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EFFECT OF NUCLEO CMP FORTE ON THE REGENERATION OF SCIATIC NERVE IN DOGS

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Abstract

The objective of the present research was to evaluate the efficacy of Cytidine Monophosphate on the regeneration of sciatic nerve injury of dogs. Eight healthy adult dogs were used to complete this study. They were randomly divided into two equal groups, control group and Cytidine Monophosphate group. The sciatic nerve axotomy was done on the right hind limb and then the nerve sutured immediately by nylon suture 0 - 5 with end-to-end anastomosis, using simple interrupted suture, and then the Cytidine monophosphate group the dogs were injected with Cytidine monophosphate 5 mg/day intramuscular intake for 30 day post operation. The clinical examination (motor and sensory function) showed occurrence of onset, gait, and knuckling that were evaluated weekly from the starting until the end of the study (sixteen week). The conductive velocity was measured at the end of study and macroscopic evaluation demonstrated a degree of nerve stump coaptation, adhesion, thickness and the presence of neuroma. The histopathological examination of regenerated nerve sections (three parts of 1 cm long each) were collected from the proximal, middle (injured site) and distal segments and used to determine the degree of the sciatic nerve regeneration. The clinical assessment of motor and sensory nerve functions revealed significant differences between the Cytidine monophosphate and control group, the Cytidine monophosphate more effective than Platelet rich plasma group. Moreover, the electrophysiological studies were carried out by isolation of the sciatic nerve. The conductive velocity on 16^{th} weeks postoperative, the results showed significant differences in the same group between right sciatic nerve (operated) and left sciatic nerve and significant differences between groups at ($p<0.05$) were the best conductivity in Cytidine monophosphate group compared with other groups. The neurohistopathological results that showed the number of Schwann cells proliferation and orientation of regenerative nerve fibers were observed most clear and improved in Cytidine monophosphate.

Key words: Nerve regeneration, Cytidine Monophosphate, Sciatic nerve and Dogs.

1. Introduction

Peripheral nerves are organs that expand throughout the body, forming a complex arborization that very much resembles that found in blood vessels, sharing with it developmental pathways (Zacchigna *et al*., 2008). The process of regeneration is dependent upon the severity of injury and site of the lesion. In a mixed nerve there was no difference between growth and maturation of the sensory and motor fibers (Moldovan *et al*., 2006).

Schwann cells are represent glial cells in the peripheral nervous system, and their main function is to provide support to the axons through the release of growth factors and isolation of the axon through formation of the myelin sheath (Pfister *et al.*, 2007). Schwann cells (SCs) also are

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able to produce extracellular matrix such as laminin and type IV collagen (Hoke, 2006).

Nucleo cytidine monophosphate (CMP) mainly used for peripheral neurological disorders like trigeminal neuralgia, diabetic neuropathy and lumbosciaticalgia, but it central roles remains to be elucidated, Nucleo CMP improve neural growth and never repair, improvement, regeneration of myelinated nerve fiber, delay spinal pain transmission, enhance spinal density and acceleration of hippocampal dependent working memory in animal model study also; Nucleo CMP contain uridine monophosphate, uridine diphosphate and uridine triphosphate, which together with cysteine monophosphate induce biosynthesis of neuronal glycolipid, phospholipids, Nucleo CMP crosses the blood brain barrier and then phosphorylated into uridine triphosphate that lead to triggering of neurotransmitter modulation (Flierl *et al.,* 2015).

Plasma and intracellular concentrations of uridine are regulated by the catabolic activity of Uridine Pase and by two transport mechanisms, facilitated diffusion and Na+ dependent active transport, Uridina Pase catalyzes the reversible phosphorolysis of uridine and to a lesser degree of thymidine (Martin *et al.,*1989). The presence of folic acid and vitamin B12 plays a key role in accelerating and boosting neural recovery processes *in vivo* (Larm and Ruckert, 2008). The combination Uridine monophosphate + vitamin B12 + folic acid is accelerating the regeneration of peripheral neuropathy. It leads to significant reductions not only in the total pain detect score but also in intensity of pain, number of areas affected and extent of pain radiation (Negrao *et al.,* 2014). Electromyography (EMG) is an instrument used in medical field for recording and assessing the electro activities originated by the skeletal muscles, accordingly considered diagnostic and identification method for those electro activities (Kamen, 2004). The aim of study was to evaluation the effect of the Cytidine monophosphate on the regeneration of peripheral nerves injury.

