Original Article

Synergistic Effect of Vitamin B12 and Mesenchymal Stem Cells to Alleviate Paclitaxel-Induced Sciatic Neuropathy in Albino Rats Via Down-Regulation of NLRP3 Inflammasome Pathway: Histological and Immunohistochemical Study

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ABSTRACT

Introduction: Taxanes are a wide group of anticancer drugs. Paclitaxel (PTX) induced peripheral neuropathy is the main long-lasting side effect of paclitaxel. This harmful impact has a significant NLRP3 inflammasome pathway integration. Vitamin B12 combined with BMMSCs may have an alleviating role.

Objectives: This study aims to assess the possible synergistic influence of vitamin B12 and bone marrow mesenchymal stem cells (BMMSCs) to alleviate sciatic neuropathy and define the related anti-NLRP3 inflammasome pathway role.

Study design: About 50 adult albino rats were allocated into 5 groups. Group I (control): no treatments, group II treated with paclitaxel (PTX neuropathy): injected (i.p) with PTX (2.0 mg kg−1) on days 1, 3, 5, and 8, group III (PTX+ vit B12): was treated as group II, then on day 10 rats were injected with vit B12 (10 mg /kg) per every other day (i.m) for 28 days, group IV (PTX+MSCs) as group II, then on day 10, injected (i.v) with a single dose of BMMSCs ($1x10^6$ cells) in 1.0 ml saline. Finally, group V (PTX + vit B12+ MSCs) was treated as group II, then injected with vitamin B12 and BMMSCs at the same doses mentioned before. After sacrificing, sciatic samples were collected, processed, and examined histopathologically, immunohistochemically, and with the electron microscope.

Results: PTX group showed marked histological distortion, congested vasculature, decreased Schwann cells, degenerated fibers, vacuolated axons, upregulated CD68, NLRP3, and caspase-1 immunomarkers. PTX+ vit B12 group showed mild histological improvement, and mild downregulated CD68, NLRP3, and caspase-1 immunomarkers. PTX+MSCs group showed moderate histological improvement and moderate downregulated CD68, NLRP3, and caspase-1 immunomarkers. PTX+ vit B12+ MSCs showed apparent histological improvement, the myelinated fibers appeared nearly normal with apparent downregulated CD68, NLRP3, and caspase1 immunomarkers.

Conclusion: Combined vitamin B12 and BMMSCs therapy synergistically alleviated PTX-neuropathy with obvious NLRP3 inflammasome pathway inhibition.

Received: 29 September 2023, **Accepted:** 23 November 2023

Key Words: BMMSCs, neuropathy, NLRP3 inflammasome, paclitaxel, vitamin B12.

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ISSN: 1110-0559, Vol. 47, No. 2

INTRODUCTION

Chemotherapy-induced peripheral neuropathy (CIPN) is considered a severe and long-lasting side effect of many chemotherapeutic agents, such as taxanes, platinum-based compounds, and vinca alkaloids[1]. These neurotoxic chemotherapeutic agents can induce structural damage in the peripheral nerves[2]. One of these chemotherapeutic agents is paclitaxel, which is used to treat people with breast, ovarian, and lung tumors. The pharmaco-toxicological profile of paclitaxel mostly includes hair fall, allergic reactions, diarrhea, bone marrow suppression, and lung inflammation[3].

The existing bottleneck in the therapeutic use of paclitaxel is that it causes advanced and often irreversible damage to the peripheral nervous system in 60–70% of patients getting paclitaxel resulting in abnormal effects on the sensory and motor functions[4]. A key factor in this neuronal damage and neural degeneration is the process of neuroinflammation[5].

It is well known that numerous neuropathological disorders are influenced significantly by the crosstalk between the immune and nervous systems[6]. Macrophages are considered an important cause of inflammation in innate immunity and so, may act as a potential culprit to cellular

 DOI: 10.21608/ejh.2023.239622.1955

senescence stimulation[7]. During the neuroinflammatory pathway, macrophages and microglia are the most plentiful immune cells activated[8]. Macrophages move to the damaged area and release inflammatory cytokines, starting an inflammatory cascade reaction[9].

The term "inflammasome" describes supramolecular structures in the cytoplasm of stimulated immune cells that trigger the proteolytic activation of proinflammatory caspases, promoting inflammation and other systemic immunological responses[10]. The most thoroughly investigated inflammasome complex among the numerous inflammasomes discovered in mammals is the NOD-like receptor protein 3 (NLRP3) inflammasome[11].

Following activation by inflammatory stimuli, the NLRP3 inflammasome is mostly expressed in immunological and inflammatory cells such as neutrophils, mast cells, and proinflammatory macrophages[12].

The inactive NLRP3 inflammasome is a tripartite protein complex that is put together in response to a wide variety of external pathogens or internal danger signals, which causes the production of pro-inflammatory cytokines and pyroptotic cell death[13]. It is composed of three protein subunits: a sensor molecule (NLRP3), an adaptor protein (ASC), and an effector protein (pro-caspase-1) which functions to switch on the inflammatory process[14,15]. Activated caspase-1 in turn converts pro-IL-1β into active IL-1β, which is then released to enhance more inflammation[16,17].

To date, stem cell technology has undergone great evolution, and it may be used to treat various diseases related to the nerves, lung, heart, and liver[18]. Mesenchymal stem cells (MSCs), a varied subpopulation of stromal stem cells, can be acquired from different tissues, such as bone marrow, dental pulp, peripheral blood, umbilical cord, and adipose tissue[19].

Characterized by little immunogenicity, great anti-inflammatory, immunoregulatory functions and regenerative capacity the use of MSCs in cell treatments and tissue engineering is extremely promising[20,21].

A key aim is to increase the potency and therapeutic advantages of MSCs[22]. Combining MSC therapy with pharmaceutical drugs can be a potential new way to maximize its benefits and minimize its drawbacks.

Based on the above evidence, we aim to target the neuroinflammation of the paclitaxel model of peripheral neuropathy and explore whether vit B12 combined with MSC alleviated neuroinflammation and enhanced axonal regeneration by regulating NLRP3 inflammasome activation, aiming to provide a new avenue for chemotherapy-induced neuropathy treatment.

Drugs

Paclitaxel 100 mg ampoule (6 mg/ml). Product name Taxol was factory-made by Corden Pharma Latina S.P.A. (Sermoneta, Latina, Italy) for Bristol-Myers Squibb Company (Roma, Italy).

Vitamin B12 (Depovit B12®) (AMRIYA Pharmaceuticals Company4, Egypt) as 1 ml ampoules of Hydroxocobalamin 1000 μ/ml.

Animals

For this study, a total of fifty adult albino rats were included. Rats started the study with a body weight of 175-200 grams and were allowed to become accustomed to the experimental animal habitat for 7 days. Throughout the experimental time, the rats were housed in plastic cages (5 per cage) at a temperature of 25–27°C and a humidity of 35–65%, with a 12-h light/dark cycle and fresh air changes/hour, and were allowed free access to rat diet and water. The Research Ethical Committee at the Faculty of Medicine, Benha University approved the research protocol; (NOM: RC 11-8-2023).

BMMSCs isolation and culture[23]

Five rats were anesthetized and were then cervical dislocated to death. We acquired femurs and tibias. After being briefly submerged in 70% isopropanol, dissected femurs and tibias were transferred to PBS media. Afterward, it was put into a 10 cm dish with DMEM. Each bone's ends were severed, and the marrow was extracted and injected into a 50ml falcon tube using a 22G needle and 3ml of DMEM. Each bone received two to three flushing repetitions. To remove the bone fragments and blood clumps, cells were suspended and then run through a 70-micron cell strainer. The obtained cells were spun down at 2000g for 5 minutes, with the supernatant aspirated out. Cells were re-suspended in 25ml of MSC media, which is composed of antibiotic- and 10% FBS-DMEM. Two 10cm culture dishes with a total of 10 ml of cell suspension each were seeded for 1-2 weeks at 37°C and 5% CO2. Every 2 to 3 days, the medium was changed. Analysis of the cultured stem cells was achieved using an inverted microscope provided with a digital camera (Olympus CKX41SF, Tokyo, Japan).

Immunocytochemistry identification of BMMSCs[24]

To identify MSCs, the main antibodies mouse anti-human CD44+ and mouse anti-CD34 were employed as CD markers. The cells were cultured again in multi-well tissue culture plates (8 wells) in MEM supplemented with 20% FBS after being dispersed with trypsin-versene and suspended in growth media. The plates were incubated to develop a monolayer of adherent cells within 3 days, after which the media was removed and the cells were fixed with 4% paraformaldehyde for 10 min. The sections were handled in accordance with the instructions provided by the kit's manufacturer (Santa Cruz Biotechnology, USA), placing them in the humid compartment at room temperature (20–25 °C) at each step. The cells were treated with 1% hydrogen peroxide followed by three 5-minute PBS washes. 1.5% blocking serum was aliquoted and applied to the cell section for an hour before being removed. After being washed for around 30 minutes, 1.2 ml of biotinylated secondary antibody was added, and the 1ry antibody was

incubated for an hour at room temperature or overnight at 4 °C at a ratio of 1:50. Cell section received an addition of 650 l of AB enzyme reagent. Each step before this one ended with washing. Cells were treated with three drops of peroxidase substrate until the appropriate stain intensity appeared. Cell sections were stained with hematoxylin for 5–10 seconds, and then they were promptly rinsed with distilled water. Finally, 1-2 drops of permanent mounting media were added, and L/M analysis was performed

Labeling of BMMSCs with PKH-26 fluorescent dye[25]

BMMSCs were labeled per the manufacturer's instructions with a few minor adjustments using the cell linker (PKH-26) (red fluorescence) (Sigma Chemical). A 2 ml injection of fetal bovine serum stopped the reaction after $2x10^7$ MSCs had been labeled for 4 minutes at room temperature with 2x105 to 2x106 mol/l PKH26. After two washes in 5 ml of Dulbecco's adjusted Eagle's medium, cells were then transferred to animals. Using a fluorescence microscope (Olympus, model: BX50F4, No. 7M03285, Japan), segments of the sciatic nerve from the MSCs-injected groups were studied.

Experimental design

To search the role of NLRP3 inflammasome in the sciatic peripheral neuro-inflammation, and its inhibition by combined vitamin B12 and MSCs therapy, fifty male adult albino rats were allocated into 5 groups. I (Control group), II (PTX-neuropathy group), III (PTX+ vitamin B12 group), IV (PTX+MSCs group), and V (PTX + vit B12+ MSCs group).

Rats of group I: received no treatments.

Peripheral neuropathy was induced in rats of group II by intraperitoneal (i.p) injection of paclitaxel (2.0 mg kg−1) on four alternative days (days 1, 3, 5, and 8)[26].

Rats of Group III: were treated as group II, then were injected on day 10 with vit B12 at a dose of 10 mg /kg/ per every other day intramuscular (i.m) for 28 days[27].

Rats of Group IV: were treated as group II, then on day 10 rats were injected with a single dose of MSCs ($1x10^6$ cells) in 1.0 ml saline intravenously (i.v)[28].

Rats of Group V: were treated as group II, then were injected with vitamin B12 and MSCs at the same doses mentioned before.

The rats were sacrificed by carbon dioxide exposure. Rats of group II were necropsied on day 10, while rats of groups I, III, IV, and V were necropsied on day 39. Sciatic nerve segments (4 cm above the knee joint) were taken and separated into two groups. Samples of one group were fixed in 10% formalin and processed following standard protocol for paraffin block preparation[29]. Sections were handled for histopathological and immunohistochemical examination, while, the other group of samples was fixed in a mixture of glutaraldehyde (2.5%) and paraformaldehyde (2.5%) and processed for electron microscopic examination.

Immunohistochemical study

All steps for immunostained section preparation were done according to standard practice[30]. Depraffinized 5 microns thick sciatic nerve sections were cut and prepared, and tissue sections were treated with 3% hydrogen peroxide for 20 Mins. Washed by PBS, then incubated with mouse anti-CD68 monoclonal antibodies (Kp-1, 1:100; DAKO), anti-NLRP3 (GTX00763 - 1:100; Genetex Co) and anti Caspase-1 ((14F468) NB100-56565 - 1:100; Novus bio Co) overnight at 4C; washed by PBS followed by cultivation with secondary antibody (1:350) HRP Envision kit (DAKO) 15 minutes; rinsing by PBS and incubated with diaminobenzidine about 10 minutes. Washing by PBS then counterstaining with hematoxylin, dehydrated and clearing in xylene then cover slipped for microscopic analysis.

Transmission electron microscopy (TEM) sample preparation

Small parts of sciatic nerves ($1mm^3$) were fixed in a mixture of glutaraldehyde (2.5%) and paraformaldehyde (2.5%) and sent to the Electron Microscope unit (Faculty of Science, Alexandria University, Alexandria, Egypt) for processing and examination. Then samples were post-fixed in osmium tetroxide (1%), dehydrated, and embedded in resin to get semithin sections which were stained with toluidine blue. Selected areas from the semithin sections were cut into ultrathin (80 nm) sections according to the provided methods[31]. Ultrathin sections were examined using a JEM-1400 plus TEM.

IHC analysis

A full HD microscopic camera operated by the Leica application module was used for sciatic slide analysis (Leica Microsystems GmbH, Wetzlar, Germany). At least 4 random non-overlapping fields from each sample were scanned and analyzed for calculation of the mean number of CD68 positive macrophages, and mean area percent of NLRP3 and caspase-1 immunohistochemical expression. Only threshold and brightness modifications were made to the entire image during image manipulation. All fields were examined at magnification power x400. Negative controls are prepared by the same steps with omission of the primary antibody which is replaced by PBS.

