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**THE AMERICAN
UNIVERSITY IN CAIRO**
الجامعة الأمريكية بالقاهرة

School of Sciences and Engineering

**THERAPEUTIC POTENTIAL OF CURCUMIN IN A SPINAL CORD INJURY
MODEL: LOCAL APPLICATION VERSUS DIETARY SUPPLEMENT**

A Thesis Submitted to
The Biotechnology Graduate program

In partial fulfillment of the requirements for
the degree of Master of Science

By
Sarah Kamel F. Abu-Hussein

Under the supervision of
Dr. Ahmed Abdellatif
Assistant Professor, Biology Department

July 2017

American University in Cairo
School of Sciences and Engineering

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By

Sarah Kamel F. Abu Hussein

This thesis has been successfully defended.

Thesis Supervisor: Ahmed Abdellatif, MD, PhD, Assistant Professor, Biology, AUC

Signature: _____ Date: _____

First reader: Hamdino Attia, MD, PhD

Associate Professor & Chair, Anatomy & Embryology Department

Faculty of Medicine, Al-Azhar University, Damietta.

Signature: _____ Date: _____

Second reader: Asma Amleh, PhD

Associate Professor, Biology, American University in Cairo

Signature: _____ Date: _____

Moderator: Karim Seddik, PhD

Associate Professor, Electronics & communications Engineering, AUC

Graduate Program Director: Asma Amleh, PhD.

Signature: _____ Date: July/2017

DEDICATION

To the most important people in my life

Dad, I am blessed for being your daughter, I miss your presence.

Mum, you are all the love in my life; none of this would have been possible without your faith in me

My Husband, Thank you for always being there for me and for truly believing in me

My Brother and Sister, Thank you for being extremely supportive and caring all the way

My Sons, you are the air that I breathe, looking into your eyes makes everything look perfect

ACKNOWLEDGEMENT

I would like to express my gratitude to all the people who have helped, supported and encouraged me throughout the work. First and foremost I would like to thank my advisor, Dr. Ahmed Abdellatif for his immeasurable guidance and support since the very beginning of the project. Thanks to him, my thesis was a rewarding learning experience. His door was always open in case of any trouble throughout the whole work process. He has been most encouraging and supportive. Thanks to his guidance and support that I was able to attend the “Society for Neuroscience Conference in Chicago”. Dr. Abdellatif was an advisor in every definition that word could hold. I would never be able to thank him enough.

I would like to extend my appreciation to Dr. Asma Amleh and her outstanding lab members. They were extremely supportive and helpful whenever needed.

I would also like to thank all of my friends and colleagues that have encouraged and supported me throughout this tough but rewarding journey. I express my great appreciation to Eman El Zeneini , Noha Moataz, Sara Samy, Hagar Nofal, Hana Heiba, Diana George, Yomn Abdullah. I would like also to thank Nahla Osama who has offered me help through the work and with lab supplies as well. I express my deep appreciation to all my friends and colleagues.

A special thanks goes to Amgad Ouf who was always there answering all of our questions and providing great advice. He has helped me greatly throughout my work either with advice or through offering great help in the practical part. I really cannot thank him enough.

I would like to acknowledge Dr. Rania Siam and Dr. Ahmed Moustafa and all the biology department members for their continued support. I would like to express my appreciation to the AUC for providing the graduate research grant that funded my thesis project. I extend my deep appreciation to all my professors in the Biotechnology graduate program; your guidance and mentorship were definitely immeasurable.

THERAPEUTIC POTENTIAL OF CURCUMIN IN A SPINAL CORD INJURY MODEL: LOCAL APPLICATION VERSUS DIETARY SUPPLEMENT

ABSTRACT

Spinal cord injury is a debilitating disability that is characterized by a sequence of tragic events that occur following the primary impact aggravating the condition, collectively called secondary spinal cord injury. Oxidative damage and inflammatory surge are two hallmarks of the secondary spinal cord injury cascade. Curcumin is a polyphenolic compound extracted from the rhizome of *Curcuma longa* that has been well known to possess antioxidant and anti-inflammatory properties. The objective of this study was to evaluate the potential of curcumin as an antioxidant and anti-inflammatory agent following spinal cord injury, and to compare its therapeutic effects following local application directly to the injury versus its effect when given as a dietary supplement in a spinal cord hemisection model at T9-T10 level of the spinal cord. Female Sprague Dawley rats were randomized into a control group, injury groups (1 day and 7 days), local treatment groups single dose of *Curcuma longa* extract immediately on the injury site (1 day and 7 days) and a Dietary supplement group. Crude Curcumin was added to the animals' feed (10% of daily feed) one week before and week after injury. Oxidative stress parameters used for detection the effect of Curcumin before and after treatment were Malondialdehyde (MDA) by Thiobarbituric acid assay (TBA) and total antioxidant capacity (TAC). Expression of tumor necrosis factor alpha (TNF α) and interleukin 6 (IL-6) was detected using Enzyme Linked Immunosorbent Assay (ELISA). Our results show that at 7 days, although Dietary supplement was effective in increasing TAC levels and lowering TNF α expression levels, yet it did not affect MDA levels (IL-6 data not measured). Local treatment regimen has shown to be more effective on all four parameters measured as the 7 days. Our results demonstrate that local Curcumin application directly on the injury site might be more efficacious in alleviating oxidative damage and reducing inflammation following spinal cord injury. Further analysis is needed to evaluate the effect of Dietary treatment regimen on IL-6 and detect the effect of different Curcumin treatment regimens on other anti-oxidant and anti-inflammatory markers to investigate the role of curcumin in alleviating oxidation and inflammation.

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LIST OF ABBREVIATIONS

AACR	American Association for cancer research
AMPARs	α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor
AN	Athymic nude
ANOVA	Analysis of Variance
AP-1	Activator Protein 1
ASIA	American Spinal Injury Association
AUC	Area under the curve
BSCB	Blood-spinal cord barrier
CAT	Catalase
CO ₃ ⁻	Carbonate Free Radical
COX	Cyclooxygenase
CNS	Central nervous system
CSPG	Chondroitin Sulphate Proteoglycan
CTGF	Connective tissue growth factor
DAMP	Damage associated molecular pattern
DHA	Docosahexaenoic Acid
DMSO	Dimethylsulfoxide
DSPG	Dermatan Sulphate Proteoglycan
ECM	Extracellular Matrix
EGFR	Epidermal growth factor receptor
ELISA	Enzyme Linked Immunosorbent Assay
ERKs	Extracellular signal regulated Kinases
GABA _A	Gamma-Amino Butyric Acid
GAP 43	Growth Associated Protein 43
GAG	Glycosaminoglycan
GCL	Glutamate Cysteine Ligase

G-CSF	Granulocyte Colony Stimulating Factor
GFAP	Glial fibrillary acidic protein
GLU	Glutamate
GM-1	mono-sialo-tetra-hexosyl-ganglioside
GSH	Glutathione
H ₂ O ₂	Hydrogen Peroxide
hESC	Human Embryonic Stem Cells
4-HNE	4-Hydroxynonenal
HO-1	Hemeoxygenase 1
HO	Heterotopic ossification
HSC	Hepatic stellate cells
HSPG	Heparan Sulphate Proteoglycan
I/R	Ischemia Reperfusion
IFN γ	Interferon Gamma
IL-1 β	Interleukin 1 β
IL-4	Interleukin 4
IL-6	Interleukin 6
IL-10	Interleukin 10
IL-13	Interleukin 6
JAK-STAT	Janus Kinase/Signal Transducer and Activator of Transcription
JNK	c-Jun N-terminal kinase
Keap 1	kelch-like ECH associated protein 1
KSPGs	Keratan Sulphate Proteoglycans
LOX	Lipoxygenase
LPS	Lipopolysaccharides
MAG	Myelin associated glycoprotein
MAPK	Mitogen activated protein kinase
MBP	Myelin Basic Protein
MDA	Malondialdehyde

MMP-9	Matrix Metalloproteinase 9
MP	Methylprednisolone
NF- κ B	Nuclear Factor kappa B
NG2	Neural/Glial antigen 2
NgR1	Nogo Receptor 1
NOS	Nitric oxide synthase
NQO1	Nicotinamide Adenine Dinucleotide Phosphate Quinone Oxidoreductase
Nrf2 /ARE	Nuclear Factor E2 Related Factor-2/ Antioxidant Response Element
OPC	Oligodendrocyte progenitor cells
PAMP	Pathogen associated molecular pattern
PBS	Phosphate Buffered Saline
PGE2	Prostaglandin E2
PMA	Phorbol 12-Myristate 13-Acetate
PON	Peroxynitrite
PPAR- γ	Peroxisome Proliferator- Activated Receptor Gamma
ROS	Reactive oxygen species
SCI	Spinal cord injury
SD	Sprague Dawley
SLCP	Solid Lipid Curcumin Particle
SOD	Superoxide dismutase
STAT	Signal transducer and activator of transcription
TAC	Total Antioxidant Capacity
TBA	Thiobarbituric Acid Assay
TBI	Traumatic brain injury
TGF β	Transforming Growth Factor Beta
TLR4	Toll like receptor 4
TNF	Tumor necrosis factor
TNFR1	Tumor Necrosis Factor Receptor 1
TRPA-1	Transient Receptor Potential Ankyrin -1

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CHAPTER 1: LITERATURE REVIEW

1.1. Spinal Cord Injury

1.1.1 Introduction

Spinal cord injury (SCI) is a debilitating tragic disability that affects a lot of people worldwide. About 2.5 million people have SCI with about 130,000 new injuries affecting people annually (Thuret et al. 2006). SCI has been described in an ancient Egyptian physician textbook called “The Edwin Smith papyrus” since 1700 BC as an “ailment not to be treated” (Porter, 2001)

It influences not only the life of the individual but also the surrounding family members socially, emotionally and financially. The cost of being enrolled in a clinical trial per person is about 50,000 to 100,000 dollars in Europe and United States. On the other hand the cost of preliminary phase 2 human efficacy trials for a drug is about 5 to 10 million dollars (Tator, 2006). In Spite of all of this, available treatments give modest benefit that’s why more effort is needed to come up with more effective SCI treatment regimens (Ali and Bahbahani 2010)

Many of the complications of SCI are permanent and eventually cause serious consequences, including; respiratory complications which are a frequent cause of death after SCI (Berney et al., 2011), Bladder dysfunction (Hagen, 2015), and heterotopic ossification (HO) (Banovac et al., 2004), which leads to reduction in joint motion range.

Unfortunately, to date the approaches taken to handle or treat SCI can be still regarded as sedative, or palliative approaches trying to prevent further progression, handle dysautonomia and complications of loss of sensory abilities and aiming at teaching patients on how to cope up with their disabilities (Jablonska et al. 2010, Silva et al., 2014).

1.1.2 Incidence and Prevalence:

In most countries, SCI patients are below 30 years of age (Singh et al., 2014; Rhee et al., 2013). A higher male to female ratio is demonstrated and traffic accidents were the most known cause for SCI followed by falls in elderly. The highest SCI prevalence was (906 /million) in the United States of America and lowest (250-280/million) in France and Finland (Singh and Tetreault et al., 2014).

1.1.3 Anatomy of the Spinal Cord:

The spinal cord extends from the medulla oblongata in the brainstem through the foramen magnum of the skull till lumbar vertebra L1 in humans or L3 in rats. The spinal cord provides connection between the brain and the peripheral nerves. It is protected by the bony vertebral column and by the three meninges namely: Dura, Arachnoid and Pia matter. Additional protection is provided by the subarachnoid space (between pia and arachnoid) which is filled with cerebrospinal fluid as well as, the epidural space between dura and periosteum; which is filled with adipose connective tissue and loose fibrous tissue (Vander et al., 2001).

The gray matter is located centrally and is composed of neurons, interneurons, dendrites and cell bodies of motor neurons, glial cells and the entering fibers of the sensory neurons. The white matter on the other hand is mainly composed of myelinated axons which are organized in functional groups called tracts; descending to the periphery or ascending to the brain transmitting information in both sides, as well as propriospinal (local) tracts which connect and coordinate different levels of the spinal cord. These tracts are regarded as a crucial communication means between the brain and the spinal cord (Silva et al., 2014).

Descending tracts (pyramidal or extrapyramidal) control motor functions as shown in figure 1. Pyramidal tracts are so named due to the fact that they decussate (cross from one side to the other) in the medulla. These tracts terminate at the lower motor neurons in the grey matter of the spinal cord. Extrapyramidal tracts include: rubrospinal, reticulospinal, tectospinal and vestibulospinal tracts. These tracts are mostly responsible for complex movements, fine locomotion tuning, reflexes and postural control.

Ascending tracts shown in figure 1 convey information from the periphery to the cerebral cortex. The dorsal column fibers carry proprioceptive, vibratory senses and fine touch. The anterolateral system (anterior & lateral spinothalamic tracts) carries crude touch, temperature and pain. The spinocerebellar tracts aid in fine tuning and coordination of motor tasks and carry unconscious proprioceptive data (Lee and Thumbikat, 2015).

Crossing of fibers carrying proprioception, motor function and light touch in the medulla means that the SCI patient will experience an ipsilateral (same side) deficit while decussation of fibers conveying information for temperature and pain occurs at their vertebral level of entrance into the spinal cord leading to a contralateral deficit upon injury (Lee and Thumbikat, 2015).

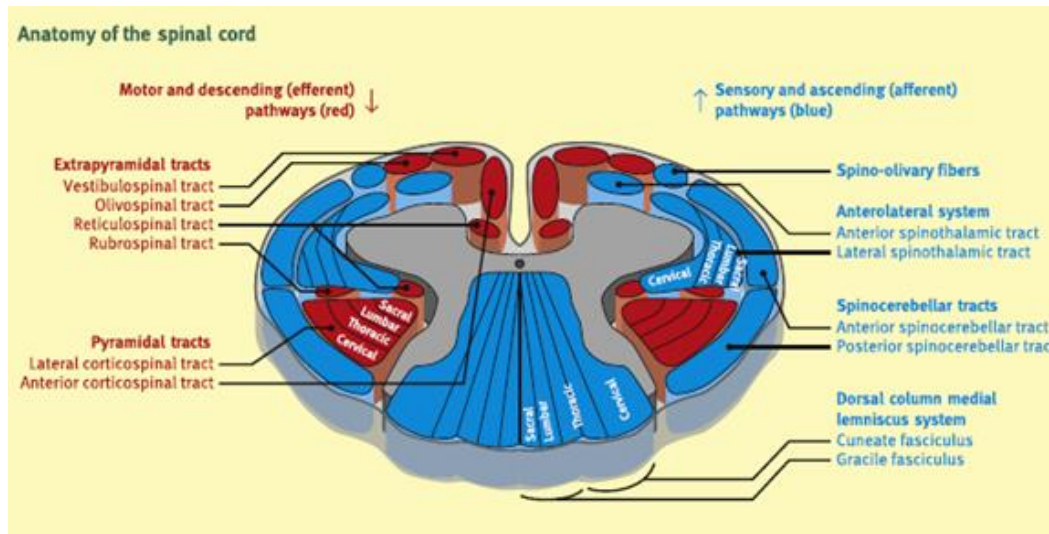


Figure 1: Ascending and Descending tracts of the Spinal Cord. Reproduced from Lee and Thumbikat 2015 with permission.