2. Materials and Methods

The study was conducted on eight adult dogs that aged from 1 - 3 years with body weight 20 – 30 kg. The animals were kept in cages for 15 days for acclimatization, the animal administration of antimicrobial and anthelmintic drugs. Moreover, animals were accommodated in a same laboratory conditions by keeping them in cages (one animal per cage).

The animals were divided randomly into two groups, four animals were included in each group. Cytidine monophosphate group similar procedure as in control group were used, but it injected with CMP 5 mg/day intramuscular intake for 30 day. The right sciatic nerve was transected and immediately sutured. Furthermore, the clinical signs were daily recorded post operation until the end of the experiment. The neurohistopathological examination was done.

The dogs were fasted for 8 hrs and water withdraw 2 hrs before operation. The site of operation was clipped and shaved carefully before operation and then given a mixture of ketamine hydrochlorid 15 mg/kg b.w and xylazine hydrochlorid 5 mg/kg b.w intramuscularly. The operation was carried out by exposing the sciatic nerve on the right side of thigh through a posterio-lateral skin incision (5 - 7 cm in length) performed parallel and behind the femur bone and separation it bluntly from the biceps femoris muscle and semimembranosis muscles by curved artery forceps. The sciatic nerve was exteriorized to the wound surface (Fig-1).

Electrophysiological examinations were carried out at 16^{th} week after nerve suture. The sciatic nerve was separated by about 3 cm and isolated from the body, then soaked in AD instruments chamber (filled with buffer solution) attached with negative, positive (recording) electrode clamps and stimulus electrodes (Fig - 2). The conductive velocity was recorded from the multiplication of distance between recording electrodes (mm) by time interval between capacity proximal and capacity distal (ms). The

stimulations were performed at a square wave of 0.2 milliseconds (ms) duration with a frequency of two pulses per second.

The microscopic examination of nerve sections were used to determine the number of Schwann cells, arrangement of nerve fibers, intraneural and extraneural scarring, vacuolated degenerative nerve fibers, the axonal alignment and density of nerve fibers.

Neurohistopathological examinations were done with routine stain (hematoxylin and eosin stain). The right sciatic nerves were isolated from each animal and the nerve samples were fixed on to plastic plate using stay sutures to keep the nerve tissues straight (Pan *et al*., 2006).then saved in special container containing 10% formalin. Three samples of 1-cm length each were harvested from the proximal, middle (site of injury) and distal portions of the the sciatic nerve (Suvarna *et al*., 2013).

