Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

GRANULOCYTE COLONY-STIMULATING FACTOR INHIBITS PONTINE ASTROGLIOSIS AND INDUCES SCIATIC REMYELINATION IN A NEUROPATHIC RAT MODEL: ROLES OF GFAP, PCNA AND S100

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Purpose: *Neuropathic pain is characterized by poor treatment response. While previous studies on granulocyte colony-stimulating factor (GCSF) have primarily explored its effects on peripheral nerves, recent evidence suggests the central nervous system, particularly the brainstem pons, plays a crucial role in neuropathic pain modulation. This study investigated the effects of GCSF on the pons and sciatic nerve to alleviate neuropathic pain.* ***Methods:*** *Four groups of rats were created: the normal control, sham group, neuropathic group induced by chronic constriction injury of the sciatic nerve (CCI), and the GCSF-treated group received 50 µg/kg of GCSF subcutaneously starting on the 7th day of CCI for 5 consecutive days. Experimental testing for heat hyperalgesia, mechanical allodynia and mechanical hyperalgesia was performed. Immunohistochemical analyses were conducted to evaluate the central (pons) expression of glial fibrillary acidic protein (GFAP), proliferating cell nuclear antigen (PCNA), and S100 protein in the peripheral (sciatic nerve) tissues.* ***Results:****GCSF significantly alleviated heat hyperalgesia, mechanical allodynia, and mechanical hyperalgesia in CCI rats. In the pons, GCSF reduced GFAP expression, indicating inhibition of astrogliosis, and enhanced PCNA expression, suggesting promotion of neurogenesis. In the sciatic nerve, S100 expression was markedly elevated, implying enhanced remyelination.* ***Conclusion:****GCSF alleviates neuropathic pain not only by modulating peripheral nerve repair but also by inhibiting pontine astrogliosis and promoting central neuronal regeneration. These findings suggest GCSF may be a promising therapeutic agent for central and peripheral components of neuropathic pain.*

Keywords: *GCSF, GFAP, PCNA, S100, Astrocytes, Pons*

INTRODUCTION

Neuropathic pain is an intractable type of chronic pain[1]. Unfortunately, only some patients would respond to the current treatments. GCSF is known as a regulator of hematopoietic stem cells. Moreover, it induces the proliferation of neural stem cells [2].

Granulocyte colony-stimulating factor (GCSF) therapy is neuroprotective and induces neurogenesis. Previous studies showed that it improved neurological functions in stroke and Alzheimer's disease. The neuroprotective effect of GCSF is mediated via activation of the GCSF receptors [3]

Few studies have suggested that GCSF can reduce neuropathic pain in sciatic neuropathy rat models [(4-7)] but these studies only assessed GCSF on the sciatic nerve with no assessment of its brain effects in the sciatic nerve constriction model. GCSF's anti-inflammatory and antiapoptotic properties have been primarily attributed to its neuroprotective effects [8].

Chao, Lu (4) suggested that GCSF ameliorates neuropathic pain through inducing the secretion of endogenous opioids in the

Received : 28/7/2025 & Accepted : 27/8/2025

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injured nerve. In addition to the reduction of IL-6 and TNF-α, there is downregulation of microglia activity in the dorsal horn [7].

Chronic constriction injury of the sciatic nerve is a widely used model for the induction of neuropathic pain that leads to a variety of molecular and biochemical changes producing neuropathic pain similar to that observed in humans [9].

The underlying mechanisms of neuropathic pain after neuronal injury include the alteration of neuronal function or the development of neuroplasticity, which may occur near or far from the site of injury [10]. It is well known that neuroplasticity, which arises from both the peripheral and central nervous system (CNS), is a major cause of neuropathic pain [11]. Following a nerve injury, both peripheral and central sensitization produce an important disease pathway, including hyperexcitability of primary sensory neurons as well as enhanced excitatory synaptic transmission or reduced inhibitory transmission in the CNS [12].

It is well recognized that the brainstem plays a crucial role in nociception, and coordination of pain signals[13]. The pons regulates the pain signals [13]. Moreover, the descending noradrenergic fibers modulating pain perception originate mainly from the pontine locus coeruleus [14]. Moreover, the locus coeruleus could be a pain generator in chronic pain conditions and selective destruction of noradrenergic neurons relieved neuropathic pain in Sprague Dawley rats [15].

Previous research mainly focused on the role of neurons in neuropathic pain [11]. In addition, non-neuronal cells, particularly glial cells, are increasingly identified as important modulators of pain sensitivity[16].

Regarding the glial cells in the CNS, both astrocytes and microglia have an important role in the regulation of neuropathic pain, primarily in the spinal cord and brain[11, 17]. Astrocytes, as the most common type of cells in the CNS, maintain CNS homeostasis[18]. Activation of astrocytes at both spinal and supraspinal levels is essential in the progression and persistence of neuropathic pain. Moreover, regulation of astrocyte activity could be a new target for managing neuropathic pain[19]. On the other side, the glial cells of the peripheral nervous system include Schwann cells, which are involved in neuropathic pain development[20].

Glial fibrillary acidic protein (GFAP) is a specific astrocyte marker in the CNS that could participate in the development of neuropathic pain[21]. To our knowledge, no studies have assessed the relationship between GCSF and GFAP in neuropathic pain.

Schwann cells release various growth factors to nourish and myelinate the large associated axons[22]. Furthermore, in case of nerve injury, Schwann cells are involved in the pathogenesis of neuropathic pain[23]. The activated Schwann cells express receptors that have the potential to regulate different pain conditions[24]. However, the effect of GCSF on Schwann cells in neuropathic pain remains poorly understood.

The S100 protein is present in Schwann cells of the peripheral nervous system[17], and its upregulation is involved in neuronal regeneration [(25)]. S100 is a multifunctional molecule that may influence neuropathic pain via different mechanisms, including NMDA receptor inhibition and reduction of inflammatory mediators [26]. The current study aims to investigate the effect of GCSF on S100 proteins in the sciatic nerve to ameliorate neuropathic pain.

Moreover, PCNA is mandatory for replication and repair of DNA. PCNA is widely expressed in both proliferating and non-proliferating cells, such as neurons[27]. Therefore, we also investigate the therapeutic effect of GCSF on PCNA proteins as a marker for neuronal regeneration.

Pons is a part of the brain involved in chronic pain conditions; only a few studies have investigated its role in neuropathy. Alterations in brain astrocytes are involved in the development of neuropathic pain. Therefore, our study examined the effect of GCSF on pontine astrocytes by detecting GFAP expression as a possible mechanism to ameliorate neuropathic pain. In addition, our study proposed that GCSF could alleviate sciatic neuropathy via inducing the regeneration of pontine neurons. Therefore, we assessed PCNA expression. Moreover, the role of Schwann cells in the sciatic nerve is important in neuronal regeneration. Hence, we explored the possible effect of GCSF on

Schwann cells of the sciatic nerve by assessing S100 expression.

MATERIAL AND METHODS

Animals

Thirty-two male adult Wistar Albino rats (200-250 g) were obtained from the Faculty of Veterinary Medicine's animal house facility. The rats were housed under a temperature of 22 ± 2°C and humidity of 55 ± 5%, and a 12-hour light/dark cycle. The animals had free access to food and water. The Ethical Committee of the Faculty of Medicine, Assiut University, approved the experimental protocol. The number of ethical approvals is 04-2024-466. All rat manipulations were performed according to the guidelines for the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and the guidelines for the care and use of experimental animals by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The injection of the GCSF and laboratory testing were done between 8:00 am and 2:00 pm.