Axons of afferent (sensory) neurons enter the spinal cord via the dorsal root with their cell bodies present in the dorsal root ganglion, while those of efferent (motor) neurons leave the spinal cord through ventral roots. Eventually dorsal and ventral roots combine to form the mixed spinal nerve (motor, sensory and autonomic) which exit from 31 vertebral levels (Cervical-8, Thoracic -12, Lumbar-5, Sacral-5 and one coccygeal nerve) (Silva et al., 2014).

1.1.4 Pathophysiology of SCI

1.1.4.1 Primary Injury:

SCI pathophysiology is biphasic in nature entailing a primary and a secondary phase. The primary mechanical injury leads to a cascade of secondary damage involving vascular, cellular and biochemical events (Simon et al. 2009; White-Schenk et al., 2015). Traumatic SCI was classified by Bunge and colleagues as “a contusion with cavity formation, massive compression or laceration” (Bunge et al., 1997).

Vascular ischemia is not an uncommon cause for SCI especially as a surgical complication during the surgery for aortic aneurysm repair (Hollier et al., 1992) where spinal cord ischemia might result from prolonged aortic cross clamping or ligation of lumbar or intercostal arteries causing ischemia (Anwar et al., 2016), that might eventually lead to paraplegia (Shimizu and Yozu 2011).

This primary phase is marked by a series of events shown in figure 2 as spinal shock, systemic hypotension, ischemia, damaged plasma membrane and ionic imbalance (Oyinbo, 2011).

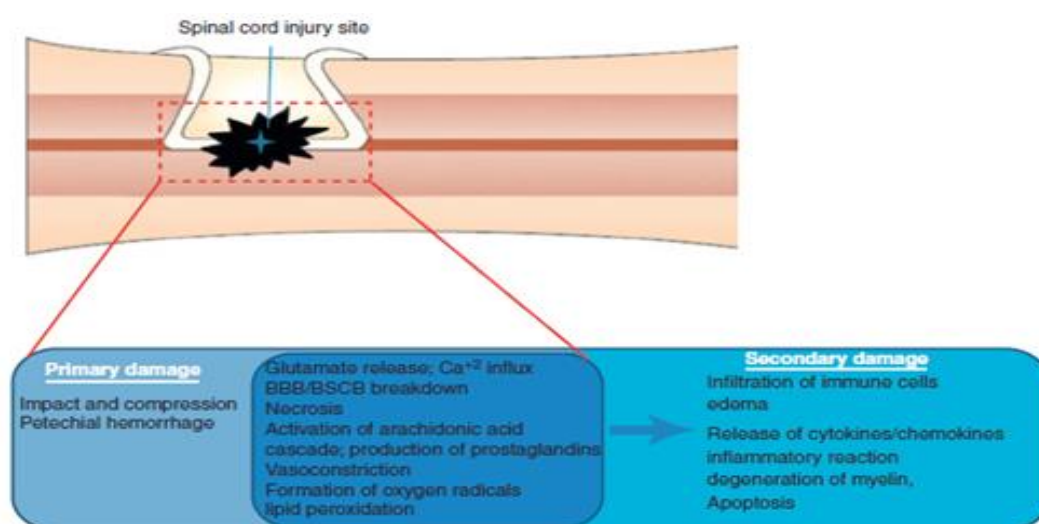


Figure 2: Pathophysiology of SCI. . A primary injury caused by impact or compression is followed by a secondary injury cascade that entails a series of cellular, molecular and biochemical events that contribute to worsening the primary injury. Reproduced from Esposito and Cuzzocrea 2011 with permission.

1.1.4.2 Secondary injury:

The secondary injury starts to take over following the primary phase and it can last for weeks (Wilson et al., 2013), the two major events that largely contribute to the secondary injury are inflammation and oxidative stress, which lead to necrosis, immune system response and apoptosis (Johnson et al., 2005), neuro-inflammation, free radicals formation, breakage of the blood-spinal cord barrier (BSCB), ischemic dysfunction, neuronal injury

and glial scar formation which limits the SCI recovery (Ormond et al., 2014). It also includes other functional problems, such as bladder, rectal and anal incontinence due to autonomic dysfunction, neuropathic pain and impotence (Singh and Tetreault et al., 2014).

Unfortunately in most cases the aggravating effects of the secondary injury commence before expert intervention starts (Oyinbo 2011). That's why treatment strategies are focusing on combating this sequence of events (Fehlings and Nguyen 2010), and new strategies would help enhance axonal regeneration and neuronal function, and restore the disrupted functions that follow the injury.

Finally the chronic phase commences which may last for years post SCI, causing continued demyelination, apoptosis and cavitation well as syringomyelia which lead to permanent neurological impairment (Yiu and He 2006; Oyinbo, 2011).

1.1.5 Main Events in Secondary Spinal Cord Injury

1.1.5.1 Inflammation

Following SCI, the net result of the immune system response through its cellular (immune cells) and molecular (regulatory proteins) components is inflammation (Oyinbo, 2011). Immune cells secrete pro-inflammatory cytokines as tumor necrosis factor α (TNF- α), interleukin 1 β and Interleukin-6 increasing the extent of inflammation, to remove cellular debris, unfortunately, over-activation of this inflammatory response aggravates the injury and damages the healthy tissues (Oyinbo, 2011).

The role of some immune cells in SCI is regarded as controversial (Donnelly and Popovich, 2008). Macrophages and microglia are thought to be a key component for neural regeneration while others suggest that these cells are responsible for oligodendrocytes damage (Merrill et al., 1993), demyelination and neuronal death (Rhoney et al., 1996). The

infiltration and activation of glial cells and leukocytes becomes the main source of reactive oxygen species (ROS) (Bains and Hall, 2012). The inflammatory cascade following SCI is shown in figure 3.

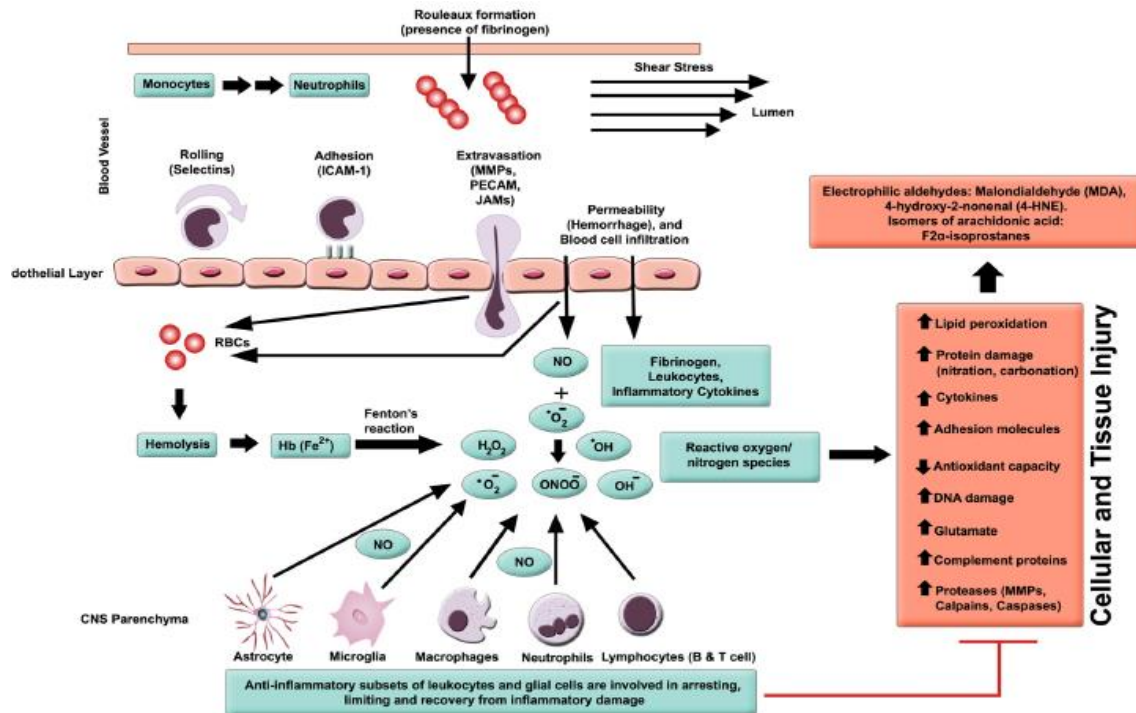


Figure 3: Inflammatory environment following SCI. The primary injury causes hemorrhage and increase in vascular permeability. Leukocyte extravasation and infiltration then occurs breaking up inter-cellular proteins and extracellular matrix. ROS are then released from activated microglia and leukocytes causing protein modification, lipid peroxidation and gene expression modulation eventually leading to cellular and tissue damage causing necrosis and apoptosis in neuronal and glial cells within the spinal cord parenchyma. Yet, the anti-inflammatory subsets of glial cells and leukocytes potentiate anti-inflammatory mechanisms. Reproduced from Anwar et al., 2016 with permission.

1.1.5.2 Neutrophils and Macrophages:

Following SCI, neutrophils arrive first at the injury site guided by the vascular endothelial cells. They enhance recovery by removing tissue debris and microbial invaders, and recruit macrophages (Kigerl et al., 2006). They also release proteases, free radicals, cytokines, and

activate other inflammatory and glial cells ultimately causing neuronal and glial toxicity and death (Joy et al. 2005, Shamash et al., 2002).

Depletion of neutrophils (Noble et al., 2002), inhibition of their adhesion (Hamada et al., 1996) or blocking of their related enzyme proteolytic activities (Noble et al., 2002) were correlated with neuroprotection (Kigerl et al., 2006).

Neutrophils and macrophages synthesize a variety of pro-inflammatory cytokines e.g. TNF- α and IL-1 β as part of their function in normal cellular communication (Probert and Selmaj 1997) ; however, the sustained release of these molecules in SCI triggers inflammation and disrupts the cytokine release pattern, potentially exaggerating glutamate excitotoxicity causing neuronal and oligodendrocyte death (Shamash et al., 2002).

Glutamate is the main excitatory neurotransmitter within the CNS (central nervous system), and excess amounts of it are normally removed by astrocytes and to a lesser extent by microglia (Rimaniol et al., 2000). However, after SCI its metabolism by astrocytes is disturbed and its clearance is prohibited by tumor necrosis factor alpha (TNF- α) or interleukin 1beta (IL- 1 β) (Takahashi et al., 2003). Neurons and oligodendrocytes are particularly sensitive to glutamate excitotoxicity as they demonstrate high numbers of glutamate receptors (Oyinbo, 2011). Blocking TNF- α or IL-1 β enhances neuroprotection in SCI, traumatic brain injury (TBI) and stroke models (Sharma et al., 2003). Moreover, interleukin-6 (IL-6) overexpression was shown to potentiate leukocyte infiltration and impair both locomotor activity and axonal regrowth (Lacroix et al., 2002).

Stellwagen and colleagues demonstrated that TNF α causes an elevation in α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPA α s) specifically non glutamate (non-GLU2-AMPA α s) through tumor necrosis factor receptor 1 (TNFR1) rendering them more calcium permeable. At the same time TNF α causes fewer expression of surface gamma-aminobutyric acid (GABA α) receptors, consequently causing alleviation

in the inhibitory synaptic strength and enhancing excitotoxicity (Stellwagen et al., 2005). Potassium (K^+) and Calcium (Ca^{2+}) may also have a role in neuronal destruction (Jung et al., 2013).

Neutrophils and Macrophages also release superoxide and Nitric oxide which both react and combine releasing the highly toxic species, peroxynitrite (Xiong et al., 2007). After injury, depolarization causes opening of voltage dependent Ca^{2+} , K^+ and sodium (Na^+) channels followed by Ca^{2+} overload leading to peroxynitrite (PON) derived free radicals production as shown in figure 4. These peroxynitrite radicals react with amino acids causing protein Carbonylation and damage the unsaturated fatty acids causing cellular damage (Xiong et al., 2007).

1.1.5.3 Lymphocytes:

After injury, Activated Lymphocytes carry out an autoimmune response that exaggerates the effect of macrophages causing more toxicity to the neurons and damage to the blood-brain barrier (Sriram and Rodriguez, 1997). T-lymphocytes appear to play a dual role in injury and repair depending on the surrounding microenvironment and the signals that recruited them (Trivedi et al., 2006). Chemokines are responsible for T-cell migration and activity modulation at inflammation sites (Sallusto et al., 2000). Some studies correlated their activation with increased cytokine release (Jones et al., 2005), fibrosis and scarring (Wynn, 2004).

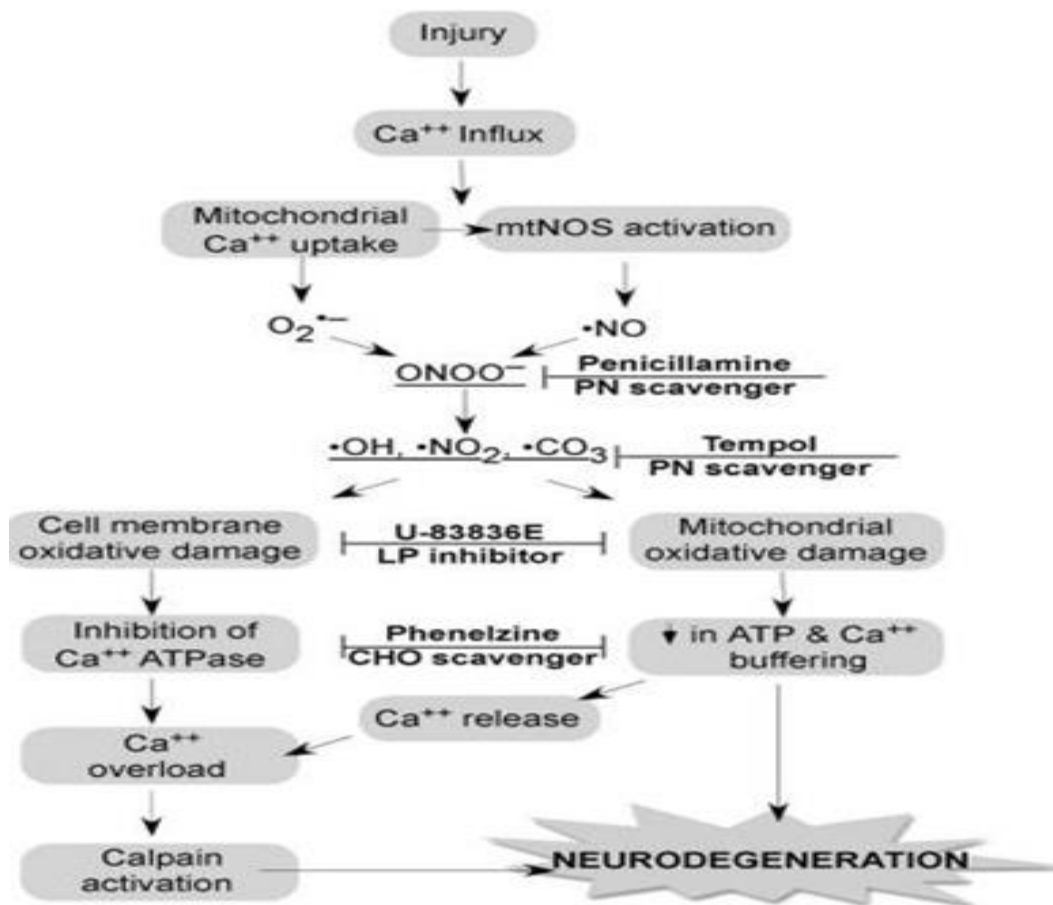


Figure 4: Oxidative damage following SCI. . Calcium overload is caused via glutamate receptor and voltage dependent channels. Mitochondrial oxygen (O₂) uptake causes nitric oxide synthase (NOS) activation and eventually peroxynitrite radicals are formed causing damage and promote cytoskeletal proteins proteolysis and neurodegeneration. Reproduced from Bains and Hall 2012 with permission.