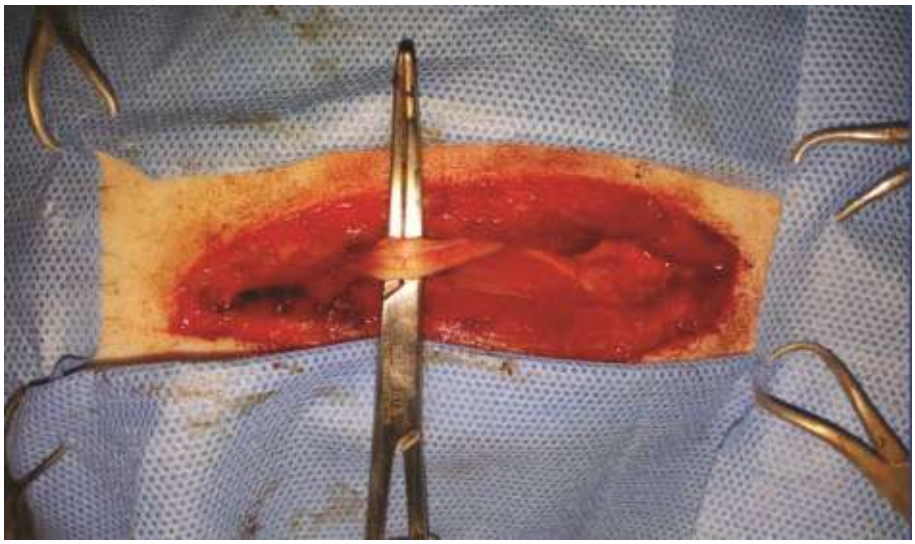


Figure - 1: Sciatic nerve exteriorization before section

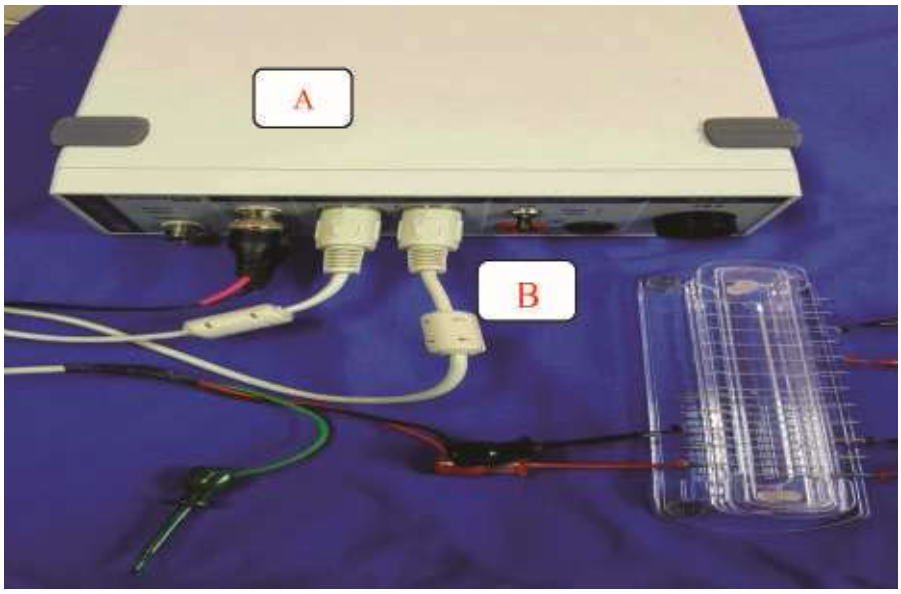


Figure - 2: AD Instrument (A) and Isolated nerve chamber (B)

3. Results

Control group

After recovery, the animals of this group showed paralysis (loss of sensory and motor function) of the right hind limb. Furthermore, pain and swelling were also recorded during the 15 day post-operative and then disappeared. The affected leg was return to the right position at the end of day 11 pos-operation. The animals had been shown severe knuckling (Flexion of fetlock joint) in the affected leg, that started from the first day after operation until the 40 day were became moderate and then progressively disappeared until the 60 day which were completely disappeared.

During the first twenty day, the muscle contraction of the affected leg was flaccid (no contraction). Moreover, the muscle had been shown a mild contraction at the 30 day pos-operation and then the muscle contraction became moderate at the 45 day pos-operation, while during the 60 day the muscle contraction became strong, furthermore muscle contraction became near normal at the 85 day pos-operation.

The CMP group in the hind limb the paralysis was also found, in addition to the appearance of the pain and swelling at the site of operation during the first days post-operation. Severe Knuckling also appeared during walking, knuckling became moderate and then disappeared early compared with other groups. The affected leg was return to the right position at the end of day 3 post-operation. The muscle contraction was also flaccid during the first day and then begins to disappear progressively and became near normal early in comparison with other group.

Macroscopic Finding

Control group

After killing the animals and opening the muscles of affected hind limb for isolation the sciatic nerve for histopathological and nerves conductive studies at the end of experiment 16 weeks. At the operated site there were many macroscopic observations were noted such as moderate cooptation of the proximal and distal end of nerve stump. Moreover, severe adhesions were observed too on other hand, thickness and neuroma were not present.

Nucleo CMP Forter group

A good coaptation observed, adhesion present in one animal, neuroma not appeared, thickness was not presented.