Statistical Study

The Statistical Package for Social Science software program, variety 26 (SPSS, Inc., Chicago, USA), was used to evaluate the obtained data. While non-parametric data were displayed as median & range (minimum-maximum), parametric data were provided as mean and standard deviation. For assessments between more than two groups of parametric data, one-way analysis of variance (ANOVA) and post-hoc Tukey were used; for assessments between more than two groups of non-parametric data, Kruskal-Wallis and post-hoc Dunn's were used. Statistical significance was defined as a *P value* less than 0.05.

RESULTS

Identification of BMMSCs

An inverted microscope was used for BMMSCs identified in culture. They appeared as colonies of rounded cells on day 0, and in subculture on day 7 as spindle-shaped cells with multiple processes (Figure 1A). Cells were also identified by immuno-cytochemical reaction for specific CD surface markers of BMMSCs, CD34 is a negative marker and CD44 is a positive marker. The results are shown in (Figure 1B). BMMSCs were detected using chromogen accumulation on the secondary antibody of CD44 and showed a dark brown color, while cells negative for CD34 were still blue (Hematoxylin color only, with no brown stain). These findings indicated bone marrow origin (+ve for CD44) but not hematopoietic origin (-ve for CD34) of the mesenchymal stem cells. After sciatic neuropathy induction, PKH26-labeled BMMSCs were injected in both groups IV and V. Stem cells were tracked in *vivo* by fluorescent microscopy which showed the homing of these labeled cells in sciatic nerve tissues of both groups (Figure 1C).

Combined vit B12 and BMMSCs alleviated sciatic neuropathy in histopathological examination of sections from experimental groups

In H&E stained longitudinal sections of sciatic nerves, the control group showed normal histological morphology including normal axons, myelin sheaths, and Schwann cells (Figure 2a). After PTX toxicity, sciatic sections showed distorted histological architecture, with marked axonal degeneration, vacuolated fibers, marked congested blood vessels, inflammatory cell infiltrate, and myelin debris were seen (Figures 2b,c). Group III (PTX+ vit B12) showed slightly improved histological appearance involving less degenerated fibers and less congested blood vessels (Figure 2d). Group IV (PTX+MSCs) showed moderately improved histological morphology. Some areas showed proliferated Schwann cells. Büngner bands were detected as large denervated Schwann cells arranged in a linear pattern indicating a sign of regeneration (Figure 2e). Group V (PTX+ vit B12 +MSCs) showed apparent improved histological morphology. Most axons were intact surrounded by normal Schwann cells, except for some focal areas of vacuolization (Figure 2f).

Combined vit B12 and BMMSCs down-regulated CD68, NLRP3 and caspase-1 inflammasome pathway immune markers in experimental groups

In the sciatic sections of the control group, we observed minimal CD68/ NLRP3/ caspase-1 immunoexpression (Figures 3a, 4a, 5a) respectively. Activation of the inflammasome-NLRP3 pathway is involved in PTX-induced sciatic neuropathy. The average number of CD68 positive proinflammatory macrophages was increased, and the mean areas of NLRP3 and caspase-1 expression were greater compared to the control group (Figures 3b, 4b, 5b). Immunoexpression of the three markers was identified as cytoplasmic brown color. Treatment with each vit B12 and BMMSCs separately slightly down-regulated the immunoexpression of these inflammasome pathway markers indicating cessation of inflammation in these groups (Figures 3c, 4c, 5c) and (Figures 3d, 4d, 5d) respectively. On the other hand, CD68/ NLRP3/ caspase-1 positive immunoreactions were sparsely observed in (PTX+ vit B12+ MSCs) group (Figures 3e, 4e, 5e) compared to the pathological group and groups treated with each therapy alone, indicating higher cessation of inflammation.

Quantitative Analysis

To determine the exact expression of pathological changes of CD68/NLRP3/Caspase-1 inflammasome markers, four pictures were photographed under x400 magnification for each slide to encompass the immunoexpression. The statistical analysis was represented in (Figures 3f, 4f, 5f) for CD68, NLRP3, and caspase-1 respectively. In the PTX-neuropathy group, CD68, NLRP3, and Caspase-1 were highly significantly upregulated ($p<0.05$) compared to the control group. This up-regulation was slightly reversed in groups treated with vitamin B12 and BMMSCs separately compared to the pathological group ($p<0.05$). Higher significant downregulation of inflammasome pathway markers was observed in group treated with combined therapy compared to other treated groups ($p<0.05$) (Table 1, Table 2).

Combined vit B12 and BMMSCs alleviated sciatic ultrastructure damage in experimental groups

Semithin sections

Moreover, in toluidine blue stained semithin sections, the control group showed multiple axons variable in shape and size, surrounded by compact myelin sheaths and embedded in connective tissue endoneurium (Figure 6a). After PTX treatment most axons became necrotic and surrounded by degenerated myelin sheaths with splitting of their lamellae. The endoneurium was filled with necrotic debris. Mononuclear cellular infiltrate and large congested blood vessels were observed (Figure 6b). In vit B12 treated group the axons were slightly improved in structure compared to the neuropathy group, but many myelin sheaths showed vacuolations. Few myelinated axons were necrotic (Figure 6c). The MSCs-treated group showed most axons with their myelin sheaths appeared more or less than normal. Few axons showed irregular myelin sheaths with invaginations and evaginations and thin myelin sheaths (Figure 6d). Combined vit B12+MSCs group showed apparent improved histological morphology. Most axons were intact with their myelin sheaths appearing nearly normal (Figure 6e).

Ultrathin sections

The control group (Figure 7) showed multiple myelinated axons surrounded by endoneurium connective tissue. Schwann cells wrapped around myelinated axons with euchromatic nuclei, rough endoplasmic reticulum,

glycogen rosettes, and prominent basal lamina. Each myelinated axon was surrounded by a compact myelin sheath. Myelin sheaths revealed a lamellar appearance. The outer layer of Schwann's cytoplasm was apparent. The axoplasm displayed neurofilaments and mitochondria. PTX- neuropathy group (Figure 8) showed loss of the nerve fibers integrity compared to the control group. Multiple necrotic myelinated axons were prominent with vacuolated, shrunken, and atrophied axoplasm. The myelin sheaths displayed shrinkage and focal splitting of their lamellae. Collagen deposition was obvious in the endoneurium. PTX+ vit B12 group (Figure 9) showed slightly improved general histological morphology compared to the neuropathy group. However, the axons still with irregular outlines and distorted myelin sheaths. Some axons displayed vacuolated mitochondria. PTX+ MSCs group (Figure 10) showed improved histological morphology compared to the neuropathy group. Some axons appeared nearly normal with improved myelin sheaths. Some others showed small areas of focal lamellar splitting or displaying evaginations and invaginations. Schwann's cell appeared normal with euchromatic nuclei. The axoplasm showed some normal and some distorted mitochondria. PTX+ vit B12+ MSCs group (Figure 11) showed apparent improved histological structure. Most axons appeared more or less than normal with healthy myelin sheaths except for small

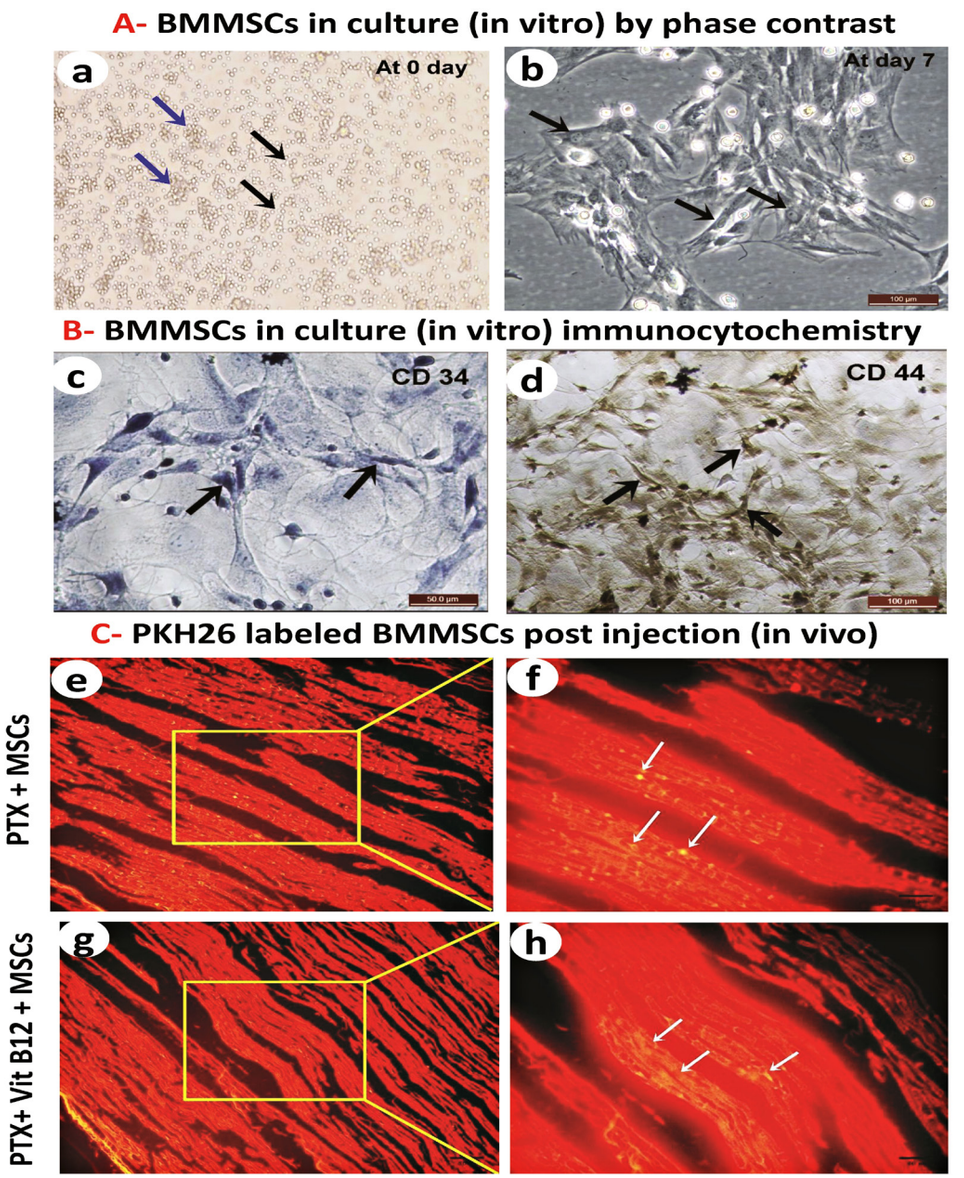

Fig. 1: Panel (A): phase contrast microscopy images of stem cells in culture media at 0 days showing many stem cells (black ↑) and cell colonies (blue ↑) (Fig. a). On the 7th day, many stem cells adherent to the culture dish appeared with spindle-shaped and displayed nuclei and multiple thin interdigitating processes (↑) (Fig. b). Panel (B): Immunocytochemistry identification of BMMSCs showing negative cells for CD34 (Fig. c) and positive cells for CD44 (Fig. d). Panel (C): Fluorescent microscopy images of PKH26 pre-labeled BMMSCs in *vivo* harvested from recipient rats of PTX+ MSCs group and PTX+ vit B12 +MSCs group (Figs: e, f) respectively showing homing of these labeled cells in sciatic nerve tissue. The yellow boxed areas are magnified and show the distribution and differentiation of PKH26-positive cells (↑) in the sciatic nerve tissues.