Neuro-antigen reactive T and B lymphocytes that act against myelin protein as Myelin Basic Protein (MBP) have noticeable pathological potential in Multiple sclerosis (Von Büdingen et al., 2001). Transgenic rats and mice vaccinated to enhance MBP-reactive T cells, were shown to cause axonal impairment and demyelination causing neuronal loss (Jones et al., 2004).

Athymic nude (AN) rats manifested better locomotor activity than injured Sprague Dawley rats (SD) one week post injury in a study aiming at detection of T lymphocyte role in SCI . Moreover, injured SD rats showed acute elevation in death related genes suggesting a secondary wave of damage caused by T-cells. This suggests that a combination of T-cell inhibition and other neuroprotective strategies might have a promising therapeutic potential (Satzler et al., 2015).

Nevertheless, Schwartz and colleagues propose a protective role for T-cells specifically reactive to MBP in Lewis rats, in enhanced neuroprotection after spinal cord contusion or crushing optic nerve injury, but not in complete transaction model (Schwartz and Kipnis 2001).

B-lymphocytes also present in the injury site within hours post SCI and last for up to 1 week (Popovich et al., 2001). Enhanced activation of B cells was also evident following SCI, with elevated CNS antibodies in the serum of SCI individuals (Hayes et al., 2002) and in a mouse model of spinal cord contusion injury (Ankeny et al., 2006). B-lymphocytes also enhanced demyelination in an experimental allergic encephalomyelitis model (Chavarria and Alcocer Varela 2004).

1.1.5.4 Microglial cells:

Microglial cells exist as two main phenotypes, M1 the pro-inflammatory phenotype and M2 the anti-inflammatory phenotype which are two polarized states whose activation depends on external signals (Durafour et al., 2012). Following SCI, microglia sense the initiation of tissue damage via toll-like receptors (Heiman et al., 2014), and undergo morphological changes, they attain an amoeboid morphology rather than the ramified appearance they maintain in their Quiescent state (Lee et al., 2007; Witcher et al., 2015).

Following SCI, M1 phenotype is activated and initiates a series of damaging side effects to neurons, axons, oligodendrocytes (Nguyen et al., 2012). This cascade is mediated through various signaling pathways, such as, inflammatory cytokines (TNF- α , IL-6) (Pineau and Lacroix, 2007), ROS (Barger et al., 2007), Chemokines (Mantovani et al., 2004) and Glutamate (Barger et al., 2007). These cytokines are expressed within two hours following injury (Pineau and Lacroix, 2007).

Moreover, the M1 phenotype has been found to express cell surface and intracellular receptors such as, CD45 (lymphocyte common antigen), CD11b (integrin family member) and inducible nitric oxide synthase 2 (iNOS2) which aids in the pro-inflammatory mechanism. On the other hand M2 phenotype releases anti-inflammatory cytokines as interleukin 4 (IL-4), interleukin 10 (IL-10) and interleukin 13 (IL-13) (Witcher et al., 2015). Thus, it appears that microglia play a vital role in the inflammatory cascade following SCI that can be either pro inflammatory or anti-inflammatory.

1.1.5.5 Astrocytes and Glial Scarring:

Following injury, the blood spinal cord barrier is disrupted (for up to 2 weeks) as a result of both mechanical forces and the influx of inflammatory mediators (Renault-Mihara et al., 2008). The activation and recruitment of astrocytes to the injury leads to reactive astrogliosis, and glial scarring where astrocytes hypertrophy occurs with the production of intermediate filaments as glial fibrillary acidic protein (GFAP) and vimentin (Wilhelmsson et al., 2004), extracellular molecules such as, chondroitin sulfate proteoglycan (CSPG) which inhibit axonal regeneration (Schwab et al., 2006). In this manner, Astrocytes build a wall around the injury site preventing axonal regrowth as shown in figure 5 (Anwar et al., 2016).

Central cavitation takes place days to weeks after the primary SCI (Oyinbo, 2011). It is manifested by an expansion of the SCI lesion size leading to the formation of a cavity much larger size than the initial scar (Fehlings and Nguyen 2010).

Reactive astrocytes role is rather confusing. On one hand, they are implicated in tissue integrity preservation (Faulkner et al., 2004), regulation of excess neurotransmitters production and in the release of extracellular matrix (Von Boxberg et al., 2006). While, On the other hand, reduction of reactive astrogliosis by inactivation of CSPG (Menet et al., 2003) or blocking Calcium channels (Lee et al., 2000), decreases the obstruction of the glial scar against axonal sprouting and regrowth (Von Boxberg et al., 2006).

Wide arrays of genes are upregulated upon reactive astrogliosis (Zamanian et al., 2012), e.g. intermediate filament proteins as GFAP, nestin, vimentin, Cytokine, and s100 (Zamanian et al., 2012). These genes are shown to form a complex enhancing damaged membranes repair (Rezvanpour et al., 2011).

1.1.5.6 Inhibitory Molecules:

Two main groups of molecules inhibit axonal regeneration; the first is the proteoglycans. These are composed of a protein core linked through four sugar moieties to a glycosaminoglycan (GAG) chain which contains repeated disaccharide units (Johnson-Green et al., 1991). These molecules have demonstrated an inhibitory profile following CNS injury. They are mainly expressed by astrocytes, meningeal cells and oligodendrocyte precursors (Fawcett and Asher, 1999). They are composed of four main groups; Dermatan sulphate proteoglycans (DSPGs), Heparan Sulphate proteoglycans (HSPGs), Keratan sulphate proteoglycans (KSPGs) and Chondroitin sulphate proteoglycans (CSPGs) (Jhonson-Green et al., 1991). CSPGs are the most studied group and it includes: Brevican, Aggrecan, Neural/Glial antigen 2 (NG2), Versican, Neurocan and Phosphacan.

On one hand, these molecules are important for pathfinding and guidance (Fukuda et al., 1997), but on the other hand, CSPGs rich areas can inhibit axonal growth (Faissner and Steindler, 1995).

The second group is the myelin associated molecules. Nogo A is one example of this group. It is a membrane protein which is expressed by oligodendrocytes and some neurons, it causes growth cone collapse and growth inhibition, when it binds to its specific receptor named NgR1 in the neuron's membrane (Schwab, 2004). Another molecule in this group is the myelin associated glycoprotein (MAG) which is stored in Myelin that surrounds the CNS axons and is also originally produced by oligodendrocytes (Silva et al., 2014). It is regarded as one of the strongest inhibitors of white matter regeneration (Silva et al., 2014).

1.1.5.7 Apoptotic cell death:

Cell death after the mechanical trauma to the spinal cord is either due to necrosis which follows inflammation and cell membrane rupture (Oyinbo 2011), or due to apoptosis (Johnson et al., 2005), which begins within hours following SCI (Oyinbo 2011). The key players in apoptosis are caspases (Green 1998). Caspase 8 and 9 are the initiator caspases (Adjan et al., 2007), leading to further activation of caspase 3 (Wu et al., 2003). This in turn cleaves several downstream substrates that are crucial for apoptosis (Zhang et al., 2012). TNF- α acts as a mediator of apoptosis, through its death domain on its surface receptor TNFR1 (Zhang et al., 2012). However, there are conflicts regarding its capacity as being both pro and anti-apoptotic (Genovese et al., 2009).

1.1.5.8 Demyelinating Surviving Axons:

Oligodendrocytes cell death by excitotoxicity, apoptosis, inflammatory cytokines and free radicals can cause demyelination of the axons which survived the initial trauma (Oyinbo 2011) as shown in figure 5. Demyelination is evident in the subacute and chronic SCI phases (Guest et al. 2005) and it is due to oligodendrocytes loss in white matter rostrally and caudally weeks post SCI (Grossman et al. 2001).

1.1.6 Oxidative damage in SCI:

The spinal cord and CNS in general has high lipid content (in Myelin) and high oxygen need, rendering it easily susceptible to cell damage by lipid peroxidation (Tian et al., 2004). Following SCI, ROS overwhelm the antioxidant buffering mechanisms causing lipid peroxidation, cell lysis, and organelle damage and calcium imbalance as shown in figure 6. Thus antioxidant therapies such as high dose methyl prednisolone (MP) are currently the only useful medical intervention to enhance neuroprotection in SCI patients (Shan et al., 2010).

Free radicals cause a chain reaction which starts by absorbing an electron from a lipid molecule, eventually leading to membrane lysis, necrotic cell death and mitochondrial dysfunction (Sullivan et al., 2007), with an increase in intracellular calcium levels activating proteases and consequently breaking down cytoskeletal proteins as shown in figure 7 (Xiong et al., 2007).

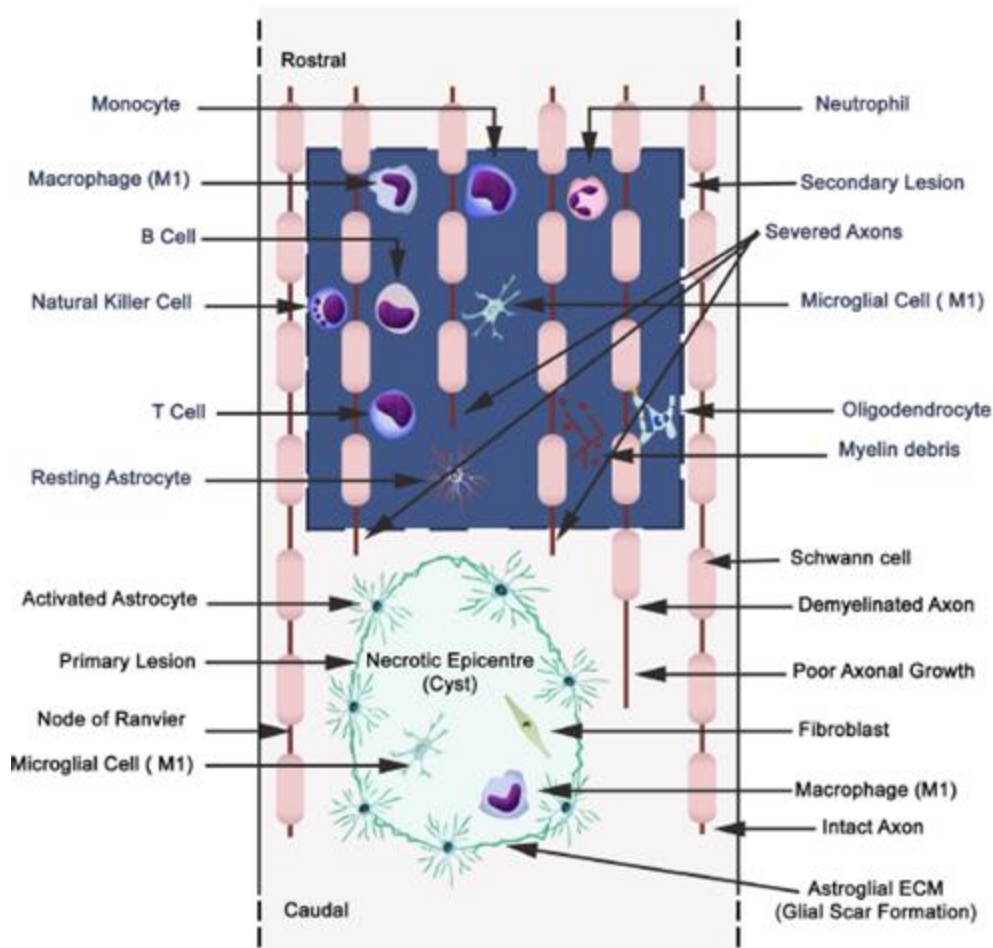


Figure 5 : Key players in SCI and glial scar formation. Demyelination, necrosis and axonal destruction takes place along with recruitment of microglia and astrocytes, leukocytes (neutrophils, macrophages, lymphocytes, natural killer cells). Reproduced from Anwar et al., 2016 with Permission.

These lipid peroxidation end products can be inactivated by a number of enzymes as glutathione-s-transferase, aldehyde reductase and aldehyde dehydrogenase (Ayala et al., 2014). ROS are also inactivated by other antioxidant defense systems such as glutathione peroxidase, catalase and superoxide dismutase, and other antioxidant mechanisms as vitamins A, E, and C , flavonoids and carotenoids as well (Bains and Hall, 2012) .

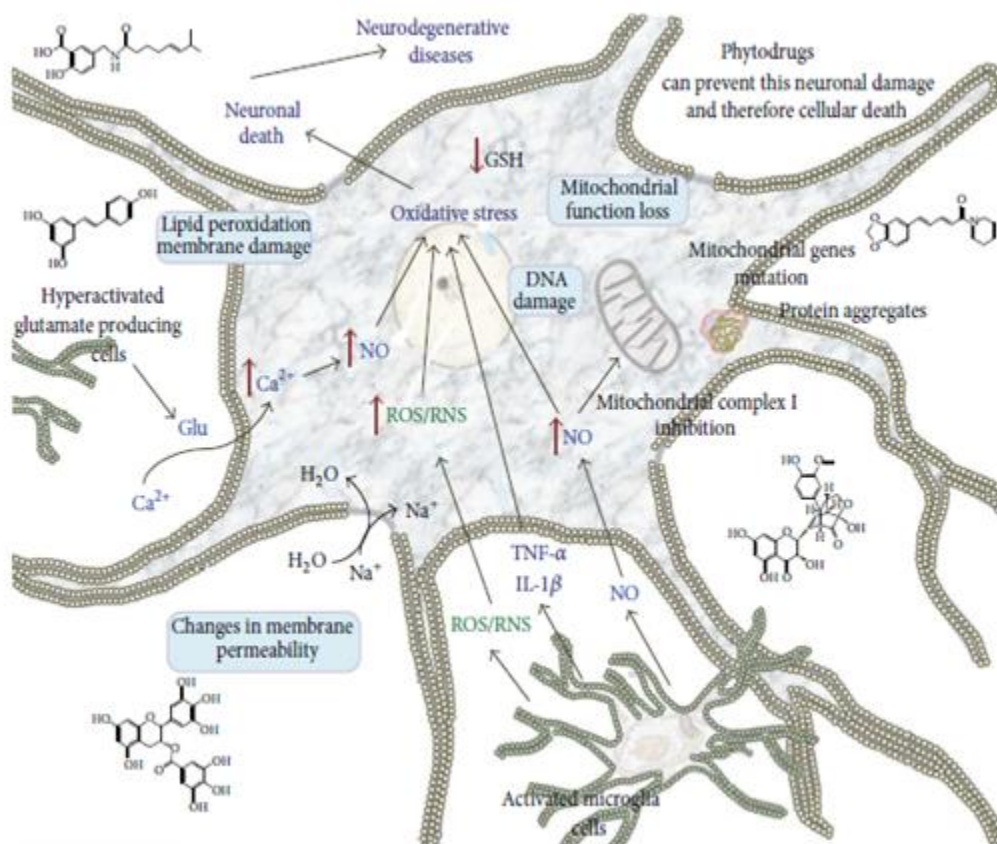


Figure 6 : Damage caused by oxidative stress following SCI. Oxidative stress causing neuronal death through DNA damage, mitochondrial dysfunction, protein aggregation and apoptosis. This damage can be prevented by phytodrugs. Reproduced from (Pérez-Hernández et al., 2016 with Permission.