Conductive velocity at 16 week post-operation

The conductive velocity of right (operative) limb on post-operative week 16 presented significant differences between control and CMP groups at $P<0.05$. However, significant differences were observed in the same group between left sciatic nerve and right sciatic nerve (post-operation) as in Table - 1. The best conductivity was in CMP group compared with control (Fig - 3 & Fig - 4).

The histopathological examination of longitudinal section in control group of the proximal part of sciatic nerve at 16^{th} week post-operative showed several vacuolated nerve fibers (Fig - 5). The histological changes at the middle transverse part of sciatic nerve presented a numerous number of vacuolated degenerated nerve fibers associated with Schwann cells, irregular and degenerated myelin sheath associated with clump formation of degenerate myline in axoplasim, disarrangement of nerve fibers with severe Wallerian degeneration (Fig - 6). The longitudinal section of distal part of nerve sections revealed several swelling vacuolated degenerated nerve fibers and few numbers of Schwann cells (Fig-7). The histopathological examination of longitudinal section of the proximal part of sciatic nerve at 16^{th} week postoperative showed occasional vacuolated nerve fibers (Fig - 8). The middle transverse part of the nerve showed highly increase number of Schwann cells and regeneration of neural tube (Fig -9). The distal longitudinal section of the nerve showed regular and occasional vacuolated nerve fibers (Fig - 10).

Table - 1: Statistical analysis of conductive velocity of control and CMP group at 16 weeks post-operation

Groups		At 16 weeks
Control	RSN (OP)	21.72±1.37*
	LSN	39.97±1.78
CMP	RSN	25.47±2.61*
	LSN	34.12±3.56

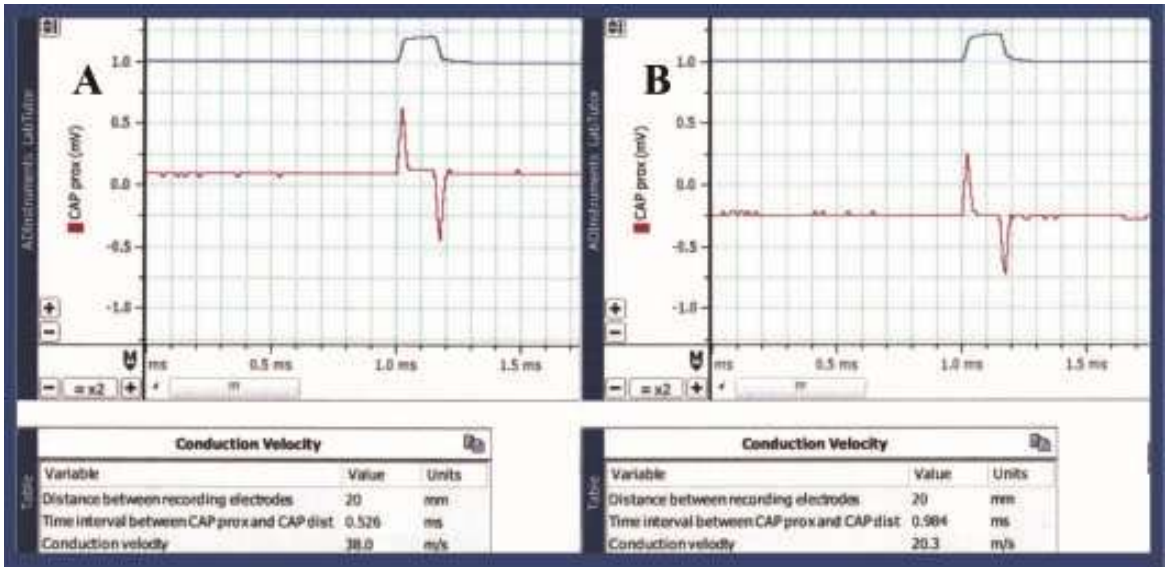
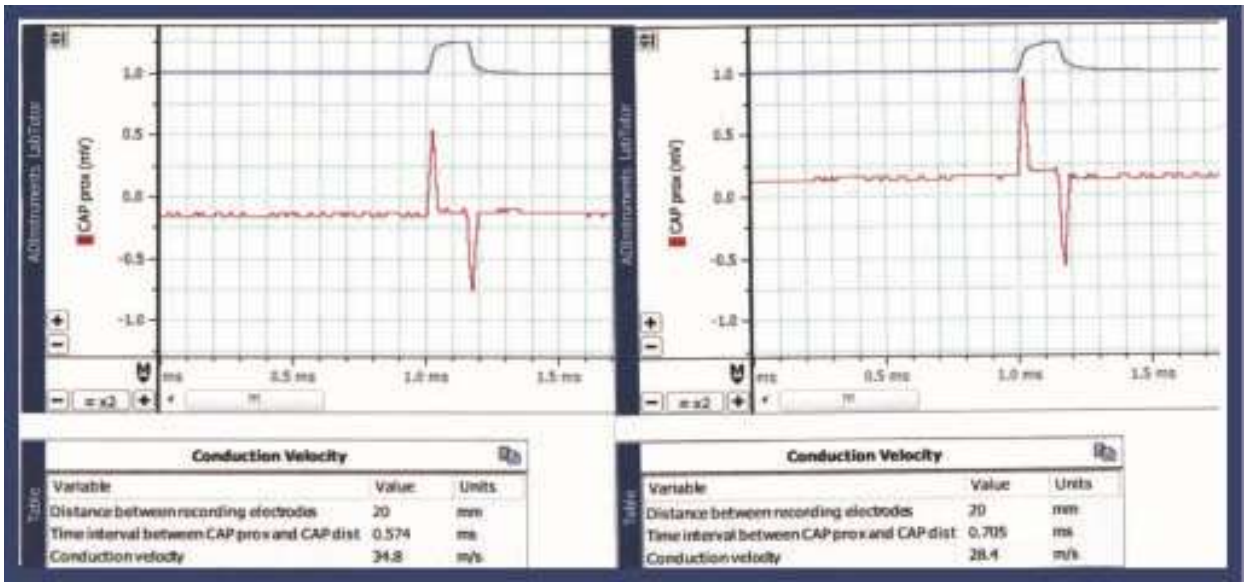


