Effect of Selective Cox-2 inhibitor celecoxib in spinal cord injury: a morphological study.

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CERTIFICATE

This thesis, submitted by **Zulfiqar Ali** is accepted in its present form by the Department of Animal science, Faculty of Biological sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirement for the degree of Master of Philosophy in Animal science.

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DEDICATED TO

I solemnly dedicate my work to
My Affectionate parents, dear daughter, brothers and sisters
Who's Love and Prayers are
A Constant source of
Inspiration and Guidance for me.

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Zulfiqar Ali

LIST OF ABBERVIATION

ALS	Amyotrophic lateral sclerosis
BDNF	Brain-derieved neurotrophic factor
BMSC	Bone marrow stromal cells
CNTF	Ciliary neurofilament factor
DG	Distil graft
DRG	Dorsal root ganglian
EAN	Experimental autoimmune neuritis
ESCs	Embryonic stem cell
Hsps	Heat shock protein
Hscs	Heat shock cognate proteins
LSRS	Lumbosacral radicular syndrome
MMP	Matrix metalloproteinase
NF	Neurofilaments
NGF	Nerve growth factor
NP	Nucleus pulposus
NPCs	Neural precursor cell
NSAIDs	Nonsteroidal anti-inflammatory drugs

PG	Proximal graft
PGA	Polyglycolic acid
PGs	Prostaglandin
RAGE	Receptere for age
SCI	Spinal cord injury
TNF-α	Tumor necrosis factors-α
WD	Wallarian degeneration

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ABSTRACT

Effect of Selective Cox-2 inhibitor celecoxib in spinal cord injury: a morphological study.

Abstract

This study was designed to observe the effects of Selective Cox-2 inhibitors celecoxib in spinal cord injury: a morphological study. In the present study effect of celecoxib was observed on the sham operated sciatic nerve of rabbit after one, two and three weeks of treatment. Celecoxib (30mg/kg/day) was given to rabbits orally. The results show that the density of normal myelinated fibers significantly ($P< 0.001$) decreased in sham operated group compared to sham control group and treatment with celecoxib caused a significantly ($P< 0.001$) increase in density of normal myelinated fibers compared to sham operated group after one, two and three weeks of treatment. The axon diameter of normal myelinated fibers significantly ($P < 0.001$) decreased in sham operated group compared sham control and treatement with celecoxib caused significant ($P< 0.001$) increase in the diameter of normal myelinated fibers compared to sham operated group ($P< 0.001$) after one, two and three weeks of treatment. Mean myelin myelin sheath thickness of normal myelinated fibers was significantly ($P< 0.001$) decrease in sham operated group compared to sham control group and treatment with celecoxib significantly ($P< 0.001$) increased myelin sheath thickness of normal myelinated fibers compared to sham operated group after one two and three weeks of treatment. In present study the greater damages in sham operated group was observed in thickness of epineurium, disintigeration of myelin sheath and degeneration of normal myelinated fibers. In celecoxib treated group there was increase in cluster of normal regenerated myelinated fibers with smaller diameter after 1^{st} , 2^{nd} and 3^{rd} weeks of treatment. The blood vessels also improved in celecoxib treated group. Macrophages were observed in celecoxib treated group. It was concluded that celecoxib significantly reduces the pathological changes induced by nucleus pulposus, but it showed the effect on promoting regeneration of sciatic nerve and showed the inflammatory effects.

INTRODUCTION

INTRODUCTION

Sciatica is usually defined as pain radiating along the sciatica nerve to one or both legs i.e. from the thigh and into the leg, and involving the foot (Andresson, 1991). Sciatica caused by a lumber herniated disc is the most common cause of radicular leg pain in adult working population (Frymoyer, 1998).

The lumbosacral radicular syndrome (LSRS or LRS; also called sciatica) is typically characterized by radiating pain in the dermatome of a lumbar or sacral spinal nerve root. Occasionally more than one root is involved. Contained in the syndrome pain may be accompanied with lumbar fixation, reflex abnormalities motor and sensory disturbances. The diagnosis includes stenosis of the spinal and/or root canal, infection, multiple sclerosis, autoimmune or metabolic neuropathy, and tumour. Herniations at the lowest three lumbar disc levels, since these represent the most common sites. In the vast majority of cases LSRS is the result of a herniated disc (Van *et al* 1990). At present it is not possible to identify these latter groups of patients in an early stage of their disease by means of intensity of pain, neurological deficit, root irritation signs, or diagnostic imaging. For this reason it is not helpful to perform early diagnostic imaging (CT or MRI), unless a disease entity different from disc herniation is considered. After the indication for surgery has been set diagnostic imaging is helpful in defining the exact site of disc herniation and its anatomical relationship with the nerve root involved. Mixter and Barr have demonstrated the success of surgery for the treatment of LSRS (Mixter and Barr, 1934). Post-traumatic inflammatory reactions may play an important role in the secondary injury processes after spinal cord injury (SCI). Peripheral nerves injury is often accompanied by chronic neuropathic pain, and in both human patients and animal models the pain may be exacerbated by, or maintained by, sympathetic nervous system stimulation. There are a number of possible sites of interaction between the sensory and sympathetic nervous systems which may account for this phenomenon, and a new possibility was added when described sprouting of sympathetic axons from blood vessels supplying the dorsal

root ganglion (DRG) into the regions of the DRG containing the cell bodies of primary sensory neurons following a complete sciatic nerve injury (McLachlan *et al.*, 1993). Back pain is strongly associated with degeneration of the intervertebral disc (Luoma *et al.*, 2000). Disc degeneration, although in many cases asymptomatic (Paassilta *et al.*, 2001) is also associated with sciatica and disc herniation or prolapse. It alters disc height and the mechanics of the rest of the spinal column, possibly adversely affecting the behaviour of other spinal structures such as muscles and ligaments. In the long term it can lead to spinal stenosis, a major cause of pain and disability in the elderly. Its incidence is rising exponentially with current demographic changes and increased aged population. Discs degenerate earlier than do other musculoskeletal tissues; the first unequivocal findings of degeneration in the lumbar discs are seen in the age group 11–16 years (Pincus *et al.*, 2002). About 20% of people in their teens have discs with mild signs of degeneration; degeneration increases steeply with age, particularly in males, so that around 10% of 50-year-old discs and 60% of 70-year-old discs are severely degenerate (Kang *et al.*, 1997).

A past and current hypothesis is that, in symptomatic individuals, the nerves are some how sensitized to the pressure (Cavanaugh, 1995) ,possibly by molecules arising from an inflammatory cascade from arachodonic acid through to prostaglandin E2, thromboxane, phospholipase A2, tumour necrosis factor-α, the interleukins and matrix metalloproteinase (MMPs). These molecules can be produced by cells of herniated discs (Kang *et al.*, 1996) and because of the close physical contact between the nerve root and disc herniation they may be able to sensitize the nerve root (Kawakami *et al.*, 1996). Nucleus pulposus (NP) sensitizes primary afferent neurons when applied to the dorsal root ganglion (DRG) (Takebayashi *et al.*, 2001), and NP applied to the nerve root or DRG sensitizes spinal dorsal horn neuronal responses (Anzai *et al.*, 2002). However, whether this sensitizing effect of NP on responses of dorsal horn neurons reflects a central sensitization, is secondary to a peripheral sensitization, or a combination of both. (Onda *et al.*, 2003).

The discs bulge or rupture (either partially or totally) posteriorly or posterolaterally, and press on the nerve roots in the spinal canal. Although herniation is often thought to be the result of a mechanically induced rupture, it can only be induced in vitro in healthy discs by mechanical forces larger than those that are ever normally encountered; in most experimental tests, the vertebral body fails rather than the disc (Adams and Hutton 1981).The herniation-induced pressure on the nerve root cannot alone be the cause of pain because more than 70% of 'normal', asymptomatic people have disc prolepsis pressurizing the nerve roots but no pain (Boos *et al.,* 1995). If pain due to disc herniation, protrusion, bulge, or disc tear is due to chemical radiculitis pain, then prior to surgery it may make sense to try an anti-inflammatory approach. Often this is first attempted with non-steroidal anti-inflammatory medications, but the long-term use of Nonsteroidal anti-inflammatory drugs (NSAIDs) for patients with persistent back pain is complicated by their possible cardiovascular and gastrointestinal toxicity; and NSAIDs have limited value to intervene in TNF-mediated processes (Carragee 2005) Disc degeneration, although in many cases asymptomatic so associated with sciatica and disc herniation or prolapse. It alters disc height and the mechanics of the rest of the spinal column, possibly adversely affecting the behavior of other spinal structures such as muscles and ligaments (Boden *et al.,* 1990).

The mechanical functions of the disc are served by the extra cellular matrix; its composition and organization govern the disc's mechanical responses. The main mechanical role is provided by the two major macromolecular components. The collagen network formed mostly of type I and type II collagen fibrils and making up approximately 70% and 20% of the dry weight of the annulus and nucleus, respectively, provides tensile strength to the disc and anchors the tissue to the bone (Eyre and Muir 1977).

Aggrecan, the major proteoglycan of the disc (Stone and Bayliss 1995), is responsible for maintaining tissue hydration through the osmotic pressure provided by its constituent chondroitin and keratan sulphate chains (Urban *et al.,* 1979).The proteoglycan and water content of the nucleus (around 50% and 80% of the weight, respectively) is greater than in the annulus (approximately 20% and 70% of the weight, respectively). In addition, there are many other minor components, such as collagen, small proteoglycans such as lumican, biglycan, decorin and fibromodulin; and other glycoproteins such as fibronectin and amyloid (Roberts *et al.,* 2001).

The matrix is a dynamic structure; its molecules are continually being broken down by proteinases such as the matrix metalloproteinases (MMPs) and aggrecanases, which are also synthesized by disc cells (Sztrolovics *et al.,* 1997).However there are significant differences between the two tissues, one of these being the composition and structure of aggrecan. Disc aggrecan is more highly substituted with keratan sulphate than that found in the deep zone of articular cartilage. In addition, the aggrecan molecules are less aggregated (30%) and more heterogeneous, with smaller, more degraded fragments in the disc than in articular cartilage (80% aggregated) from the same individual (Donohue *et al.,* 1988).

The most significant biochemical change occur in disc degeneration is loss of proteoglycan aggrecan molecules become degraded, with smaller fragments being able to leach from the tissue more readily than larger portions. The results in the loss of glycosaminoglycans; this loss is responsible for a fall in the osmotic pressure of the disc matrix and so a loss of hydration (Lyons *et al.,* 1981).

Less is known of how the small proteoglycan population changes with disc degeneration, although there is some evidence that the amount of decorin, and more particularly biglycan, is elevated in degenerate human discs as compared with normal ones. Although the collagen population of the disc also changes with degeneration of the matrix, the changes are not as obvious as those of the proteoglycans. The absolute quantity of collagen changes little but the types and distribution of collagens can alter (Inkinen *et al.,* 1998).

In addition, the fibrillar collagens, such as type II collagen, become more denatured, apparently because of enzymatic activity. As with proteoglycans, the triple helices of the collagens are more denatured and ruptured than are those found in articular cartilage from the same individual; the amount of denatured type II collagen increases with degeneration.However, collagen cross-link studies indicate that, with proteoglycans, new collagen molecules may be synthesis the normal and degenerate lumbar intervertebral disc. The annulus lamellae surrounding the softer nucleus pulposus are clearly visible (Antoniou *et al.,* 1996).

The biochemistry of disc degeneration indicates that enzymatic activity contributes to this disorder, with increased fragmentation of the collagen, proteoglycan and fibronectin populations. Several families of enzymes are capable of breaking down the various matrix molecules of disc, including cathepsins, MMPs and aggrecanases. Cathepsins have maximal activity in acid conditions (e.g. cathepsin D is inactive above pH 7.2). Aggrecanases have also been shown to occur in human disc but their activity is apparently less obvious, at least in more advanced disc degeneration (Sztrolovics *et al.,* 1997; Roberts *et al.,* 2000).

Necrotic and Apoptotic mechanisms of cell death after Spinal Cord Injury.

Both necrotic and apoptotic mechanisms of cell death after SCI have been well and extensively characterized in animal SCI models. It has been thought that microglial cells might be the source of cytotoxic cytokines, such as tumor necrosis factor-alpha (TNF-α) that kill oligodendrocytes .Within 1 h after spinal cord injury, increased synthesis or secretion of TNF-α is detectable at the injury site. Demonstrated that 6 h post-acute SCI there were an increased neuronal expression of (TNF-α and its receptors. They also demonstrated that at 1 h after acute SCI, TNF-αlevels in the CSF were significantly higher than those found in the sham-injured animals, indicating the release of this cytokine into the interstitial fluid (Harrington *et al.,* 2005).

TNF-α, has an important role in several pathologies such rheumatoid arthritis. TNF-αbinds to two receptors, the type 1 TNF receptor (p55) and the type 2 TNF receptor (p75) is expressed on many types of the cells. The biologic activity of TNF-αcan is attenuated by soluble TNF receptors (Bazzoni and Beutler, 1996).

Studies in animals have provided key evidence that antagonizing TNF-αis a viable therapeutic strategy. Subsequent studies in patients with rheumatoid arthritis indicated that blocking TNF improved symptoms. Biological agents that are currently available include 3 agents that decrease the activity of tumor necrosis factor-alpha (infliximab, adalimumab, and etanercept), (Kapadia *et al.,* 1995).

