريجابالين (30 ملجم/ كجم من وزن الجسم/ يومًا) لمدة ستة أسابيع. المجموعة الخامسة كانت الجرذان مصابة بداء السكري وتم معاملتها بريجابالين وزيت بذور الكتان معًا لمدة ستة أسابيع. لقد أظهرت النتائج أن داء السكري أدى لنقص ملحوظ في وزن الجسم ونقصًا ملحوظًا فى نشاط كلًا من SOD وCAT وزيادة ملحوظة في مستوى MDA. كذلك أظهر الفحص المجهري للعصب الوركي للجرذان العديد من التغيرات الباثولوجية والتركيبية الدقيقة. ولكن عند معالجة الحيوانات المصابة بداء السكري بالبريجابالين ظهر تحسن طفيف يشمل التغيرات البيوكيميائية فقط. بينما استعادت الحيوانات المعالجة بالبريجابالين وزيت بذور الكتان معًا الصورة النسيجية الطبيعية والتركيبية الدقيقة للعصب الوركي وسجلت تحسنًا ملحوظًا في القياسات الكيميائية الحيوية. خلُصت هذه الدراسة إلى أن زيت بذور الكتان قام بتحسين التأثير الوقائي العصبي لعقار البريجابالين ضد الاعتلال العصبي السكري بسبب خصائصه المضادة لفرط سكر الدم والمضادة للأكسدة والمضادة للالتهابات.