Figure - 3: The sciatic nerves waves (A) left (B) right, and conductive velocity for control group at 16 weeks post operation, the C.V measured by multiplucated the distance between recording electrodes on time interval



Figure(4): Shows the sciatic nerves waves (A) left (B) right, and conductive velocity for CMP group at 16 weeks PO, the C.V measured by multiplucated the distance between recording electrodes on time interval

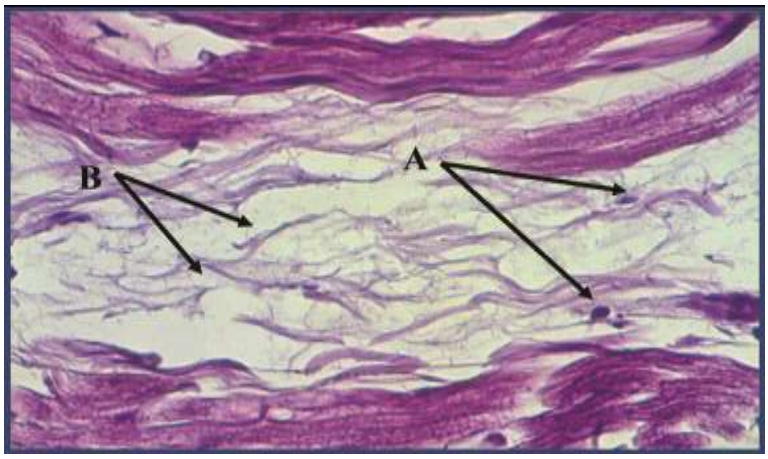


Figure - 5: The proximal longitudinal section of sciatic nerve at 16 weeks post operation of control group (A) Schwann cells. (B) Vacuolated degenerative fibers (H & E40 X)