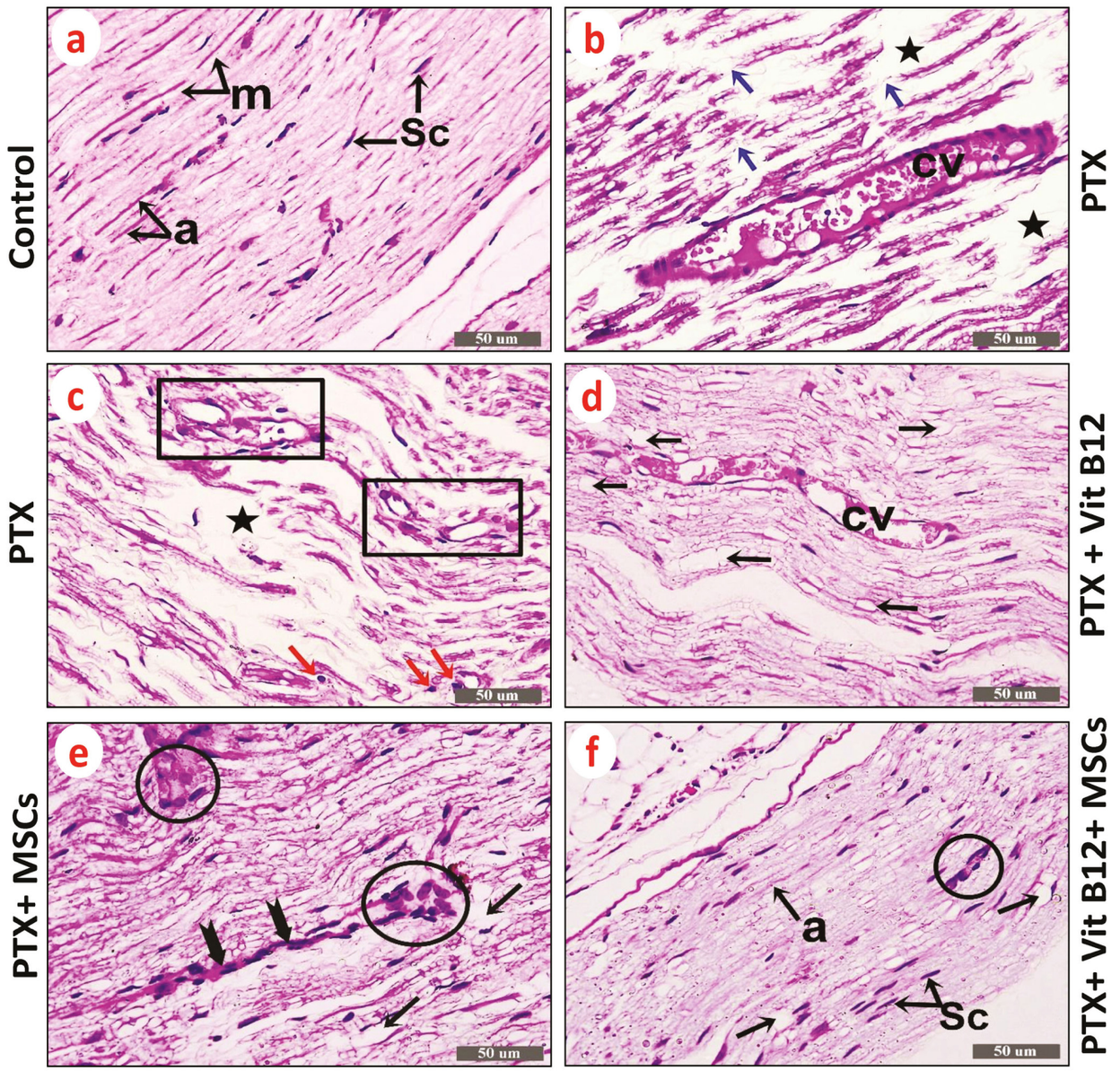

Fig. 2: Hematoxylin and eosin stained longitudinal sections of sciatic nerves from different experimental groups showing: (a) control group: Nerve fibers are tightly and regularly arranged. Each nerve fiber is formed of the central axon (a) surrounded by a poorly stained myelin sheath (m). The nuclei of Schwann cells can be demonstrated (Sc). (b & c) PTX-neuropathy group: (b) loss of the normal histological architecture, with marked axonal degeneration (blue ↑) with foci of complete nerve fibers loss (star). Marked congested blood vessels are seen (CV). (c) Another section shows inflammatory cellular infiltrate (red ↑) and degenerated fibers with myelin debris "digestion champers" (rectangles). Notice: apparent decreased number of Schwann cells. (d) PTX+ vit B12group: slightly improved histological appearance. Congested blood vessels are still observed (CV). Some nerve fibers are showing vacuolization (↑). (e) PTX+ MSCs group: moderately improved histological morphology. Some areas are showing proliferated Schwann cells (circles). These large denervated Schwann cells can be arranged in linear pattern forming (Büngner band) (bifid arrows) as a sign of regeneration. Few nerve fibers are showing vacuolizations (↑). (f) PTX+ vit B12+MSCs group: apparent improved histological morphology. Most axons (a) are intact surrounded by normal Schwann cells (Sc), except for some focal areas of vacuolization (↑). Some proliferated Schwann cells can be observed (circle). (H&E stain; Scale bar= 50µm; magnification: 400x).

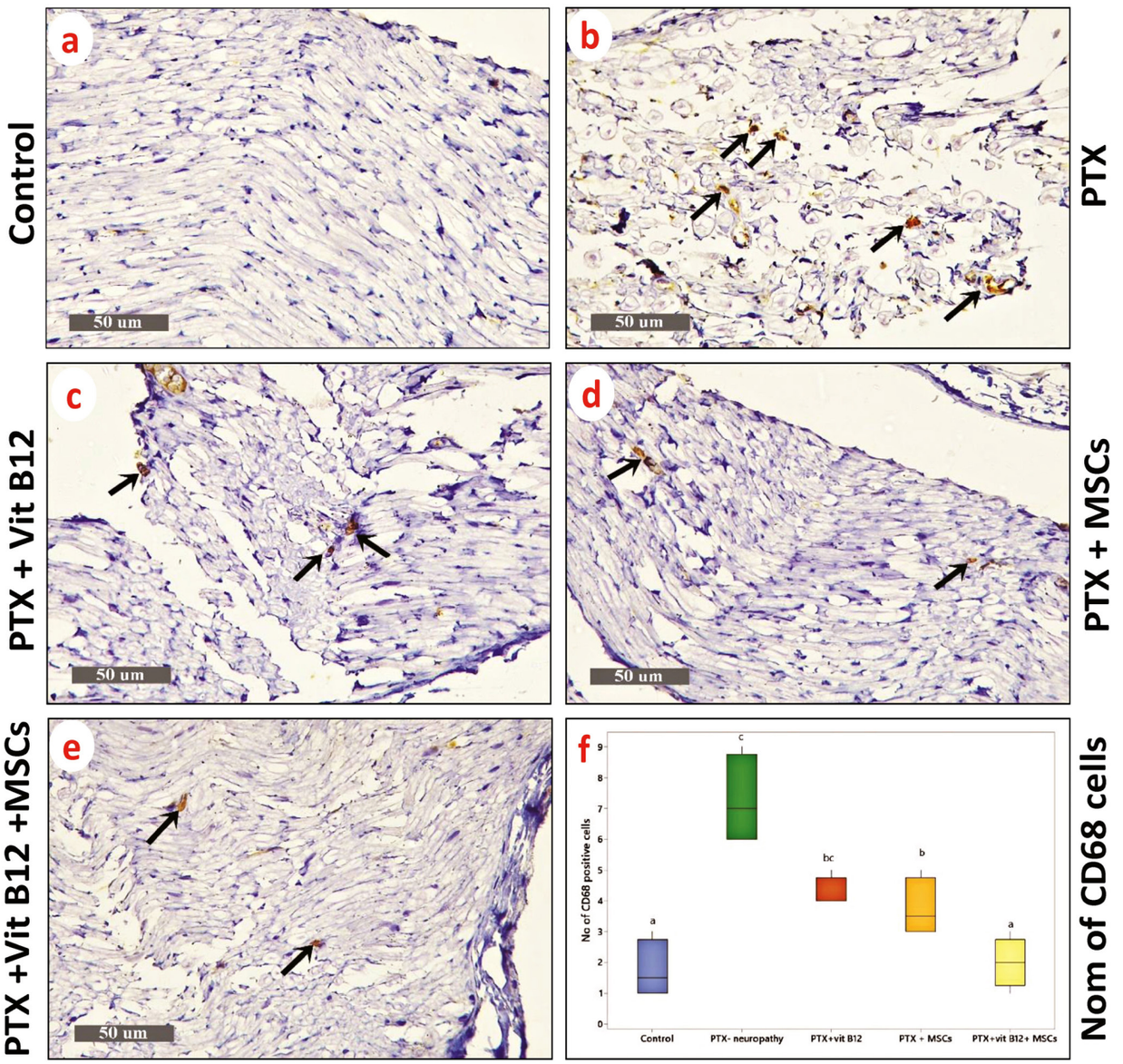

Fig. 3: Representative immunohistochemical results of anti-CD68 stained longitudinal sections of sciatic nerves from different experimental groups. The cytoplasmic brown color is considered a positive reaction. (a): nearly negative reaction of anti-CD68 in the control group. (b): Multiple CD68 positive macrophages (↑) in the PTX-neuropathy group indicating inflammatory reaction. (c): After vitamin B12 treatment, the sciatic nerve showed moderate anti-CD68 immunoreaction within macrophages cytoplasm (↑). (d) The MSCs-treated group showed decreased anti-CD68 expression (↑) compared to the pathology group. (e): Combined vitamin B12 and MSCs group showed minimal reaction (↑) compared to the pathology group. (f): Number of CD68 positive macrophages in experimental groups is expressed as median and range (minimum-maximum). Statistical analysis was done with Kruskal-Wallis followed by post-hoc Dunn's test. Different superscript small alphabetical letters indicate a significant difference. (Anti-CD68: Scale bar= 50µm; magnification: 400x).

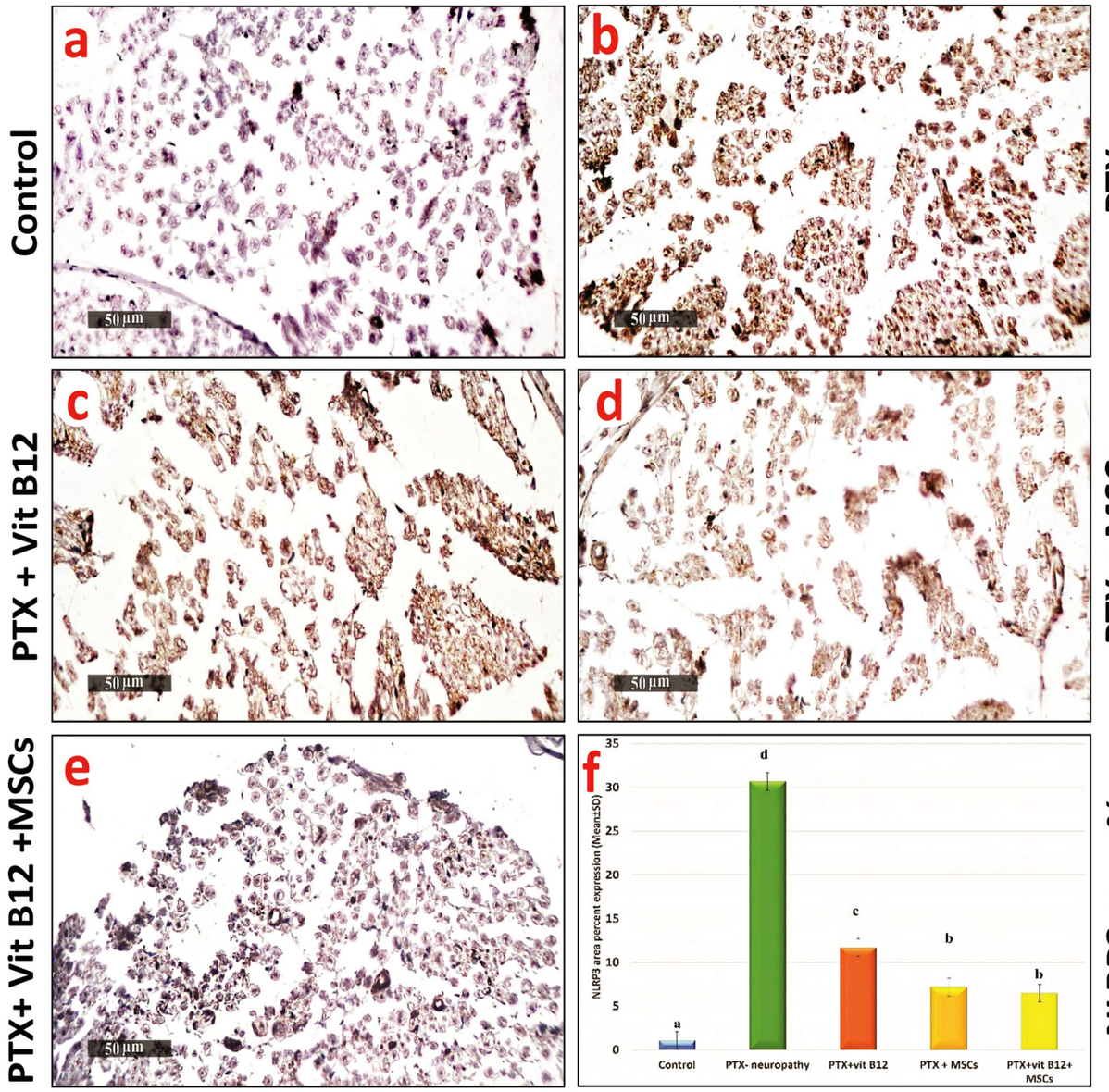

Fig. 4: Representative immunohistochemical results of anti-NLRP3 stained cross sections of sciatic nerves from different experimental groups. The cytoplasmic brown color is considered a positive reaction. (a) The control group showed a negative reaction to anti-NLRP3. (b): Intense positive reaction of anti-NLRP3 in PTX-neuropathy group indicating apparent inflammatory reaction. (c): After vitamin B12 treatment, the sciatic nerve showed moderate anti-NLRP3 immunoreaction. (d) The MSCs-treated group showed mild anti-NLRP3 immunoreaction compared to the pathology group. (e): Combined vitamin B12 and MSCs group showed mild anti-NLRP3 immunoreaction compared to the pathology group. (f) Mean area percentage of anti-NLRP3 immunoexpression between experimental groups. Statistical analysis was done with one-way ANOVA followed by a post-hoc Tukey multiple comparison test. Different superscript small alphabetical letters indicate a significant difference. (Anti-NLRP3: Scale bar= 50 µm; magnification: 400x)