Disc morphology

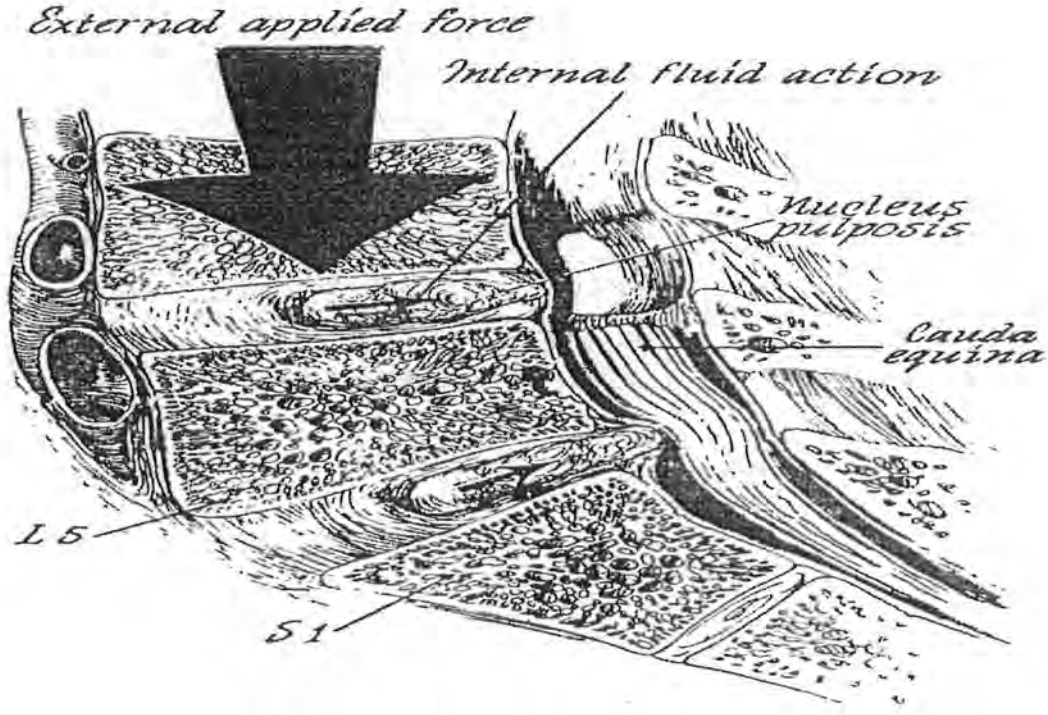
The normal disc

Major role is mechanical, as they constantly transmit loads arising from body weight and muscle activity through the spinal column. Provide flexibility to this, allowing bending, flexion and torsion. They are approximately 7–10mm thick and 4 cm in diameter (anterior–posterior plane) in the lumbar region of the spine (Twomey *et al.*, 1987; Roberts *et al.*, 1989).

The intervertebral discs are complex structures that consist of a thick outer ring of fibrous cartilage termed the annulus fibrosus, which surrounds a more gelatinous core known as the nucleus pulposus; the nucleus pulposus is sandwiched inferiorly and superiorly by cartilage end-plates. The central nucleus pulposus contains collagen fibres, which are organised randomly (Inoue, 1981) and elastin fibres, which are arranged radially (Yu *et al.*, 2002) these fibers are embedded in a highly hydrated aggrecan-containing gel. Interspersed at a low density approximately 5000/mm^3 (Maroudas *et al.*, 1975) are chondrocyte like cells, sometimes sitting in a capsule within the matrix. Outside the nucleus is the annulus fibrosus, with the boundary between the two regions being very distinct in the young individual (<10 years). The annulus is made up of a series of 15–25 concentric rings, or lamellae (Marchand and Ahmed 1990), with the collagen fibres lying parallel within each lamella. The fibers are orientated at approximately 60°C to the vertical axis, alternating to the left and right of it in adjacent lamellae. Elastin fibres lie between the lamellae, possibly helping the disc to return to its original arrangement following bending, whether it is flexion or extension. They may also bind the lamellae together as elastin fibers pass radially from one lamella to the next (Yu *et al.*, 2002). The cells of the annulus, particularly in the outer region, tend to be fibroblast-like, elongated and thin aligned parallel to the collagen fibres. Toward the inner annulus the cells can be more oval. Cells of the disc, both in the annulus and nucleus, can have several long, thin cytoplasmic projections, which may be more than 30 mm long (Errington *et al.*, 1998)

features are not seen in cells of articular cartilage (Errington *et al.*, 1998). and communicators of mechanical strain within the tissue (Errington *et al.*, 1998).

The third morphologically distinct region is the cartilage end-plate, a thin horizontal layer, usually less than 1mm thick, of hyaline cartilage. This interfaces the disc and the vertebral body. The collagen fibres within it run horizontal and parallel to the vertebral bodies, with the fibres continuing into the disc (Roberts *et al.*, 1989).The healthy adult disc has few blood vessels, but it has some nerves, mainly restricted to the outer lamellae, some of which terminate in proprioceptors (Roberts *et al.*, 1995).The cartilaginous end-plate, like other hyaline cartilages, is normally totally a vascular and a neural in the healthy adult. Blood vessels present in the longitudinal ligaments adjacent to the disc and in young cartilage end-plates (less than about 12 months old) are branches of the spinal artery (Goldwasser *et al.*, 1991). Nerves in the disc have been demonstrated, often accompanying these vessels, but they can also occur independently, being branches of the sinuvertebral nerve or derived from the ventral rami or grey rami communicantes. Some of the nerves in discs also have glial support cells, or Schwann cells (Johnson *et al.* 2001).



Degenerated discs

During growth and skeletal maturation the boundary between annulus and nucleus becomes less obvious, and with increasing age the nucleus generally becomes more fibrotic and less gel-like. With increasing age and degeneration the disc changes in morphology, becoming more and more disorganized. Often the annular lamellae become irregular, bifurcating and inter digitating, and the collagen and elastin networks also appear to become more disorganized (Buck Walter 1995). Nerves and blood vessels are increasingly found with degeneration (Roberts *et al.,* 1995). Cell proliferation occurs, leading to cluster formation, particularly in the nucleus (Johnson *et al.,* 2001).Cell death also occurs, with the presence of cells with necrotic and apoptotic appearance (Trout *et al.,* 1982).These mechanisms are apparently very common, it has been reported that more than 50% of cells in adult discs are necrotic (Trout *et al.,* 1982).

The morphological changes associated with disc degeneration were recently by, an age-associated change in morphology, with discs from individuals as young as 2 years of age having some very mild cleft formation and granular changes to the nucleus (Boos *et al.,* 2002).

Similar to embryonic stem cells (ESCs), adult neural precursor cells (NPCs) have shown an extensive capacity for self-renewal and multipotency in vitro (Reynolds and Weiss, 1992), and importantly, they obviate the potential ethical issues surrounding the use of ESCs for regenerative therapies. The population of NPCs residing in the forebrain and spinal cord is maintained for life and can be considered as a potential source of transplantable cells for individuals with SCI or other myelin disorders. (Richards *et al.,* 1992).Single adult neural stem cells can be isolated in vitro in the presence of growth factors that enable the proliferation and formation of clonally derived colonies of cells. These free-floating colonies called neurospheres, comprised <1% neural stem cells (Morshead *et al.,* 2002) and >99% progenitor cells. The ability of brain-derived adult NPCs to sub serve SCI repair has not been fully characterized.

Glial activation leads to the release of numerous inflammatory agents such as cytokines, growth factors, kinins, purines, and amines (Scholz and Woolf, 2002). Inflammatory agents have been shown to activate and enhance the sensitivity of primary afferents and spinal cord neurons (Reeh, 1994; Shu and Mendell, 1999; Woolf and Costigan, 1999).Synaptic transmission increase is sustained by transcriptional changes within the neuron, resulting in induction of genes such as Cox-2, which leads to PGE2 production (Watkins and Maier, 2000).The inflammatory agents alter neuronal excitability by facilitating pre- and post-synaptic signaling of excitatory neurons and by reducing inhibitory transmission (Yamagata *et al.*, 1993; Kaufmann *et al.*, 1996; Woolf and Salter, 2000; Scholz and Woolf, 2002).

Nonsteroidal anti-inflammatory drugs (NSAIDs)

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to treat inflammatory disorders, such as rheumatoid arthritis, by inhibiting cyclooxygenases (COX), enzymes that play a key role in the formation of proinflammatory prostaglandins (Simmons *et al.*, 2004). The classical view that Cox-1 was constitutive and that Cox-2 was exclusively a proinflammatory inducible enzyme (Vane *et al.*, 1998) was challenged because both isoforms were found to be present in different tissues and sites of inflammation and induced differentially (O'Neill and Ford-Hutchinson, 1993; Harris *et al.*, 1994).Cox-2 protein was found to be up regulated in a number of non-neuronal cell types such as macrophages, human monocytes, and synoviocytes, including microglia in CNS inflammation (Bauer *et al.*, 1997; Minghetti *et al.*, 1999).Cox-2 is strongly involved in different processes of central nervous modeling and regulated by different signaling pathways. The explicit role of the constitutive enzyme in the pain and inflammatory processes remains to be determined (Hoffmann, 2000).With increasing age comes an early work showed that systemically delivered NSAIDs were effective inhibitors of cyclooxygenase (COX) (Smith and Willis, 1971) and that peripheral prostanoids could sensitize the peripheral terminal. This suggested that hyperalgesia arose from a peripheral afferent sensitization.

Two COX enzymes (COX-1 and COX-2) catalyze the conversion of arachidonic acid to PGE2 (Kujubu *et al.*, 1991).COX-1 was thought to be constitutive, whereas COX-2 was upregulated as an immediate-early gene in response to injury (Kujubu *et al.*, 1991).COX-2 is inducibly expressed in cells involved in inflammation and in neoplastic tissues by proinflammatory and mitogenic stimuli, and is primarily responsible for the synthesis of prostanoids involved in acute and chronic inflammation (Xie *et al.*, 1997).

Celecoxib is a new generation of highly specific COX-2 inhibitors that have been approved for the treatment of rheumatoid arthritis and other inflammatory diseases. The selectivity of COX-2 inhibition is much higher than traditional COX-2 inhibitors (Penning *et al.*, 1997). Furthermore celecoxib has been shown to exert a potent anti-tumour effect. Interestingly, the anti-tumour effect by celecoxib has been both COX-2-dependent and COX-2- independent mechanisms (Grosch *et al.*, 2001). Cell cycle arrest and apoptosis of various kinds of cells induced by celecoxib appeared to be COX-2- independent effects (Hsu *et al.*, 2000).

The spinal cord, both COX-1 and COX-2 are expressed constitutively (Beiche *et al.*, 1996). Because classic NSAIDs exhibit no preferential inhibition of both COX isozymes (Meade *et al.*, 1993), Showed that intrathecally delivered COX-2 inhibitors reduce paw carrageenan-evoked hyperalgesia to the same degree as nonspecific NSAIDS (Dirig *et al.*, 1998).A wider role for spinal COX-2 has been proposed based on the presence of constitutive COX-2 in the spinal cord and its up-regulation in animal models of inflammation (Ichitani *et al.*, 1997) and the fact that COX-2 inhibitors given intrathecally can inhibit both inflammation-induced thermal hyperalgesia and substance P-evoked prostaglandin release (Yaksh *et al.*, 2001).

Prostaglandins are lipidic acids that are locally synthesized by the cyclooxygenase enzymes after focal tissue injury and that sensitize peripheral nerve endings and enhance pain behavior in both animals and humans (Masferrer *et al.*, 1994).

Prostaglandins (PGs) are well established as mediators of several components of the inflammatory response. Particularly, the oedema resulting from increased microvascular permeability is a consequence of the vasodilator of eject PGs potentiating the microvascular ejects of other mediators such as bradykinin, substance P and histamine (Williams and Morley.1973). These mediators also induce pain in inflammatory sites and this component is also potentiated by PGs (Ferreira, 1972). Although PGs are not direct algesic agents as are bradykinin, substance P and histamine, they never the less induce a state of hyperalgesia in which previously non-painful stimuli are now perceived as painful in both animal models and in human subjects (Ferreira, 1972).Therefore, inhibitors of PG biosynthesis should reduce PG-induced hyperalgesia to basal levels of pain perception, rejecting the algesic action of the directly acting agents, but not reducing pain perception beyond the normal threshold. The observed anti-oedema and analgesic ejects of the non- steroidal anti-inflammatory drugs (NSAIDs) were attributed over 30 years ago to inhibition of the biosynthesis of PGs catalysed by cyclo-oxygenase (COX) (Vane, 1971).

Most of the NSAIDs already used clinically (aspirin, indomethacin, ibuprofen, diclofenac, etc.) are non-selective inhibitors of COX, ejecting both isoforms to a variable extent (Vane *et al.,* 1998). The selective inhibitors of COX-2 has lead to treatments that are as effective as conventional nonsteroidal anti-inflammatory drugs in treating pain due to inflammation but without the GI toxicity associated with COX-1 activity (Cannon, 2000).

There are a number of selective COX-2 inhibitors on the market including celecoxib (Penning *et al.,* 1997), rofecoxib, (Prasit *et al.,* 1999), and valdecoxib (Talley *et* al., 2000), and although all are effective at treating conditions such as osteoarthritis and rheumatoid arthritis (Clemett and Goa, 2000), the overall response rate and relief of pain is not maximal (Everts *et al.,* 2000), and they have little efficacy in neuropathic pain conditions. Most COX inhibitors are relatively insoluble in aqueous media and

must be dissolved in organic solvents, such as dimethyl sulfoxide, which limits their intrathecal (IT) use. Ketorolac, a member of the pyrrolo-pyrrole group of nonsteroidal anti-inflammatory drugs (NSAIDs), is a potent anti-inflammatory drug and inhibits both COX-1 and COX-2, but, in contrast to other NSAIDs, there are three crystal forms of ketorolac, all of which are water-soluble. These selective COX-2 inhibitors exhibit NSAID-like ejects in human disease and, in animal models of inflammation; they decrease oedema and hyperalgesia (Chan *et al.,* 1999).

Local injury yields the release of a variety of factors that alter sensitivity of the primary afferent fibers innervating the damaged tissue, and among these factors that are important components of this peripheral sensitization are prostaglandins.COX-2 is expressed in neurons and endothelial cells in healthy brain. In rats with EAE, the expression of COX-2 was reported to be up regulated in endothelial cells in inflammatory lesions (Schaible and Grubb, 1993).