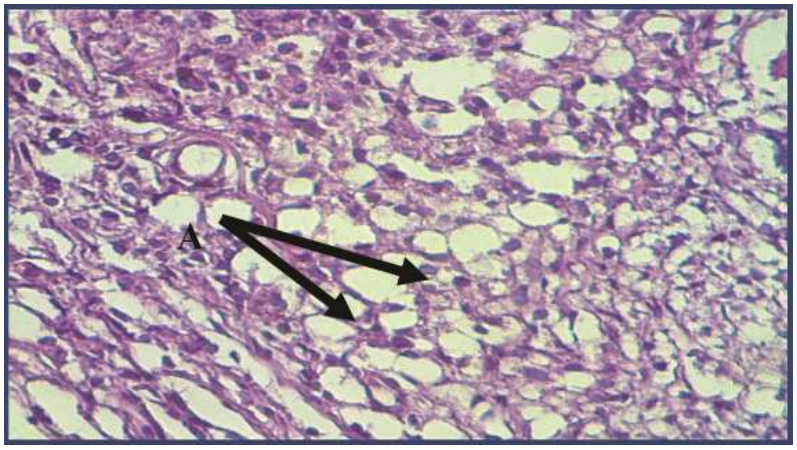


Figure - 6: The transverse section of middle part of sciatic nerve of control group (A) Wallarian Degeneration (H & E40 X)

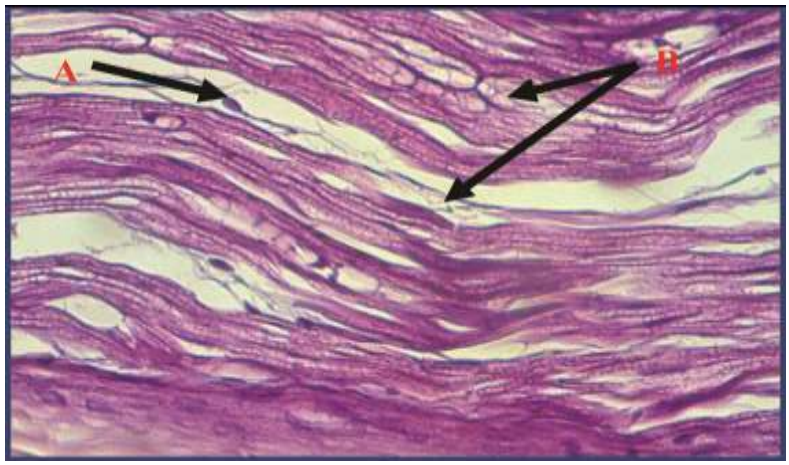


Figure-7: The distal longitudinal section of sciatic nerve of control group (A) few number of Schwann cells (B) Degenerative vacuolated of nerve fibers (H&E40X)

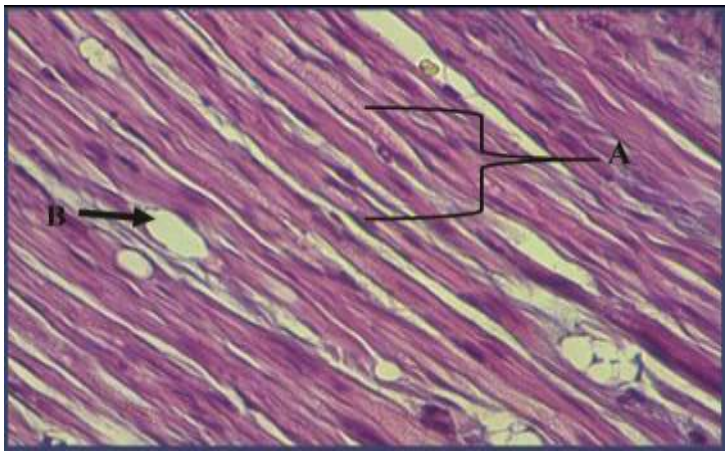


Figure - 8: The proximal longitudinal section of sciatic nerve of CMP group, (A) regular arrangement of nerve fibers, (B) Few vacuolated nerve fibers (H & E40 X)