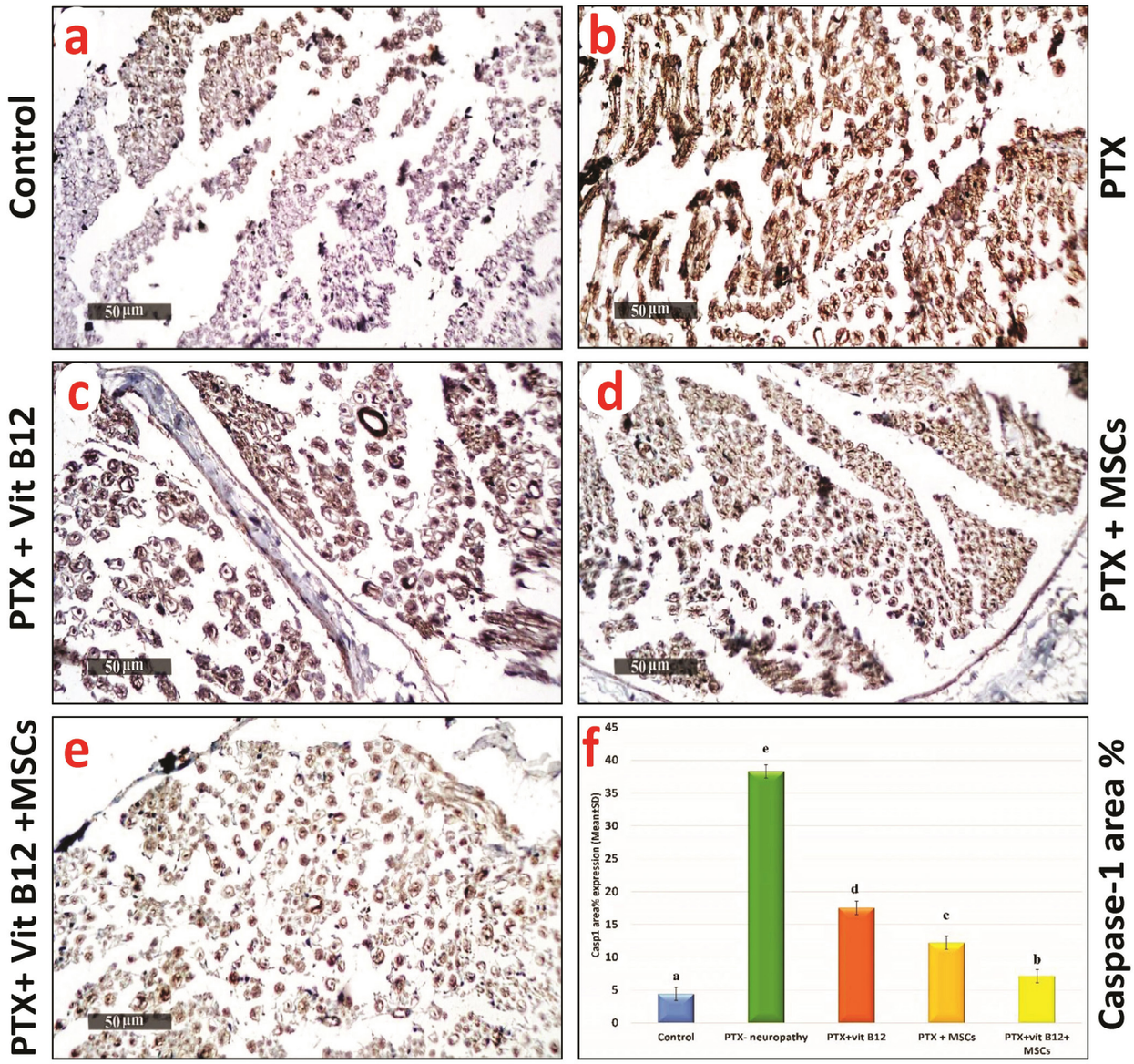

Fig. 5: Representative immunohistochemical results of anti-caspase-1 stained cross sections of sciatic nerves from different experimental groups. The cytoplasmic brown color is considered a positive reaction. (a): Minimal reaction of anti-caspase-1 in the control group. (b): Highly positive reaction of anti-caspase-1 in PTX-neuropathy group indicating severe inflammatory reaction. (c): After vitamin B12 treatment, the sciatic nerves showed moderate anti-caspase-1 immunoreaction. (d): The MSCs-treated group showed decreased anti-caspase-1 immunoreaction compared to the pathology group. (e): Combined vitamin B12 and MSCs group showed mild immunoreaction compared to the pathology group. (f) Mean area percentage of anti-caspase-1 immunoexpression between experimental groups. Statistical analysis was done with one-way ANOVA followed by a post-hoc Tukey multiple comparison test. Different superscript small alphabetical letters indicate a significant difference. (Anti-caspase-1: Scale bar= 50 µm; magnification: 400x)

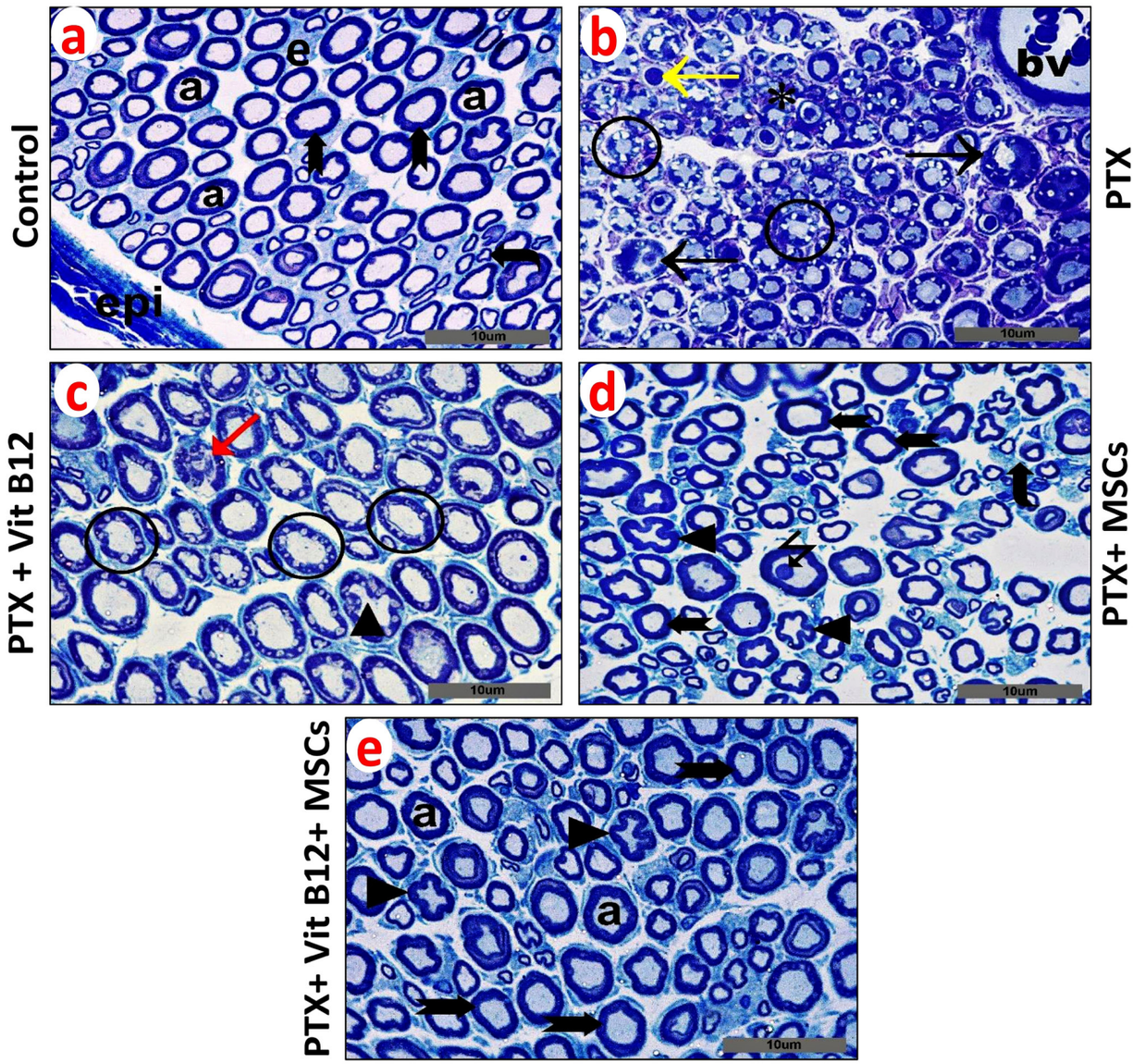

Fig. 6: Toluidine blue stained cross sections of sciatic nerves from different experimental groups showing: (a) Control group: outer connective tissue epineurium surrounding the nerve (epi). Multiple axons (a) are variable in shape and size, surrounded by compact myelin sheaths (bifid arrows) and embedded in connective tissue endoneurium (e). There is a small amount of small myelinated nerve fibers (bent arrow). (b) PTX-neuropathy group: most axons are necrotic and surrounded by vacuolated myelin sheaths (circles). Some axons with their myelin sheaths are completely distorted (thin arrow). The endoneurium is filled with necrotic debris (asterisk). Mononuclear cellular infiltrate (yellow arrow) and large congested blood vessels (bv) can be observed. (c) PTX+ vit B12 group: The axons are slightly improved in structure compared to the neuropathy group, but most myelin sheaths still show vacuolations (circles). Few myelinated axons are still necrotic (red arrow) or showing invaginations and evaginations (▲). (d) PTX+ MSCs group: most axons with their myelin sheaths appear more or less than normal (bifid arrows). A few axons are showing irregular myelin sheaths with invaginations and evaginations (▲), while others are showing focal separation of the myelin sheath (zigzag arrow). Many thin myelinated axons are obvious (bent arrow). (e) PTX+ vit B12+MSCs group: apparent improved histological morphology. Most axons are intact (a) with their myelin sheaths appearing nearly normal (bifid arrows). A few axons are showing irregular myelin sheaths with invaginations and evaginations (▲). (Toluidine blue: Scale bar= 10 µm; magnification: 1000x)

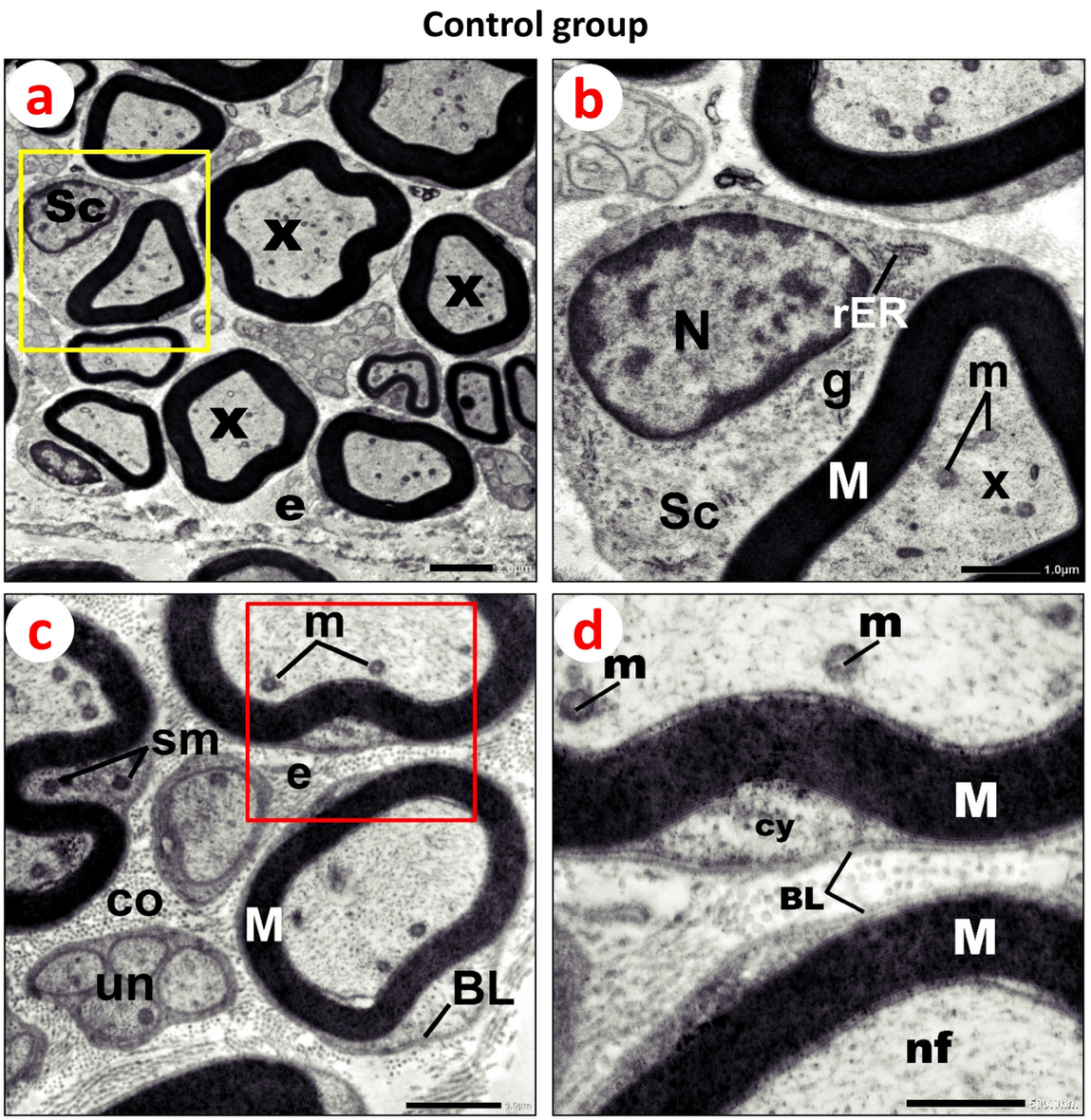

Fig. 7: Electron micrographs of Sciatic nerve isolated from the control group showing: (a) multiple myelinated axons (x) surrounded by endoneurium connective tissue (e). One Schwann cell (Sc) wraps around a single myelinated axon. The yellow boxed area is magnified in figure (b): showing a large Schwann cell (Sc) wrapping around a myelinated axon, with oval euchromatic nucleus (N), rough endoplasmic reticulum (rER), glycogen rosettes (g). The axon (x) is surrounded by a compact homogenous myelin sheath (M) and displays mitochondria (m). The thickness of the myelin sheath is proportionate to the width of the axonal diameter. (c) Another field showing some nerve fibers surrounded by myelin (M), and some unmyelinated fibers (un). Schwann cells display prominent basal lamina (BL) and mitochondria (sm). The axoplasm also displays mitochondria (m). The connective tissue endoneurium (e) contains deposited collagen fibers (co). The red boxed area is magnified in Figure (d), showing compact myelin sheaths (M). The axoplasm displays mitochondria (m), and neurofilaments (nf). An outer small area of Schwann cell cytoplasm (cy) surrounded by basal lamina (BL) can be seen.