Surgical procedure

Unilateral CCI of the right sciatic nerve was used to induce neuropathic pain as described by [28]. In brief, rats received intraperitoneal thiopental sodium (50 mg/kg, i.p.) to induce anesthesia. At the level of the mid-thighs, the right sciatic nerve was exposed. Four loosely knotted ligatures (4.0 silk) were carried out around the nerve. Then the incision was soaked with iodine and sealed with silk sutures. Sham animals were operated similarly except for not ligating the sciatic nerve.

Experimental design

Rats were randomly divided into 4 groups, eight animals per group: normal control, sham-operated, neuropathy control group, and neuropathy group treated with GCSF. A power analysis using KISS approach for calculation sample size in animal study, shows that the sample size of 8 rats/group (8/ group) has 80% power to detect a hypothesized standardized effect size of 1.5 SDs between control and treated group to identify the potential therapeutic effect of GCSF in sciatic neuropathy, assuming a 5% significance level and a two-sided test[29].

On the seventh day of CCI, the fourth group (the treated group) received (GCSF) (filgrastim) (Roche, Egypt) 50 µg /kg subcutaneously for 5 consecutive days [30]. The normal control, Sham-operated, and CCI rats were given saline subcutaneously. On the 7th day of the experiment, the pain response was assessed to confirm the occurrence of neuropathy after CCI. After assessment of the pain response on the twelfth day of CCI, all rats were anesthetized with IP thiopental sodium (50 mg/kg). The hearts were then exposed, and isotonic saline was infused via the left ventricle until the flowing blood was cleared[31]. Then, the hearts were perfused with formalin fixative. After that, the brains were extracted, and sciatic nerves were dissected.

Assessment of the Rat Groups' Pain Response

Von Frey Test (assessment of tactile allodynia)

It assesses the painful sensation triggered by a non-noxious mechanical stimulation using Von Frey filaments, Bioseb, France[32] . The rat was placed into a 20 × 25 × 615 cm wooden box with a metal mesh bottom. Then it was given 20 minutes to become accustomed to it. Next, a set of calibrated Von Frey filaments (20 filaments total, weighing between 0.008 and 300 g) was placed perpendicularly to the hind paw's plantar surface for 6 seconds. The stiffness of the filaments was gradually raised in an ascending sequence.

Flinching or withdrawing one's paws was regarded as a positive reaction. A filament of the next-lower force was applied when a reaction was detected. A filament of next-greater force was added if there was no reaction. For each animal, five measurements were taken at intervals of five minutes. The threshold value in grams was calculated according to the filament that caused the hind paw to respond positively three times out of five[33].

Randall and Selitto Test

The Randall and Selitto test assessed mechanical hyperalgesia via an analgesia meter (Ugo Basile, Italy). In this test, linearly increasing forces were applied to the rat's hind

paw by a cone-shaped presser. To protect tissue, a cutoff pressure of 160 g was employed. The weight in grams (g) needed to cause paw flexion as a nociceptive response was used to measure the pain threshold [34].

Assessment of Thermal Hyperalgesia

The reaction to thermal stimulation was evaluated using the hot-plate test. Rats were set on the hot plate, with a temperature setting of 55°C. The time of onset of a hind-paw licking or jumping was calculated. Thirty seconds was the cutoff time to avoid tissue injury [35].

Histopathological examination

Light microscope (LM) examination

The coronal sections of the pons and transverse sections of the nerve trunk were processed for light microscopic and immunohistochemical staining. The paraffin sections were stained with hematoxylin and eosin (H&E) for general histological examination[36].

Immuno-histochemical methods

Pons and sciatic nerve trunk paraffin sections (5-µm) were taken on the poly-L-lysine-coated slides, deparaffinized in xylene, and rehydrated in graded alcohols. Slides were incubated in 0.01 M citrate buffer (pH 6.0) at 95 °C for 20–30 min for antigen retrieval. Immunohistochemical staining was performed for PCNA (to demonstrate cell proliferation**),** GFAP (to demonstrate glial cells) (Chongqing Biospes Co., Ltd. China)**,** and S100 (to demonstrate Schwann cells) (Abcam, ab231303, USA). an avidin-biotin peroxidase method was used [37]. Primary antibodies were diluted 1:100 and 1:200 in phosphate buffer solution, and the sections were incubated at room temperature overnight.

Sections were stained with an avidin-biotin-peroxidase and diaminobenzidine used as a chromogen. To counterstain the sections after their rinsing in distilled water, Mayer's hematoxylin was used. PCNA-positive cells have brownish nuclei, GFAP-positive glial cells have brownish cytoplasm and processes, and S100-positive Schwann cells have brownish cytoplasm.

Some sections were incubated with phosphate buffer solution instead of the primary antibody for negative control staining. No immunoreactivity was present in negative control. For positive control staining, sections from rat skin were used for PCNA[38]. Sections of astrocytoma were used for GFAP [39], Sections of schwannoma were used for S100 [40].

Morphometric studies

Morphometric experiments were done using Image J. From each group, 5 non-overlapped sections/5 rats were used. Each group's parameters were measured in micrometers by an arbitrary distance approach and a 40X objective lens. The number of blood capillaries in the pons was counted. The number of positive (+ve) PCNA immune-stained cells and GFAP-positive astrocytes was counted in the pons. The percentage of surface area expression of S-100 in the nerve trunk was measured.

Data Analysis

The Statistical Package of Social Sciences (SPSS) software (27th version, Chicago, USA) was used. A normality test (Kolmogorov-Smirnov & Shapiro-Wilk test) was done. The data of behavioral results were normally distributed and were expressed by the mean ± SE. One-way analysis of variance (ANOVA) was used to assess the significance of differences between groups, and for multiple comparisons, the post hoc Dunnett's test was employed. The Effect size was calculated for ANOVA using Partial Eta Squared.

The data of immunohistochemical analyses were not normally distributed. Continuous data were expressed as minimum and maximum, mean and standard deviation (mean ± SD). Differences between the two groups were detected using the Mann-Whitney U test for non-parametric data, and differences between more than two groups were detected using Kruskal-Wallis H for non-parametric data. The Effect size was calculated for the Kruskal-Wallis test using Eta-squared. When $p < 0.05$, differences were determined to be statistically significant.

RESULTS AND DISCUSSION

Behavioral assessment

Results of Von Frey Test (assessment of tactile allodynia)

The results of the Von Frey test were displayed in **table 1** and **Fig. 1**. The results of

the Von Frey test were displayed in **Fig. 1**. One Way ANOVA test revealed a significant difference in the mechanical nociceptive threshold between all groups on the 7th day and the 12th day following CCI (F=65.14, 31.64 respectively; p<0.001 for both). Our study findings demonstrated that, compared to the control and the Sham group, CCI neuropathic rats exhibited substantial mechanical allodynia (p < 0.001) on the 7th day following CCI. On the 12th day, compared to CCI rats, the mechanical nociceptive threshold of rats treated with GCSF after CCI showed a highly significant increase (p < 0.001). GCSF treatment effect size using Partial Eta Squared equals 0.80. At the baseline (day 0), the mechanical nociceptive threshold showed a non-significant difference among all rat groups.

Results of the Randall and Selitto Test (assessment of mechanical hyperalgesia)

The results of the Randall and Selitto test were displayed in **table 1** and **Fig. (2)**. The pain withdrawal threshold displayed a significant difference between all rat groups on the 7th, and 12th day following CCI (F=69.71, 15.25 respectively; p<0.001 for both) . CCI model rats showed a sustained decrease in the pain withdrawal threshold on days 7 and 12 relative to the control and sham groups (p < 0.001). A single dose of GCSF 50 ug/kg for five days significantly increased the pain withdrawal threshold in the treated group compared to the CCI model rats (p < 0.001). GCSF treatment effect size using Partial Eta Squared equals 0.65. At the baseline (day 0), the pain withdrawal threshold showed a non-significant difference among all rat groups.