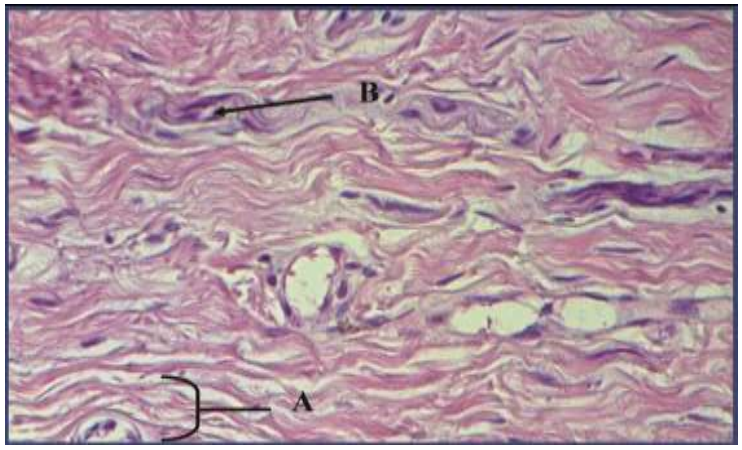


Figure - 9: The transverse section of middle part of sciatic nerve of CMP group, (A) Regenerative of neural tube, (B) High number of Schwann cells (H&E40X)

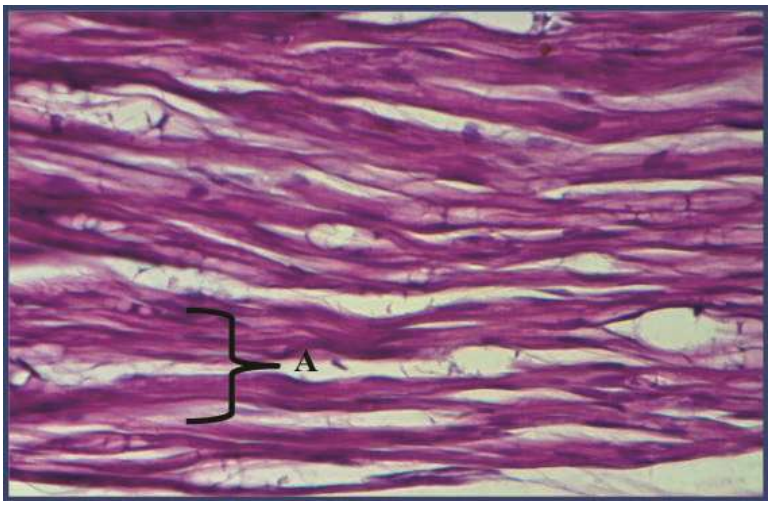


Figure-10: The distal longitudinal section of sciatic nerve of CMP group, (A) Good orientation of nerve fibers (H&E40X)

4. Discussion

In the present study, after transaction of sciatic nerve, the animal show several sings such as paralysis (sensory and motor dysfunction), since this sing was disappeared gradually. Furthermore, the paralysis was disappeared earlier in the CMP group compared with controls. Moreover, may be used in the treatment of injuries to nerve, tendon, ligament, muscle, bone and joint with success at pain reduction and return to desired level of activity (Kristin and David, 2010).

On the other hand, the paralysis was disappeared faster in the CMP group compared with control group. Nucleo CMP mainly used for peripheral neurological disorders, improve neural growth and never repair, improve regeneration of myelinated nerve fiber, Nucleo CMP contain uridine monophosphate, which together with cytidine monophosphate induce biosynthesis of neuronal glycolipid, phospholipid, RNA and DNA, Nucleo CMP crosses the blood brain barrier and then phosphorylated into uridine triphosphate that lead to triggering of neurotransmitter modulation (Flierl *et al*., 2015).

Pain and swelling were also recorded in the operated leg in all three groups, were they disappeared early in CMP group compared with controls, while they disappeared rabidly in CMP group compared with control group. Similar results were recorded by Negrao *et al*. (2014) who mentioned that CMP more effective on pain redaction, decrease number of effective areas and extent of pain radiation. Furthermore, nucleotide receptors have been shown to have a potent antinociceptive effect in neuropathic pain models (Ando *et al*., 2010).