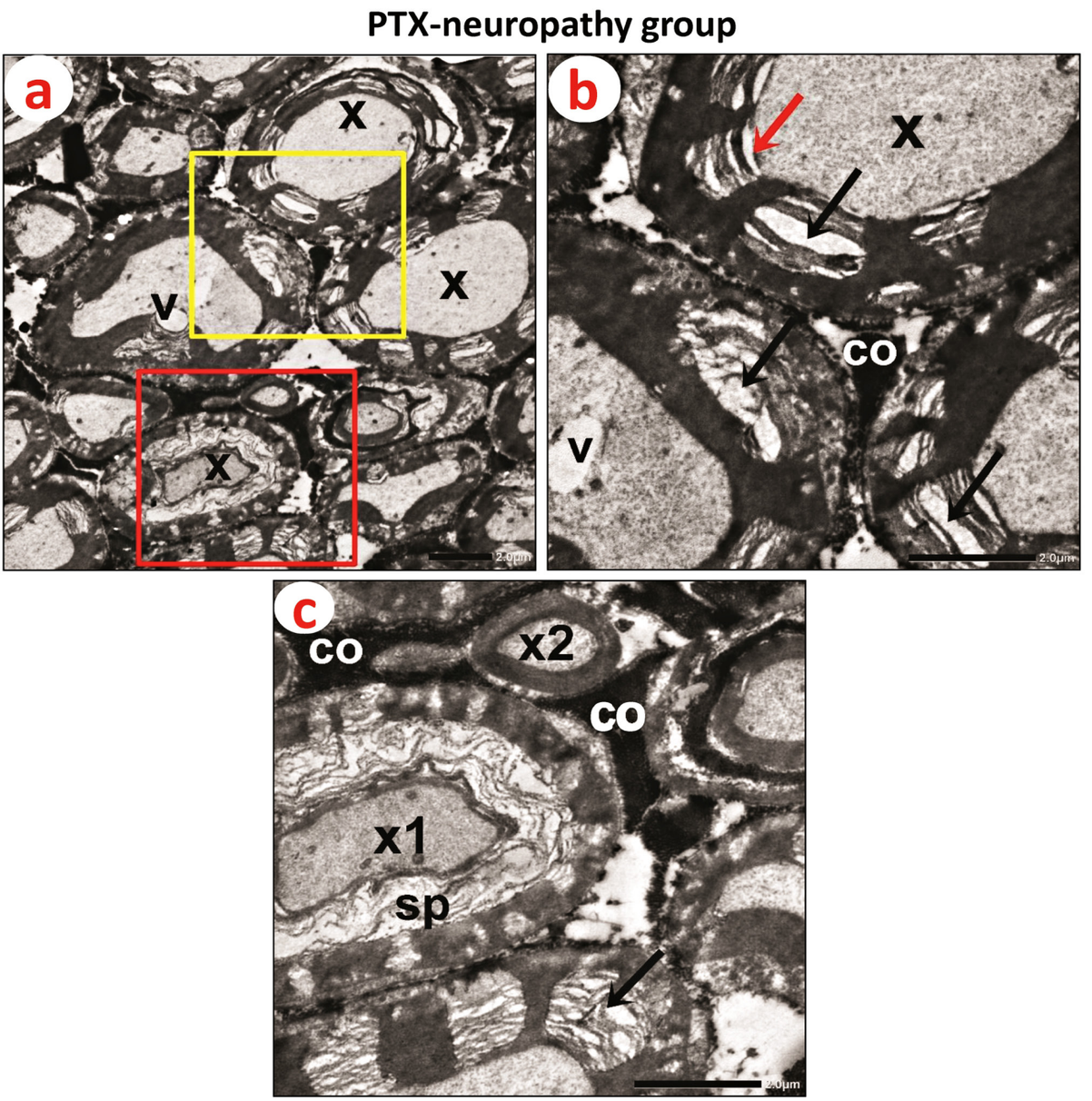

Fig. 8: Electron micrographs of Sciatic nerve isolated from PTX-neuropathy group showing: (a) loss of the nerve fibers integrity compared to the control group. Multiple necrotic myelinated axon fibers (x) are prominent. Axoplasm displaying vacuoles (v). The yellow boxed area is magnified in Figure (b); showing axons (x) with focal cracks and splitting in their myelin lamellae (↑), vacuolated axoplasm (v), and focal separation of the axoplasm from the surrounding sheath (red ↑). The red boxed area in Figure (a) is magnified in Figure (c); most fibers are showing focal splitting in their myelin lamellae (↑), some shrunk axons (x1) with totally separated myelin lamellae (sp), some other atrophied small axons (x2) with thin myelin sheaths. A prominent amount of collagen fibers in the endoneurium forms collagen pockets (co) between fibers.

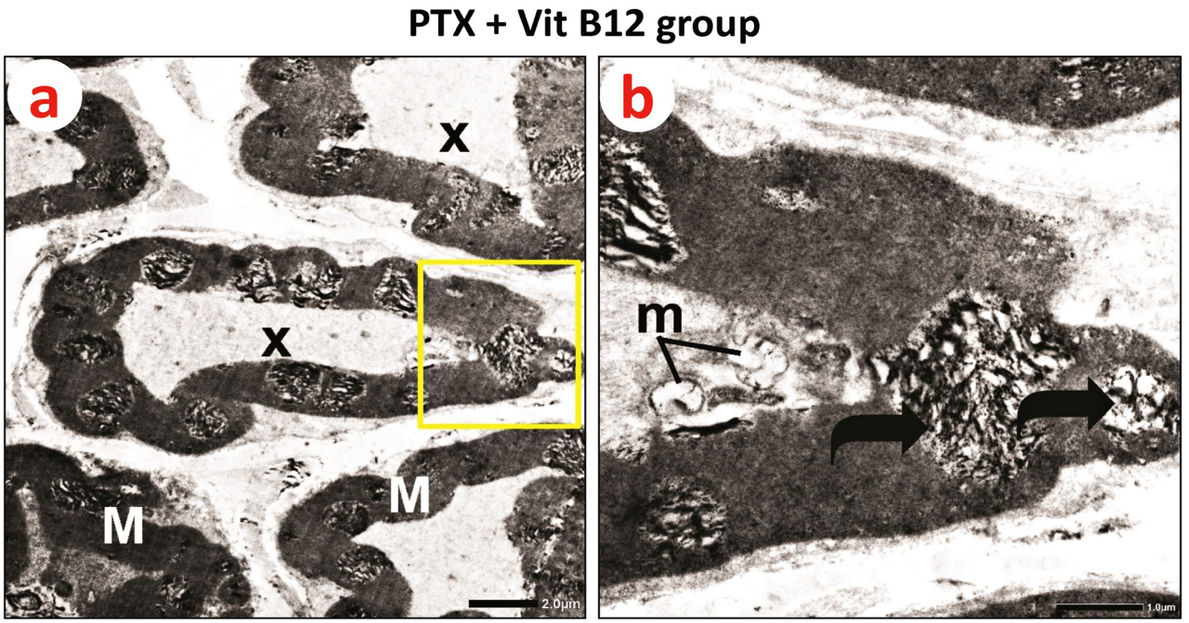

Fig. 9: Electron micrographs of Sciatic nerves isolated from PTX+ vit B12 group showing: (a): Slightly improved histological morphology compared to the PTX-neuropathy group. However, the axons (x) have irregular outlines with distorted myelin sheaths (M). The yellow boxed area is magnified in Figure (b); showing the axon with myelin sheath displaying bulging segments with splitting myelin containing debris (bent arrows). The axoplasm shows swollen mitochondria with destructed cristae (m).

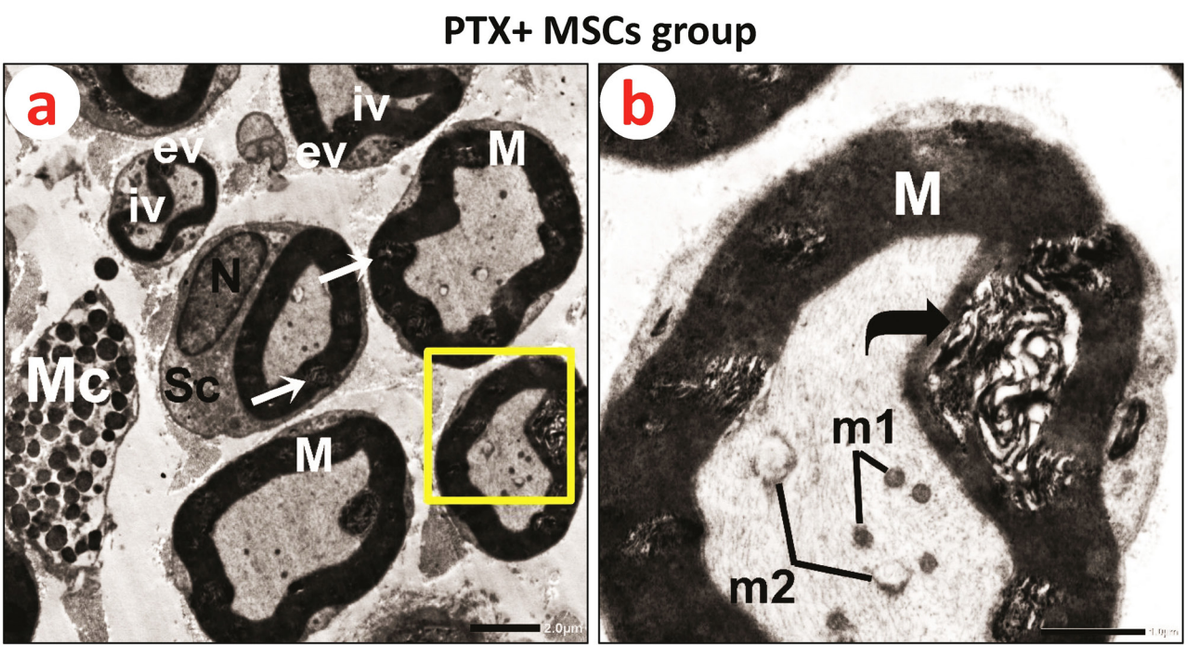

Fig. 10: Electron micrographs of Sciatic nerve isolated from the PTX+ MSCs group showing: Improved histological morphology compared to the PTX-neuropathy group. Some axons appeared with improved myelin sheaths (M) except for slightly separated lamellae (↑), some myelin sheaths are showing alternating invaginations (iv) and evaginations (ev). Schwann cell (Sc) appears normal with euchromatic nucleus (N). Notice: Mast cells with dense granules can be seen (Mc). The yellow boxed area is magnified in Figure (b) showing an axon with myelin sheath (M) displaying an area of lamellar splitting filled with debris (bent arrow). The axoplasm shows some normal mitochondria (m1) and some distorted mitochondria with destructed cristae (m2).