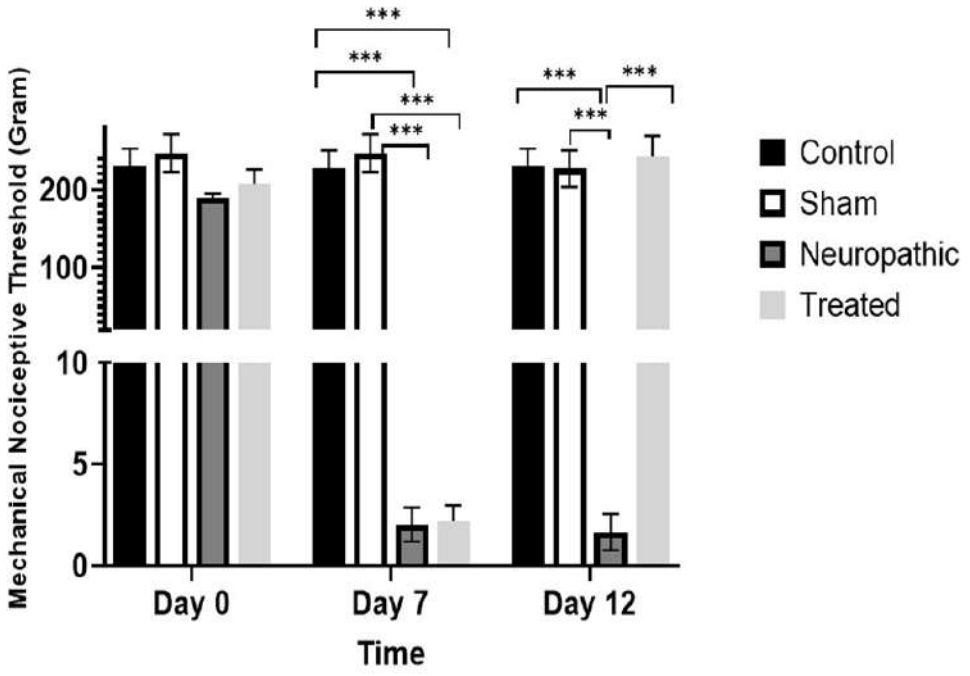

Fig. 1: Results of Von Frey Test (assessment of Mechanical Allodynia). Number of rats in each group is 8 rats. For every animal, five measurements were taken at intervals of five minutes. The threshold value in grams was the filament that caused the hind paw to respond positively three times out of five. Differences were evaluated by analysis of variance with post hoc Dunnett's test. The data were displayed as mean ± SEM. ***: p value < 0.001.

Table 1: Changes in Mechanical Nociceptive Threshold, Pain Withdrawal Threshold, And Thermal Reaction Latency of Normal Control, Sham, Neuropathy and GCSF-Treated Rats.

	Control group	Sham group	Neuropathy group	Treated group
Mechanical nociceptive threshold (Gram)				
Day 0	213.33±17.64; [167.99- 258.67]	246.67±24.04; [184.88-308.46]	190.00±4.47; [178.50-201.50]	206.67±19.09 [157.59-255.74]
Day 7	226.67±23.48; [166.32-287.01]	246.67±24.04; [184.88-308.46]	2.03±0.85#$; [-0.14-4.21]	2.20±0.80#$; [0.15-4.25]
Day 12	230.00±22.36; [172.52-287.48]	226.67±23.48; [166.32-287.01]	1.67±0.89#$; [-0.62-3.95]	243.33±25.517^; [177.74-308.93]
Pain withdrawal threshold (Gram)				
Day 0	225±9.91; [199.5-250.49]	230.±5.77; [215.16-244.84]	213.33±10.21; [187.06-239.6]	218.33±6.01 [202.89-233.78]
Day 7	241.67±4.77; [229.4-253.94]	235±6.71; [217.76-252.25]	160±5.77#$; [145.16-174.84]	148.33±6.01#$; [132.89-163.87]
Day 12	226.67±12.02; [195.77-257.49]	236.67±5.58; [222.33-251]	160±5.77#$; [145.16-174.84]	215±9.92^; [189.5-240.49]
Thermal reaction latency (Seconds)				
Day 0	15.17±0.48; [13.94- 16.39]	15.33±0.49; [14.06-16.60	14.50±0.76; [12.54-16.46]	14.33±0.49; [13.06-15.60]
Day 7	14.83±0.48; [13.61-16.06	14.83±0.40; [13.80-15.87	7.83±0.60#$; [6.29-9.38	8.83±0.31#$; [8.04-9.62]
Day 12	15.17±0.83; [13.02-17.31	15.83±0.87; [13.59-18.08]	9.67±0.56#$; [8.23-11.10]	15.00±0.37^; [14.06-15.94]

N: 8 rats for each group. Data are expressed as mean ±SEM; confidence interval 95%. # has a significant difference versus healthy control rats, $ has a significant difference versus sham rats, ^ has a significant difference versus neuropathic rats.

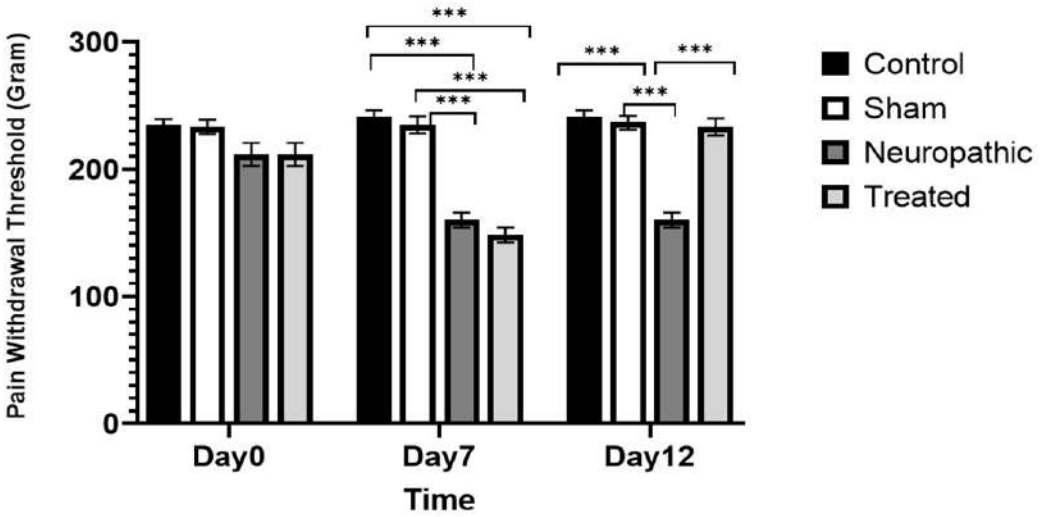

Fig. 2: Results of Randall and Selitto Test (assessment of mechanical hyperalgesia via an analgesia meter). Number of rats in each group is 8 rats. The weight in grams (g) needed to cause paw flexion as a nociceptive response was used to measure the pain threshold. Differences were evaluated by analysis of variance with post hoc Dunnett's test. The data were displayed as mean ± SEM. ***: p value < 0.001.

Results of Hot Plate Test (assessment of thermal hyperalgesia)

Results of the Hot Plate test were displayed in **table 1 and Fig. (3**). ANOVA test demonstrated the thermal latency had a significant difference between all rat groups on the 7th, and 12th day following CCI (F= 67.50, 17.17 respectively; p<0.001 for both). In comparison to the control and sham groups, the thermal reaction latency in the hot plate test decreased in the CCI model on days 7 and 12 (p<0.001). After a single dose of GCSF 50 ug/kg for five days, the thermal reaction latency was higher than in the treated group relative to the CCI model (p< 0.001). The thermal reaction latency displayed a non-significant difference among all rat groups at day 0. GCSF treatment effect size using Partial Eta Squared equals 0.67.