Non-selective COX-2 inhibitors have been reported to moderately ameliorate EAE (Prosiegel *et al.,* 1989), suggesting that COX-2 may have an important role in the pathogenesis of EAE (Deininger and Schluesener, 1999).Furthermore, demonstrated that COX-2 inhibitors suppress experimental autoimmune neuritis (EAN), a model of Guillain–Barre′ syndrome, which is also characterized as a CD4+-Th1 T-cell-mediated autoimmune neurological disease model similar to EAE (Miyamoto *et al.,* 1998).

Inflammation is also a feature of several neurodegenerative diseases including Alzheimer's disease (P.L. McGeer and E.G. McGeer 2002b), Parkinson's disease (McGeer *et al.,* 2001), and amyotrophic lateral sclerosis (ALS) (Kawamata *et al.,* 1992) NSAIDs have been shown to enhance the heat shock response, a reaction to hyperthermia and other toxic conditions characterized by the induction of heat shock proteins (Hsps). Constitutively expressed Hsps and the heat shock cognate proteins (Hscs) function under basal conditions as chaperones during protein synthesis, intracellular trans- port, and degradation (Morimoto 1998).

Regeneration of Nerve fibers.

The development and proper function of peripheral nerves in vertebrate depends on the intimate interaction and continued signaling between Schwann cells and associated nerve fibers. In recent years, much progress has been made in identifying components of cell-cell interaction necessary in early stages of peripheral nerve development (Jessen and Mirsky, 1999). In contrast, very little is known about extra-cellular signals and intra-cellular signal pathways that initiate myelination. The myelinated programmer of Schwann cells is under the control of associated axon and correlated with axonal diameter (Aguayo *et al.,* 1976 and Voyvodic, 1989).The nature of axonal signal that drives myelination by Schwann cells remain unclear (Eldridge *et al.,* 1989, Bunge *et al.,* 1990). Most of the Schwann cells are derived early in embryonic development from the neural crest, a small transient population of cells that breaks away from the neural tube as it closes (Le Douarin, 1986). The crest cell goes through two steps, first developing into a precursor cell, which consequently transforms into an immature Schwann cell until birth (Jessen *et al.,* 1994). After birth, the immature cells become myelinating Schwann cells or non-myelinating Schwann cells. The immature cells first go through the myelination pathway at birth and then go through the non-myelin pathway later on in development (Jessen and Mirsky, 2005).The pathologies associated with Schwann cells can be divided into injury response, demyelinating disorders and tumor disorders. After an injury occurs in the peripheral nervous system, there tends to be disruption in the conductance of neuronal signals due to axonal damage (Mirsky, 2005).The Schwann cell helps to phagocytize the damaged end of the axon and then forms the regeneration tube of the axon that is connected to the cell body. While forming this tube, Schwann cells proliferate rapidly and provide the axon with a path to grow along. This trail is formed by the Schwann cells (Bungner's Band) that are still present in the distal stump. Schwann cells are also able to attract injured neurons by secreting neurotrophic factors such as nerve growth factor (NGF) to aid axon elongation.

The Schwann cell also promotes elongation by secreting F-Spondin, a develop axons of neurons as well as damage to the neuron as a whole. While endogenous Schwann cells are also recruited following spinal cord injury, the transplantation of Schwann cells into targeted areas may improve healing. The transplantation of Schwann cells derived from bone marrow stromal cells (BMSC) into a transected rat spinal cord results in axonal regeneration as well as regained hind limb function (Kamada *et al.*, 2005).Transplanting autologous Schwann cells in higher animals such as the rhesus monkey (Bachelin *et al.*, 2005). Human Schwann cells can be cryopreserved and later transplanted into rats to promote remyelination of the dorsal column (Kohama *et al.*, 2001). Schwann cell transplantation is also enhanced by neurotrophic factors and stimulating the cAMP cascade (Pearse *et al.*, 2004). These encouraging early stage studies suggest that Schwann cells may eventually be used therapeutically to facilitate axon regeneration after trauma. A number of diseases cause demyelination, the most common of which is multiple sclerosis.

After one, two and three weeks of celecoxib treatment, morphometric analysis showed that mean density of normal myelinated fibers of Ls nerve root irritation by nucleus pulposus in rabbits of sham control group was grater as compare to sham operated group and medicine group). Medicine group shows the greater density of myelinated fibers as compared to sham operated group but this increase in density is statistically significant. Sham control group showed greater density of myelinated fibers as compared to sham operated group but increase in density is statically significant. In present study significant increase in normal density of myelinated fiber was also observed in sham control group, increase in density of normal myelinated fibers was highly significant and sham operated group, increase in density of normal myelinated fibers was highly significant. But in medicine group, mean density of normal myelinated fibers was highly increasing significantly after one, two and three group of Celecoxib treatment.

The mechanism of sciatica or lumber disc disorder, many models of the lumber disc herniation and experiment has showed that nucleus pulposus can produce damage without mechanical compression of the nerve root (Olmarker and Myers, 1998, Kawakami *et al.,* 2000). Celecoxib plays an important role in the regeneration of peripheral nerves after spinal cord injury was tested. Treatment within sham control, sham operated sciatic nerve model of rabbits for three weeks resulted in the nerve regeneration as assessed by the density and diameter of normal myelinated fibers, myelin sheath thickness and histomorphology of sciatic nerve. Peripheral nerve is damaged, the distal axons will undergo Wallerian degeneration, which is characterized by myelin breakdown and the recruitment of inflammatory cells such as macrophages, lymphocytes, mast cells and neutrophils to invade the degenerating regions, (Brown *et al.,* 1991) Recruited inflammatory cells, macrophages are the principal effectors cells involved in the process. Macrophages not only clear up degraded nerve debris but also facilitate axonal regeneration by producing and releasing growth factors, cytokines and pro-inflammatory mediators. However, in addition to the beneficial effects in promoting axonal regeneration, most products of Wallerian degeneration are also pro-nociceptive. It has been shown that inflammatory mediators including PGE2, serotonin, bradykinin and histamine sensitize both injured and intact axons to mechanical innocuous and noxious stimuli following nerve injury (Michaelis *et al.,* 1998).

COX2 expressing cell profiles were present in injured sciatic nerve 2 and 4 weeks following PSNL and most of these COX2 expressing cells were identified as macrophages (Ma and Eisenach, 2002). Since macrophage invasion of the degenerating nerve is a common event following peripheral nerve injury, therefore hypothesized that up-regulation of COX2 or over-production of PGs occurs universally in injured nerve tested this hypothesis in various nerve injury models and observed that 2 weeks following SNL, CCI and CSNT, COX2 was markedly up-regulated in cell profiles in injured nerves and the majority of COX2-IR cell profiles were identified as macrophages. Some COX-2 expressing cells did not co-express

ED1, suggesting that other types of inflammatory cells may up regulate COX-2 expression as well.

The NSAIDs reduce the pathological change of rabbits sciatic nerve induced by autologus nucleus pluposus. It is suggested that the Celecoxib is a nonsteriod anti-inflammatory drug that exhibits anti-inflammatory, analgesic, and anti-pyretic activities. Celecoxib is believed to be due to inhibition of prostaglandin synthesis primarily via inhibition of cyclooxygenases in human, celecoxib dose not inhibit the cyclooxygenase-1 (Cox-1) isoenzyme. Celecoxib cannot be expected to substitute for corticosteroids or to treat corticosteroid insufficiency. Abrupt discontinuation of corticosteroid may lead to exacerbation of corticosteroid-responsive illness. Patients on prolonged corticosteroid therapy should have their therapy tapered slowly if a decision is made to discontinue corticosteroid. Hepatic effects (liver disease develop, or if systemic manifestations occurs (e.g eosinophilia, rash, etc), celecoxib should be discontinued. renal effects (NSAIDs has resulted in renal papillary necrosis and other renal injury), Hematological effect (treatment with celecoxib should have their hemoglobin or hematocrit checked if they exhibits any sign and symptoms of anemia or blood loss). Chan *et al* (2001) observed that sciatic nerve treated with brain-derived neurotrophic factor (BDNF) not only produced an increase in the number of myelinated axons, but also an enlargement of myelin sheath itself. This increase in size as compared to control nerve is due to an increase in the number of lamellae in the myelin internodes. The role of BDNF in nerve injury experiment has documented an increase in the number of peripheral nervous system myelin (Zhang, *et al.*, 2000; Namiki, *et al.*, 2000). In current study, the animal model of sham operated sciatic nerve is used to observe the effect of celecoxib on the pathological changes of sham operated sciatic nerve of rabbit after one, two and three weeks of treatment. Its aim is to evaluate that wether celecoxib reduces histological and structural changes of nerve induced by nucleus pulposus. Whether celecoxib treatment has any effect on regeneration of sham operated nerve.

MATERIAL AND METHOD

MATERIAL AND METHOD

Forty-five rabbits weighing 1100-1260g (Both male and female) were used for the conducting experiment to find out the effect of selective Cox-2 inhibitor celecoxib on spinal cord injury: a morphological study. The rabbits were purchased from the National Institute of Health (NIH). These rabbits were kept in the cage in Animal house of Department of Animal Sciences Quaid-i-Azam University Islamabad. The rabbits were kept in controlled conditions of temperature and lighting and with no restriction on food or water.

Materials

The following injection and chemical were used.

1. Injection Ketamax (Ketamine HCL) 500mg/10ml (Reg.No.60189 TRITTU Germany).
2. Injection Diazepam 10mg/2ml (Reg.No.0.03821,Star laborites Lahore)
3. Injection Gentamycin 80 mg/ml (Reg.No.017429 Sinochem Ningo, China)
4. Capsule. Celbex (Celecoxib) 200mg (Reg.No.028693 Getz Pharma Karachi Pakistan.
5. Normal saline (0.9%)
6. 1% Toludine Blue.
7. 1% Borax.
8. 1% Pyronine G.
9. Phosphate Buffer.
10. Acetone.
11. 1% Osmic acid.
12. Durcupan A/M Epoxy Resin (Sigma Chemical Co. USA).

Equipments:

1. Surgical Instruments (The facilities of operation room. Animal House Quaid-i- Azam University Islamabad Pakistan).
2. Light Microscope (Nikon, Japan)
3. Micrometer.
4. Ultra microtome LKB (2088 Ultrotom®V) Bromma.

Experimental Procedure:

The Olmarker's method (Olmarker's & Myers, 1998) was model of sciatica nerve root irritated by auto logous nucleus pulposus. The 45 rabbits weighting 1100-1260g (male and female) were used 15 in Sham control group, 15 in Sham operated group and 15 in Medicine group.

- The rabbit were anaesthetized with 100 mg of Ketamax and 5mg of Diazepam by intramuscular injection through midline incision, the throacolumbar fascia was incised just right to the spinous process of the 5^{th} and 6^{th} lumbar vertebrae.
- The erector spine muscle was gently moved laterally to expose the facet joint between the 5^{th} and 6^{th} lumbar vertebrae by this procedure. The ligamentum flavum and intervertebral disc could be identified.
- A 24 Gauge needle was used to puncture the exposed disc by gently injecting some air through the needle and manipulating the spinal, a small disc herniation could be produced.
- To ensure contact between the nucleus pulposus and the nerve root, some nucleus pulposus was transferred with the tip of the needle to the 5^{th} lumber nerve root.
- The wound was closed in the layer using silk sutures.
- For the purpose of preventing infection, intramusculature injection of Gentamycin 80 mg was given daily on left buttock, for 4 days.
- After 4 days of operation the treatment were started.

Method

1. Animal Model.

For this study 45 rabbits were used which were divided into three groups, each with 15 animals as fallow:

- Sham (control) group (n=15).
- Sham operated group (n=15).
- Celbex (Celecoxib) 200mg, Medicine group (n=15).

Sham Control group

In the Sham control normal animals were used without operation, Sham control group was further categories into three groups, according to treatment period, each group contained five each animals.

Groups	Period (Without operation)
▪ Group.1	One week.
▪ Group.2	Two week.
▪ Group.3	Three week.

Sham Operated group

Five rabbits of each group were operated, the spinal cord was opened and caused disc herniation, and then the wound was closed in the layer using silk sutures. For the purpose of preventing infection, intramusculature injection of Gentamycin 80mg was given daily on left buttock, for 4 days. After the operation, these rabbits were not given any treatment, Sham operated group was further categories into three groups, according to treatment period, each group contained five rabbits each.

Groups	Period (Operated but not given any treatment)
▪ Group.1	One week.
▪ Group.2	Two week.
▪ Group.3	Three week.

Medicine group (Celecoxib).

Five rabbits of each group were operated, the spinal cord was opened and caused disc herniation then the wound was closed in the layer using silk sutures. For the purpose of preventing infection, intramusculature injection of Gentamycin 80 mg was given daily on left buttock, for 4 days. After 4 days of operation the treatment was started. Celbex (Celecoxib) 200mg (Medicine) group was also further categorized into three groups according to the duration of exposure to drug (Celecoxib). Each group contained five animals.

Groups	Duration of Exposure
▪ Group.1	One week.
▪ Group.2	Two week.
▪ Group.3	Three week.

Weight of animals

Forty five rabbits weighing 1100-1260g (male and female) were used 15 in Sham control group 15 in Sham operated group, and 15 in Medicine group.

Weights of the selective Rabbits for the Experiments (One week study).

R. NO:	Sham Control	Sham Operated	Celecoxib treated group
01	1200g (Female)	R6: 1100g (Female)	R11: 1150g (Female)
02	1250g (Male)	R7: 1200g (Male)	R12: 1250g (Male)
03	1150g (Male)	R8: 1150g (Male)	R13: 1200g (Female)
04	1100g (Female)	R9: 1260g (Male)	R14: 1200g (Male)
05	1100g (Male)	R10:1190g (Female)	R15: 1190g (Male)

R = Rabbits

Weights of the selective Rabbits for the Experiments (Two week study).