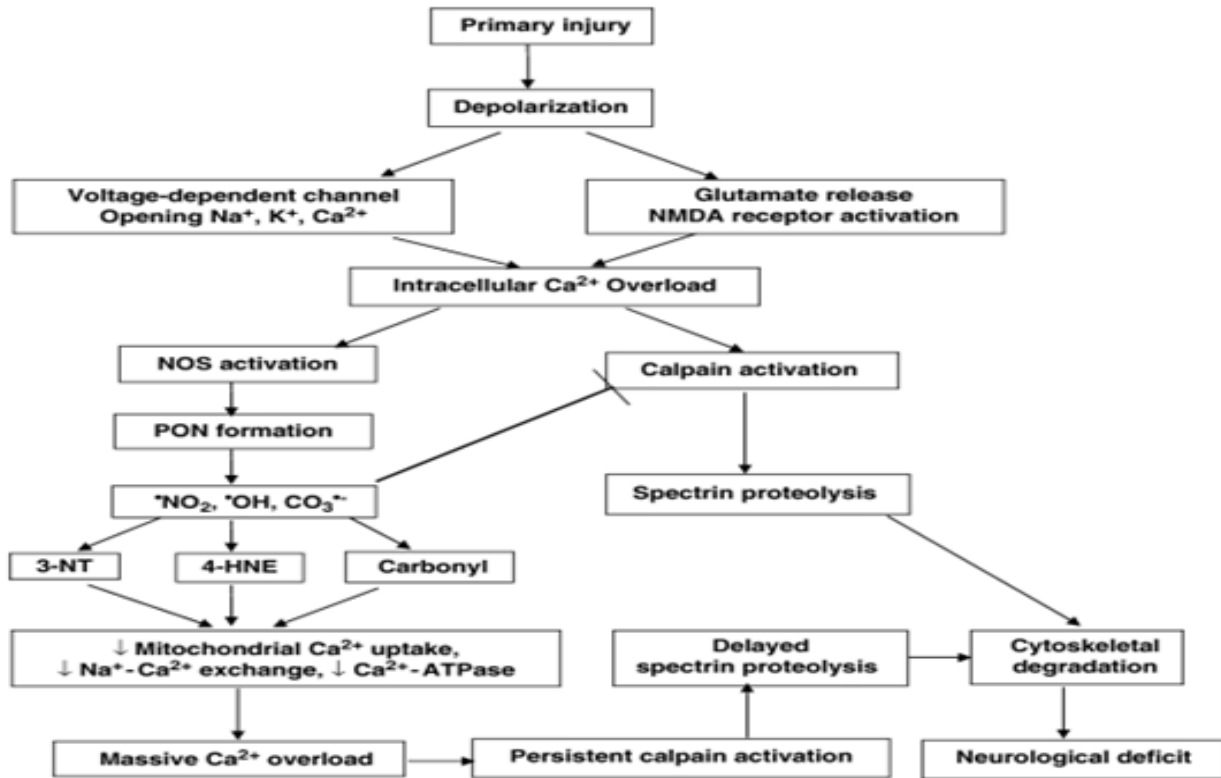


Figure 7: Oxidative damage cascade leading to Calcium influx causes rapid calpain activation. Excess calcium is taken up by mitochondria elevating mitochondrial nitric oxide synthase (NOS) which compete for O₂ radical forming peroxynitrite (PON). PON derived radicals partially inhibit calpain initially but then, sustained oxidative stress potentiate cellular damage outweighs the initial calpain inhibition causing further cytoskeletal degradation. Reproduced from Xiong et al., 2007 with permission.

1.1.7 Role of TNF alpha following SCI:

Tumor necrosis factor alpha (TNF- α) is one of the most well characterized cytokines. However, to date there is no obvious agreement or consensus on its role in acute CNS injury (Oyinbo, 2011). Its levels are elevated following SCI reaching its peak within one hour after primary injury (Dinomias et al., 2009). Most studies suggest that its production at the site of injury exacerbates tissue damage in SCI (Pan et al. 2003, Paterniti et al. 2009). TNF α potentiates Wallerian degeneration of axons and activates recruitment of

macrophages and Schwann cells at the injury site (Sebastian and Schroeter 2003). It also increases the level of hyperalgesia and central sensitization through stimulation of synaptic transmission (Kawasaki et al., 2008).

Yakovlev and Faden showed that TNF- α mRNA levels were elevated 30 minutes following SCI at the injury site and that the extent of injury was proportional to the level of TNF- α message (Yakovlev and Faden, 1994). Paterniti et al. demonstrated that elevation of TNF α in a spinal cord trauma model which was accompanied by an elevation in myeloperoxidase activity (MPO) – a marker for polymorphonuclear leukocyte accumulation 24 hours post injury (Paterniti et al. 2009).

1.1.8 Current Interventions in Spinal Cord Injury:

As the primary injury to the spinal cord cannot be prevented it can only be minimized through surgical intervention by means of surgical decompression or stabilization of the vertebrae. Currently there are no generalized standards of care regarding decompression surgery regarding indications and timing of interventions (Li & Walker et al., 2014). Many questions remain unanswered in this regards, as the optimal timing of intervention to gain neuroprotective effect or even if the intervention itself is recommended or not (Silva et al., 2014).

Many strategies have been developed to minimize the extent of secondary injury, and enhance the therapeutic outcomes. Methylprednisolone is the most widely prescribed pharmacological agent for SCI yet it is the most controversial. It has many advantages as its ability to inhibit lipid peroxidation, limit inflammatory response, sustain the blood-spinal cord barrier and enhances blood flow (Tator, 1998). Other approaches include altering neuro-inflammation by means of immune-modulators such as minocycline (Schwartz and Yoles 2006) or promoting M2 macrophage phenotype through administration of granulocyte colony stimulating factor (G-CSF) within 72h following SCI (Guo et al., 2013).

Other mechanisms were adopted to enhance regeneration and myelination following SCI via creating a more suitable environment for axonal regeneration (Thuret et al. 2006). Nogo-A suppression (Freund et al., 2006), stem cells (Webb et al., 2010), and human embryonic stem cells (hESC) derived oligodendrocyte progenitor cells (OPCs) transplantation (Keirstead et al., 2005). Using Neurotrophins (Sharma, 2007), Autologous Activated Macrophages (Bomstein et al., 2003), reducing neuro-inflammation (Bracken 2012, Das et al., 2011), preventing free radical damage (Bains and Hall, 2012), and excitotoxicity (Mazzone and Nistri, 2011), and improving blood flow (Ritz et al., 2010), as well as targeting the local immune response (Varma et al., 2013), have all been used following injury with great variability in improving motor function, decreasing lesion volume, enhancing axonal regeneration and myelination.

Very few studies were advanced to clinical trial stage, e.g. the Sygen Multi Center Acute SCI study, which used variable doses (200, 300, and 600 mg IV) of monosialotetrahexosylganglioside GM-1 for 56 days. This study did not show a significant clinical efficacy of GM-1 but proposed a trend in favor of (GM-1) regarding American Spinal Injury Association (ASIA) motor, pin prick and light touch scores, contribution to neurological recovery and enhancing bowel / bladder function and improving sacral sensation as well (Chinnock and Roberts, 2005; Geisler et al., 2001). Rho antagonist (BIOAXONE BIOSCIENCES INC, Cethrin®) is another example for a clinically tested drug in SCI that was found to enhance neuronal growth (Dergham et al., 2002). Thus more research and clinical trials are needed to validate the benefit and efficacy of current proposed treatments for SCI.

1.1.9 SCI Models

Models of SCI aim at simulating human SCI injuries as much as possible (Cheriyian et al., 2014). The following are some examples of these models shown in figure 8.

Compression and Contusion models: It induces acute transient SCI via weight drop apparatus, air gun devices, or electromagnetic impactors (Cheriyian et al., 2014). This

model is clinically relevant as most of human SCI are caused by rapid movements, bone fractures and consequent impact of bone fragments (Allen, 1911). It is characterized by rostral and caudal spread of tissue damage from lesion epicenter, disruption of white matter axons and cell death of grey matter neurons, intra-parenchymal hemorrhage, glial activation, macrophage recruitment and oligodendrocyte apoptosis (Anwar et al., 2016). In some cases contusion is followed by sustained compression of the spinal cord. Experimentally this is simulated through clip compression (Rivlin and Tator 1978), balloon compression (Vanický et al., 2001) or calibrated forceps compression (Plemel et al., 2008). It induces vascular derangements, ischemia and hemorrhagic necrosis (Anwar et al., 2016).

Distraction model: it entails stretching of the spinal cord to induce tension forces that mechanically tear the spinal tissues leading to SCI (Silberstein and McLean 1994).

Dislocation: Involves displacement of the vertebra causing trauma to the cord which leads to astrocytic activation, vascular impairment in grey matter and apoptosis (Anwar et al., 2016). No surgical exposure is needed for this type of SCI model; however, more research is required to validate its reproducibility (Cheriyian et al., 2014).

Transection models: Complete or partial transaction is not regarded as clinically pertinent models; however, they have been vastly used to study neuronal outcomes following injury (Chriyan et al., 2014). These models are useful in studying axonal degeneration and regeneration following SCI (Anwar et al., 2016).

Chemical model: Such as injection of phospholipase A2 to induce inflammation (Liu et al., 2006), or inducing oxidative damage through injection of hydroxyl peroxynitrite into the rat's gray matter. (Bao et al., 2003)

Ischemia reperfusion injury: induced by aortic cross clamping causing neuronal and vascular damage, it is regarded as a non-reproducible model (Anwar et al., 2016).

Spinal Cord Injury

Distraction: Stretching of cord

Compression of cord: Burst fractures

Dislocation: Displacement of vertebra

Contusion: Displacement and damage of cord

Ischemia-reperfusion injury: aortic cross-clamp

Transection of the T4 spinal cord: Partial or complete separation

Collagenase (intraspinal hemorrhage): Neuronal damage, neutrophil infiltration and ED 1- positive macrophages/microglia

Figure 8 : Different models of rat SCI. . Reproduced from Anwar et al., 2016 with Permission.

1.2 Curcumin

Phytodrugs represent an important approach in CNS disorders due to their neuroprotective abilities. Many plants' raw extracts or their pure isolated compounds have demonstrated effective neuroprotective properties in neurodegenerative diseases (Pérez-Hernández et al., 2016). Silymarin administration (400mg/kg/day for 3 days) increased glutathione and superoxide dismutase (SOD) levels in aged rats brains (Galhardi et al., 2009). While daily doses (15 mg/kg) of Vincamine in rats reduced iron levels by 50% thus reducing oxidative stress resulting from iron deposition in neurodegenerative diseases (Fayed, 2010).

Resveratrol a Mediterranean traditional phytophenol present in grapes has also shown neuroprotective traits. In focal cerebral ischemia model resveratrol significantly minimized infarction size suggesting a potent neuroprotective effect due to its antioxidant, anti-platelet and vasodilating effect in focal cerebral ischemia model (Huang et al., 2001).

Gingerol, a compound of Ginger which is extracted from the root of *Zingiber officinale* was shown to inhibit LPS induced cyclooxygenase 2 (COX-2) level of expression in U937 cells subjected to lipopolysaccharides, thus suggesting a role as an anti-inflammatory inhibiting prostaglandin E2 (PGE2) (Lantz et al., 2007).

Curcumin was first isolated by Vogel and Pelletier in 1815 in an impure form, later on both its chemical structure and synthesis were confirmed in 1910 and 1913 by Lampe et al. The first study for its use in human disease was published in 1937 (Oppenheimer, 1937). Throughout the last 60 years, over 3000 studies have described its variable biological activities as an antioxidant, antifungal, antibacterial anti-proliferative, pro-apoptotic, antiviral and other activities, consequently exhibiting medicinal benefit in neurodegenerative diseases, cardiovascular diseases, diabetes, psoriasis and lung fibrosis and other diseases (Aggarwal and Harikumar, 2009). This pleiotropic nature of curcumin has been attributed to its ability to interact with multiple targets including inflammatory cytokines, growth factors, transcription factors, apoptosis related proteins, adhesion molecules, kinases and others (Zhou et al., 2011).

Curcumin is a bis α , β unsaturated β diketone. Curcumin is one of turmeric constituents. Turmeric powder consists of 4% curcuminoids, 70% carbohydrates, 6% proteins, 5% resins, 4% fat and 10% moisture as shown in figure 9. Curcumin has also been used over the past centuries in Ayurveda or Ayurvedic medicine where it showed a great therapeutic efficacy in different respiratory conditions, liver disorders, diabetic wounds, anorexia and rheumatism (Araujo and Leon 2001). In Chinese medicine it has been used for abdominal pain (Aggarwal, Takada & Oommen, 2004) while in Hindu medicine it was applied for swellings and sprains (Araujo and Leon 2001). In the Orient it was mainly used as an anti-inflammatory (Aggarawal et al. 2003).

Many other therapeutic effects of Curcumin have been documented, such as its role as an antioxidant (Liu et al., 2013; CONEAC et al., 2017), anti-inflammatory (Machova et al., 2015; Gokce et al., 2016), hepatoprotective (Palipoch et al., 2014), anticarcinogenic (Rao

et al. 1995), hypoglycemic (Chuengsamarn et al., 2012), renoprotective (Ueki et al., 2013), in cardiovascular diseases (Ahuja et al., 2011) and anti-arthritis (Dcodhar et al. 2013).

Studies show that Curcumin can promote neurogenesis in the adult hippocampus (Kim et al. 2008), inhibition of astrocyte expression, and preventing death of hippocampal cells by Kainic acid (Shin et al. 2007). Curcumin is characterized by a good safety profile demonstrated in animal (Qureshi et al. 1992) or human studies (Lao et al. 2006).