Histopathological results

Light microscopic results

H and E-stained sections of rat's pons from the normal and sham control groups showed the consistent structure of rat's pons. It consisted of pontine nuclei that have Nissl granules in their cytoplasm and a large centrally located nucleus with a prominent nucleolus (arrows) surrounded by longitudinal and transverse pontine fibers **(fig.4A & B)**. In the neuropathic group, the pontine neurons had ill-defined nuclei **(fig. 4C)**, and congested blood capillaries were also detected **(fig. 4D)**. In the treated group the pontine neurons had well-defined nuclei **(fig. 4E)**. Moreover, there was a significant increase in the number of blood capillaries in neuropathic group (42.6±8.59) in comparison to normal (20.6±4.81) and sham control (21.4±5.32) groups and treated group (23.4±6.15), P-value <0.001 **(fig.4F)** with treatment effect size estimated by Eta square η^2= 0.45. The nerve trunk in the normal control, sham control group, neuropathic and treated groups consisted of large fascicles of peripheral nerves surrounded by a thin layer of connective tissue called perineurium. Myelinated nerve fibers formed of axons surrounded by unstained area of dissolved myelin, nuclei and neurilemmal sheath of Schwann cells and separated by endoneurium **(fig.5)**. In the neuropathic group, we observed the presence of cellular infiltration around the perineurium **(fig. 5C)**, but in the normal control, sham control and treated groups, there was no cellular infiltration around the perineurium **(fig. 5A, B&D)**.

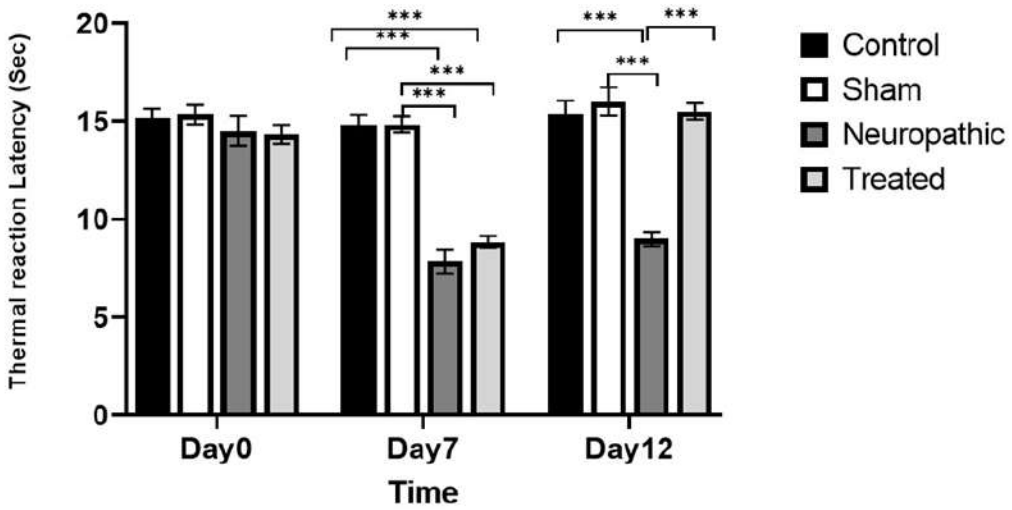

Fig. 3: Results of Hot Plate Test (assessment of thermal hyperalgesia). Number of rats in each group is 8 rats. The temperature setting was at 55°C. The time of onset of a hind-paw lick or a jump was recorded. Differences were evaluated by analysis of variance with post hoc Dunnett's test. The data were displayed as mean ± SEM. ***: p value < 0.001.

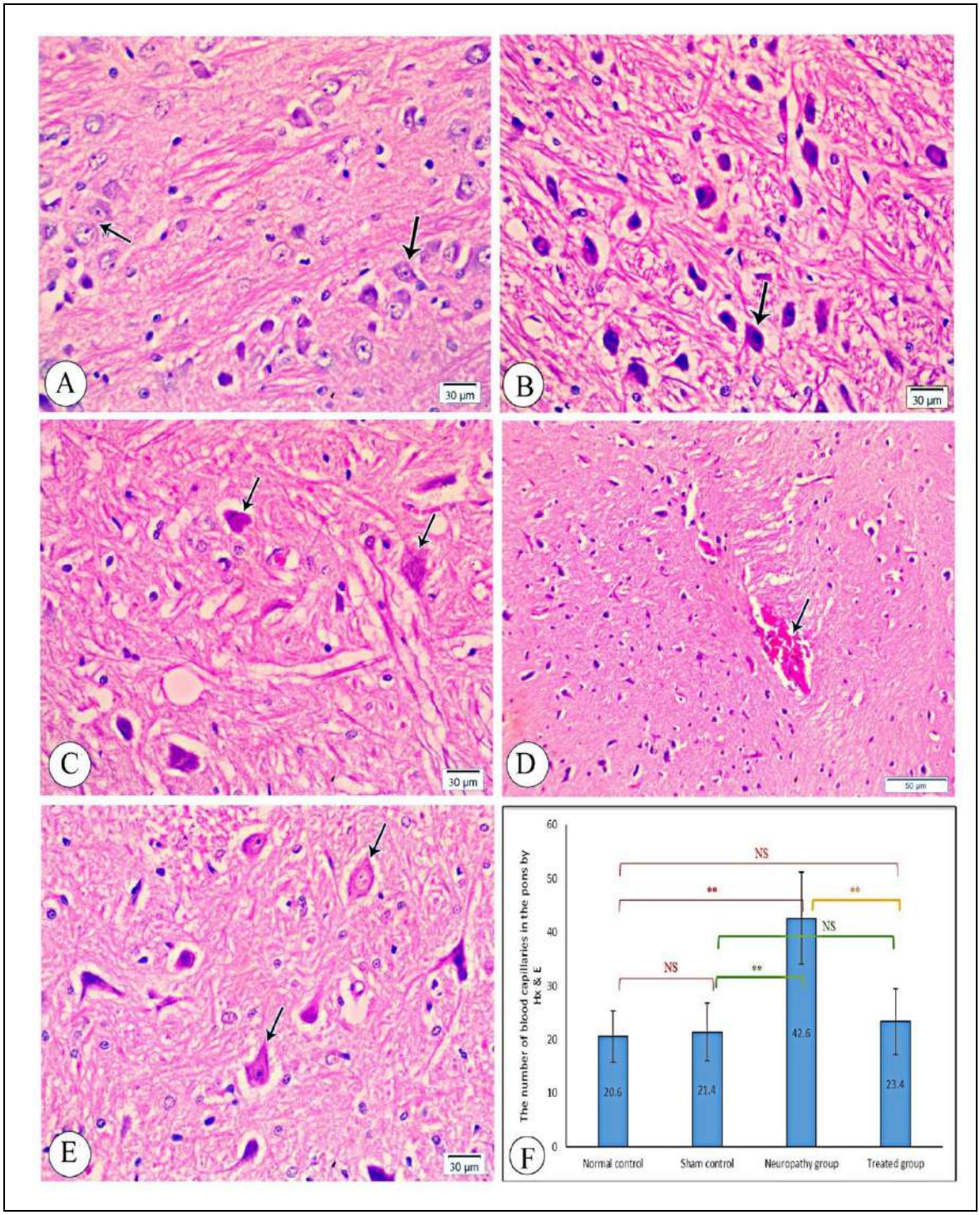

Fig. 4: Photomicrographs of rat pons stained with H&E. Normal and sham control groups showing **(A &B)** Neuronal cell bodies of pontine nuclei that have Nissl granules in their cytoplasm and a large centrally located nucleus with prominent nucleolus (arrows) (x400). Longitudinal and transverse pontine fibers are also seen between pontine nuclei. Neuropathic group showing **(C)** pontine neuronal cell bodies with ill-defined nuclei and irregular outlines (arrows) (x400). **(D)** Pons with dilated congested blood capillary (arrow) (x200). Treated group showing **(E)** pontine neuronal cell bodies with well-defined vesicular nuclei and Nissl granules in their cytoplasm (arrows) (x400). **(F)** The histogram shows a significant increase in the number of blood capillaries in neuropathic groups compared to normal, sham control groups and treated group, P-value <0.001.