R. NO	Sham Control	Sham Operated	Celecoxib treated group
16	1100g (Female)	R21: 1140g (Male)	R26: 1100g (Female)
17	1150g (Male)	R22: 1250g (Female)	R27: 1100g (Female)
18	1250g (Male)	R23: 1200g (Male)	R28: 1200g (Female)
19	1200g (Female)	R24: 1100g (Female)	R29: 1200g (Male)
20	1180g (Female)	R25: 1180g (Male)	R30: 1180g (Female)

Weights of the selective Rabbits for the Experiments (Three week study).

R.NO	Sham Control	Sham Operated	Celecoxib treated group
31	1200g (Female)	R36: 1180g (Female)	R41: 1180g (Male)
32	1250g (Male)	R37: 1100g (Male)	R42: 1200g (Male)
33	1260g (Male)	R38: 1200g (Male)	R43: 1250g (Female)
34	1180g (Female)	R39: 1100g (Female)	R44: 1250g (Male)
35	1250g (Female)	R40: 1180g (Female)	R45: 1180g (Female)

R = R Abbits.

TREATMENTS

Sham (control) group (n=15):

The rabbits of sham control group neither operated nor treated.

Sham Operated group (n=15):

After operation, these rabbits were not given any treatment.

Celbex (Celecoxib) 30mg/kg treatment (n=15):

After 4 days of operation, oral dose of celecoxib 30mg/kg dissolved in saline was given orally to rabbit of group 1 for one week, group 2 for two weeks and group 3 for three weeks.

Collection of Tissues:

After 1,2,3 weeks of treatment, rabbits of Sham control groups, Sham operated group, and celecoxib groups, were sacrificed 5-8 mm of the sciatica nerve was removed from 5-7 mm above (proximal part) and under (distal part) the crushed point. The segments of the experimental nerve were fixed in Karnowosky solution (Karnovosky, 1965), for 2-3hrs.

Composition of Karnovosky solution

Paraformaldhyde	2gm
Glutaraldehyde	8mg
Distilled water	25ml
0.1 N NaOH	few drop
Phosphate Buffer (pH: 7.4)	67ml

Composition of Phosphate Buffer:

0.1 M Na_2HPO_4	53.6ml.
0.1 M KH_2PO_4	13.4ml

Fixation was followed by dehydration with different grades of Acetone.

1. 30% Acetone	30 min
2. 50% Acetone	30 min
3. 70% Acetone	30 min
4. 80% Acetone	30 min
5. 90% Acetone	30 min
6. 100% Acetone	2×30 min
7. Propylene Oxide	2×15 min

Tissues were then post fixed in 1% Osmic acid solution for 90 min.

Osmic acid 1% solution contained:

Osmic Acid	2%
KH_2PO_4	0.2 M
$NaHPO_4$	0.2 M
Sucrose	0.205g

After post fixation, tissues became black and stiff and then embedded in epoxy resin. For embedding Durcupan (sigma) resin kit was used, then kit contained three components.

Durcupan Component A	26 ml.
Durcupan Component B	24 ml.
Durcupan Component C	1 ml.

For infiltration propylene oxide and Durcupan solution were mixed in ratio as

	Durcupan Mixture: Propylene oxide	
A.	1 : 1	for one hrs.
B.	1 : 3	for 24 hrs.

C. Pure Durcupan mixture

Durcupan mixture was poured in Capsules and then tissues were placed carefully in capsule, then Capsules were placed in incubator for Polymerization at different temperatures for different time duration.

A. 38 °C 24 hrs.
B. 43°C 24 hrs.
C. 63°C 48 hrs.

After polymerization, the blocks were ready for trimming to make 1µm. semi thin sections.

Trimming and Semi thin section cutting

The apoxy resin block with the immed and tissue was exposed with sharp blade then the trimmed block was fixed in ultra-microtome, set the ultra-microtome on 1µm thickness after that smoothly cut the sections, then put the semi thin sections on glass slides in a drop of distal water after that place the slide on hot plate to dry the section.

Composition and preparation of stains

Toludine Blue

One gram of Toludine Blue and 1g of Borax was dissolved in 100 ml distilled water, to prepare 1% solution.

Pyronine G.

One gram of Pyronine G powder was dissolved in 100 ml of distilled water, to prepare 1% solution.

Then mixed both stains in the ratio 3:1 i.e. 3 volume of Toludine Blue and 1 volume of Pyronine G to make working solution. The solution is filtered before use.

Staining Procedure

- Place the prepared slide on a hot plate at 60°C.
- Then pour Toludine blue and Pyronine G mixed stain on the sections and stained for one minute.
- Then removed the stains from slide and washed with distilled water.
- Examine under light microscope.

Light Microscopic study

Semi-thin sections (1μm thick) were prepared with ultra-microtome. Under microscopic examination with 20 objective and oil immersion, the eye piece magnification was 10; the pathological changes in the sciatica nerve were examined, particularly, to find evidence of axonal demyelination, remyelination, and regeneration.

Intact myelinated fibers were counted with objective 20 x eye piece 10 magnification; three areas of (/0.126mm²) fields in each cross section were used for counting intact myelinated fibers, two continuous cross section were used for this purpose.

In this way six (/0.126mm²) fields' are as (/0.126mm²) were totally counted in each animal. In each animal, five slides and in each slide two continuous sections were used to measure diameter and density of intact myelinated.

Diameter and thickness of myelinated fiber was measure with eye piece, micrometer calibrated with stage micrometer with object oil immersion X 10 magnification

For oval type of myelinated fibers, diameter along their width and length were measured; the mean of the two was used as diameter of oval type myelinated fibers. In each animal 20 myelinated fibers were randomly measured for diameter of oval and round type. Thus, the diameters of 100 myelinated fibers were measured in all the 5 rabbit in each treated group.

Statistical Analysis

Statistical analysis using Student's *t* test were applied to compare mean differences of density, diameter and myelin sheath thickness of normal myelinated fibers in rabbits treated with medicine (Celecoxib), Sham operated and Sham Control group. Mean variance and standard error also calculated for different variables to assess the difference in the variables used in this study. The way analysis of t-test was used to compare Sham Operated and Sham Control groups with treatment groups.

RESULTS

RESULTS

The present study was carried out to assess the "effect of Selective Cox-2 inhibitor celecoxib in spinal cord injury: a morphological study". To evaluate the effect of celecoxib following parameters were studied.

i) Morphometry of normal myelinated fibers.

ii) Histomorphology of the sciatic nerve.

i) MORPHOMETRY OF NORMAL MYELINATED FIBERS.

Morphometry of normal myelinated fibers in sham control, sham operated and Celecoxib treated group included the study of following.

a) Mean density of normal myelinated fibers

b) Mean diameter (μm) of normal myelinated fibers.

c) Axon diameter of normal myelinated fibers.

d) Thickness of the myelinated sheath of nerve fibers.

a) Mean density of normal myelinated fibers.

The density of normal myelinated fibers of L_5 nerve root irritation of nucleus pulposus was noted in the cross section area (/0.126mm^2) of sham control (without operation), sham operated (operated but without treatment) and medicine (operated with celecoxib treatment) to find out the effect of celecoxib on density of myelinated fibers in all the three groups with or without the respective treatment, after one, two and three weeks of experiment.

Mean density of normal myelinated fibers (One week study).

Mean density of normal myelinated fibers in sham control, sham operated and celecoxib treated group is given in Table 1 and Figure 1. Mean density of normal myelinated fibers in sham control was 15554±1.6 after one week of operation. In case of sham operated mean density was 467.0±0.44 after one week of experiment, but in celecoxib treated group there was improvement in density of myelinated fibers which was 616±0.89.

After one week of experiment there was highly significant ($t_{(8)}$ = 531; $P < 0.001$) decrease in density of normal myelinated fibers in sham operated group and celecoxib treated group ($t_{(8)}$ =376.66; $P< 0.001$) compared to sham control group. Celecoxib treatment to sham operated caused a significant ($t_{(8)}$ = 149.0; $P < 0.001$) increase in the density of normal myelinated fibers compared to sham operated group.

Mean density of normal myelinated fibers

(Two weeks study).

Mean density (/0.126mm²) of normal myelinated fibers in sham control, sham operated and celecoxib treated group is given in Table 1 and Figure 1. Mean density of normal myelinated fibers in sham control was 1563.2±0.9165 after two weeks of operation. In case of sham operated group mean density was 472±0.836 after two weeks of experiment, while medicine (Celecoxib) treatment caused improvement in density of myelinated fibers which was 704.8±0.86.

After two weeks of experiment there was highly significant ($t_{(8)}$ = 622.8; $P< 0.001$ decrease in density of normal myelinated fibers in sham operated group and celecoxib treated group ($t_{(8)}$ = 4837.9; $P< 0.001$) compared to sham control group. However, sham operated and celecoxib treated group comparison showed a significant ($t_{(8)}$ = 194.00; $P < 0.001$) increase in the density of normal myelinated fibers in celecoxib treated group.

Mean density of normal myelinated fibers

(Three weeks study).

Mean density (/0.126mm²) of normal myelinated fibers in sham control, sham operated and celecoxib treated group is given in Table 1 and fig 1. Mean density of normal myelinated fibers in sham control was 1560.4±1.288 after three weeks of operation. In case of sham operated group mean density was 482±0.66 after three weeks of treatment. In celecoxib treated group there was improvement in the density of myelinated fibers which was 753.8±0.678.

After three weeks of experiment there was highly significant ($t_{(8)} = 555.54$; $P < 0.001$) decrease in density of normal myelinated fibers in sham operated group and celecoxib treated group ($t_{(8)} = 410.68$; $P< 0.001$) compared to sham control group. However, sham operated and celecoxib treated group comparison showed a significant ($t_{(8)} =290$; $P< 0.001$) increase in the density of normal myelinated fibers in celecoxib treated group.

Table 1: Mean density (/0.126mm²) of myelinated fibers of L5 Nerve root irritated by Nucleus pulposus in sham control , sham operated and celecoxib treated group after first, second and third weeks of experiment.

Weeks of treatment	Sham control group	Sham operated group	Celecoxib treated group
1	1555.4±1.6	$467\pm0.4^{a**}$	$616\pm0.8^{b**c**}$
2	1563.2±0.9	$472\pm0.8^{a**}$	$704.8\pm0.8^{b**c**}$
3	1560.4±1.2	$478\pm0.6^{a**}$	$753.6\pm0.6^{b**c**}$

Values (Mean ± SEM), t-test.

a = Sham control group Vs Sham operated group.

b = Sham control group Vs Celecoxib treated group.

c = Sham operated group Vs Celecoxib treated group.

$P< 0.001^{**}$

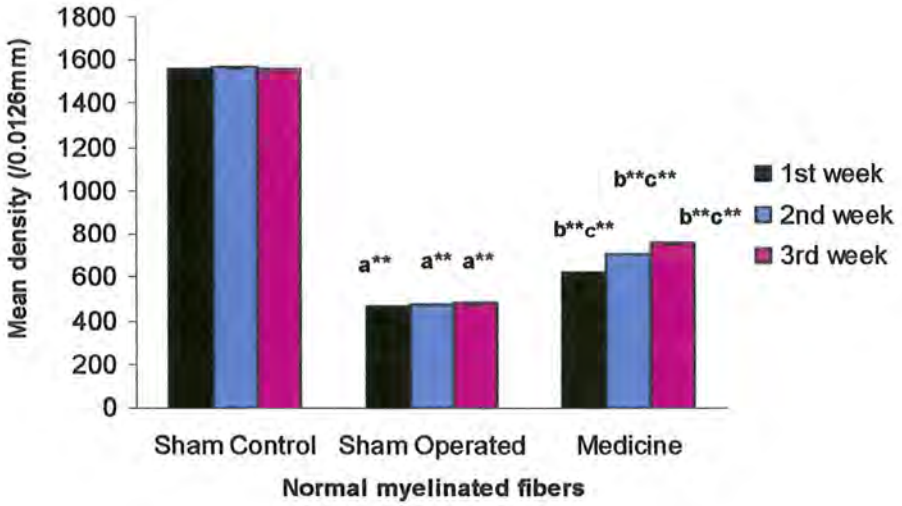


Figure 1: Mean density (/0.126mm²) of myelinated fibers of L5 Nerve root irritated by Nucleus pulposus in sham control group, sham operated and Celecoxib treated group in first, second and third weeks after experiment.

Values (Mean ± SEM),t-test.

a = Sham control Vs sham operated group.

b = Sham control Vs Celecoxib treated group.

c = Sham operated Vs Celecoxib treated group.

$P < 0.001^{**}$

b) Mean diameter (μm) of normal myelinated fibers,

(One week study).

Mean diameter (μm) of normal myelinated fibers of sham control, sham operated and celecoxib treated group is given in Table 2 and Figure 2. Mean diameter of normal myelinated fibers in sham control was 18.6±0.19 μm after one week of operation. In case of sham operated group mean diameter was 12.7±0.23 μm after one week of treatment, in celecoxib treated group there was some improvement in diameter of normal myelinated fibers which increased to 15.1±0.22 μm.

After one week of experiment there was highly significant ($t_{(8)} = 13.7$; $P< 0.001$) decrease in diameter of normal myelinated fibers in sham operated group compared to sham control. There was significant ($t_{(8)} = 8.5$; $P< 0.001$) decrease in diameter of normal myelinated fibers in celecoxib treated group compared to sham control group. There was significant ($t_{(8)} = 5.3$; $P< 0.001$) increases in diameter of normal myelinated fibers of celecoxib treated group compared to sham operated group.

Mean diameter (μm) of normal myelinated nerve fibers.

(Two weeks study).

Mean diameter (μm) of normal myelinated fibers of sham control, sham operated and celecoxib treated group is given in Table 2 and Figure 2. Mean diameter of normal myelinated fibers in sham control was 18.8±0.05 μm after two weeks of operation. In case of sham operated group mean diameter was 11.7±0.17 μm after two weeks of experiment, in medicine group there was some improvement in mean diameter of normal myelinated fibers which increased to 14.1±0.20 μm.