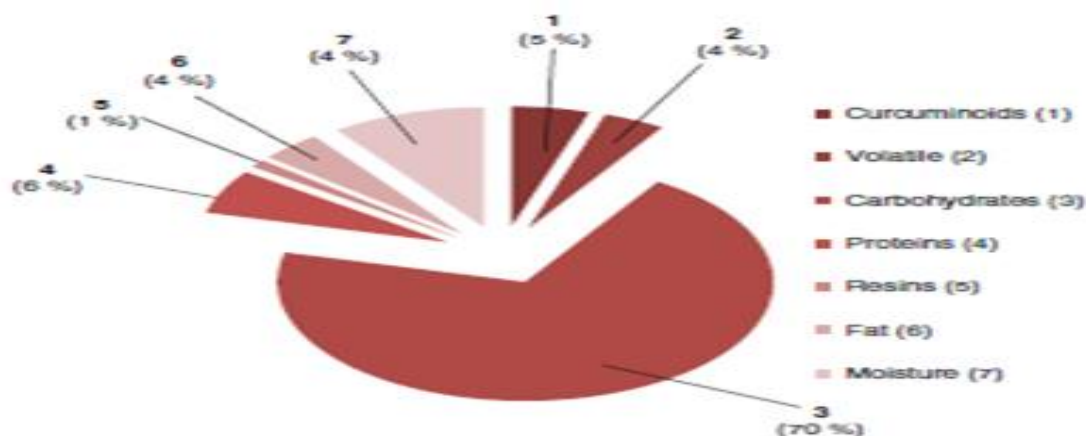


Figure 9 : Composition of turmeric powder. . Curcuminoids constitute about 5 % of turmeric components while the major constituent of turmeric is carbohydrates (70%) Reproduced from Farooqui 2013 with Permission.

1.2.1 Curcumin Structure:

The main constituents of commercially available curcumin are; Curcumin I (77%), Curcumin II called demethoxycurcumin (17%) and Curcumin III called bisdemethoxycurcumin (3%) as shown in figure 10, where these curcuminoids collectively are found in 3-5% of Turmeric (Goel et al. 2008). This yellow complex has multiple names, e.g. yellow ginger, Indian saffron, yellow root or kacha haldi.

Curcumin powder is soluble in organic solvents as dimethyl sulfoxide, ethanol and acetone but insoluble in water or ether, it has a melting point of 183°C, a molecular weight of 368.37 g/mol and a molecular formula of $C_{21}H_{20}O_6$ (Goel et al. 2008).

Curcumin is more stable at acidic PH while unstable at basic and neutral PH where it is degraded to ferulic acid and feruloylmethane .If placed in phosphate buffer systems of PH 7.2, it is degraded rapidly within 30 minutes (Wang et al., 1997).

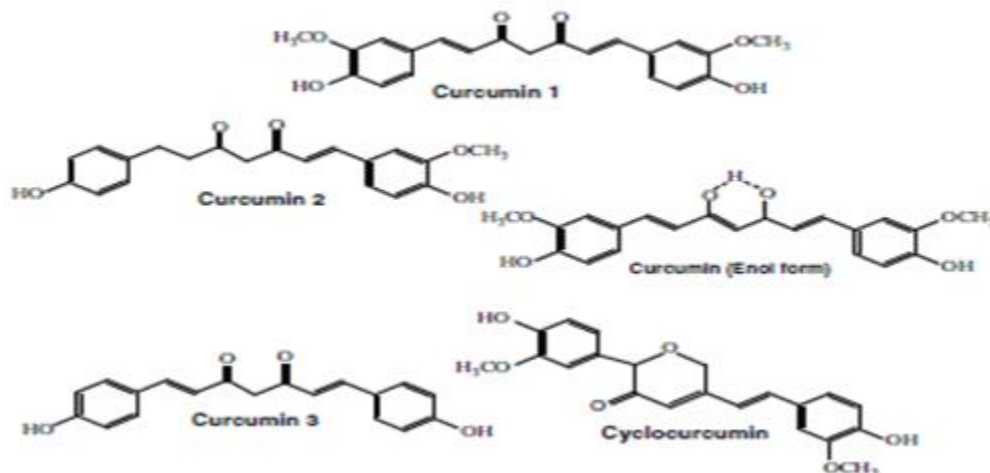


Figure 10 : Different chemical structures of Curcuminoids. Curcumin I compose about (77%) of the total curcuminoids, Curcumin II (demethoxycurcumin) and Curcumin III (bisdemethoxycurcumin) are present in smaller ratios. Reproduced from Farooqui 2013 with permission.

Curcumin degradation is slow at PH 1-6 which mimics the stomach PH. While one of the major Curcumin metabolites called tetrahydrocurcumin is more stable at neutral or basic PH (Pan et al. 1999), keeping its antioxidant abilities (Somparn et al., 2007).

Curcumin has shown to exhibit poor bioavailability for three main reasons: its poor absorption, rapid metabolism in the liver and rapid systemic elimination due to its short half-life (Bisht and Maitra 2009). Curcumin has shown to be highly tolerable. Phase 1 and 2 clinical trials of Curcumin have been carried out on patients with advanced colorectal cancer for 4 months at variable doses (500, to 12000 mg/day) showed no toxicity (Cheng et al. 2001). It showed that the serum concentration peaks at 1-2 hours following oral intake of Curcumin, followed by gradual decline within 12 hours (Jiao et al., 2009).

Curcumin metabolites do not have the same power or activity as the parent molecule where it has been reported that the reduced or conjugated forms of Curcumin do not have the same inhibitory effect on inflammatory enzymes expressed in human colon cells culture (Ireson et al. 2001).

To make the best use of its therapeutic properties, many approaches have been proposed to enhance Curcumin's bioavailability, e.g. using an adjuvant as Piperine which interferes with the process of glucuronidation in the liver and intestinal metabolism, and complexing Curcumin with phospholipids. (Indena, Meriva®) - A phosphatidylcholine-Curcumin complex- showed plasma concentration and area under the curve (AUC) were 5 times higher than free Curcumin (Marczylo et al., 2007), hence showing an enhanced bioavailability pattern.

Also, preparing Curcumin liposomes and nanoparticles have been reported to remarkably increase free Curcumin's bioavailability (Gota et al., 2010). Where mean peak plasma concentration of 650 mg of solid lipid Curcumin nanoparticle (SLCP) was 22.43 ng/ml while the same quantity of the unformulated Curcuminoid extract (95%) was undetectable; however, the study reported remarkable inter-individual variability in the two tested groups proposing complex absorption kinetics (Gota et al., 2010).

1.2.2 Curcumin as an Antioxidant and Anti-Inflammatory:

Curcumin is a highly pleiotropic molecule that affects variable molecular targets involved in neuro-inflammation, oxidative stress and neuroplasticity (Farooqui 2010). Curcumin possesses anti-inflammatory and anti-oxidative powers by downregulating enzymes as lipoxygenase (LOX), COX2 and inducible nitric oxide synthase (iNOS), these enzymes enhance the generation of several inflammatory mediators as leukotrienes, cytokines and TNF α (Farooqui et al. 2013).

Curcumin was shown to protect biological membranes including neural membranes against peroxidative damage, due to its ability to scavenge free radicals resulting from peroxidation (Menon and Sudheer, 2007). This unique ability is due to the presence of functional groups including carbon-carbon double bonds, β diketo group and phenyl rings to which hydroxyl and methoxy groups are attached (Menon and Sudheer, 2007; Indira Priyadarsini et al., 2003). This remarkable antioxidant capability is comparable to Vitamin E (Nimse et al., 2015)

A study was performed to evaluate the antioxidant potential of Curcumin in rat SCI model demonstrated that SOD level was higher in the Curcumin group compared with MP group. Malondialdehyde (MDA) exhibited the lowest levels in Curcumin group. It was concluded that Curcumin exhibits an antioxidant capacity against oxidative damage following SCI (Kavakli et al., 2011)

Neuro-inflammation is a natural defense mechanism aiming at isolating the injured tissue from the healthy tissue; unfortunately it might cause the destruction of the injured and healthy tissues (Correale and villa 2004). Upon CNS injury, the main mediators of inflammation are astrocytes and microglial cells. Upon injury, these cells enhance the rapid release of a number of cytokines/ chemokines which degrade the neural membrane, help release arachidonic acid and lyso-phosphatidylcholine (Farooqui 2013). Arachidonic acid is consequently oxidized to the pro-inflammatory eicosanoids as prostaglandins, leukotrienes, and thromboxanes by the help of COX and LOX. On the other hand, lysophosphatidylcholine enhances the synthesis of the pro-inflammatory platelet activating factor. Thus this cascade of events intensifies an aggressive neuro-inflammatory process (Farooqui 2013).

Inflammation is finally terminated by a process of resolution through the release of anti-inflammatory mediators as IL-10, resolvins, protectins and lipoxins (Farooqui 2010) and

this process is a turning-off mechanism held by neural cells for the purpose of limiting tissue injury (Farooqui 2013).

Curcumin has displayed a remarkable anti-inflammatory potential. It decreases linoleic acid conversion to arachidonic acid (Farooqui 2013). It also blocks the ability of macrophages to uptake arachidonic acid therefore limiting its ability for eicosanoids production. Curcumin can also inhibit cyclooxygenases and lipoxygenases and thus inhibit prostaglandins and leukotrienes synthesis (Rajasekaran, 2011).

Previous studies also demonstrated that dietary Curcumin decreased phospholipase A2, phospholipase C and prostaglandin E2. Moreover products of the lipoxygenase and cyclooxygenase as prostaglandin E2 and 15 (S) hydroxyeicosatetraenoic acid from arachidonic acid was reduced in colonic mucosa and tumors in Curcumin fed animals versus control group (Rao et al., 1995).

Curcumin also showed the ability to decrease COX at the transcriptional level. A study by the American Association for cancer research (AACR) on human colon cancer cells concluded that Curcumin reduced COX-2 expression on the mRNA level in a time and dose dependent manner and inhibited cell growth as well (Goel et al. 2001).

1.2.3 Molecular Targets of Curcumin:

Curcumin has proved to be a highly pleiotropic molecule that can interact with multiple molecular targets. Curcumin can bind directly to these targets or act indirectly through modulation of their activities (Zhou et al., 2011).

From the proteins that act directly with Curcumin: DNA polymerase (Takeuchi et al. 2006), lipoxygenase (Skrzypczak et al. 2003) and tubulin (Gupta et al. 2006). It has been proven

that Curcumin can bind to different divalent metal ions as well as Fe, Cu, Mn and Zn (Ishihara and Sakagami 2005).

Curcumin might activate or attenuate the activity of different transcription factors depending on the particular target. For instance, a Curcumin analogue has shown an ability to suppress the activity of nuclear factor kappa B (NF- κ B) by inhibiting its nuclear translocation in Mouse RAW 264.7 and its downstream inflammatory cytokines as tumor TNF α , Interleukin 1 β (IL-1 β) and interleukin 6 (IL-6) in macrophage cells and it was found to be even more potent than Curcumin (Olivera et al., 2012). Therefore, inhibiting the activation of many genes responsible for proliferation and cell survival as Cyclin D1, IL-6, COX-2 and matrix metalloproteinase (MMP 9) , thus arresting the cell cycle, induces apoptosis and inhibiting cell proliferation (Aggarwal & Takada et al., 2004; Wang et al., 2015).

Curcumin loaded solid-lipid nanoparticles reduced the expression of pro-inflammatory cytokines in serum as TNF- α , IL-6 and IL-1 β , increased expression of anti-inflammatory IL-10 cytokine (Wang et al., 2015).

In a study that aimed at revealing the key players behind the anti-inflammatory mechanism of Curcumin, Curcumin suppressed COX-2, inducible nitric oxide synthase (iNOS) mediators of inflammation. The study suggested that this effect was in part due to Janus Kinase/Signal Transducer and Activator of Transcription (JAK-STAT) inflammatory cascade inhibition in the brain microglial cells (Kim et al., 2003). Curcumin also suppressed (STAT-3) phosphorylation significantly and showed anti-proliferative effect assessed by Cyclin D1 in lung adenocarcinoma cells (Alexandrow et al., 2012).

Peroxisome proliferator- activated receptor gamma (PPAR- γ) is a member of a large and diverse group of proteins factors called the nuclear receptor family of transcription factors. It regulates different functions as inflammation, apoptosis, cell differentiation and lipid

metabolism (Farooqui 2013). Its main role is to downregulate macrophage, microglial activation and cytokine expression by inhibiting the activity of some transcription factors as NF- κ B, activator protein 1 (AP-1) and STAT proteins (Ricote et al. 1998; Aggarawal et al., 2007). Xu et al have shown that Curcumin induces PPAR- γ gene expression in activated hepatic stellate cells (HSCs) (Xu et al., 2003). In another study, Curcumin activated PPAR- γ cells inhibited Moser cells (human colon cancer cell line) and could suppress cyclin D1 and epidermal growth factor receptor (EGFR) gene expression (Chen and Xu 2005).

The limited regenerative ability of the injured axons following damage or injury to the brain and spinal cord in the adult mammalian CNS is due to the inhibitory effect of various neurite outgrowth inhibitors at the injury site consequently impairing axonal regeneration and causing functional deficits (Kohta et al., 2009). Besides the myelin associated inhibitory molecules, extracellular matrix molecules also play a growth impeding role as well. Astrocytic activation and the release of extracellular matrix inhibitory molecules as CSPGs (Kohta et al., 2009), causes further impediment to axonal regeneration (Yamashita et al., 2005; Yiu and He, 2006).

Zheng and Chen concluded that Curcumin could enhance PPAR- γ activity in HSCs thus inducing apoptosis and suppressing cell proliferation and extracellular matrix (ECM) gene expression. They also concluded that PPAR- γ activation by Curcumin could interrupt transforming growth factor (TGF- β) pathway leading to the inhibition of connective tissue growth factor (CTGF) expression which is originally responsible for ECM overproduction (Zheng and Chen, 2006). Yuan et al. also demonstrated a correlation between TGF- β inhibition by Curcumin and decreasing the deposition of CSPG of the extracellular matrix, reducing glial scar formation and enhancing innervations in SCI (Yuan et al. 2015), this renders the environment more suitable for axonal regeneration following injury.

Nuclear factor E2 related factor-2 (Nrf2) is a transcription factor that controls basal levels of expression of a number of antioxidant genes as well as enhancing the expression and upregulation of a number of detoxifying, antioxidant and cytoprotective genes that reduce tissue injury (Element 2004). Moreover, it is proposed that Nuclear Factor E2 Related Factor-2/ Antioxidant Response Element (Nrf2/ ARE) activation within the astrocytes enhances neuroprotection to neurons through mediating the expression of genes essential for maintaining redox potential and scavenging of free radicals (Kraft et al. 2004).

Curcumin leads to increasing Nrf-2 level thus enhancing its binding to ARE and increasing the expression of heme – oxygenase 1 (HO-1) in both neural and non-neural cells through activating UDP-glucuronosyltransferase (UGT) which is responsible for detoxification of many toxins present in our daily diet where HO-1 plays a pivotal role in cytoprotection from toxins of either endogenous or exogenous origin thus enhancing neuroprotection (Hatcher et al. 2008; Scapagnini et al., 2011).

Growth factors together with their receptors play a crucial role in the normal differentiation and growth process. Any irregularities in these molecules function or expression can cause abnormalities in development which might lead to malignant transformation (Witsch et al., 2010). Curcumin can regulate the expression of these growth factors thus demonstrating anti-invasive, anti-proliferative and anti-angiogenic effects (Zhou et al., 2011).

The epidermal growth factor receptor (EGFR, ErbB, and Her 1 in humans) is an integral protein kinase of the plasma membrane that comprises multiple autophosphorylation sites (Scaltriti and Baselga 2006). It was recently demonstrated that EGFR inhibition blocked pro-inflammatory cytokine secretion from reactive astrocytes and inhibited reactive astrogliosis as well in a scratch wound model in vitro, and reduced neuronal loss and demyelination which was concurrent with improvements in hind limb motor function and in bladder function as well in SCI rat model, thus proposing that EGFR inhibition might be a promising potential after CNS injury (Li & Li et al., 2014). Thus, if Curcumin has an

inhibitory effect on EGFR, this might provide a new mechanism of how Curcumin can impede the undesirable effects of reactive astrogliosis and enhance axonal regeneration.