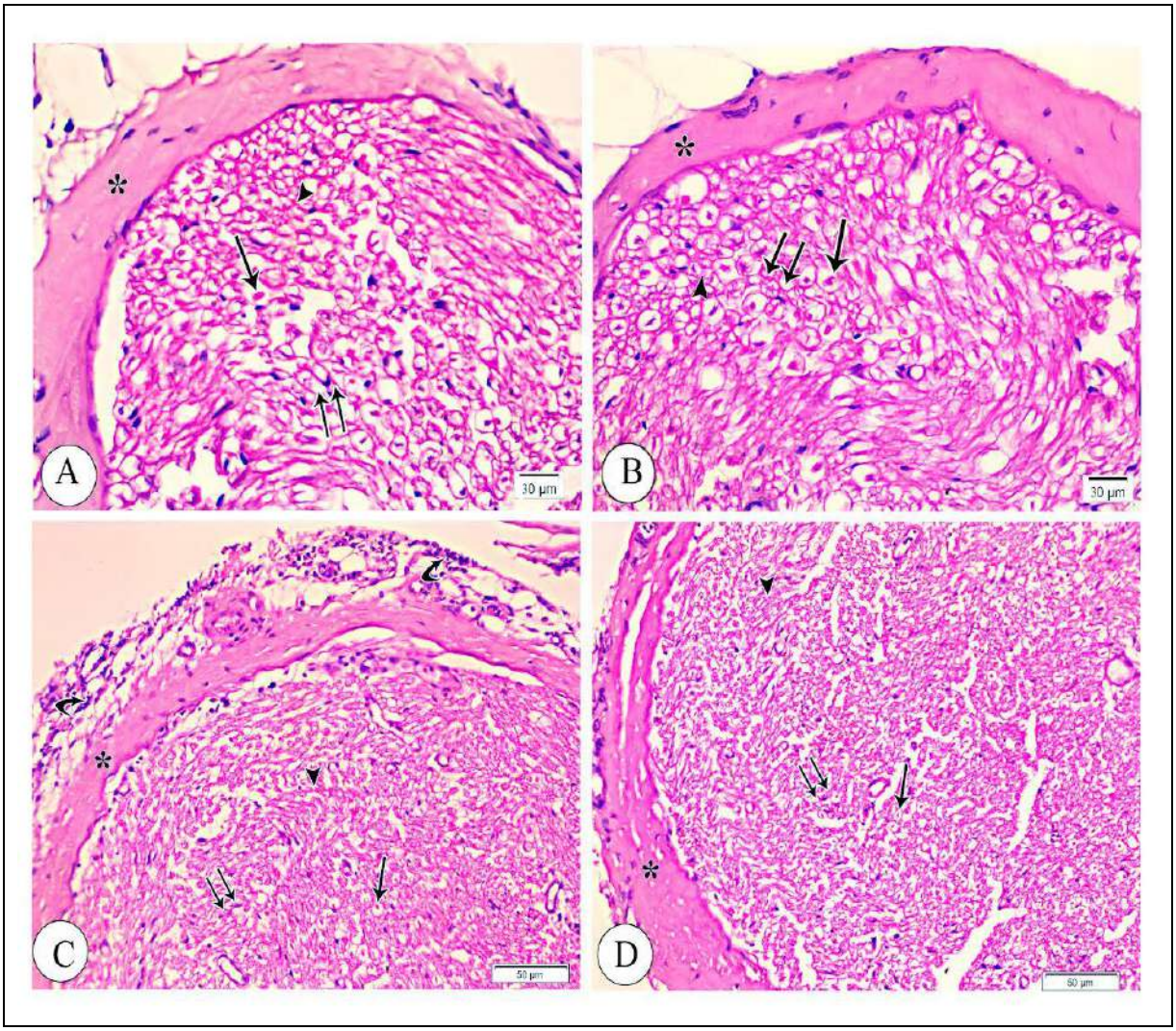

Fig. 5: Photomicrographs of rat nerve trunk of the normal control group, sham group, neuropathic group, and treated group, respectively, stained with H&E show (A, B, C,&D) large fascicles of the peripheral nerves surrounded by perineurium (asterisk). Myelinated nerve fibers are formed of axons surrounded by an unstained area of dissolved myelin (arrow) and separated by endoneurium (arrowhead). Nuclei and neurilemmal sheath of Schwann cells (double arrow) are also detected (x400, x400 x200 & x200). Note: cellular infiltration (curved arrow) around the perineurium in the neuropathic group **(fig.2C).**

Immunohistochemical results

Sustained cell proliferation was further visualized by immunohistochemical staining of PCNA **(fig.6)**, confirming a significant increase in proliferating cells in the pons within the GCSF group (27.8±4.8) when compared with normal (5.1±2.64) and sham control groups (5.2±2.82) and neuropathic groups (5.1±2.51), P-value <0.001 **(fig. 6E)** with treatment effect size Eta squared η^2= 0.48. Additionally, GFAP immunostaining revealed positive reaction for astrocytes **(fig. 7)**. In the neuropathic group, there was a significant increase in the number of pontine positive-GFAP astrocytes (66.1±5.92) in comparison to normal control (16.6±3.47), sham control (17.2±3.52) and treated groups (55.7±3.89), P-value <0.001 **(fig.7E)** with Eta squared η^2=0.75. While immunohistochemical staining section of S100 in all groups showed positive immune reaction around axons **(fig.8)**, but there was a significant decrease in the % of surface area expression of S100 in the nerve trunk of neuropathic groups (1780±204.4) in comparison to normal (4070±160.21) and sham control groups (4115±214.8) and treated groups (3710±246.98), P-value <0.001 **(fig. 8E)** with Eta squared η^2=0.68.