After two weeks of experiment there was highly significant ($t_{(8)} = 32.2$; $P < 0.001$) decrease in diameter of normal myelinated fibers in sham operated group compared to sham control group. There was significant ($t_{(8)} = 18.4$; $P< 0.001$) decrease in diameter of normal myelinated fibers in celecoxib treated group compared to sham control group. There was significant ($t_{(8)} = 6.7$ $P< 0.001$) increases in diameter of normal myelinated fibers of celecoxib treated group compared to sham operated group.

Mean diameter (μm) of normal myelinated nerve fibers.

(Three weeks study).

Mean diameter (μm) of normal myelinated fibers of sham control, sham operated and celecoxib treated group is given in Table 2 and Figure 2. Mean diameter of normal myelinated fibers in sham control was 18.7±0.20 μm after three weeks of operation. In case of sham operated group mean diameter was 11.4±0.45 μm after three weeks of experiment, in celecoxib treated group there was some improvement in mean diameter of normal myelinated fibers which increased to 13.6±0.35 μm.

After three weeks of experiment there was significant ($t_{(8)}$ =11.06; P <0.001) decrease in diameter of normal myelinated fibers in sham operated group compared to sham control group. There was significant ($t_{(8)}$ = 9.10; P< 0.001) decrease in diameter of normal myelinated fibers compared to sham control group. There was no significant ($t_{(8)}$ = 2.7; P< 0.05) differences in diameter of normal myelinated fibers of celecoxib treated group compared to sham operated group.

Table 2: Mean diameter (µm) of normal myelinated fibers of L_5 Nerve root irritated by Nucleus pulposus in sham control, sham operated and celecoxib treated group after first, second and third weeks of experiment.

Weeks of treatment	Sham control	Sham operated	Celecoxib treated group
1	18.6±0.19	$12.7\pm0.23^{a**}$	$15.1\pm0.22^{b**c*}$
2	18.8±0.05	$11.7\pm0.17^{a**}$	$14.2\pm0.20^{b**c*}$
3	18.7±0.20	$11.4\pm0.45^{a**}$	$13.6\pm0.35^{b**}$

Values (Mean ± SEM), t-test.

a = Sham control Vs sham operated group.

b = Sham control Vs Celecoxib treated group.

c = Sham operated Vs Celecoxib treated group.

$P < 0.001^{**}$, $P < 0.05^{*}$

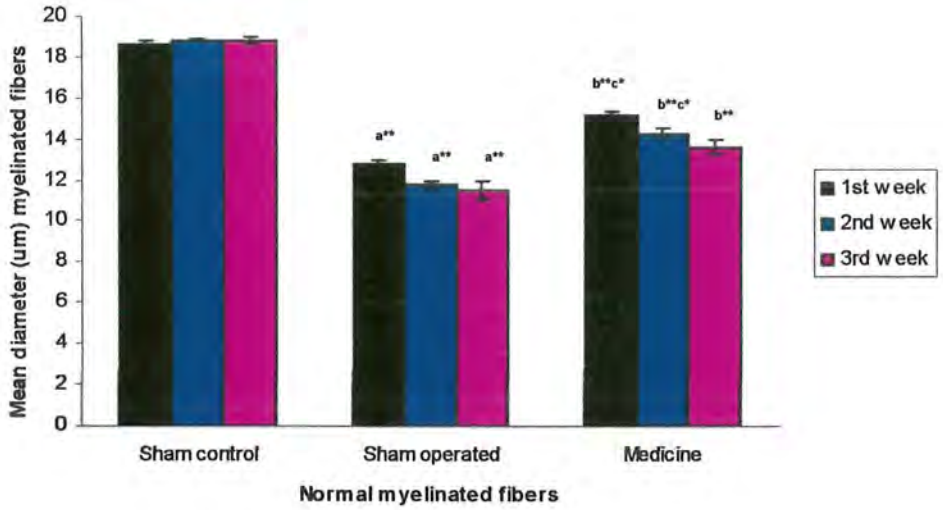


Figure 2 : Mean diameter (μm) normal myelinated fibers of L_5 Nerve root irritated by Nucleus pulposus in sham control , sham operated and celecoxib group in first, second and third week after experiment.

Values (Mean ± SEM), t-test.

a = Sham control Vs sham operated group.

b = Sham control Vs Celecoxib treated group.

c = Sham operated Vs Celecoxib treated group.

$P < 0.001^{**}$, $P<0.05^{*}$

c) Axon diameter of normal myelinated fibers.

The diameter (μm) of normal myelinated fibers of L_5 nerve root irritation of nucleus pulposus was measured after one, two and three weeks of the sham control (without operation), sham operated (operated but without treatment) and medicine (with celecoxib treatment) groups. To find out the effect of celecoxib on diameter of axon and myelin sheath thickness of myelinated fibers in all three groups with or without the treatment, after one week, two weeks and three weeks of experiments was measured.

Mean axon diameter (μm) of normal myelinated fibers,
(One week study).

Mean axon diameter (μm) of normal myelinated fibers of sham control, sham operated and celecoxib treated group is given in Table 3 and Figure 3. Mean diameter of normal myelinated fibers in sham control was 15.7±0.19 μm after one week of operation. In case of sham operated group mean diameter was 11.02±0.25 μm after one week of treatment, in celecoxib treated group there was some improvement in axon diameter of myelinated fibers which was 13.21±0.20 μm.

After one week of experiment there was highly significant ($t_{(8)} = 9.8$; $P < 0.001$) decrease in diameter of normal myelinated fibers in sham operated group and celecoxib treated group ($t_{(8)} = 5.53$; $P< 0.001$) compared to sham control group. In comparison between sham operated and celecoxib treated group showed highly significant ($t_{(8)} =5.34$; $P< 0.001$) increase in the axon diameter of normal myelinated fibers was observed in celecoxib treated group.

Mean axon diameter (μm) of normal myelinated fibers,
(Two weeks study).

Mean axon diameter (μm) of normal myelinated fibers of sham control, sham operated and celecoxib treated group is given in Table 3 and Figure 3. Mean diameter of normal myelinated fibers in sham control was 15.9±0.06 μm after two weeks of operation.

In case of sham operated group mean diameter was 10.0±0.017 μm after two weeks of experiment, in celecoxib treated group some improvement in axon diameters of myelinated fibers which was 11.8±0.20 μm.

After two weeks of experiment there was highly significant ($t_{(8)} = 32.5$; $P < 0.001$) decrease in diameter of normal myelinated fibers in sham operated group and celecoxib treated group ($t_{(8)} = 15.6$; $P < 0.001$) compared to sham control group. In comparison between sham operated and celecoxib treated group showed highly significant ($t_{(8)} = 6.39$; $P < 0.001$) increases in axon diameter of normal myelinated fibers was observed in celecoxib treated group.

Mean axon diameter (μm) of normal myelinated fibers,
(Three weeks study).

Mean axon diameter (μm) of normal myelinated fibers of sham control, sham operated and celecoxib treated group is given in Table 3 and Figure 3. Mean diameter of normal myelinated fibers in sham control was 15.8±0.20 μm after three weeks of operation. In case of sham operated group mean diameter was 9.7±0.44 μm after three weeks of experiment, in celecoxib treated group some improvement in axon diameters of myelinated fibers which was 11.0±0.33 μm.

After three weeks of experiment there was highly significant ($t_{(8)} = 9.41$; $P < 0.001$) decrease in diameter of normal myelinated fibers in sham operated group and celecoxib treated group ($t_{(8)} = 9.09$; $P < 0.001$) compared to sham control group. In comparison between sham operated and celecoxib treated groups showed significant ($t_{(8)} = 3.25$; $P < 0.01$) increases in axon diameter of normal myelinated fibers was observed in celecoxib treated group.

Table 3: Mean axon diameter (μm) of myelinated fibers of L_5 Nerve root irritated by Nucleus pulposus in sham control , sham operated and celecoxib treated group after first, second and third week of experiment.

Weeks of treatment	Sham Control group	Sham Operated group	Celecoxib treated group
1	15.8±0.23	$10.9\pm0.23^{a**}$	$13.2\pm0.20^{b**c*}$
2	15.9±0.06	$9.0\pm0.13^{a**}$	$11.8\pm0.20^{b**c*}$
3	15.8±0.20	$9.6\pm0.45^{a**}$	$11.9\pm0.23^{b**c*}$

Values (Mean ± SEM), t-test.

a = Sham control group Vs sham operated group.

b = Sham control group Vs Celecoxib treated group.

c = Sham operated Vs Celecoxib treated group.

$P< 0.001^{**}$, $P< 0.01^{*}$

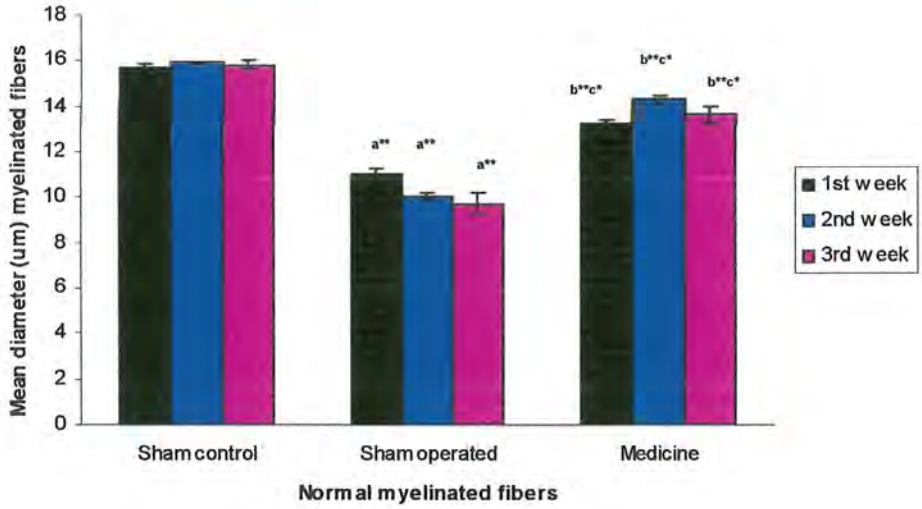


Figure 3: Mean axon diameter (μm) of myelinated fibers of L_5 Nerve root irritated by Nucleus pulposus in sham control , sham operated and celecoxib treated group after first, second and third weeks of experiment.

Value (Mean ± SEM), t-test.

a = Sham control group Vs sham operated group.

b = Sham control group Vs Celecoxib treated group.

c = Sham operated group Vs Celecoxib treated group.

$P< 0.001^{**}$, $P< 0.01^{*}$

e) Thickness of myelinated sheath of nerve fibers

(One week study).

Mean myelin sheath thickness (μm) of normal myelinated fibers of sham control, sham operated and celecoxib treated group is given in Table 4 and Figure 4. Mean diameter of normal myelinated fibers in sham control was 2.9±0.01 μm after one week of operation. In case of sham operated group mean diameter was 1.7±0.01 μm after one week of experiment, in celecoxib treated group some improvement of mean myelin sheath thickness of myelinated fibers which was 2.4±0.05 μm.

After one week of experiment there was highly significant ($t_{(8)} = 60$; $P<0.001$) decrease in thickness of myelin sheath of the myelinated fibers in sham operated group compared to sham control group. There was significant ($t_{(8)} = 8.3$; $P< 0.001$) decrease in thickness of myelin sheath of the myelinated fibers in celecoxib treated group compared to sham control group. There was significant ($t_{(8)} = 11.6$ $P< 0.001$) increases in thickness of myelinated fibers of celecoxib treated group compared to sham operated group.

Thickness of myelinated sheath of nerve fibers

(Two weeks study).

Mean myelin sheath thickness (μm) of normal myelinated fibers of sham control, sham operated and celecoxib treated group is given in Table 4 and Figure 4. Mean diameter of normal myelinated fibers in sham control was 2.9±0.04 μm after two weeks of operation. In case of sham operated group mean diameter was 1.72±0.01 μm after two weeks of experiment, in celecoxib treated group some improvement of mean myelin sheath thickness of myelinated fibers which was 2.44±0.06 μm.

After two weeks of experiment there was highly significant ($t_{(8)} = 24$; $P < 0.001$) decreases in thickness of myelin sheath of the normal myelinated fibers in sham operated group compared to sham control group. There was significant ($t_{(8)} = 5.0$; $P < 0.001$) decrease in thickness of myelin sheath of the myelinated fibers in celecoxib treated group compared to sham control group. There was significant ($t_{(8)} = 23.3$; $P < 0.001$) increase in thickness of myelinated fibers of celecoxib treated group compared to sham operated group.

Thickness of myelinated sheath of nerve fibers

(Three weeks study).

Mean myelin sheath thickness (μm) of normal myelinated fibers of sham control, sham operated and celecoxib treated group is given in Table 4 and Figure 4. Mean diameter of normal myelinated fibers in sham control was 2.9±0.01 μm after three weeks of operation. In case of sham operated group mean diameter was 1.72±0.02 μm after three weeks of experiment, in celecoxib treated group some improvement of mean myelin sheath thickness of myelinated fibers which was 2.6±0.04 μm.

After three weeks of experiment there was highly significant ($t_{(8)} = 40$; $P < 0.001$) decrease in thickness of myelin sheath of normal myelinated fibers in sham operated group compared to sham control. There was significant ($t_{(8)} = 6.0$; $P < 0.001$) difference in thickness of myelinated fibers of celecoxib treated group compared to sham control group. There was significant ($t_{(8)} = 15.0$; $P < 0.001$) increase in thickness of myelinated fibers of celecoxib treated group compared to sham operated group.

Table 4: Mean myelin sheath thickness (μm) normal myelinated fibers of L_5 Nerve root irritated by Nucleus pulposus in sham control, sham operated and celecoxib treated group after first, second and third week of experiment.