In human astroglioma cell lines, Curcumin strongly suppressed phorbol 12-myristate 13-acetate (PMA) induced phosphorylation of extracellular signal-regulated kinases (ERK) , c-Jun N-terminal kinase (JNK) and p38 mitogen activated protein Kinase (MAPK) thus suppressing MMP-9 enzymatic activity, mRNA and protein level as well as shown in figure 11 (Woo et al.,2005). This suggests that Curcumin can suppress AP-1 and NF- κ B through inhibiting MAPKs pathway consequently adding to Curcumin's potent anti-cancer and anti-inflammatory effects (Zhou et al. 2011).

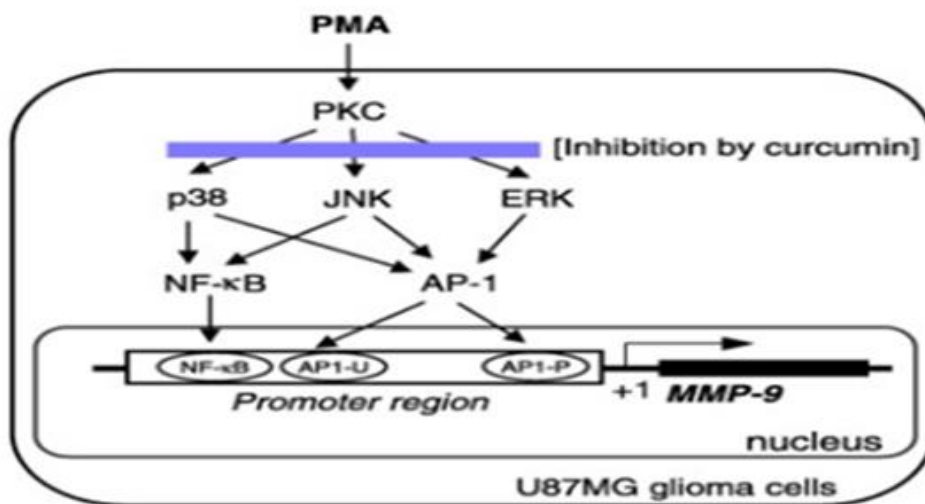


Figure 11 : Curcumin effect on MAPK signaling pathway in human astroglioma cells. The figure shows MMP-9 signaling pathways in PMA treated U87MG cells. Curcumin inhibits (PMA) induced phosphorylation of ERK, JNK and p38 MAP kinase thus suppressing (MMP-9) enzymatic activity, mRNA and protein level, Reproduced from Woo et al., 2005 with permission.

Pro-inflammatory cytokines as TNF- α , IL-1 β and IL-6 are molecules produced excessively after severe injury or infection and they participate significantly in the development of systemic and local inflammation which might result in organ failure (Munford and Pugin 2001). The gene and protein cytokine expression is tightly regulated in the producing cells,

and one of the critical steps in this regulation is gene transcription, thus manipulating and regulating some of the transcription factors as NF- κ B is a suggested strategy in controlling the inflammatory response (Baeuerle and Henkel 1994). Several research groups have demonstrated the ability of Curcumin to act as a potent anti-inflammatory by modulation of various inflammatory cytokines production (Chen et al., 2008). Both in vitro and in vivo studies have shown that Curcumin has a profound inhibitory effect on TNF- α production (Zhou et al., 2011). Curcumin prevented the release of pro-inflammatory cytokines, PGE2 and nitric oxide (NO) in a dose dependent manner. Curcumin also attenuated inducible NO synthase and cyclooxygenase-2 expression on mRNA and protein levels (Park, 2007).

Another group of inflammatory cytokines are the interleukins which play a crucial role in the regulation of the inflammatory response and in the signaling pathways as NF- κ B and STATs which are involved in angiogenesis and tumor invasion (Dinarello, 2006). In an experiment done on TNF- α treated cells, Curcumin could attenuate expression of TNF α induced interleukin 1 β (IL-1 β) and interleukin 6 (IL-6) by inhibiting activation of NF- κ B and MAPKs pathway (JNK, p38, MAPK, ERK) (Cho et al., 2007). A summary of Curcumin various targets is highlighted in figure 12.

Curcumin is able to form chelates with transition metals due to the presence β unsaturated β -diketone moiety. Metal chelates of Curcumin with Cu²⁺, Fe²⁺, Mn²⁺, Pb³⁺ Have been reported (Gupta et al., 2011). A study showed that Curcumin mononuclear complex with copper has anti-oxidant properties superior to Curcumin. The complex protected the cells against superoxide dismutase and glutathione peroxidase initially induced by radiation (Kunwar et al., 2007). Manganese complexes with Curcumin and its derivatives showed a potential capacity of protecting brain lipids from peroxidation (Sumanont et al., 2004).

Curcumin was found to bind to DNA via guanine and adenine N7 (major groove) and thymine O2 (minor groove) and to the phosphate (PO2O backbone as well. Curcumin binding to RNA was through H-bond to uracil O2 besides guanine and adenine N7 binding

as well as to the phosphate backbone (Nafisi et al., 2009). Further investigation is needed to explore the outcomes of Curcumin DNA binding especially regarding its noticeable nuclear localization and presence in tumor cells (Gupta et al., 2011).

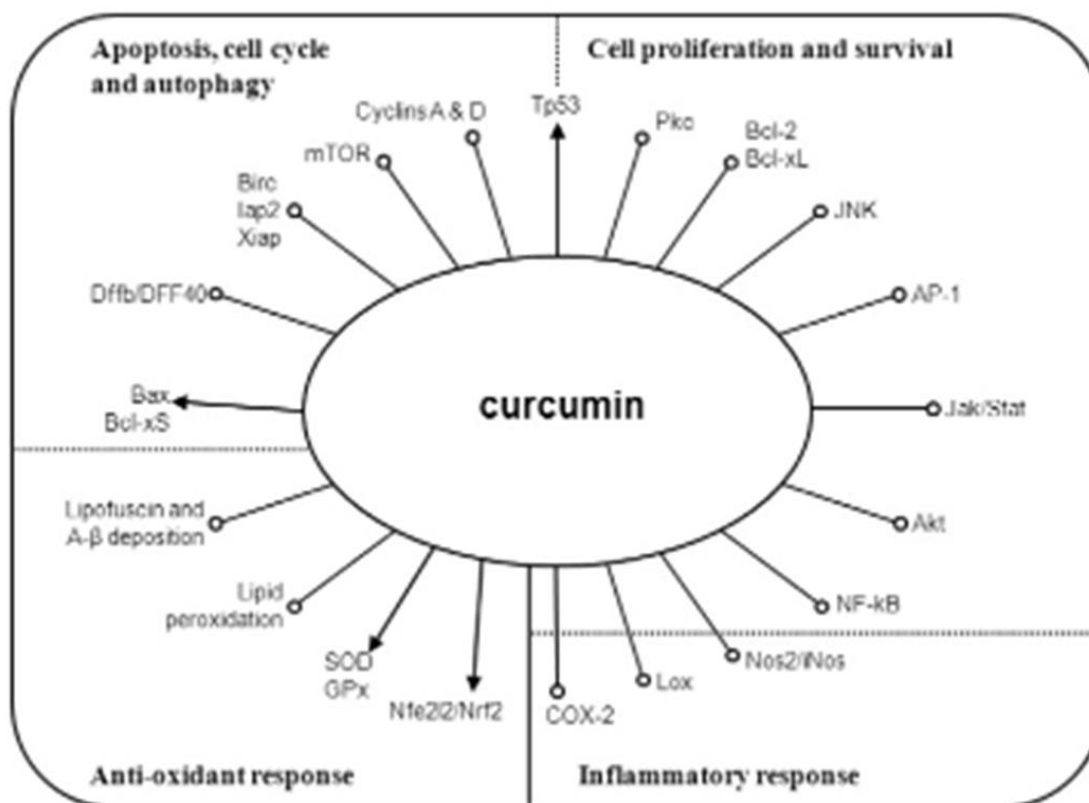


Figure 12 : Molecular targets of Curcumin. Curcumin can either upregulate or down regulate various molecular targets as Nrf2 and NF-kB respectively inducing a number of biological effects as antioxidant and anti-inflammatory. It also targets a number of cytokines, enzymes and growth factors affecting several biological processes. Reproduced from Shen et al., 2013 with permission

The role of Curcumin with DNA and other molecules still looks confusing and definitely more investigation in this arena is a necessity to clarify exactly the risk benefit ratio of Curcumin as a therapeutic agent either alone or in combination.

STUDY OBJECTIVES

The aim of this study is to investigate the role of Curcumin on the inflammatory and oxidative components of the secondary injury cascade following primary SCI in a rat model. We specifically aimed at comparing the effect of local application of Curcumin directly on the injury site with that of Dietary Curcumin supplement, by comparing the change in oxidative stress and inflammatory markers before and after Curcumin treatment.

CHAPTER 2: Materials and Methods

2.1 Establishment of SCI model:

A randomized controlled animal study using Female Sprague Dawley rats weighing approximately 150gm-200 gm were purchased from Ain Shams University, Cairo. The animals were allowed free access to food and water before surgical procedures. All experiments were performed according to the NIH (PHS Policy on Humane Care and Use of Laboratory Animals, 2015 (US Department of Health and Human Services 2015) and international guiding principles for biomedical research involving animals December 2012. All rats were anesthetized with ketamine (75 mg/kg) intraperitoneally. After shaving and cleaning the skin with Betadine, skin and muscle incision was made over the mid-dorsal section of the back. Laminectomy was performed at T9-T10 thoracic vertebral levels. The dura was incised longitudinally and spinal cord injury was performed by right side lateral hemisection using micro-iris scissors at T9-T10 level, Tail wagging reflex and hindlimb retraction proposed successful model establishment. Muscles and skin were then sutured in layers. All animals received analgesia (Voltaren 15 mg/kg) and local with systemic antibiotics (10 mg/kg) (Nair et al., 2016) were given post injury to prevent either surgical or urinary tract infections. Bladder evacuation was performed as needed during the experiment.

Two time points were investigated at our study and animals were grouped as follows::

- Control group n=15 normal rats and n=15 sham surgery rats (skin and muscle incision with no injury to the cord).
- Two Injury groups n=15 (at each time point) with lateral hemisection level at days 1 and 7 (SCI 1d and SCI 7d)
- Two local treatment groups n=15 (at each time point) with Curcumin (*Curcuma longa* extract, Herb Pharm, Williams, OR) 200 mg/kg single dose (Yuan et al., 2015) at the injury site immediately after injury (SCI+CUR 1d and SCI+CUR 7d)

- Dietary supplement group n=15 (SCI+ Diet 7d) where crude Curcumin (Imtinan, Egypt) was mixed with diet for one week before and one week after injury as 10% of daily feed.

At the time of analysis, an average of 4-6 rats were used for each marker where rats were sacrificed with IP ketamine overdose after 1 day or 7 days for sample collection, the spinal cord was exposed at the injury site where a segment including the injured area was removed and properly preserved.

2.2. Oxidative Stress Parameters:

2.2.1 Malondialdehyde test by Thiobarbituric Acid Assay

Malondialdehyde (MDA) is an end product of lipid peroxidation. The level of Malondialdehyde was determined using Thiobarbituric acid (TBA) assay (BIODIAGNOSTIC). TBA reacts with Malondialdehyde (MDA) at 95°C for 30 minutes forming a colored reactive product that can be measured.

Spinal cord samples were homogenized in ice cold Phosphate buffered saline (PBS, pH 7.4) supplemented with protease cocktail inhibitor (Sigma-Aldrich). Samples were centrifuged at 10000 g for 15 minutes at 4°C. The supernatant was then removed to be used in the assay at -80 °C. Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) at 95°C for 30 minutes forming a reactive product of a pink color that can be measured at 534 nm. so samples were incubated with TBA for 30 minutes in boiling water and absorbance was measured at 534 nm. Samples were run in duplicates. MDA is expressed as nmol/gram wet tissue.

2.2.2 Total Antioxidant Capacity:

The total antioxidant capacity in spinal cord samples (TAC) was determined using colorimetric assays (BIODIAGNOSTIC). This test is used as a measure of the ability of antioxidant systems in a sample to buffer increased reactive oxygen species (ROS).

The principle of the test is that exogenous H_2O_2 is added where a part of it is removed by the power of the antioxidants in the sample and the residual amount is determined in a colorimetric manner through an enzymatic reaction that converts 3,5-dichloro-2-hydroxybenzene sulphonate into a colored product.

Spinal cord tissue samples were homogenized in ice cold Phosphate buffered saline (PBS, pH 7.4) supplemented with protease cocktail inhibitor (Sigma-Aldrich). On performing the assay samples were centrifuged at 10000 g for 15 minutes at 4°C. The supernatant was then removed to be used in the assay at -80 °C. Samples were run in duplicates. TAC is expressed as mM/L. TAC and MDA assays were analyzed at Cairo University Research Park, Faculty of Agriculture, Cairo University using STAT LAB SZSL60-Spectrum.

2.3 Inflammatory Markers Testing by Enzyme Linked Immunosorbent Assay (ELISA):

Spinal cord samples (including the injury epicenter) were homogenized using ice cold PBS, PH7.4 supplemented with protease cocktail inhibitor (Sigma -Aldrich) and centrifuged at 10000 g for 15 minutes at 4°C. The supernatant was then removed to be evaluated for TNF- α enzyme linked immunosorbent (ELISA) assay kit (WKEA MED SUPPLIES CORP, New York, USA), and IL-6 (R&D systems, R6000B) according to the manufacturer's guidelines.

Briefly, the microplate is coated with an antibody specific to TNF α or IL-6 Standard dilutions were prepared according to the manufacturer's guidelines, samples were diluted 1:5 using calibrator diluent, wells were washed and conjugate (HRP enzyme catalyzed conjugate) was added followed by incubation. Finally substrate solution was added and incubated followed by stop solution where OD readings were determined. All samples were run in duplicates. Finally, the concentrations of the samples were calculated from the constructed standard curve represented as Pg/ml.

2.4 Neurological evaluation

In our study, a neurological assessment using modified Tarlov Criteria (Shi et al., 2010) was performed. The criteria were as follows: 0 (no movement), 1 (minimal movement), 2 (good movement), 3 (stand and walk but cannot hop) and 4 (normal). A behavioral assessment using the grid walk (sensorimotor test) and the inclined plane test (motor test) was also conducted by 7 days treatment compared to simple injury group.

2.5 Histological Staining

Following anesthesia, rats were sacrificed and the spinal cord sections surrounding the injury epicenter were removed, dehydrated, embedded in paraffin and cryosectioned into 5 μm thick sections. Pathologic changes of the spinal cord were detected using hematoxylin and eosin staining. Histological examination of spinal cord sections for local treatment and dietary treatment groups to be completed.