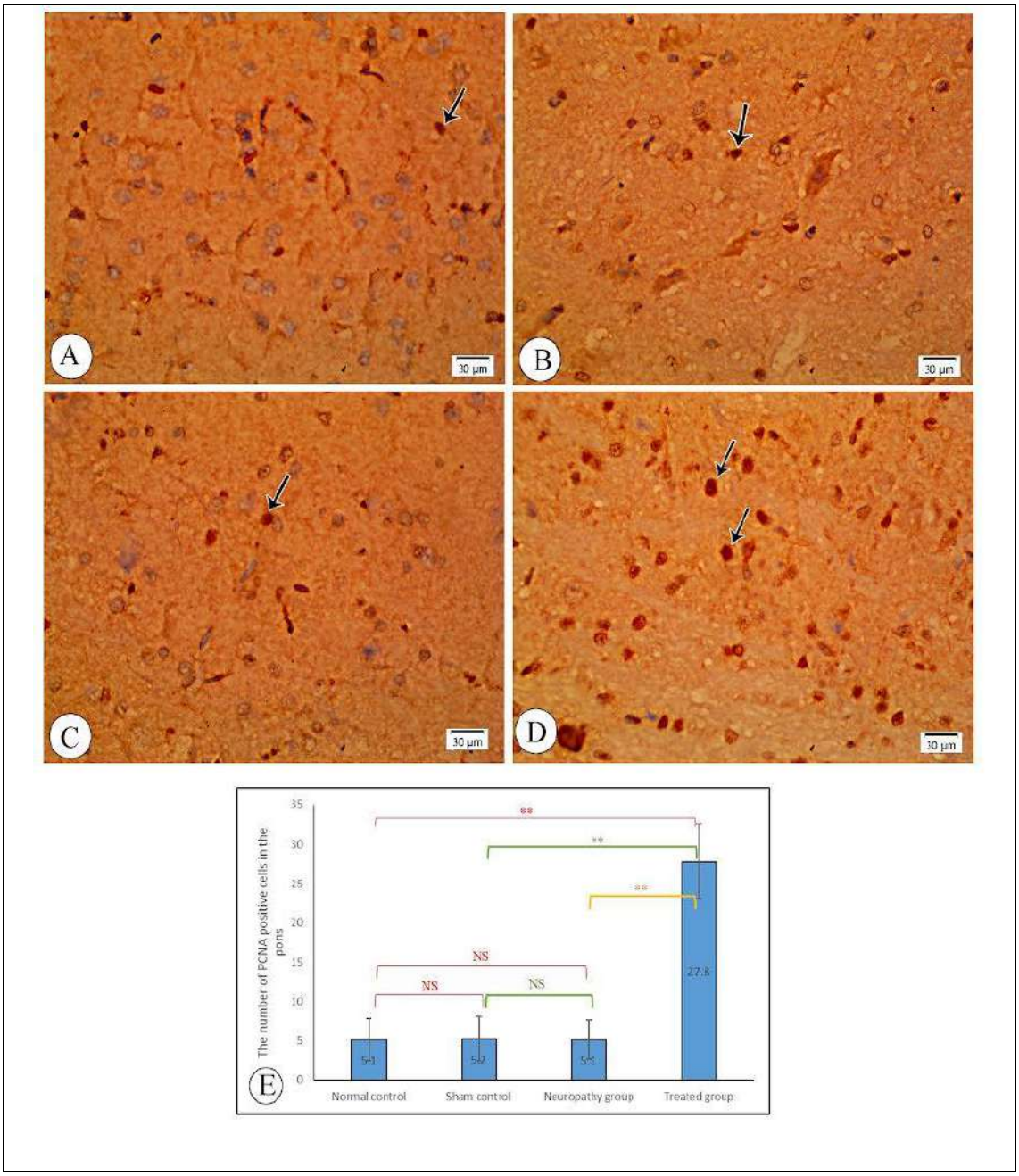

Fig. 6: Immunohistochemical staining of rat pons with PCNA (x400). Normal and sham control groups **(A&B**) and neuropathic group **(C)** show a few PCNA immunopositive reactions (arrow). The treated group **(D)** shows increased PCNA-positive immune-stained cells (arrows). **(E)** The histogram shows a significant increase in the PCNA immune-positive cells in the treated group in comparison to normal, sham control groups ($P<0.001$) and the neuropathic group ($P<0.001$).

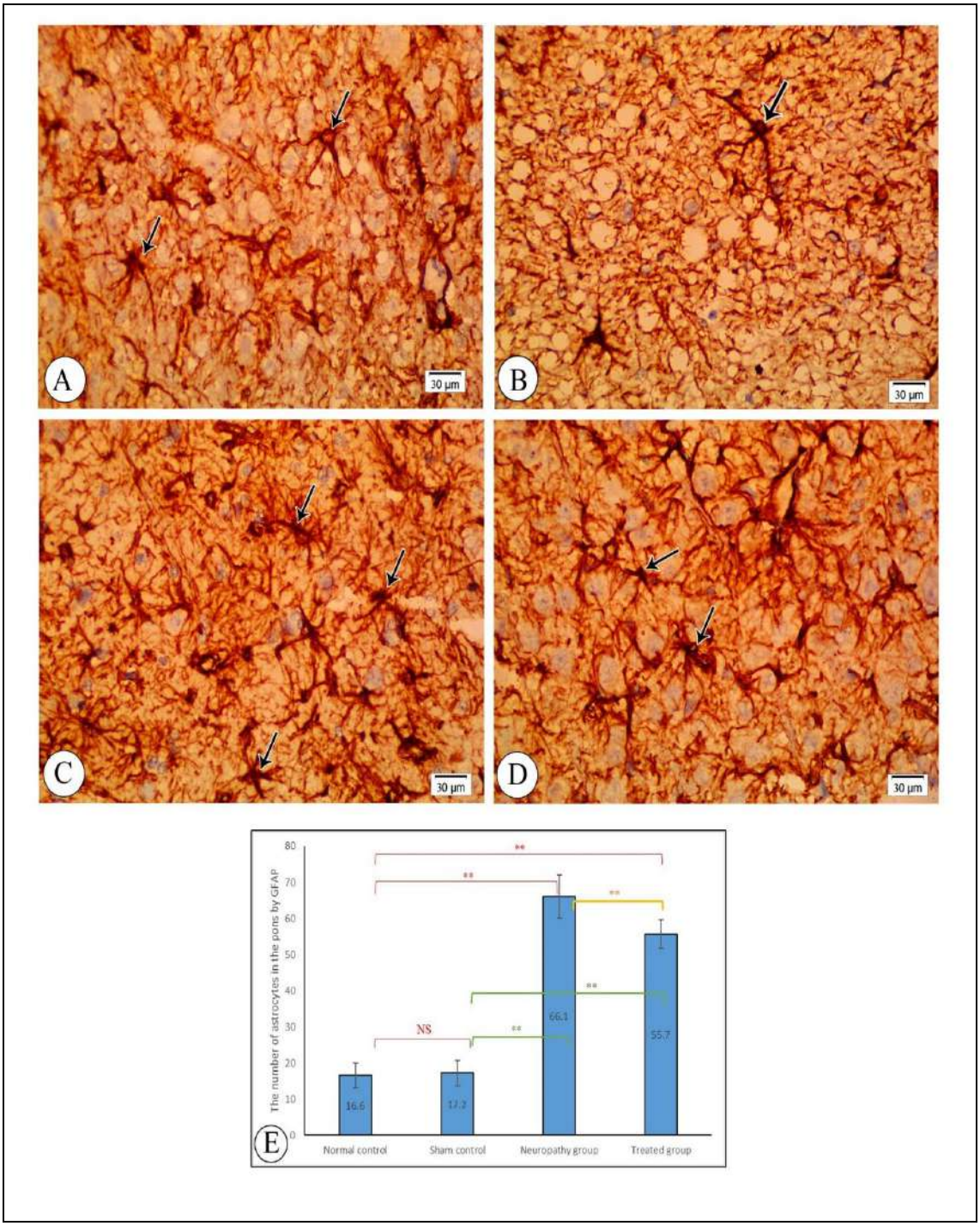

Fig. 7: Immunohistochemical staining of rat pons with GFAP (x400). Normal and sham control groups **(A&B)** show positively stained astrocytes (arrows) with long branching processes. The neuropathic group **(C)** shows numerous positively stained astrocytes (arrows) with long branching processes. Treated group **(D)** shows positively stained astrocytes (arrows) with long branching processes. **(E)** The histogram shows a significant increase in the number of GFAP-positive astrocytes in the neuropathic group in comparison to normal, sham control groups ($P<0.001$) and the treated group ($P<0.001$).