Weeks of treatment	Sham Control group	Sham Operated group	Celecoxib treated group
1	2.9±0.01	1.7±0.01^{a**}	2.4±0.05^{b**c**}
2	2.9±0.04	1.7±0.01^{a**}	2.4±0.06^{b**c**}
3	2.9±0.01	1.7±0.02^{a**}	2.6±0.04^{b**c**}

Values (Mean±SEM), *t*-test.

a = Sham control Vs sham operated group.

b = Sham control Vs Celecoxib treated group.

c = Sham operated Vs Celecoxib treated group.

$P < 0.001^{**}$

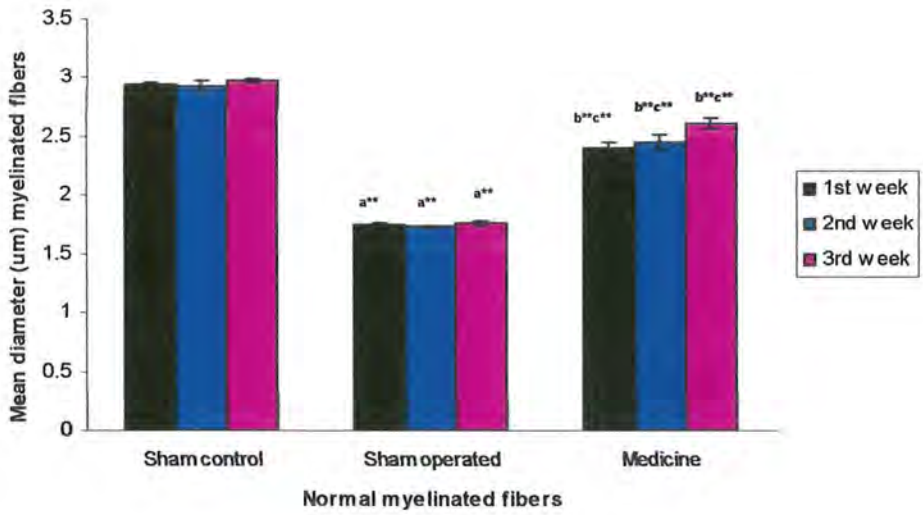


Figure 4: Mean myelin sheath thickness (μm) normal myelinated fibers of L_5 Nerve root irritated by Nucleus pulposus in sham control , sham operated and celecoxib treated group after first, second and third week of experiment.

Values (Mean ± SEM), *t*-test.

a = Sham control Vs sham operated group.

b = Sham control Vs Celecoxib treated group.

c = Sham operated Vs Celecoxib treated group.

$P<0.001^{**}$

ii) HISTOMORPHOLOGY OF SCIATIC NERVE

Effect of selective Cox-2 inhibitor celecoxib in spinal cord injury: a morphological study.

One week study:

The study of 1μm thin cross section of plastic embedded sciatic nerve of rabbits (sham control) by light microscope demonstrated that each of the nerve fascicle is encircled by a sheath of connective tissues (epineurium, perineurium and endoneurium), the central core of fiber is the axon and surrounding the axon is a myelin sheath (Figure5 and 6 a, b). The fibers are of variable sizes (Figure5 and 6 a, b). After one week of operation some regeneration was observed in sham operated group (Figure7 a, b and 8 a, b). The unmyelinated nerve fibers with myelin debris were clearly seen in the cross sections (Figure7 a, b and 8 a, b), which indicate the greater damages in sham operated group due to lack of any treatment (Figure7 a, b and 8 a, b). Few thin myelinated fibers were also seen in sham operated group (Figure7 a, b and 8 a, b). In sham operated group, after nerve injuries, nerve ischemia produced wallerian degernation (distruction of distil segment of nerve). The degenerated axons were swollen, empty and were darkly stained (Figure7 a, b and 8 a, b). Normal myelinated fibers with normal axons were also present in the nerve (Figure7 a, b and 8 a, b). In celecoxib treated group, regeneration was observed (Figure9 a, b and 10 a, b) after one week of experiment. There were present a few normal regenerated myelinated fibers with smaller diameter and less debris as compared to sham operated group (Figure9 a, b and 10 a, b). Celecoxib treatement effected the myelinated fibers with the result that there was increase in the diameter of normal myelinated fibers (Figure9 a, b and 10 a, b) and decrease in large size myelinated fibers in celecoxib treated group as compared to the sham operated group (Figure9 a, b and 10 a, b). In celecoxib treated group diameter regenerating fibers shifted to smaller size and in the unmyelinated fiber few lipid droplets were also seen (Figure9 a, b and 10 a, b).

In celecoxib treated group, myelin sheath thickness of normal myelinated fibers was increased after one week with the treatment of celecoxib (Figure9 a, b and 10 a, b). The numbers of small myelinated fibers was more than sham operated group and macrophages were observed in celecoxib treated group (Figure9 a, b and 10 a, b).

Two weeks study:

In the semi-thin cross section of normal rabbits sciatic nerve (Sham control), there were many myelinated nerve fibers; there was not much variation in size. Among these fibers, there are some small myelinated fibers as well (Figure6 c, d). In sham operated group no regeneration was observed and unmyelinated nerve fibers with myelin debris were clearly seen in the cross section of sham operated group (Figure8 c, d), which indicate the greater damages in sham operated group due to lack of any treatment (Figure8 c, d). No macrophages were seen in the sham operated group (Figure8 c, d). In sham operated group, it was observed that many axons were lost due to wallerian degeneration, the myelin sheaths were swollen, loose, brocken and became a mass of myelin (Figure8 c, d). Among these abnormal structures, there were few regenerated small myelinated fibers and very few of normal size myelinated fibers (Figure8 c, d). In the normal myelinated fibers of sham operated group there was decrease in fiber diameter, myelin sheath and axon diameter compared to sham control (Figure8 c, d).

After two weeks of celecoxib treated group, myelin sheath was thin and axon had smaller diameter compared to those in normal nerves in sham control group (Figure 10 c, d). There was an increased in the number of small myelinated fibers in celecoxib treated group as compared to sham operated group (Figure10 c, d). In celecoxib treated group increase in the number of small diameter fibers and corresponding reduction in number of large diameter fibers was observed (Figure10 c, d). Celecoxib treatement caused greater increase in the myelin sheath thickness (Figure10 c, d) and axon diameters nerve fibers with irregular (Figure10 c, d).

However there were increased and myelin sheath was thicker, but there were still irregularly shaped variable diameter axons (Figure10 c, d). Many macrophages were observed in celecoxib treated group (Figure10 c, d).

Three weeks study:

The structure of nerve fibers in sham control group after three weeks of experiment showed some histomorphological features as observed after one and two weeks of experiment (Fig 6 e, f). In sham operated group no regeneration was observed (Fig8 e, f). Macrophages were not seen in the sham operated group therefore greater damages were seen in sham operated group (Figure8 e, f). The celecoxib treated groups revealed the presence of the greater clusters of small myelinated fibers in celecoxib treatment group compared to sham operated group. In celecoxib treated group increased number of macrophages and Schwann cells were present, which were not prominent in sham control and sham operated groups (Figure10 e, f). Myelin sheath of normal myelinated fibers became thicker after three weeks of celecoxib treatment (Fig10 e, f). But in sham operated group these structures were absent and showed axonal degeneration with the presence of degenerated axons with myelin debris (Fig8 e, f).

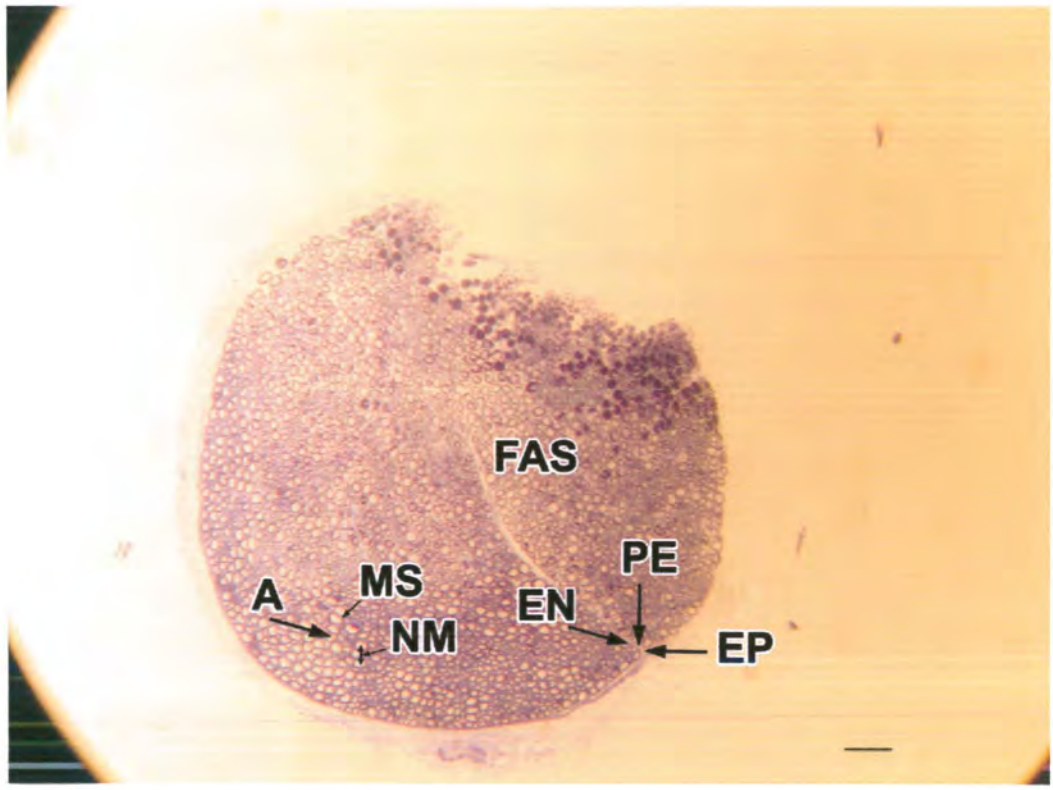


Figure 05: **Light micrograph of sciatic nerve of sham control group. Cross section showed epineurium (EP), perineurium (PE), endoneurium (EN), normal myelinated fibers (NM), myelin sheath (MS), axons (A) and fascicle (FAS). scale bars represent 100μm. Toluidine Blue and Pyeronin G.**

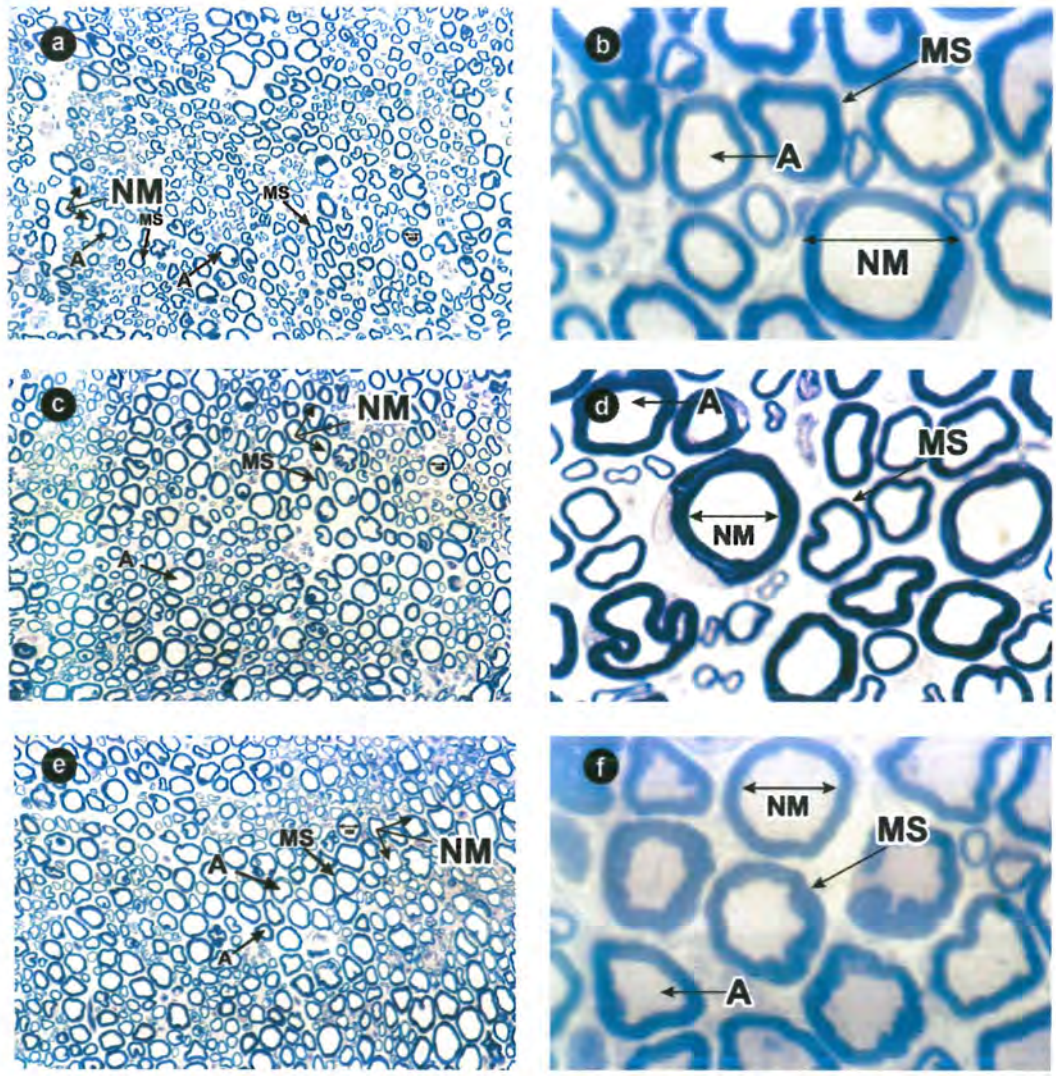


Figure 06: Light micrograph of sciatic nerve of sham control group after (a, b) one week, (c, d) two weeks and (e, f) three weeks of experiments. Cross section of sham control (normal sciatic nerve) showed the normal myelinated fibers (NM), axon (A) and myelin sheath (MS), a, c, e 190 X magnification, b, d, f 952 X magnification. Toluidine blue and Pyronin G.