The knuckling is a condition of permanent flexion of the fetlock joint and bending of digits due to paralysis of the extensor and flexor muscles (Raffe, 1985). In the present study, adhesions of sciatic nerve that recorded in control group was sever, while does not appeared in the CMP and control groups except one animal which showed moderate adhesion. These results were agreed with Ozay *et al.* (2007) and Aslan *et al.* (2011). They mention that CMP reduce nerve adhesion and tethering of the nerve to the surrounding tissue and enhance nerve separately.

During the week sixteen postoperative, significant differences in conductive velocity were observe between CMP and control groups at $p \leq 0.05$. On other hand, significant differences also observed in the same group between left and right sciatic nerve. Furthermore, the best conductivity was in the CMP group compared with control group. This results are consistent with the findings of Cansev *et al*. (2005) who mentioned that the using of CMP increasing the synthesis of neuronal membrane phospholipid and neurogenesis that improve transmission of nerve impulses.

The regeneration of peripheral nerves axons require neurotrophic support, they could benefit from the presence of a growth factors delivery cell system capable of responding to stimuli of the local environment during axonal regeneration (Amado *et al*., 2010). In a normal integrated peripheral nerve, trophic factors which affect nerve tissue are generated in the target organ and transported in a retrograde fashion along the cell body. Schwann cells (SCs) are not only the actors of Wallerian degeneration that occurs following the nerve damage but are also responsible for the production of trophic factors that regulate regeneration. The growth factors generated by Schwann cells, such as nerve growth factor, brain - derived neurotropic factor, ciliary neurotropic factor and glial cell line-derived neurotropic factor are involved in the modulation of recovery. Neurotrophins diffusely distribute around the damaged axons after releasing from the Schwann cells. Regenerating axons tend to extend towards the distal segment with a high concentration of neurotrophin (Yu *et al*., 2011).

In the present study, results of the neurohistopathological examination of the longitudinal and transfers section of sciatic nerve demonstrated presence of swollen vacuolated degeneration nerve fibers in all groups but in variable degrees. According to Stoll and Muller

(1999) demonstrate axotomy or crush of a peripheral nerves leads to degeneration of the distal nerve stump referred to as Wallerian degeneration (WD). Decrease number of the swollen vacuolated degenerative nerve fibers and prominent presence of Schwann cells proliferation and increase number of regenerative nerve fibers in the middle and distal part of nerve stump and loss of fibrous tissue were showed in the CMP group compared with control group. On other hand, this histopathological change such as increase in Schwann cells and nerve fibers, decrease swollen vacuolated degenerative nerve fibers were more better in CMP group compared with control group. Schwann cells down-regulate their normal proteins such as myelin basic protein (MBP), peripheral myelin protein-22 (PMP-22), myelin associated glycoprotein (MAG), P0 and connexin-32 (Trapp *et al*., 1988) in order to convert the pre-myelinating cells phenotype (Hall, 2005).

The differentiated Schwann cells up regulate nerve growth factor (NGF) expression, cytokines, neurotrophic factors. The latter are important in preventing neuronal apoptosis in response to injury and potentiate the migration and adhesion of Schwann cells to axonal projections (Boyd and Gordon, 2003). Nucleo CMP is useful in the regeneration of nerve cells by stimulating the synthesis of phospholipids and sphingolipids (the major components of neuronal cell membranes and myelin sheath). Furthermore, CMP has essential role in the activation of Schwann cells (Durany, 2005; Martianez *et al.*, 2012). Moreover, successful regeneration of peripheral nerve after nerve injury depends on activated Schwann cells and their supportive role in the production of neurotrophic and neurotropic factors (such as nerve growth factors) that enhance neural recovery (Oyama *et al.*, 2004). The healing cascade of the nerve fibers is initiated and controlled by bioactive proteins found in platelets, plasma and white blood cells. Increasing the concentration of these bioactive proteins, may accelerate the healing process of the regeneration of axons (Fernandez *et al.*, 2005).

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