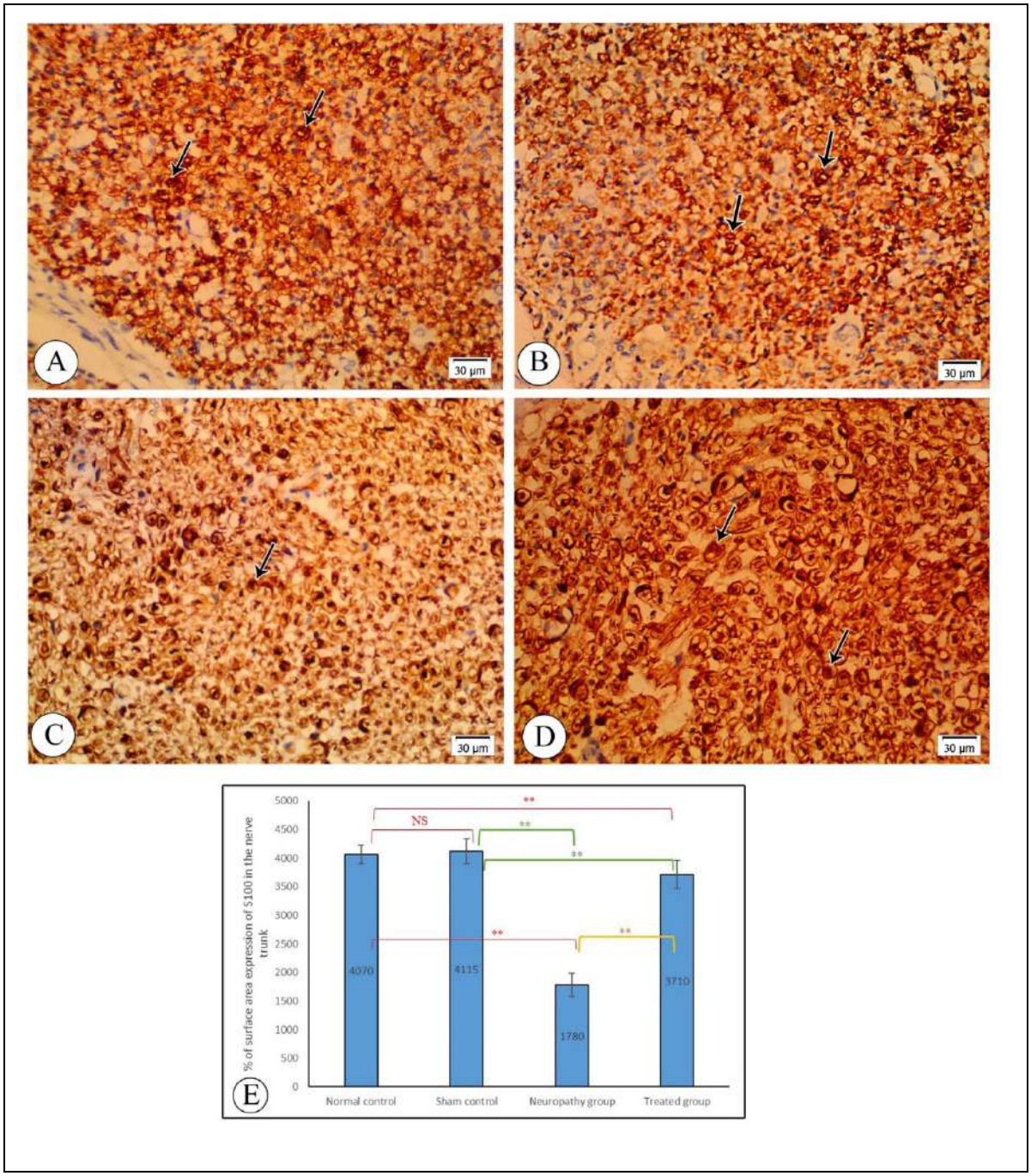

Fig. 8: Immunohistochemical staining of rat nerve trunk of the control group, neuropathic group, and treated group, respectively, with S100 showing **(A, B, C& D)** positive S100 immune reaction (arrows) around axons. (x400). **(E)** The histogram shows a significant decrease in % of surface area expression of S100 in the nerve trunk in the neuropathic group in comparison to normal, sham control groups (P<0.001) and the treated group (P<0.001).

Discussion

Granulocyte colony-stimulating factor (GCSF) is a protein that induces stem cell differentiation into different types of cells, including neurons. In addition, it inhibits cell death and stimulates neuronal regeneration (Hung-Chuan et al., 2008). Few studies have investigated the peripheral effects of GCSF in CCI for nerve repair[4, 6, 7]

However, no previous studies explored the central mechanisms of GCSF in alleviating neuropathic pain; the current study is the first to assess the role of GCSF in the pons in sciatic CCI. Additionally, the current study is the first to investigate the effect of GCSF on S100

expression in Schwann cells of the sciatic nerve.

GCSF-based therapy is readily available and may hold promise for treating chronic pain [41]. Our findings indicate that GCSF has central and peripheral therapeutic effects on the injured nerves and subsequently relieves pain.

Hyperalgesia and allodynia are the most characteristic features of neuropathic pain (Challa, 2015). Firstly, we observed the mechanical and thermal hyperalgesia in the neuropathic group on the 7th day of CCI. GCSF was injected subcutaneously in this study for 5 consecutive days to treat neuropathic pain in rats. On the twelfth day, it was found that treated rats with GCSF demonstrated a significant elevation in thermal and mechanical nociceptive thresholds. Our findings suggest that early treatment with GCSF significantly ameliorates mechanical and thermal hyperalgesia in neuropathic rats.

Then, we studied the histological changes in the pons, as it relays and regulates the signals that give the pain sensation from anywhere below the neck[13]. In the pontine neurons of the neuropathic group, we observed that neurons had irregular outlines and ill-defined nuclei. In addition, we found dilated congested blood capillaries. After GCSF treatment, the neurons appeared normal with well-defined nuclei. In addition, we found a significant increase in the number of blood capillaries in the neuropathic group compared to normal control, sham, and treated groups. These findings may be a compensatory protective mechanism during the regeneration of pontine tissue in the neuropathic group. Previous studies showed a direct impact of neuronal blood flow on regeneration in peripheral nerve injuries[42, 43].

Furthermore, PCNA is a nuclear protein mandatory for cell division, and it identifies newly divided brain cells[44]. PCNA is used as a marker of cellular proliferation and is involved in DNA replication and repair. It is also used as an indicator of neural stem cells' proliferation[45].

By immunohistochemistry, we observed a significant increase in the number of pontine PCNA-positive cells in the treated group compared to both control and neuropathic groups. According to our findings, GCSF treatment resulted in upregulation of PCNA expression in the pons in the early stage of nerve injury. The upregulation of PCNA indicated proliferating neurons in the central nervous system, which attenuated neuropathic pain. In agreement with our result Kotb, Abedalmohsen (34) reported that injection of mesenchymal stem cells in sciatic neuropathy rat model produced recent proliferation of PCNA-positive stem cells in the cerebral cortex.

Our study detected a significant elevation in the number of GFAP-positive astrocytes in the neuropathic group. This indicated the presence of reactive astrogliosis in response to neuropathic pain. These results are in agreement with a previous study, which showed that noxious stimulation and nerve injury induced changes in the shape, function, and expression of astrocytes, known as reactive astrogliosis. It is an initial defense mechanism for repairing damage [18].

In the CNS, GFAP is a particular astrocytic marker and increased expression of GFAP can produce CNS damage. Furthermore, the control of neuropathic pain perception and transmission is linked to the activities of cerebral cortical neurons and astrocytes[46]. Moreover, a previous study reported that the activation of astrocytes in the pontine locus coeruleus was involved in neuropathic pain[47]. Our study revealed that the subcutaneous injection of GCSFs significantly decreased the pontine expression of GFAP. These findings also contributed to the central analgesic effect of GCSFs that is directly related to the inhibition of astrocytic activation.