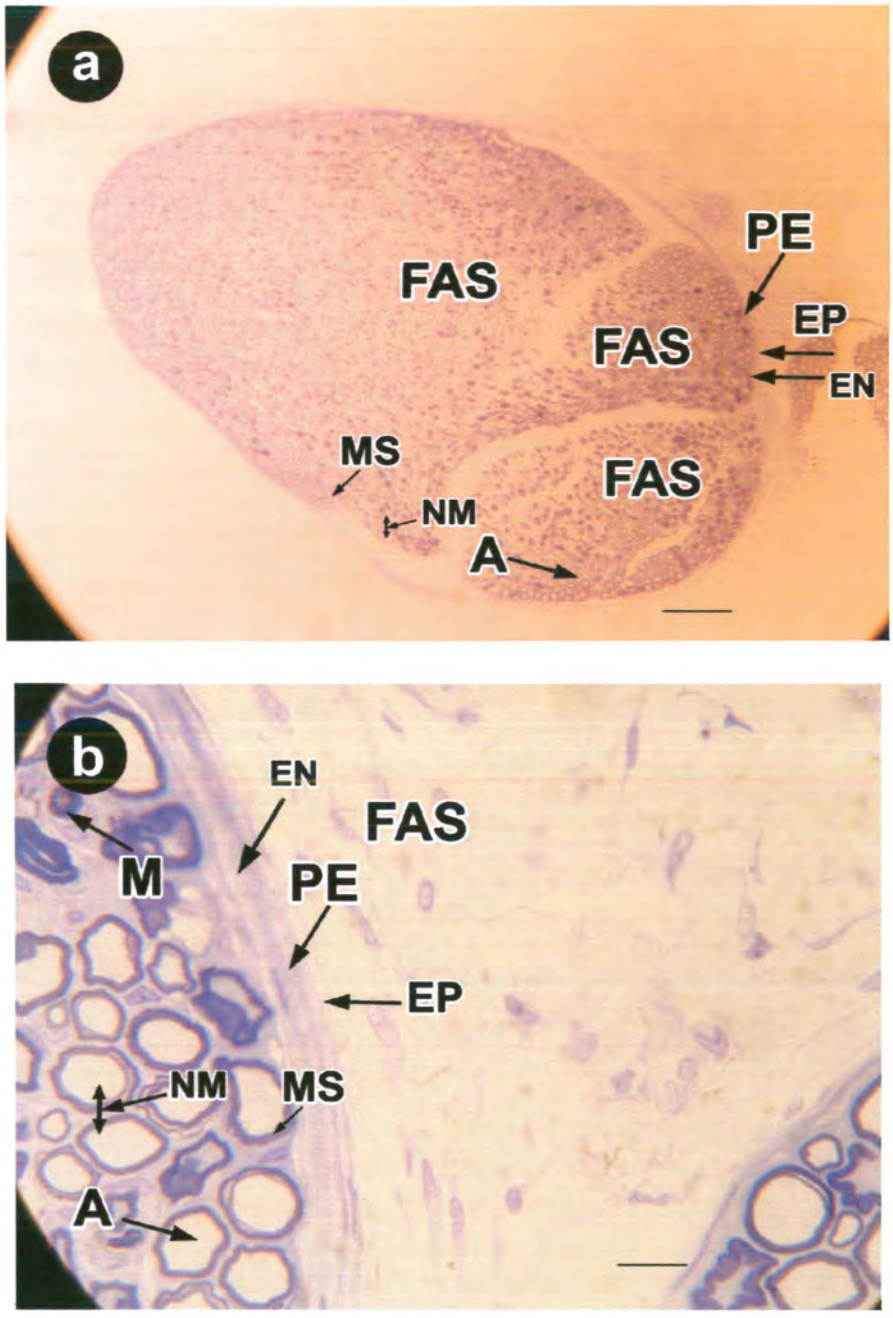


Figure 07: **Light micrograph of sciatic nerve of sham operated group. (a, b) Cross section showed epineurium (EP), perineurium (PE), endoneurium (EN), normal myelinated fibers (NM), myelin sheath (MS), axons (A), wallerian degeneration (WD), thin myelinated fibers (*), myelin debris (M) and fascicle (FAS) Scale bars represents (a) 100µm, b 40 µm. Toluidine Blue and Pyeronin G.**

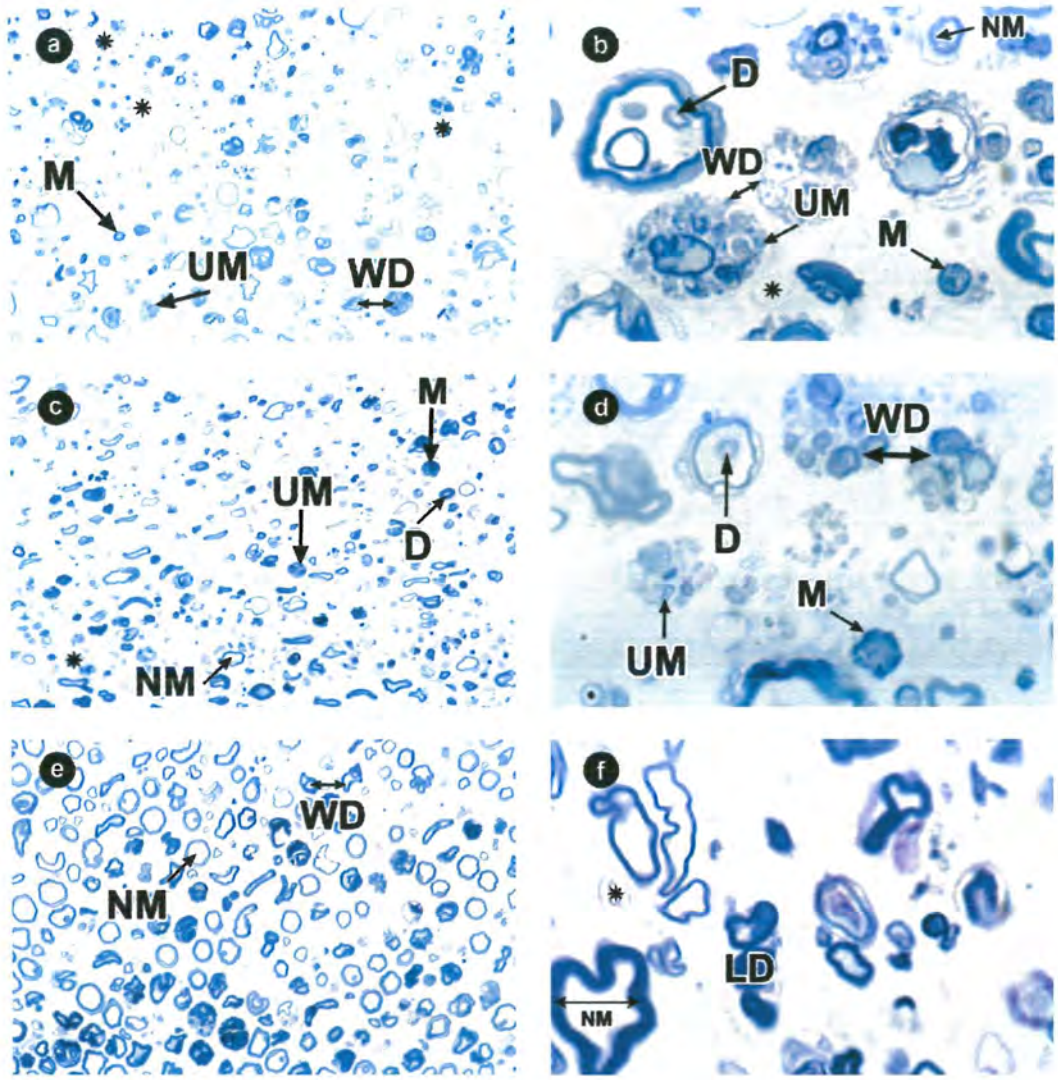


Fig 08: Light micrograph of sciatic nerve of sham operated group (operated but without treatment) after (a, b) one week, (c, d) two weeks and (e, f) three weeks of operations. Cross section of Sham Operated showed wallarian degeneration (WD). There is myelin debris (M), little amount of degeneration (D), thin myelinated fibers (*), lipid debris (LD) and unmyelinated nerve fibers (UM). a, c, e 190 X magnification, b, d, f 952 X magnification. Toluidine blue and Pyronin G.

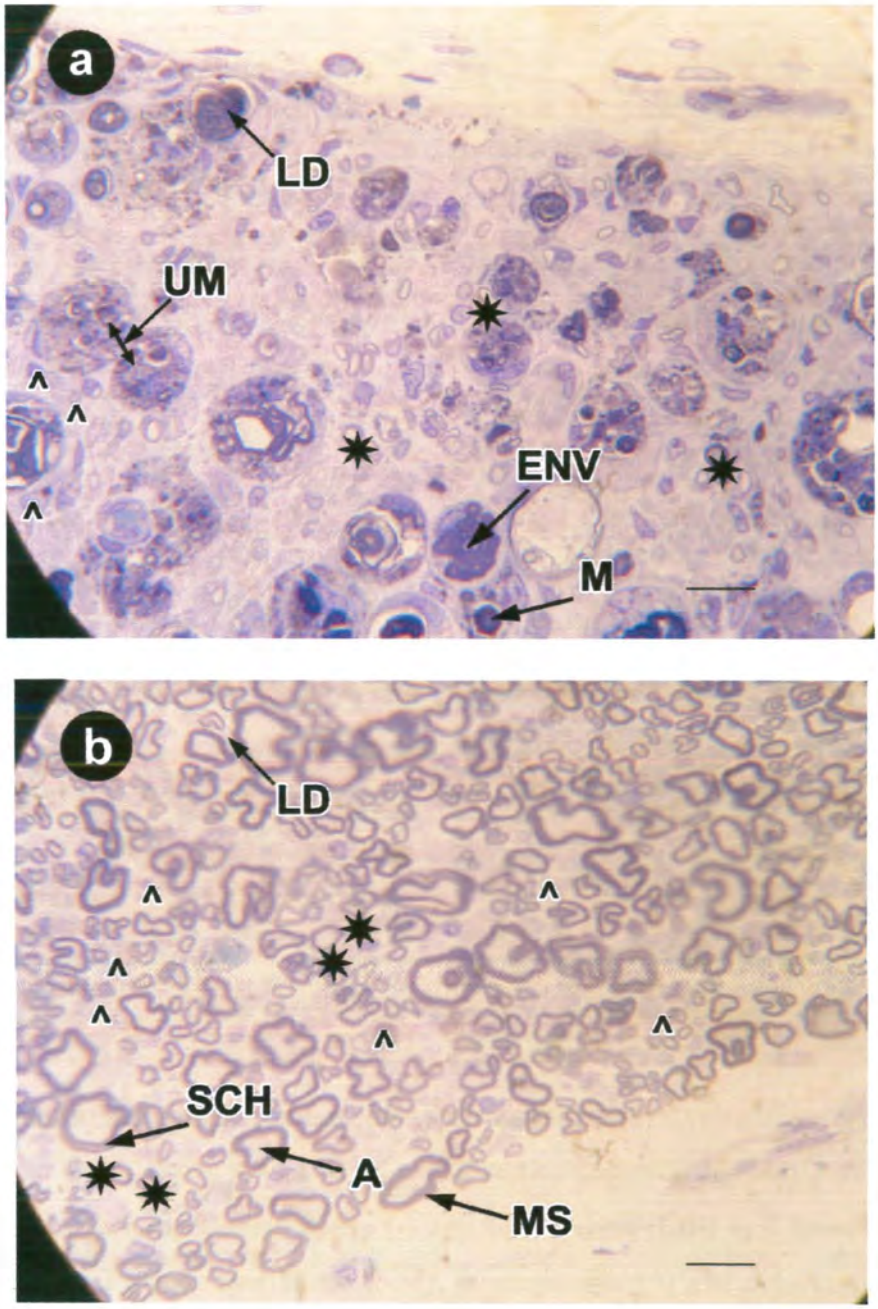


Figure 09: Light micrograph of sciatic nerve of celecoxib (a, b,) cross section showed fibers cross sections showed normal myclinated fibers (NM), thin myelinated fibers (*) and lipid debris (LD), macrophages (^) and Schwann cell (SCH). Scale bars represent (a) 40µm, (b) 40µm. Toluidine Blue and Pyeronin G.

DISCUSSIONS

DISCUSSION

Present study has revealed the effect of celecoxib on the regeneration of sciatic nerves after spinal cord injury. Celecoxib treatment to sham operated group (opaerated but witout treatment) of sciatic nerve of rabbits for one, two and three weeks caused nerve regeneration. More over effect of celecoxib was also studied on the density, axon diameter of normal myelinated fibers, myelin sheath thickness, and histomorphology of sciatic nerve. Studies aimed to understand the mechanism of sciatica or lumber disc disorder have used many models of the lumber disc herniation which have shown that nucleus pulposus can produce damage without mechanical compression of the nerve root (Olmarker and Myers, 1998, Kawakami *et al.* 2000).