2.6. Statistical Analysis:

All results were tabulated and statistical analysis was performed using IBM SPSS program (SPSS Inc., Chicago, IL, USA) , version 20, and all graphs were done using Microsoft Excel 2010. Quantitative data was represented as Mean \pm Standard Deviation.

One-way ANOVA test was used to compare means of quantitative variables. If ANOVA test was positive, Dunnett post Hoc test was used to identify the variable that made the significant change. Independent t-test was used to compare means of quantitative variables within SCI and SCI+CUR groups. All p -values are two-sided, and difference was considered statistically significant if $p \leq 0.05$ (95% confidence interval).

CHAPTER 3: RESULTS

3.1 Effect of Local and Dietary Curcumin on Oxidative Stress Markers in Spinal Cord Tissue

To study the effects of Curcumin on oxidative damage following SCI, we measured MDA and TAC levels in spinal cord tissue at 1 and 7 days post injury after local treatment (200 mg/kg) with *Curcuma longa* extract (Herb pharm, Williams, Oregon). For the dietary supplement with crude Curcumin MDA and TAC levels were measured only at 7 days post injury.

In this section results are demonstrated for:

- 1 day after SCI: Control group, Injury (SCI group) and Local treatment group (SCI+CUR).
- 7 days after SCI: Control group, Injury (SCI group), Local treatment group (SCI+CUR) and Diet Supplement group (SCI +Diet).

Groups will be referred to as Control, injury (SCI), local treatment (SCI+CUR) and Dietary supplement (SCI + Diet).

3.1.1 Local Curcumin Significantly Decreases MDA Levels 1 Day after SCI

Malondialdehyde (MDA) was measured in spinal cord tissue samples in all groups; control, injury at 1 and 7 days (SCI 1 day and 7 days), local treatment (200 mg/kg SCI + CUR 1 day and 7 days), and Dietary supplement group (SCI+ Diet) using Thiobarbituric Acid assay (TBA) assay and expressed as nmol/g wet tissue. As shown in figure 13, 1 day after SCI, MDA levels in SCI group were significantly elevated compared to control group ($P < 0.01$). Following local treatment (SCI+CUR), MDA levels were significantly lower when compared to SCI group $p < 0.05$. Tables are represented in appendix.

3.1.2 Local Curcumin Significantly Decreases MDA levels 7 Days after SCI

At day 7 following local treatment (SCI+CUR), MDA levels were significantly lowered when compared to SCI $p < 0.05$. However, diet supplement (SCI +Diet) did not induce a significant reduction in MDA levels when compared to SCI group as shown in figure 14. Tables are represented in appendix.

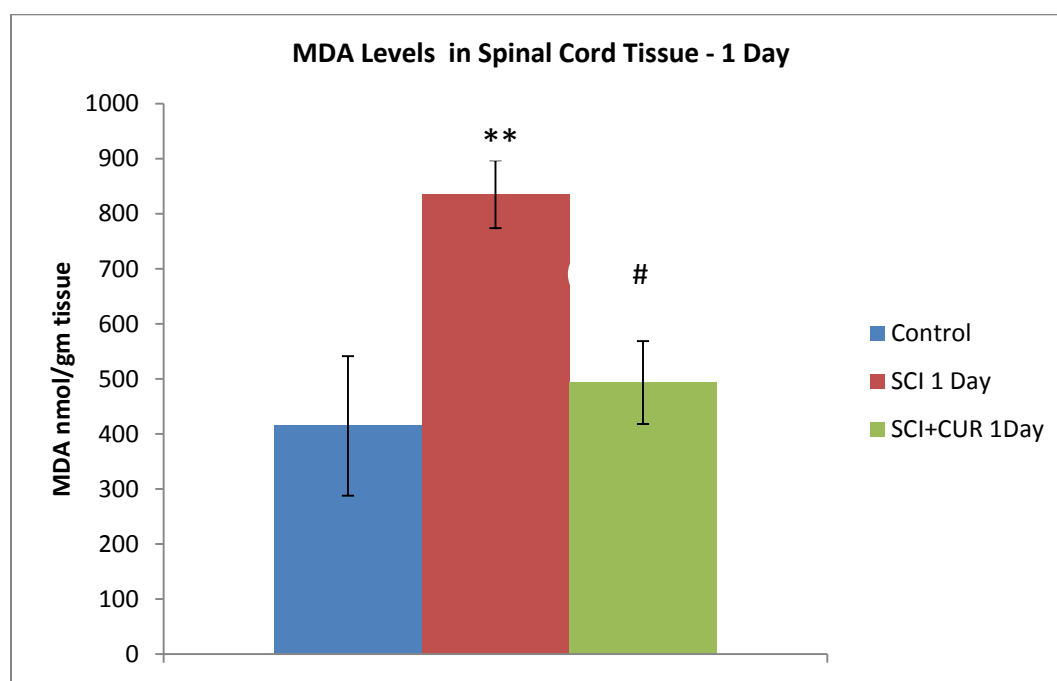


Figure 13: MDA levels in spinal cord tissue 1 day after SCI. MDA levels for Control, SCI, SCI+CUR in homogenized spinal cord tissue. MDA represented as nmol/g wet tissue weight. $n = (4-6)$, all samples were run in duplicate. Error bars are standard deviation (** $p < 0.01$ vs. control group and # $p < 0.05$ vs. SCI group). (One way ANOVA, Dunnett post hoc, Independent t-test).

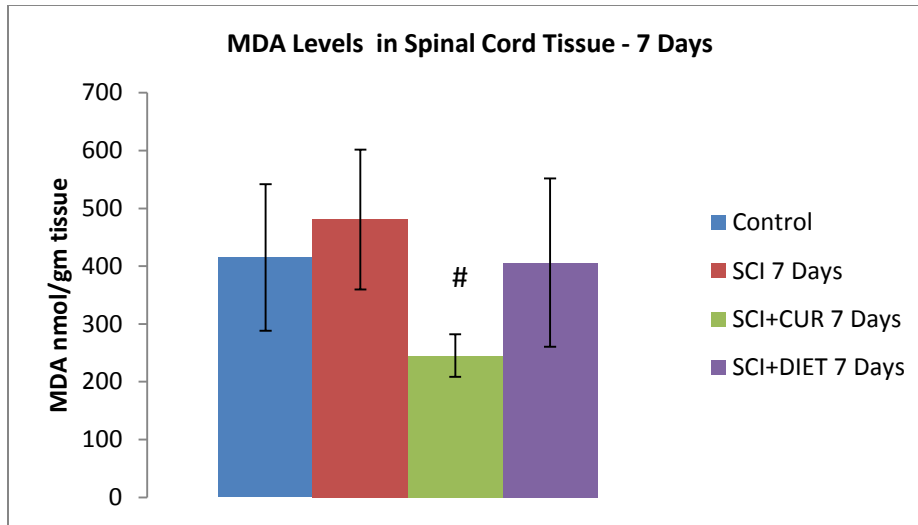


Figure 14 : MDA levels in spinal cord tissue 7 days after SCI. MDA levels measured for Control, SCI, SCI+CUR and SCI+Diet groups in homogenized spinal cord tissue. MDA represented as nmol/g wet tissue weight. n = (4-6), all samples were run in duplicate. Error bars are standard deviation (#p<0.05 vs SCI group). (One way ANOVA, Dunnett post hoc).

3.1.3 Total Antioxidant Capacity 1 Day after SCI and Local Curcumin Application

Total antioxidant capacity (TAC) was measured to detect the effect of Curcumin as an antioxidant following SCI. TAC was measured as mM/L of homogenized tissue samples. An elevation in TAC might be an indication of an antioxidant capacity exerted by Curcumin treatment. As shown in figure 17 at day 1 after SCI, TAC levels were significantly lowered in all injury and treatment groups compared to control $p < 0.01$ as shown in figure 15. After local treatment (SCI + CUR), no significant elevation in TAC levels was detected when compared with SCI group. Tables are represented in appendix.

3.1.4 Local Curcumin and Diet supplement significantly elevate TAC 7 Days after SCI.

TAC levels were significantly decreased in SCI + local treatment group (SCI+CUR) when compared to control group $p < 0.01$ at 7 days after SCI. However, TAC levels were significantly elevated by local treatment regimen (SCI+CUR) $p < 0.05$ and by Dietary supplement group (SCI + Diet) $p < 0.01$ compared to SCI group as shown in figure 16. Moreover, TAC levels were significantly elevated by diet supplement when compared to local treatment regimen $p < 0.01$ as shown in figure 16. Tables are represented in appendix.

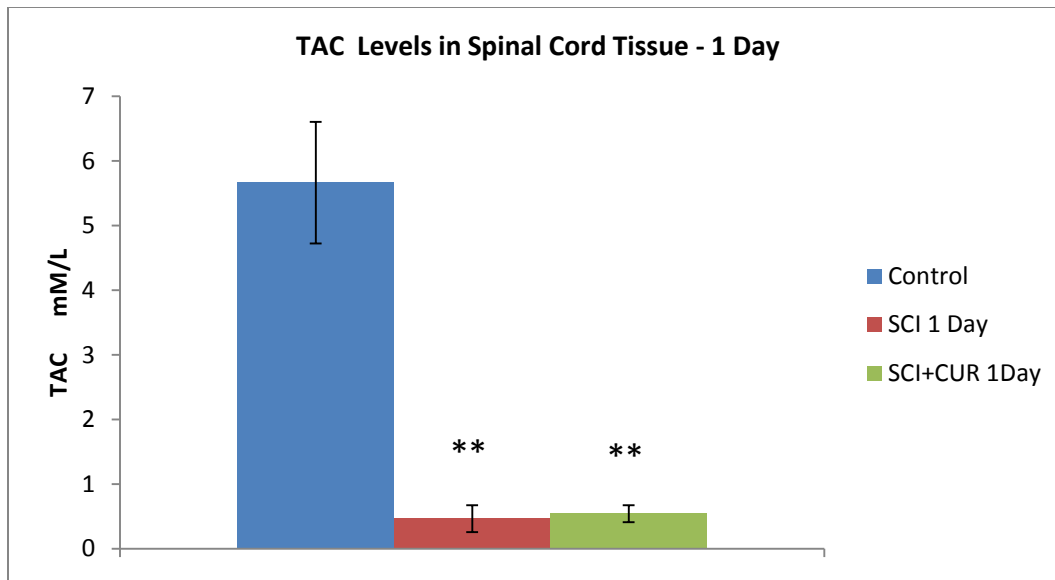


Figure 15 : TAC levels in spinal cord tissue 1 day after SCI. TAC levels measured for Control, SCI, SCI+CUR in homogenized spinal cord tissue. TAC represented as Mm/L. n = (4-6), all samples were run in duplicate. Error bars are standard deviation. (**p<0.01 vs. Control group. (One way ANOVA, Dunnett post hoc, Independent t-test))

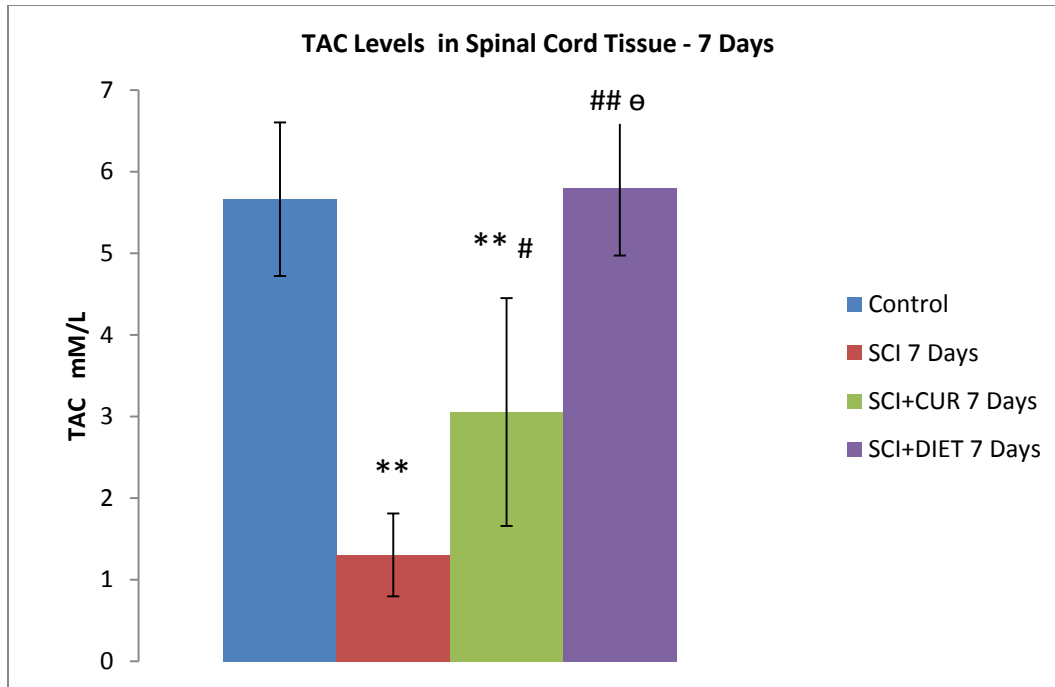


Figure 16: TAC levels in spinal cord tissue 7 days after SCI. TAC levels measured for Control, SCI, SCI+CUR and SCI+Diet in homogenized spinal cord tissue. TAC represented as Mm/L. n = (4-6), all samples were run in duplicate. Error bars are standard deviation. (**p<0.01 vs.Control, ##p<0.01 vs. SCI group, #p<0.05 vs. SCI group, θ p<0.01vs.SCI+CUR group). (One way ANOVA, Dunnett post hoc).

3.2. Effect of Curcumin on Inflammatory Cytokines Expression in Spinal Cord Tissue

To determine the effect of Curcumin on the inflammatory process following SCI, we measured TNF α and IL-6 levels in spinal cord tissue after local treatment

- TNF α levels were measured at 7 days post injury for both local treatment (SCI + CUR 200 mg/kg) with Curcuma longa extract (Herb pharm, Williams, Oregon) and dietary supplement regimen (SCI + Diet) (1 day TNF α values to be completed).
- IL-6 levels were measured for 1 day and 7 days local treatment (SCI + CUR 200 mg/kg) with Curcuma longa extract (IL-6 levels for Dietary supplement group to be completed).

3.2.1 Local Curcumin and Diet Supplement Significantly Decrease TNF α Expression Levels

TNF α levels were measured in rat spinal cord tissue in control, injury and treatment groups using Enzyme Linked immunosorbent Assay (ELISA). TNF α was measured as Pg/ml.

As shown in figure 17 at 7 days after SCI, TNF α levels were significantly elevated in SCI group $p < 0.01$ compared to control group. Upon treatment, TNF α levels were significantly lower in local treatment (SCI+CUR) and Diet supplement groups (SCI+Diet) compared to SCI group $p < 0.01$. Tables are represented in appendix.