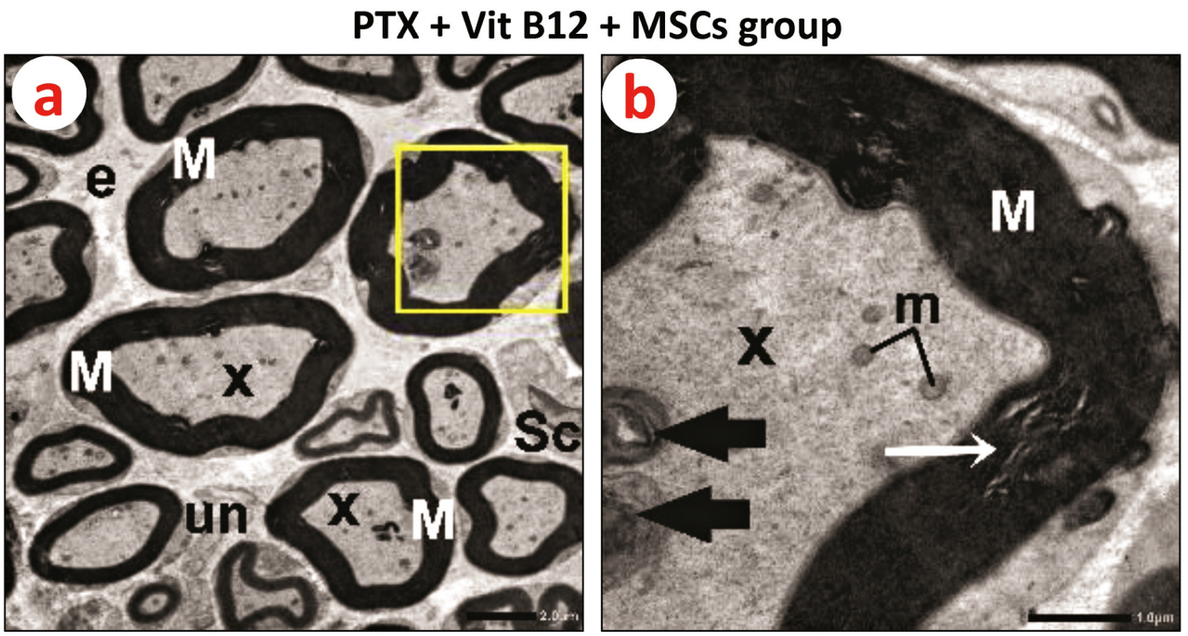

Fig. 11: Electron micrographs of Sciatic nerve isolated from PTX+ vit B12+ MSCs group showing (a): apparent improved histological structure. Most axons (x) appear more or less than normal with healthy myelin sheaths (M) within connective tissue endoneurium (e). Some unmyelinated nerve fibers (un) and Schwann cells (Sc) can be seen. The yellow boxed area is magnified in Figure (b) showing; axoplasm (x) with normal mitochondria (m) and autophagy vacuoles (thick arrows). A small area of focal splitting can be seen (↑).

Table 1: The test used: Kruskal Wallis followed by post-hoc Dunn's for comparison between groups Different superscript small alphabetical letters indicate differences in significance

	Control	PTX- neuropathy	PTX+Vit B12	PTX + MSCs	PTX+Vit B12+ MSCs	*P value*
Nom of CD68 positive macrophage cells	1.5(1.0-3.0)^{a}	7.0(6.0-9.0)^{c}	4.0(4.0-5.0)^{bc}	3.5(3.0-5.0)^{b}	2.0(1.0-3.0)^{a}	<0.001*

Data expressed as median (range), *: significance ≤0.05

Table 2: The test used: One-way ANOVA followed by post-hoc Tukey for comparison between groups Different superscript small alphabetical letters indicate differences in significance

	Control	PTX- neuropathy	PTX+Vit B12	PTX + MSCs	PTX+ Vit B 12 + MSCs	*Pvalue*
Casp1 area% expression	4.4±0.8^{a}	38.3±1.4^{c}	17.5±0.9^{d}	12.2±1.9^{c}	7.1±0.4^{b}	<0.001*
NLRP3 area % expression	1.1±0.3^{a}	30.7±1.2^{d}	11.7±0.6^{c}	7.2±0.7^{b}	6.5±0.8^{b}	<0.001*

Data expressed as mean ±SD, *: significance ≤0.05

areas of focal splitting.

DISCUSSION

The most frequent non-hematological side effect of taxane is peripheral neuropathy (PN), which may necessitate dose reduction or treatment discontinuation, which could negatively affect patient survival[32].

Our study provides additional evidence that PTX therapy may be coupled with the risk of peripheral neuropathy. We applied an established animal model of PTX-peripheral neuropathy and the obtained H&E as well as toluidine blue results showed that PTX-induced highly degenerated fibers with disrupted myelin sheaths, congested blood vessels, and infiltrated macrophages. The most striking of all the results was the ultra-structural changes observed in ultrathin sections following PTX-intoxication, which revealed vacuolated axons, shrunken axons, myelin lamellar splitting, and necrotic debris. A considerable amount of previous research demonstrated the same results[33,34].

Another crucial finding of our work is the existence of up-regulated immunomarkers of NLRP3 inflammasome pathway as indicated by significant increased (p<0.05) CD68 positive cells, significant increased (p<0.05) area % of NLRP3 and caspase-1 immune expression. These results were in parallel with previous results of previous studies[35,36].

It is well known that; axonal degeneration is a key

feature and a major hallmark in peripheral neuropathy. A wide variety of taxane-related stimuli can hasten axonal degeneration, leading to a systematic cascade of molecular mechanisms leading to axonal damage[37]. In this multifactorial pathophysiological cascade, oxidative stress, mitochondrial injury, apoptosis, altered ion channel functions, and lost myelination are all postulated[38]. Most of these physiological and molecular mechanisms implicated in PTX-induced neuropathy have been summarized in detail by prior study[39].

Among the multiplicity of the pathophysiologic mechanisms postulated for the detrimental effects of PTX-neuropathy, increasing evidence proposes that inflammasome is a direct cause of PTX-induced sciatic inflammation[35]. The expression of NLRP3 was primarily found in macrophages that infiltrate the sciatic nerve after injury[40]. Pro-inflammatory cytokines are released when the NLRP3 inflammasome is formed and activated in response to risk stimuli[41]. Specifically, PTX promotes an increase in TNF-α, IL-8, and IL-1β pro-inflammatory cytokines with the suppression of anti-inflammatory cytokines IL-4 and IL-10[42]. In addition, it has been demonstrated that abnormal NLRP3 activation impedes recovery from PTX toxicity, worsens disease pathology[43], and increases the risk of neuropathic pain[44].

To come up with a different theory, earlier research has also demonstrated that microtubules are crucial in controlling how the NLRP3 inflammasome is put together[45]. Because paclitaxel binds to -tubulin, it stabilizes the mitotic spindle and microtubules. As a result, PTX unluckily increases NLRP3 inflammasome activation[46] and decreases neuronal axonal transport of organelles, neurotransmitters, and nutrients[47,48].

All of the aforementioned information suggests that anti-inflammatory therapy targeting the NLRP3 inflammasome pathway may be a useful strategy for reducing sciatic neuropathy.

A prior paper by McCarty *et al*[49] on the NLRP3 pathway concluded that dietary supplements like N-acetylcysteine, glucosamine, taurine, folate, vitamin B12, and betaine may have the clinical potential to reduce the role of NLRP3 inflammasomes in a variety of inflammation-related insults. Precisely, group (B) vitamins are the most widely used, generally affordable, safe, and effective vitamins when taken as supplements[50]. On the other hand, lack of vitamin B12 is well recognized to produce neuropathies, which are typically accompanied by paresthesia, numbness, and ataxia[51]. Furthermore, vitamin B12 directly affects how harmful anticancer medications are. According to Esam *et al*[52], cobalamin supplementation was utilized to lessen the severity of chemotherapy-induced peripheral neuropathy, which affects about 1/3 of all chemotherapy patients. So, we tested the use of vitamin B12 in alleviating PTX-neuropathy.

The PTX-neuropathy group treated with Vitamin B12 in our study revealed slightly improved histological morphology. H&E stained sections revealed less degenerated fibers. Sciatic ultrastructure examination showed less toxic effects in myelin sheaths, however, myelin splitting and necrotic debris were still observed. Immunohistochemical examination revealed mild significantly downregulated (p<0.05) immuno-markers of NLRP3 inflammasome pathway as indicated by slightly decreased immunoexpression of proinflammatory macrophage CD68, NLRP3, and caspase-1 compared to the pathological group.

Simultaneously, another team by Yuan *et al*.[53] demonstrated an increase in the number and diameter of myelinated fibers as well as the lamellae number after using vitamin B12 for nerve regeneration in mice-induced sciatic injury. A comprehensive review[54] conducted before explained this marginal improvement and concluded that vitamin B12 has beneficial effects on nerve regeneration by stabilizing microtubules, encouraging remyelination and myelin repair, inhibiting the apoptosis of damaged neurons, and promoting neurite outgrowth by fostering a recovery-friendly environment.

Conversely to our findings, Schloss *et al*[55] found that vitamin B complex supplementation was statistically unsuccessful at preventing CIPN. In addition, vitamin B12 supplementation has been linked to an elevated risk of colon carcinomas, according to research by[56].

MSCs were the first cells discovered and researched in regenerative medicine stem cells as a form of therapy[57]. These cells are a reliable source for treating a variety of illnesses and regenerating damaged tissues through paracrine processes such as pro-angiogenesis, anti-apoptosis, and immunomodulatory effects or direct differentiation into tissue-type cells[58]. The use of stem cells to treat neuropathic insults has shown positive results, with MSCs being the greatest instrument for cell-based therapy[59]. This is crucial information for our subject.

Intravenous injection of BMMSCs treated neuropathy group showed moderate improvement in the histological morphology. More myelinated fibers with Schwann cell proliferation appeared. Less congested blood vessels, and fewer vacuolations were also noticed. Ultrastructure examination supported these results with healthier myelin sheaths, less separated lamellae, less debris, and healthy unmyelinated fibers. Two MSCs-based mechanisms explained this improvement in the current study. The first one is the recruitment and differentiation of MSCs into nerve fibers which was confirmed using PKH26-fluorescently labeled MSCs in groups treated with MSCs. According to previous theory, MSCs encourage regeneration of peripheral nerves primarily by developing into Schwann cells[60].

The second mechanism was a targeted approach to prevent toxicity-induced neuroinflammatory events cascade. The apparent suppression of the NLRP3 inflammasome pathway was confirmed immunohistochemically by

significant downregulated (p<0.05) CD68, NLRP3, and caspase-1 immunomarkers.

About this anti-inflammatory action, Fan and his team[61] declared that MSCs produce a range of soluble chemicals and regulate the host's cytokine production. Furthermore, according to research by Kouroupis *et al*[62], MSCs were also discovered to be a potent suppressor of the NLRP3 inflammasome pathway. Earlier research discussed this inhibitory effect of MSCs on macrophages[63], inflammatory cardiomyopathy[64] and, inflammatory renal disease[65]. Another work[66] concluded that transplanting MSCs into the Primary ovarian insufficiency (POI) rat model reduced pyroptosis, NLRP3 inflammasome formation, pro-inflammatory factor production, and enhanced ovarian function. This may suggest that one of MSCs' potential mechanisms for regeneration involves regulation of the NLRP3 inflammasome, which could mediate the local inflammatory response that results from PTX-neuropathy and change the milieu towards a pro-regenerative phenotype. To our knowledge, this is the first study of its kind to look into improving axonal regeneration using combined therapy of vitamin B12 and mesenchymal stem cells.

Vitamins are known to play a decisive role in eukaryotes' metabolism because they enhance anti-oxidant mechanisms[67]. Recent research investigated the relevance of vitamins to increase MSCs' therapeutic value[68].

Based on these considerations, we observed the good regenerative potential afforded by combined vitamin B12 and MSCs injection on the repair ability compared to using each therapy alone. In the combined therapy group, we demonstrated improved histological, ultrastructural, and immunohistochemical results. Axonal histology was improved with more or less healthy myelin sheaths compared to the control group. CD68, NRLP3, and caspase-1 were statistically downregulated (p <0.05) to nearly normal levels.

By our findings, Zhou and his team concluded that MSCs in combination with the probiotic formulation (VSL#3) prevented neurodegenerative changes in Parkinson's disease mice through anti-inflammatory activities possibly by inhibiting the NLRP3 inflammasome[69]. Moreover, in a previous study, Wang and his team[70] concluded that adenosylcobalamin B12-dependent hydrogels may enhance MSCs' viability in 3D culture.

Another prior study showed that vitamin B12 was helpful for in *vitro* tooth or bone regeneration by MSCs derived from rat dental pulp. Increased development of calcified nodules occurs when vitamin B12 is added to the medium[71].

CONCLUSION

The outcomes of this study highlighted that vitamin B12 and BMMSCs were able to exert anti-inflammatory effects on PTX-induced neuropathy, but better augmented effects when used together. One limitation to the work described here is that we cannot completely rule out a beneficial effect of this combined therapy on the neuropathic pain combining this inflammatory process. Moreover, the biochemical parameters accompanying the inflammatory process need to be assessed. So, future research in the area of sciatic regeneration should follow these considerations.

CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Klafke N, Bossert J, Kröger B, Neuberger P, Heyder U, Layer M, Winkler M, Idler C, Kaschdailewitsch E, Heine R, John H, Zielke T, Schmeling B, Joy S, Mertens I, Babadag-Savas B, Kohler S, Mahler C, Witt CM, Steinmann D, Voiss P, Stolz R. Prevention and Treatment of Chemotherapy-Induced Peripheral Neuropathy (CIPN) with Non-Pharmacological Interventions: Clinical Recommendations from a Systematic Scoping Review and an Expert Consensus Process. Medical Sciences. (2023); 11(1):15. DOI: 10.3390/medsci11010015
2. Al-Massri, KF; Ahmed, LA.; El-Abhar HS. Mesenchymal stem cells in chemotherapy-induced peripheral neuropathy: A new challenging approach that requires further investigations. Journal of Tissue Engineering and Regenerative Medicine. (2020); 14(1): 108-122. DOI: 10.1002/term.2972.
3. Andrei, C.; Zanfirescu, A.; Mihai, D.P.; Negres, S. Paclitaxel A Valuable Tool for Inducing Visceral Pain in Preclinical Testing? Int. J. Transl. Med. (2023); 3(1):108–119. DOI: 10.3390/ijtm3010010
4. Brandolini L, D'Angelo M, Novelli R, Castelli V, Giorgio C, Sirico A, Cocchiaro P, D'Egidio F, Benedetti E, Cristiano C, Bugatti A, Ruocco A, Amendola PG, Talarico C, Manelfi C, Iaconis D, Beccari A, Quadros AU, Cunha TM, Caruso A, Russo R, Cimini A, Aramini A, Allegretti M. Paclitaxel binds and activates C5aR1: A new potential therapeutic target for the prevention of chemotherapy-induced peripheral neuropathy and hypersensitivity reactions. Cell Death Dis. (2022); 13(5):500. DOI: 10.1038/s41419-022-04964-w
5. Buscemi L, Price M, Bezzi P, Hirt L. Spatio-temporal overview of neuroinflammation in an experimental mouse stroke model. Sci Rep. (2019); 9(1):507. DOI: 10.1038/s41598-018-36598-4
6. Molnár K, Nógrádi B, Kristóf R, Mészáros Á, Pajer K, Siklós L, Nógrádi A, Wilhelm I, Krizbai IA. Motoneuronal inflammasome activation triggers excessive neuroinflammation and impedes regeneration after sciatic nerve injury. J Neuroinflammation. (2022); 19(1):68. DOI: 10.1186/s12974-022-02427-9
7. Li J, Wang X, Yao Z, Yuan F, Liu H, Sun Z, Yuan Z, Luo G, Yao X, Cui H, Tu B, Sun Z, Fan C. NLRP3-Dependent Crosstalk between Pyroptotic Macrophage

and Senescent Cell Orchestrates Trauma-Induced Heterotopic Ossification During Aberrant Wound Healing. Adv Sci (Weinh). (2023); 10(19):e2207383. DOI: 10.1002/advs.202207383

8. Khan N, Kuo A, Brockman DA, Cooper MA, Smith MT. Pharmacological inhibition of the NLRP3 inflammasome as a potential target for multiple sclerosis-induced central neuropathic pain. Inflammopharmacology. (2018); 26(1):77–86. DOI: 10.1007/s10787-017-0401-9
9. Yan M, Song Z, Kou H, Shang G, Shang C, Chen X, Ji Y, Bao D, Cheng T, Li J, Lv X, Liu H, Chen S. New Progress in Basic Research of Macrophages in the Pathogenesis and Treatment of Low Back Pain. Front Cell Dev Biol. (2022); 20(10): 866857. DOI: 10.3389/fcell.2022.866857
10. Blevins HM, Xu Y, Biby S, Zhang S. The NLRP3 Inflammasome Pathway: A Review of Mechanisms and Inhibitors for the Treatment of Inflammatory Diseases. Front. Aging Neurosci. (2022); 14:879021. DOI: 10.3892/ijmm.2023.5238
11. Moretti J, Jia B, Hutchins Z, Roy S, Yip H, Wu J, Shan M, Jaffrey SR, Coers J, Blander JM. Caspase-11 interaction with NLRP3 potentiates the noncanonical activation of the NLRP3 inflammasome. Nat Immunol. (2022); 23(5):705-717. DOI: 10.1038/s41590-022-01192-4
12. Chen R, Yin C, Fang J, Liu B. The NLRP3 inflammasome: an emerging therapeutic target for chronic pain. J Neuroinflammation. (2021); 18(1):84. DOI: 10.1186/s12974-021-02131-0
13. Chang MX. Emerging mechanisms and functions of inflammasome complexes in teleost fish. Front Immunol. (2023); 14:1065181. DOI: 10.3389/fimmu.2023.1065181
14. O'Brien WT, Pham L, Symons GF, Monif M, Shultz SR, McDonald SJ. The NLRP3 inflammasome in traumatic brain injury: potential as a biomarker and therapeutic target. J Neuroinflammation. (2020); 17(1): 104. DOI: 10.1016/j.lfs.2022.121352
15. Li M, Jiang Y, Hou Q, Zhao Y, Zhong L, Fu X. Potential pre-activation strategies for improving the therapeutic efficacy of mesenchymal stem cells: current status and future prospects. Stem Cell Res Ther. (2022); 13(1):146. DOI: 10.1186/s13287-022-02822-2
16. Zeng QZ, Yang F, Li CG, Xu LH, He XH, Mai FY, Zeng CY, Zhang CC, Zha QB, Ouyang DY. Paclitaxel Enhances Innate Immunity by Promoting NLRP3 Inflammasome Activation in Macrophages. Front. Immunol. (2019); 29(10):72. DOI: 10.3389/fimmu.2019.00072
17. Cui M, Liang J, Xu D, Zhao L, Zhang X, Zhang L, Ren S, Liu D, Niu X, Zang YJ, Zhang B. NLRP3 inflammasome is involved in nerve recovery after sciatic nerve injury. Int Immunopharmacol. (2020); 84:106492. DOI: 10.1016/j.intimp.2020.106492
18. Wu HW, Chen HD, Chen YH, Mao XL, Feng YY, Li SW, Zhou XB. The Effects of Programmed Cell Death of Mesenchymal Stem Cells on the Development of Liver Fibrosis. Stem Cells Int. (2023); 4586398. DOI: 10.1155/2023/4586398
19. Zhang J, Zheng Y, Huang L, He J. Research Progress on Mesenchymal Stem Cells for the Treatment of Diabetes and Its Complications. Int J Endocrinol. (2023); 24:9324270. DOI: 10.1155/2023/9324270
20. Barzaghini B, Carelli S, Messa L, Rey F, Avanzini MA, Jacchetti E, Maghraby E, Berardo C, Zuccotti G, Raimondi MT, Cereda C, Calcaterra V, Pelizzo G. Bone Marrow Mesenchymal Stem Cells Expanded Inside the Nichoid Micro-Scaffold: a Focus on Anti-Inflammatory Response. Regen Eng Transl Med. (2023); 20:1-12. DOI: 10.1007/s40883-023-00296-z
21. Saadh MJ, Mikhailova MV, Rasoolzadegan S, Falaki M, Akhavanfar R, Gonzáles JLA, Rigi A, Kiasari BA. Therapeutic potential of mesenchymal stem/stromal cells (MSCs)-based cell therapy for inflammatory bowel diseases (IBD) therapy. Eur J Med Res. (2023); 28(1):47. DOI: 10.1186/s40001-023-01008-7.
22. Li W, Liang J, Li S, Wang L, Xu S, Jiang S, Song M, Meng H, Zhai D, Tang L, Yang Y, Zhang L, Zhang B. Research progress of targeting NLRP3 inflammasome in peripheral nerve injury and pain. Int Immunopharmacol. (2022); 110:109026. DOI: 10.1016/j.intimp.2022.109026.
23. Abdullah RH, Yaseen NY, Saleh SM, Mohamed MH, Al-Shammari AM. Direct and Simple Method for Mesenchymal Stem Cells Isolation, Culturing and Detection. Int J Stem Cell Res Ther. (2018); 5:054. DOI: 10.23937/2469-570X/1410054.
24. Lin CS, Xin ZC, Dai J, Lue TF. Commonly used mesenchymal stem cell markers and tracking labels: Limitations and challenges. Histol Histopathology. (2013); 28(9):1109-1116. DOI: 10.14670/HH-28.1109.
25. Shibata T, Naruse K, Kamiya H, Kozakae M, Kondo M, Yasuda Y, Nakamura N, Ota K, Tosaki T, Matsuki T, Nakashima E, Hamada Y, Oiso Y, Nakamura J. Transplantation of bone marrow-derived mesenchymal stem cells improves diabetic polyneuropathy in rats. Diabetes. (2008); 57(11):3099-107. DOI: 10.2337/db08-0031
26. Singh J, Thapliyal S, Kumar A, Paul P, Kumar N, Bisht M, Naithani M, Rao S, Handu SS. Dimethyl Fumarate Ameliorates Paclitaxel-Induced Neuropathic Pain in Rats. Cureus. (2022); 14(9):e28818. DOI: 10.7759/cureus.28818

27. Mizukami H, Ogasawara S, Yamagishi S, Takahashi K, Yagihashi S. Methylcobalamin effects on diabetic neuropathy and nerve protein kinase C in rats. Eur J Clin Invest. (2011); 41(4):442-50. DOI: 10.1111/j.1365-2362.2010.02430.x

28. Cooney DS, Wimmers EG, Ibrahim Z, Grahammer J, Christensen JM, Brat GA, Wu LW, Sarhane KA, Lopez J, Wallner C, Furtmüller GJ, Yuan N, Pang J, Sarkar K, Lee WP, Brandacher G. Mesenchymal Stem Cells Enhance Nerve Regeneration in a Rat Sciatic Nerve Repair and Hindlimb Transplant Model. Sci Rep. (2016); 6(1):31306. DOI: 10.1038/srep31306

29. Bancroft, JD. Layton, C. The hematoxylin and eosin, in: S.K. Suvarna, C. Layton, J. D. Bancroft (Eds.), Bancroft's Theory Pract. Histol. Tech, 8th ed. (2019). Elsevier, Philadelphia, pp. 126–138.

30. Sanderson T, Wild G, Cull AM, Marston J, Zardin G. Immunohistochemical and immunofluorescent techniques, in Bancroft's Theory Pract. Histol. Tech., 8th ed (2019). Elsevier, Phialdelphia, pp. 337–396.

31. Kuo. J: Electron Microscopy: Methods and Protocols (Methods in Molecular Biology, 1117). Chapter 1 (Conventional Specimen Preparation Techniques for Transmission Electron Microscopy of Cultured Cells. pp; 1-21). The 3rd ed. (2014). Springer. DOI: 10.1007/978-1-62703-776-1

32. Burgess J, Ferdousi M, Gosal D, Boon C, Matsumoto K, Marshall A, Mak T, Marshall A, Frank B, Malik RA, Alam U. Chemotherapy-Induced Peripheral Neuropathy: Epidemiology, Pathomechanisms and Treatment. Oncol Ther. (2021); 9(2):385-450. DOI: 10.1007/s40487-021-00168-y

33. Singh J, Thapliyal S, Kumar A, Paul P, Kumar N, Bisht M, Naithani M, Rao S, Handu SS. Dimethyl Fumarate Ameliorates Paclitaxel-Induced Neuropathic Pain in Rats. Cureus. (2022); 14(9):e28818. DOI: 10.7759/cureus.28818

34. Singh J, Saha L, Singh N, Kumari P, Bhatia A, Chakrabarti A. Study of nuclear factor-2 erythroid related factor-2 activator, berberine, in paclitaxel-induced peripheral neuropathy pain model in rats. J Pharm Pharmacol. (2019); 71:797-805. DOI: 10.1111/jphp.13047

35. Jia M, Wu C, Gao F, Xiang H, Sun N, Peng P, Li J, Yuan X, Li H, Meng X, Tian B, Shi J, Li M. Activation of NLRP3 inflammasome in peripheral nerve contributes to paclitaxel-induced neuropathic pain. Mol Pain. (2017); 13:1744806917719804. DOI: 10.1177/1744806917719804

36. Chen YF, Wu CH, Chen LH, Lee HW, Lee JC, Yeh TK, Chang JY, Chou MC, Wu HL, Lai YP, Song JS, Yeh KC, Chen CT, Lee CJ, Shia KS, Shen MR. Discovery of Potential Neuroprotective Agents against Paclitaxel-Induced Peripheral Neuropathy. J Med Chem. (2022); 65(6):4767-4782. DOI: 10.1021/acs.jmedchem.1c01912

37. Park SB, Cetinkaya-Fisgin A, Argyriou AA, Höke A, Cavaletti G, Alberti P. Axonal degeneration in chemotherapy-induced peripheral neurotoxicity: clinical and experimental evidence. J Neurol Neurosurg Psychiatry. (2023); 94(11):962-972. DOI: 10.1136/jnnp-2021-328323

38. Zajaczkowska, R., Kocot-Kepska, M., Leppert, W., Wrzosek, A., Mika, J., & Wordliczek, J. Mechanisms of chemotherapy-induced peripheral neuropathy. Int J Mol Sci, (2019); 20(6): 1451. DOI: 10.3390/physiologia3040042

39. Hammad ASA, Sayed-Ahmed MM, Khalifa MMA, El-Daly M. Mechanisms of Paclitaxel-Induced Peripheral Neuropathy. J. Adv. Biomed. & Pharm. Sci. (2023); 6:25 – 35. DOI: 10.21608/JABPS.2022.170238.1172

40. Donnelly CR, Chen O, Ji RR. How do sensory neurons sense danger signals? Trends Neurosci. (2020); 43(10):822–38. DOI: 10.1016/j.tins.2020.07.008

41. Zhang C, Huang Y, Ouyang F, Su M, Li W, Chen J, Xiao H, Zhou X, Liu B. Extracellular vesicles derived from mesenchymal stem cells alleviate neuroinflammation and mechanical allodynia in interstitial cystitis rats by inhibiting NLRP3 inflammasome activation. J Neuroinflammation. (2022); 19 (1):80. DOI: 10.1186/s12974-022-02445-7

42. Singh AK, Mahalingam R, Squillace S, Jacobson KA, Tosh DK, Dharmaraj S, Farr SA, Kavelaars A, Salvemini D, Heijnen CJ. Targeting the A3 adenosine receptor to prevent and reverse chemotherapy-induced neurotoxicities in mice. Acta Neuropathol Commun. (2022); 10(1):11. DOI: 10.1186/s40478-022-01315-w

43. Son S, Shim DW, Hwang I, Park JH, Yu JW. Chemotherapeutic Agent Paclitaxel Mediates Priming of NLRP3 Inflammasome Activation. Front Immunol. (2019); 10:1108. DOI: 10.3389/fimmu.2019.01108