In this study 200mg/kg body weight of Celecoxib (Celbex) was given to rabbits orally, for one, two and three weeks. The results show that mean density of normal myelinated fibers in sciatic nerve of rabbits significantly ($P < 0.001$) decreased in sham operated group compared to sham control group and treatment with celecoxib caused a significantly ($P < 0.001$) increase in density of normal myelinated fibers compared to sham operated group. Mehjabeen (2005) observed that decrease the density of sham operated group, However treatment of crushed sciatic nerve with methylcobalamin (500 microgram/kg) after one, two and three weeks increased density of myelinated fibers compared to crush control group ($P= 0.04$) but this increase in density is statistically non significant ($P= 0.11$). Canpolat *et al* (1999) observed the morphogenesis of peripheral nerve regenerating in silicone tubes, the morphometric counts consisted of the numbers of myelinated fibers in the proximal and distal stumps of the regeranated segment 3, 6 and 9 months after insertion of the silicon tube. There were more fibers in the proximal stump than in the distal stump. The numbers of fibers within the tube increased significantly compared to the control group ($P< 0.001$).

In present study significantly (P< 0.001) decreased the mean density of normal myelinated fibers was observed in sham operated group compared to sham control group, but in celecoxib treated group mean density of normal myelinated fibers was significantly (P< 0.001) increased compared to sham operated group.

Mean density of normal myelinated fibers between distal and proximal parts of model after crush injury in rabbits, proximal part were significant more than distal part (P< 0.01). No significant difference between medicine (Diclofenac sodium treatment) and model distal group (P > 0.05) La *et al.* (2005). According to Wang *et al.* (2005) at the PG (proximal graft) or DG (distil graft), both percentage of NF (neurofilaments) immunopositive areas and myelinated axon fiber density showed no significant difference between the chitosan/PGA (polyglycolic acid) and autograph group (P > 0.05), but they were significantly lower in the non-grafted group compared with the [(1→4)-2 amino-2-deoxy-ß-$_D$-glucan] chitosan/PGA graft group (P > 0.05) both are artificial nerve grafts. In present study, greater increase in density was observed after three weeks of celecoxib treatment and better results will be obtained if we increase the duration of celecoxib treatment. So celecoxib can be used clinically for the treatment of sciatica, peripheral neuropathies and neurodegenerative diseases.

Rong *et al.* (2004) observed that morphology in the distal part 3 mm beyond the site of crush was examined in transverse semi-thin sections stained with toluidine blue, decreased numbers of myelinated fibers were evident in (Receptere for age) sRAGE-treated mice compared with control-treated animals. Consistent with these observations, quantitative analysis decrease in myelinated fiber density on day 21 after crush in sRAGE-treated animals vs. vehicle; P< 0.001). Similarly in present study, in sham operated group the mean density of normal myelinated fibers was significantly (P< 0.001) decreased compared to sham control group after one, two and three weeks of experiments.

Acording to Prinz *et al.* (2003) both left and right sciatic nerve were fixed with paraformaldhyde, however nerve morphology exhibited endoneurial edema with thickening of both the endoneurium and perineurium, implying a distinct displacement of nerve fibers. In present study it was observed that in sham operated group the thickness of endoneurium and perineurium decrease, microcirculation also damaged where as in celecoxib treated group endoneurium and perineurium was thick as compared to sham operated group. The blood vessels also improved in celecoxib treated group. Mascrophages were observed in celecoxib treated group.

In current study, the results show that mean axon diameter of normal myelinated fibers in sciatic nerve of rabbits significantly ($P< 0.001$) decreased in sham operated group compared to sham control and treatment with celecoxib caused a significantly ($P < 0.001$) increase in axon diameter compared to sham operated group. In the celecoxib treated group, there was decrease in large size myelinated fibers as compared to the sham operated group. In celecoxib treated group, there was increased axon diameter which showed better regeneration of normal myelinated fiber compared to sham operated group after treatment. It was observed that the diameter of regenerated fibers was smaller and myelin sheath was thin.

Regeneration of nerve is characterized by an increase in the number of small fibers and a corresponding reduction in large diameter fibers. This change is also reflected in the distribution of axon and myelin area (Myers 1997). Present study showed better regeneration after celecoxib treatment to the sham operated group. It was observed that the diameter of regenerated fibers was smaller and myelin sheath was thin. Andrew *et al.* (2004) observed that mean axonal diameter and the size-frequency distribution of normal myelinated fibers in the sciatic nerve were measured as structural correlates to nerve conduction. After 8 weeks of untreated diabetes, mean axonal diameter was significantly ($P < 0.05$) lower than in control animals.

Ciliary neurofilament factor (CNTF) treatment partially ameliorated the decrease in diabetic animals such that mean axonal diameter in this group was not significantly different from that in either the control or diabetic animals. Diabetes was associated with a significant ($P < 0.05$) increase in the relative number of small fibers and a trend toward a decrease in the proportion of larger fibers. CNTF treatment did not have a significant impact on the axonal size-frequency distribution of either the control or diabetic animals. In this study two and three weeks treatment of celecoxib showed greater increase in small myelinated fibers with the myelin sheath as compared to large diameter fibers. So in the regenerating nerve, numbers of small fibers increased with thin myelin sheath, most of the axons were normal with no degeneration product. These small myelinated fibers were greater in three weeks treatment of celecoxib as compared to firt and second weeks of treatment.

Wang *et al.* (2002) demonstrated that the effects of Liqi Buxue Decoction (LBD is a Chinese medicine composed of milkvet root, asiabell root, teasel root, chianxiong) on the recovery rate of normal myelinated nerve, axon count, diameter of regenerated axon and thickness of myelin sheath of the injured peripheral nerve were superior and mature myelin sheath lay in the LBD treated group, more than those in the control group. Jenq and Coggeshall (1984) indicating the several regenerating axons emanated from one myelinated axon in the proximal stump. Population of myelinated fibers was larger in the regenerated nerve than in the opposite normal nerve in 21-month-old rats. There were many degenerating fibers in the contra lateral normal nerve of 21-month-old rats, but no degenerating fibers were found in the regenerated nerve of the opposite side. This could indicate that regenerated fibers maintain their function longer than the original nerve fibers. There was a significant difference between implantations of collagen sponge (or fibrin glue) and alginate gel. This shows that alginate gel is more effective than collagen sponge and fibrin glue.

La *et al.* (2005) observed that after nerve crush injury, the control, medicine (Diclofenac sodium treatment) and acupuncture groups showed structural changes in normal nerve fibers. It was observed that many axons were lost due to wallerian degeneration, the myelin sheaths were swollen, loose, broken down and became a mass of myelin. In present study no regeneration was observed in sham operated group and the unmyelinated nerve fibers with myelin debris were clearly seen in the cross sections, which indicate the greater damage in sham operated group due to lack of any treatment. No macrophages were seen in sham operated group. But regeneration was observed in celecoxib treated group after one week of experiment and showed a few normal regenerated myelinated fibers with smaller diameter and less debris as compared to sham operated group. In sham operated group animals the axon degeneration showed presence of darkly stained degeneration product, swollen and empty axon.

According to Brown *et al.* (1991) if peripheral nerve is damaged; the distal axons will undergo Wallerian degeneration, which is characterized by myelin breakdown and the recruitment of inflammatory cells such as macrophages, lymphocytes, mast cells and neutrophils to invade the degenerating regions. These recruited inflammatory cells, macrophages are the principal effectors cells involved in the process. Macrophages not only clear up degraded nerve debris but also facilitate axonal regeneration by producing and releasing growth factors, cytokines and pro-inflammatory mediators. In present study lipid debris were observed only in first week, but after two and three week's treatment of celecoxib lipid debris was absent because of activity of macrophages. Macrophages are found in celecoxib treated group. Following the nerve injuries, non-resident haematogenous macrophages invade the injury side, and their numbers increased during the intense period of phagocytic activity, associated with the Wallerian degeneration.

In current study, mean myelinated sheath thickness of normal myelinated fibers in sciatic nerve of rabbits significantly ($P< 0.001$) decreased in sham operated group compared to sham control group and treatment with celecoxib significantly ($P< 0.001$) increase the myelin sheath thickness compared to sham operated group. Chan *et* al (2001) observed that sciatic nerve treated with brain-derived neurotrophic factor (BDNF) not only produced an increase in the number of myelinated axons, but also an enlargement of myelin sheath itself. This increase in size as compared to control nerve is due to an increase in the number of lamellae in the myelin internodes. The role of BDNF in nerve injury experiment has documented an increase in the number of peripheral nervous system myelin (Zhang, *et al.*, 2000; Namiki, *et al.*, 2000). Kurtoglu *et al* (2004) observed the retarding effect of trapidil (effect of trapidil is caused by decreasing the inflammation) on myelin regeneration in recovery period because myelin sheath thickness of trapidil group was less as compared to crush group on the 15^{th} and 45^{th} days according to the control group ($P< 0.05$) and the crush group, there were statistically significant difference only between the 7^{th}, 15^{th} and 30^{th} days ($P< 0.05$). In present study sham control group showed greater mean myelin sheath thickness of normal myelinated fibers as compared to sham operated group but increase in mean myelin sheath thickness is significant ($P< 0.001$). Celecoxib treated group (celecoxib is a anti-inflammatory drugs which act by decreasing the inflammations) showed greater mean myelin sheath thickness of myelinated fibers as compared to sham operated group but this increase in mean myelin sheath thickness is statistically significant ($P< 0.001$).

Stoll and Muller (1999) indicated that the model in which the nerve is crushed by Halsted mosquito hemostat or dressing forceps is a reliable traditional method. Crushed of nerve leads to the wallerian degeneration in distil site. But the endoneurium is continuous in crushed nerve and the regeneration is better than in cut nerve. In this study after one week of spinal cord injury the cross section of sham operated group indicated that only some of axons were degenerated and abnormal structures were also visible.

A few degenerated fibers without axons degeneration were observed in sham operated group. After nerve injuries, nerve ischemia produced wallerian degernation (distruction of distil segment of nerve). Few thin myelinated fibers were also seen in sham operated group. Sun *et al.* (1998) absorved that in rat, After 21 days of crused small myelinated fibers are present, after 28 days, the new regernated fibers with myelin sheath are in abudance, but in some of them, myelin sheath are vacuolated. Mice sciatica nerve compressed by needle holder for 5 seconds shows that the wallerian degeneration of distal sites starts after 2 days of compression (Chen et *al* 1994). In current study after one week in sham operated group wallarian degernation was observed, but contains regenerating nerve fibers, small myelinated fibers and unmyelinated axons are present in the sham operated group. Mehjabeen (2005) observed that after one, two and three weeks significant decrease in myelin sheath thickness was observed in crushed control group compared with model proximal group. While no significant variation in thickness was observed in Methylcobalamin treated group compared to model proximal group. Myelin sheath thickness of Methylcobalamin treated group was observed closer to the thickness measured in model proximal group. In present study after one, two and three weeks sham operated group there were greater decrease in the mean myelin sheath thickness compared to celecoxib treated group. The thickness of normal myelinated fibers of celecoxib treated group significantly ($P< 0.001$) increased compared to sham operated group. In the celecoxib treated group, there was increased in diameter of myelin sheath thickness of normal myelinated fibers and decrease in large size myelinated fibers in celecoxib treated group as compared to the operated group. La *et al.* (2005) observed that after 11 days of crushing rabbits sciatic nerve, wallerian degeneration is still in serious stage, but there starts the regeneration, small myelinated fibers and unmyelinated axons are present in the distil site (control group). A few of unmyelinated or myelinated axons are complety normal. In present study sham operated group, it was observed that many axons were lost due to wallerian degeneration; the myelin sheaths were swollen, loose, and broken-down and became a mass of myelin. Among these abnormal structures, there were few regenerated small myelinated fibers and very few of normal size myelinated fibers.

Zhang *et al.* (2000) showed that 2, 4 or 6 weeks after rat's sciatic nerve was injured in the treated group with the (Chinese medicine) extract of Ginkgo leaf, $EGb_{24/6}$, they also found that in the number, mean diameter and section area of vessels and the myelinated nerve fibers as well as the thickness and the degree of maturation of the medullary in the $EGb_{24/6}$. These result expounded that $EGb_{24/6}$ can promote nerve regerenetion and recovery of functions. Mackinnon *et al.* 1985 demonstrated that 21 months after surgery, a fraction of regenerated nerve fibers became as large in diameter as those in the normal sciatic nerve. This indicates that myelinated fibers can gradually become mature in terms of diameter approaching the normal level in the long term. The overall population of myelinated fibers in the regenerated nerve was larger than that in the normal sciatic. In this study, Myelin sheath thickness of celecoxib treated group was observed closer to the thickness measured in sham control group. Clusters of small myelinated fibers were observed after first, second and third weeks of celecoxib treatment. But greater clusters were observed after three weeks of celecoxib treatment with increased number of macrophages and Schwann cells which were not prominent in sham control and sham operated groups.

Lundborg (1988) observed that external pressure to the peripheral nerve results in ranging from delayed venular flow in the epineurium to complete circulatory arrest according to the duration and intensity of the pressure. In a serious trauma like crush, a short period of localized total or subtotal ischemia is followed by evident increase in the endoneural fluid pressure and impairment of the normal capillary blood flow in the endoneurium. All of these results in the release of the endogenous chemical mediators increase in the vascular permeability and impairment of the blood-nerve barrier. Endoneural and intraneuraledema with inflammatory response follows this process (Lundborg, 1988; Zochodne and Ho, 1990; Podhajsky; Ambrosio and Tritto, 1999). In present study it was observed that blood vessel decreased after nerve injuries and microcirculation become damaged. This impairment in circulation may be due to the same mechanism as described earlier. But after one, two and three weeks of celecoxib treatment endoneurial vessels were increased and microcirculation became normal.

Conclusion

- It was concluded from this study that nerve regeneration in celecoxib treated group are greater compared sham operated and sham control group.

- Celecoxib is a nonsteriodal anti-inflammatory drug that exhibits anti-inflammatory, analgesic and antipyretic activities. However it used in the treatment of rheumatoid arithitis osteoarthritis, acute pain condition.

- Celecoxib showed the effect of promoting regernation of sciatic nerve injury, it can show the anti-inflammatory effects.

- Celecoxib significantly reduces the pathological changes indused by nucleus pulposus. However celecoxib effects on sciatic pain.

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