According to a growing body of evidence, the pontine locus coeruleus (LC) experiences plastic changes in neuropathic pain that go beyond neuronal firing characteristics and include glial and cellular remodeling[48, 49]. PCNA expression has been reported to increase in the brainstem regions following peripheral nerve injury. Increased PCNA expression in the pontine LC could indicate an attempt at metabolic compensation by noradrenergic neurons, which could promote adaptive remodeling as well as survival[50]. The proliferative repair (indicated by increased PCNA expression) noticed in the current study supports the view that the LC is not a site of relay but rather a site of molecular reprogramming during neuropathic pain.

Furthermore, the neuroadaptive role of pontine LC in neuropathic pain appears to be partially mediated via attenuating astrocytic reactivity indexed by GFAP. Moreover, selective activation of the LC-spinal cord pathway leads to elevated dorsal horn norepinephrine, raised mechanical/thermal thresholds, and reduced GFAP-positive area in sciatic nerve CCI mice [48].

Our findings showed that GCSF treatment can reduce the expression of GFAP in the pontine astrocytes. These data provide a novel cellular pathway linking GCSF neuroprotective effect to attenuated pontine astrogliosis in neuropathic pain. Mechanistically, a recent work clarifies how LC-derived norepinephrine can reshape synaptic networks that are responsible for central sensitization. Noradrenergic signaling acts directly through astrocytic adrenoceptors to decrease astrocytic activity, rendering astrocytes the primary NE targets capable of shifting networks away from pro-nociceptive plasticity[51].

Regarding the histopathological changes observed in the sciatic nerve trunk, we found no obvious differences between the three groups except for the presence of cellular infiltration around the perineurium in the neuropathic group. However, by immunohistochemistry, we found a significant decrease in the expression of S-100 in the nerve trunk of the neuropathic group compared to normal control, sham, and treated groups.

One of the most useful markers for definitively characterizing nerve damage is S100 [52]. It exists in Schwann cells of the peripheral nervous system[17]. Schwann cells induce axon regeneration and remyelination of damaged nerves, consequently ameliorating neuropathy. Moreover, previous studies suggested that Schwann cell transplantation relieves pain [53].

S-100 is present predominantly in myelin-forming Schwann cells, and the amount of S-100 immunoreactivity correlates with the myelin sheath thickness[54]. It is well established that demyelination is involved in the development of neuropathic pain by disturbing the precise structural and molecular features of nerve fibers[55].

Myelinating Schwann cells dedifferentiate and start the myelin sheath degradation at the site of injury. This is an essential step for the beginning of regeneration[56]. As a novelty, this work demonstrated that the expression of S-100 in the sciatic nerve of the treated group with GCSF was higher than in the neuropathic group. These results showed that GCSF promoted remyelination of the sciatic nerve. This is in agreement with previous studies. Chan, Tsai (52) reported that moderate hot compress restored the nerve cells at the site of peripheral nerve injury and increased the expression of S100. In addition, ginsenoside and curcumin enhanced the repair of sciatic nerve injury, and the underlying mechanism included increased S100 expression in the sciatic nerve [57, 58].

Previous studies reported a direct relationship between remyelination and pain relief. For example, Gabapentin alleviated mechanical and thermal allodynia and improved nerve remyelination after chronic constriction of the sciatic nerve [59]. The topical application of lysolecithin induced neuronal demyelination of peripheral nerves and produced neuropathic pain behaviors, which were reversible by nerve remyelination when cannabinoids were administered [60]. Finally, our work showed that GCSF significantly induced the remyelination process and reduced neuropathy and neuropathic pain.

The current study had some limitations. The short duration of the study did not allow for assessment of the long-term therapeutic effects of GCSF in sciatic neuropathy. Another limitation of the study is that only a single dose of GSF was selected. However, the 50 ug/kg GCSF dose for five days was according to previous studies[61-63]. An additional limitation is the absence of a comparative standard drug for the treatment of neuropathic pain.

Conclusion

The current study showed that GCSF increased the pain threshold in rat models of CCI. GCSF produced antinociceptive effects via inhibiting astrogliosis and promoting neuronal regeneration in the pons. In addition, GCSF enhanced the remyelination process of the sciatic nerve. GCSF could be a promising option in the treatment of neuropathic pain. Further studies on its central effects are recommended.

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Bull. Pharm. Sci., Assiut University, Vol., Issue-, 2025, pp. ----.

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عامل تحفيز مستعمرات الخلايا المحببة يثبط التفاعل النجمي في جسر الدماغ ويحفز إعادة تكوين الميلين في العصب الوركي في نموذج فأري لاعتلال الأعصاب: دور البروتين الحمضي الليفي الدبقي والمستضد النووى للخلايا المتكاثرة والأس ١٠٠ بروتين

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الهدف:

يُعد الألم العصبي من الحالات التي غالبًا ما تقاوم العلاجات التقليدية .بينما ركزت الدراسات السابقة حول تأثيرعامل تحفيزمستعمرات الخلاياعلى الأعصاب الطرفية، تشير الأدلة الحديثة إلى أن الجهاز العصبي المركزي، وخاصة جذع الدماغ (الجسر) ، يلعب دورًا محوريًا في تنظيم الألم العصبي .تهدف هذه الدراسة إلى استكشاف التأثيرات المركزية والطرفية لعامل تحفيز مستعمرات الخلايا المحببة في نموذج اعتلال العصب الوركي في الفئران.

الطرق:

تم تقسيم الفئران الى اربع مجموعات: مجموعة ضابطة طبيعية، مجموعة جراحية وهمية، مجموعة مصابة باعتلال عصبي ناتج عن إصابة انضغاطية مزمنة للعصب الوركى، ومجموعة عولجت بعامل تحفيز مستعمرات الخلايا المحببة. تم إعطاء عامل تحفيز مستعمرت الخلايا المحببة بجرعة ٥٠ ميكروجرام/ لكل كجم في اليوم السابع بعد الإصابة ولمدة خمسة أيام متتالية. تم تقييم السلوكيات المرتبطة بالألم باستخدام اختبارات لفرط التحسس الحرارى. والالم الناتج عن التنبيه الميكانيكى الخفيف، وفرط التحسس الميكانيكى. كما تم اجراء دراسة مناعية نسيجية لقياس مستوى البروتين الليفي الحمضي الدبقى والمستضد النووي للخلايا المتكاثرة في انسجة الجسر وقياس مستوى الاس١٠٠ بروتين في العصب الوركي.

النتائج:

أدى العلاج بعامل تحفيز مستعمرات الخلايا المحببة الى تحسن ملحوظ في فرط التحسس الحراري والتحسس الميكانيكى في مجموعة الفئران المصابة باعتلال العصب الوركي. لوحظ أيضا انخفاض ملحوظ في مستوى البروتين الليفي الحمضي الدبقى مما يشير الى تثبيط الخلايا النجمية.

بالإضافة الى زيادة في كمية المستضد النووي للخلايا المتكاثرة مما يدل على تحفيز تجـــدد الخلايـــا العصبية في الجهاز العصبي المركزي (الجسر). أما في العصب الوركى، لوحظ ارتفاع ملحوظ فـــي مستوى الاس١٠٠ بروتين مما يشير الى تعزيز إعادة تغليف الالياف العصبية بمادة الميلين.

الاستنتاج:

يعمل العامل المحفز لمستعمرات الخلايا الحبيبية على تحسين الألم العصبي من خلال اليـــات مزدوجة تشمل تثبيط الخلايا النجمية وتحفيز تجدد الخلايا العصبية المركزية في الجسر الـــى جانـــب تعزيز اصلاح الاعصاب الطرفية. تشير هذه النتائج الى ان عامل تحفيز مستعمرات الخلايا المحببة قد يمثل علاجا مؤثرا لاستهداف كل من المسببات المركزية والطرفية للالم العصبي.