44. Chen, C., Smith, M.T. The NLRP3 inflammasome: role in the pathobiology of chronic pain. Inflammopharmacol. Inflammopharmacology. (2023); 31(4):1589-1603. DOI: 10.1007/s10787-023-01235-8

45. Li X, Thome S, Ma X, Amrute-Nayak M, Finigan A, Kitt L, Masters L, James JR, Shi Y, Meng G, Mallat Z. MARK4 regulates NLRP3 positioning and inflammasome activation through a microtubule-dependent mechanism. Nat Commun. (2017); 28(8):15986. DOI: 10.17863/CAM.12594

46. Arnst KE, Banerjee S, Chen H, Deng S, Hwang DJ, Li W, Miller DD. Current advances of tubulin inhibitors as dual-acting small molecules for cancer therapy. Med Res Rev. (2019); 39(4):1398-1426. DOI: 10.1002/med.21568

47. Klein I, Lehmann HC. Pathomechanisms of paclitaxel-induced peripheral neuropathy. Toxics. (2021); 9(10):229. DOI: 10.3390/toxics9100229

48. Desforges AD, Hebert CM, Spence AL, Reid B, Dhaibar HA, Cruz-Topete D, Cornett EM, Kaye AD, Urits I, Viswanath O. Treatment and diagnosis of chemotherapy-induced peripheral neuropathy: An update. Biomed Pharmacother. (2022); 147(2):112671. DOI: 10.1016/j.biopha.2022.112671

49. McCarty MF, Iloki Assanga SB, Lewis Luján L, O'Keefe JH, DiNicolantonio JJ. Nutraceutical Strategies for Suppressing NLRP3 Inflammasome Activation: Pertinence to the Management of COVID-19 and Beyond. Nutrients. (2020); 13(1):47. DOI: 10.3390/nu13010047

50. Szklener K, Szklener S, Michalski A, Żak K, Kuryło W, Rejdak K, Mańdziuk S. Dietary Supplements in Chemotherapy-Induced Peripheral Neuropathy: A New Hope? Nutrients. (2022); 14(3):625. DOI: 10.3390/nu14030625

51. Wolffenbuttel BHR, Wouters HJCM, Heiner-Fokkema MR, Van der Klauw MM. The Many Faces of Cobalamin (Vitamin B12) Deficiency. Mayo Clin Proc Innov Qual Outcomes. (2019); 3(2):200-214. DOI: 10.1016/j.mayocpiqo.2019.03.002

52. Esam S, Naser I, ALWahidi K. Is Functional Vitamin B12 Deficiency a Risk Factor for the Development of Chemotherapy-Induced Peripheral Neuropathy in Cancer Patients? Research Square; 2022. 1-23.https://doi.org/10.21203/rs.3.rs-1667065/v1

53. Yuan Y, Shen H, Yao J, Hu N, Ding F, Gu X. "The protective effects of _Achyranthes bidentata_ polypeptides in an experimental model of mouse sciatic nerve crush injury," Brain Res Bull. (2010); 81(1):25-32. DOI: 10.1016/j.brainresbull.2009.07.013

54. Baltrusch S. The Role of Neurotropic B Vitamins in Nerve Regeneration. Biomed Res Int. (2021);4: 1-9. DOI: 10.1155/2021/9968228

55. Schloss JM, Colosimo M, Airey C, Masci P, Linnane AW, Vitetta LA. Randomized, Placebo-Controlled Trial Assessing the Efficacy of an Oral B Group Vitamin in Preventing the Development of Chemotherapy-Induced Peripheral Neuropathy (CIPN) Support. Care Cancer. (2017); 25(1):195–204. DOI: 10.1007/s00520-016-3404-y

56. Urbanski G, Hamel JF, Prouveur B, Annweiler C, Ghali A, Cassereau J, Lozac'h P, Lavigne C, Lacombe V. Strength of the Association of Elevated Vitamin B12 and Solid Cancers: An Adjusted Case-Control Study. J Clin Med. 2020 Feb 9;9(2):474. DOI: 10.3390/jcm9020474

57. Lavorato A, Raimondo S, Boido M, Muratori L, Durante G, Cofano F, Vincitorio F, Petrone S, Titolo P, Tartara F, Vercelli A, Garbossa D. Mesenchymal Stem Cell Treatment Perspectives in Peripheral Nerve Regeneration: Systematic Review. Int J Mol Sci. (2021); 22(2):572. DOI: 10.3390/ijms22020572

58. Joshi HP, Jo HJ, Kim YH, An SB, Park CK, Han I. Stem cell therapy for modulating neuroinflammation in neuropathic pain. Int J Mol Sci. (2021); 22(9):4853. DOI: 10.3390/ijms22094853

59. Lee N, Park GT, Lim JK, Choi EB, Moon HJ, Kim DK, Choi SM, Song YC, Kim TK, Kim JH. Mesenchymal stem cell spheroids alleviate neuropathic pain by modulating chronic inflammatory response genes. Front Immunol. (2022); 8(13):940258. DOI: 10.3389/fimmu.2022.940258

60. Aman M, Schulte M, Li Y, Thomas B, Daeschler S, Mayrhofer-Schmid M, Kneser U, Harhaus L, Boecker A. Benefit of Adjuvant Mesenchymal Stem Cell Transplantation to Critical-Sized Peripheral Nerve Defect Repair: A Systematic Review and Meta-Analysis of Preclinical Studies. J Clin Med. (2023); 12(4):1306. DOI: 10.3390/jcm12041306

61. Fan X-L, Zhang Y, Li X, Fu Q-L. Mechanisms underlying the protective effects of mesenchymal stem cell-based therapy. Cell Mol Life Sci (2020); 77(1):2771–2794. DOI: 10.1007/s00018-020-03454-6

62. Kouroupis D, Bowles AC, Greif DN, Leñero C, Best TM, Kaplan LD, Correa D. Regulatory-compliant conditions during cell product manufacturing enhance in *vitro* immunomodulatory properties of infra-patellar fat pad-derived mesenchymal stem/stromal cells. Cytotherapy. (2020); 22(11):677-689. DOI: 10.1016/j.jcyt.2020.06.007

63. Oh JY, Ko JH, Lee HJ, Yu JM, Choi H, Kim MK, Wee WR, Prockop DJ. Mesenchymal stem/stromal cells inhibit the NLRP3 inflammasome by decreasing mitochondrial reactive oxygen species. Stem Cells. (2014); 32(6):1553-1563. DOI: 10.1002/stem.1608

64. Miteva K, Pappritz K, Sosnowski M, El-Shafeey M, Müller I, Dong F, Savvatis K, Ringe J, Tschöpe C, Van Linthout S. Mesenchymal stromal cells inhibit NLRP3 inflammasome activation in a model of Coxsackie virus B3-induced inflammatory cardiomyopathy. Sci Rep. (2018); 8(1):2820. DOI: 10.1038/s41598-018-20686-6

65. Zhu Q, Li XX, Wang W, Hu J, Li PL, Conley S, Li N. Mesenchymal stem cell transplantation inhibited high salt-induced activation of the NLRP3 inflammasome in the renal medulla in Dahl S rats. Am J Physiol Renal Physiol. (2016); 310(7): F621–F627. DOI: 10.1152/ajprenal.00344.2015

66. Chen D, Hu N, Xing S, Yang L, Zhang F, Guo S, Liu S, Ma X, Liang X, Ma H. Placental mesenchymal stem cells ameliorate NLRP3 inflammasome-induced ovarian insufficiency by modulating macrophage M2 polarization. J Ovarian Res. (2023); 16(1):58. DOI: 10.1186/s13048-023-01136-y

67. Del Mondo A, Smerilli A, Sané E, Sansone C, Brunet C. Challenging microalgal vitamins for human health. Microb Cell Fact. (2020); 19(1):201. DOI: 10.1186/s12934-020-01459-1

68. Al-Azab M, Idiiatullina E, Safi M, Hezam K. Enhancers of mesenchymal stem cell stemness and therapeutic potency. Biomed Pharmacother. (2023); 162(3):114356. DOI: 10.1016/j.biopha.2023.114356

69. Zhou L, Han D, Wang X, Chen Z. Probiotic Formulation VSL#3 Interacts with Mesenchymal Stromal Cells To Protect Dopaminergic Neurons via Centrally and Peripherally Suppressing NOD-Like Receptor Protein 3 Inflammasome-Mediated Inflammation in Parkinson's disease Mice. Microbiol Spectr. (2023); 11(2):e0320822. DOI: 10.1128/spectrum.03208-22

70. Wang R, Yang Z, Luo J, Hsing IM, Sun F. B12-dependent photoresponsive protein hydrogels for controlled stem cell/protein release. Proceedings of the National Academy of Sciences. (2017); 114(23): 5912-5917. DOI: 10.1073/pnas.1621350114

71. Inamoto T, Yoshikawa M, Miyamoto A, Maeda H. Effects of vitamin B12 in culture medium for calcified nodule formation by rat dental pulp cells. J Dent Sci. (2022); 18(3):1079-1085. DOI: 10.1016/j.jds.2022.11.015

الملخص العربى

التأثير التآزري لفيتامين ب١٢ والخلايا الجذعية الوسيطة للتخفيف من الاعتلال العصبي الوركي الناجم عن باكليتاكسيل في الجرذان البيضاء عن طريق تثبيط المسار الالتهابي NLRP3 دراسة هستولوجية و هستوكيميائيه مناعية

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الخلفية: التاكسانات هي مجموعة واسعة من الأدوية المضادة للسرطان. ويعتبر الاعتلال العصبي المحيطي هو التأثير الجانبي الرئيسي للباكليتاكسل و المقيد للجرعة طويله الأمد. يحتوي هذا التأثير الضار على تأثير واضح في المسار الالتهابي NLRP٣. و قد يكون لفيتامين ب ١٢ مع الخلايا الجذعية الوسيطة المشتقه من نخاع العظام دور مخفف.

هدف البحث: الهدف من هذه الدراسة هو تقييم التأثير التآزري المحتمل لفيتامين ب١٢ و الخلايا الجذعية الوسيطة المشتقة من نخاع العظام في تخفيف الاعتلال العصبي الوركي وتحديد التأثير المضاد للمسار الالتهابيي NLRP٣

المواد و الطرق المستخدمة: تم توزيع حوالي ٥٠ فأرًا أبيضًا بالغًا على خمسة مجموعات. مجموعة (التحكم) لم تتلقى أي علاج، المجموعة الثانية (مجموعة الاعتلال العصبي بواسطة الباكليتاكسل): تم حقن الفئران داخل الصفاق بعقار الباكليتاكسل (٢,٠ مجم كجم−١) في الأيام ١ و٣ و٥ و٨، المجموعة الثالثة (باكليتاكسل + فيتامين ب ١٢) عولجت مثل المجموعة الثانية ، ثم في اليوم العاشر، تم حقن الفئران بفيتامين ب ١٢ (١٠ ملغم / كغم) كل يومين داخل العضل لمدة ٢٨ يومًا، المجموعة الرابعة (الباكليتاكسل + الخلايا الجذعية الوسيطة) عولجت مثل المجموعة الثانية، ثم في اليوم العاشر، تم حقن الفئران عن طريق الوريد بجرعة وحيدة من الخلايا الجذعية الوسيطة المشتقة من نخاع العظام (١x١٠٦خلية) في ١ ملم من محلول الملح. وأخيراً, المجموعة الخامسة (الباكليتاكسل + فيتامين ب ١٢+ الخلايا الجذعية الوسيطة) عولجت مثل المجموعة الثانية ، ثم تم حقنها بفيتامين ب ١٢و الخلايا الجذعية الوسيطة بنفس الجرعات المذكورة سابقاً. تم ذبح الفئران وجمع عينات من الأنسجة العصبية الوركية ومعالجتها وفحصها نسيجيا ومناعيا وكيميائيا وتحت المجهر الإلكتروني.

النتائج: أظهرت مجموعة الباكليتاكسل تشوهًا نسيجيًا ملحوظًا، واحتقان بالأوعية الدموية، وانخفاض عدد خلايا شوان، وتدهور في الألياف، وفراغات في الخلايا، وزياده في المؤشرات المناعية لبروتينات ضد CD٦٨ وNLRP٣ و caspase-١ . وأظهرت مجموعة باكليتاكسل + فيتامين ب ١٢ تحسنًا نسجيًا بسيطا، وانخفاض بسيط في المؤشرات المناعية ضد CD٦٨ وNLRP٣ وcaspase-١. أظهرت مجموعة الباكليتاكسل مع الخلايا الجذعية الوسيطة تحسنًا نسيجيًا معتدلًا و انخفاض معتدل للمؤشرات المناعية ضد CD٦٨ وNLRP٣ وcaspase-١. و أظهرت مجموعه الباكليتاكسل و فيتامين ب ١٢ و الخلايا الجذعية الوسيطة تحسنًا نسجيًا واضحًا، وبدت أغلفة الميالين طبيعية إلى حد ما مع وجود انخفاض واضح للمؤشرات المناعية ضد CD٦٨ وNLRP٣ وcaspase١.

الاستنتاج: يمكن للعلاج المشترك بواسطة فيتامين ب ١٢ والخلايا الجذعية الوسيطة أن يخفف بشكل تأزري من الاعتلال العصبي الناجم عن الباكليتاكسل مع تثبيط واضح المسار الالتهابي NLRP٣.