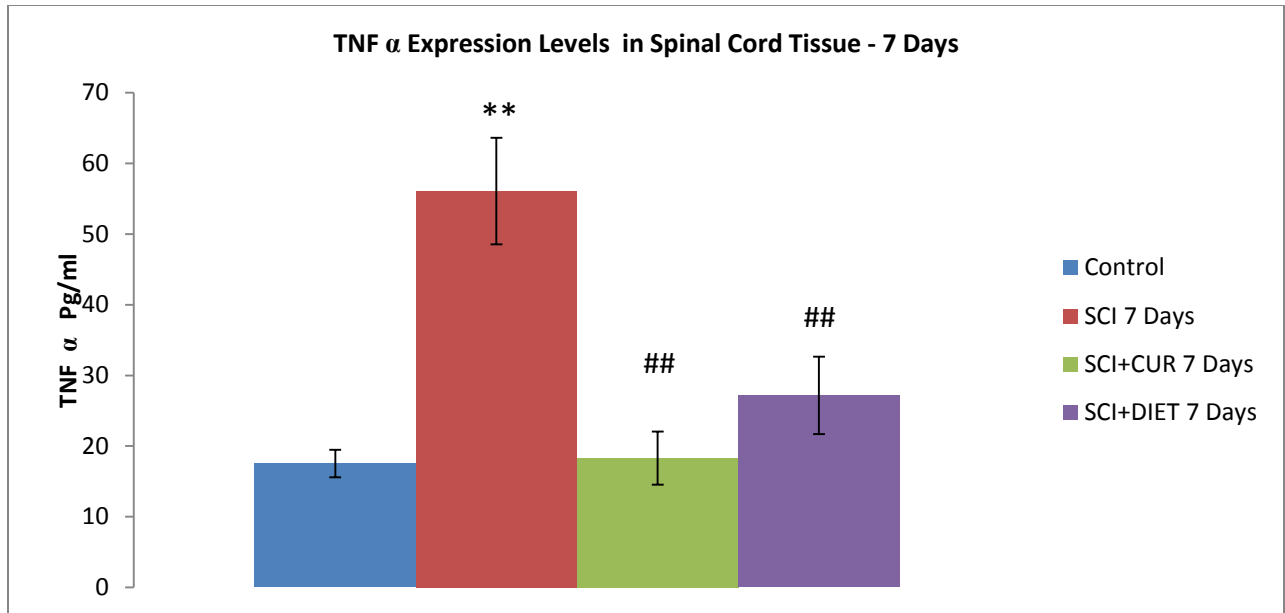


Figure 17: TNF α expression levels in spinal cord tissue 7 days after SCI by ELISA. TNF alpha levels for Control, SCI, SCI+CUR and SCI+Diet in homogenized spinal cord tissue represented as Pg/ml. n=(4-6), all samples were run in duplicate. Error bars are standard deviation. **p< 0.01 vs. control, ##p< 0.01 vs SCI. (One way ANOVA, Dunnett post hoc).

3.2.2. IL-6 Expression Levels 1 Day after SCI and Local Curcumin Application

IL-6 expression was measured in rat spinal cord homogenized tissue samples using ELISA. IL-6 is expressed as Pg /ml. At 1 day after SCI, IL-6 levels were significantly elevated in SCI and local Curcumin treatment (SCI+CUR) compared to control group $p < 0.01$ and $p < 0.05$ respectively. No significant lowering of IL-6 values was detected upon local treatment (SCI+CUR) when compared to SCI group at 1 day as shown in figure 18. Tables are represented in appendix.

3.2.3 Local Curcumin Significantly Decreases IL-6 Levels 7 Days after SCI

At 7 days, IL-6 levels were significantly elevated in SCI and local treatment group (SCI+CUR) compared to control group $P < 0.01$. Upon local treatment, IL-6 levels were significantly lowered compared to SCI group $P < 0.05$ as shown in figure 19. Tables are represented in appendix.

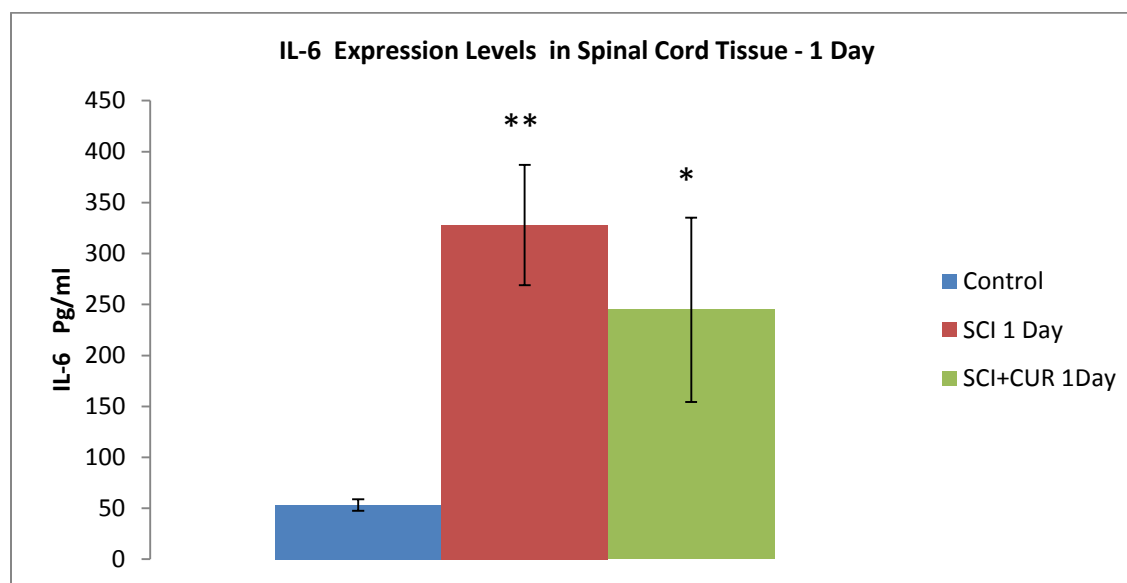


Figure 18 : IL-6 Expression levels in spinal cord tissue 1 day after SCI measured by ELISA. IL-6 levels for Control, SCI, SCI+CUR in homogenized spinal cord tissue represented as Pg/ml. $n=3$, all samples were run in duplicate. Error bars are standard deviation. (* $p < 0.05$, ** $p < 0.01$ vs. Control group). (One way ANOVA, Dunnett post hoc, Independent t-test).

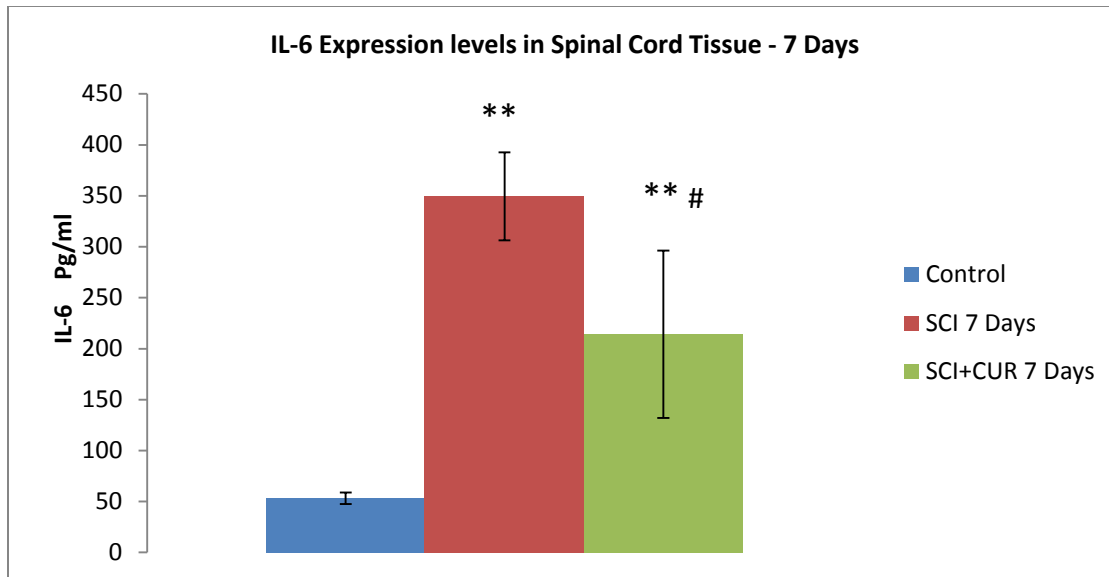


Figure 19 : IL-6 expression level in spinal cord tissue 7 days after SCI measured by ELISA. IL-6 for Control, SCI, SCI+CUR and SCI+Diet in homogenized spinal cord tissue represented as Pg/ml. n=(3), all samples were run in duplicate. Error bars are standard deviation. (**p<0.01 vs. Control, # p< 0.05 vs SCI group). (One way ANOVA, Dunnett post hoc, Independent t-test).

3.3 Neurological Function Evaluation

In our study, behavioral assessment has shown that Curcumin treatment groups exhibit a trend towards improved functional activity in treatment groups relative to injury groups. However, our results didn't evaluate the functional improvements on longer term, yet an improvement upon injury was noticed when treatment groups were compared to injury group 7 days after SCI

Thus a complete behavioral assessment was not presented. However, this is a long term study and since our main goal is to compare between dietary regimen and local regimen we assume that motor function test starting at 7 days would reflect more reliable data especially regarding diet supplement regimen. Thus, in order to validate our data we intend to complete the neurological assessment applying the scoring system between different groups on longer term to obtain a more descriptive and accurate data on the effect of Curcumin treatment on neurological function.

3.4 Histology

Spinal cord sections were examined by hematoxylin and eosin staining. Normal spinal cord sections and spinal cord sections following SCI were examined. Following injury, the normal spinal cord structure was impaired where both grey matter and white matter were damaged; pronounced cavitation was detected accompanied by neuronal degeneration. Normal and injured spinal cord sections are shown in figures 20 and 21 respectively.

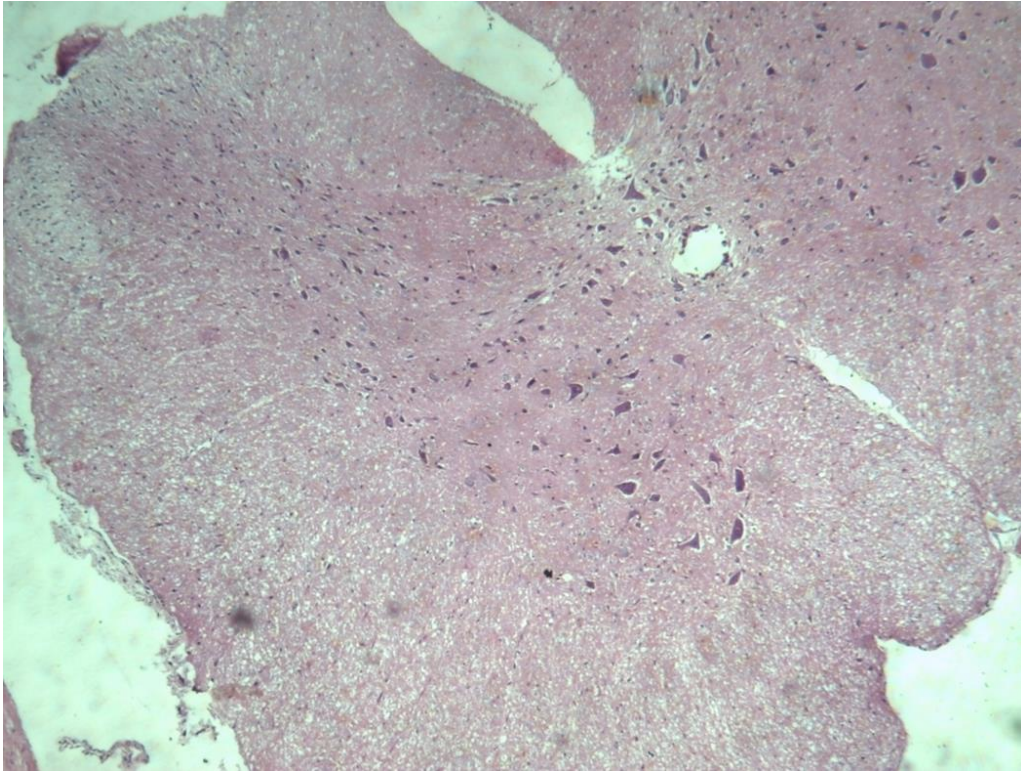


Figure 20 : Hematoxylin and Eosin Staining Sections of Normal spinal cord. Spinal cord sections around injury were removed dehydrated, embedded in paraffin and cryosectioned into 5 μ m thick sections. The normal tissue shows preserved tissue with the normal arrangement of the white matter, grey matter and neurons. (Magnification X40)

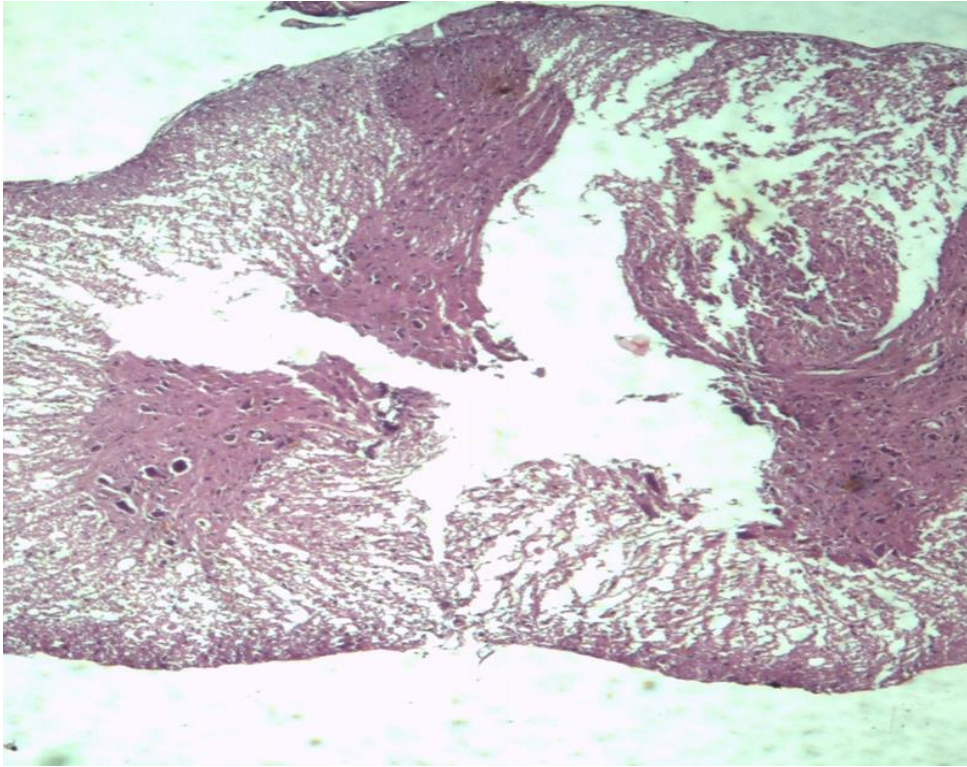


Figure 21 : Hematoxylin and Eosin staining for spinal cord tissue following SCI. Spinal cord tissue was damaged with impairment of both white matter and grey matter with noticeable cavitation and neuronal degeneration. (Magnification X40)

CHAPTER 4: DISCUSSION

Spinal cord injury is a debilitating condition characterized by a series of complex series of events, the primary injury causes tissue damage, axons demyelination, and cell membrane damage enhancing the initiation of secondary injury cascade (Kwon et al., 2004). Secondary damage involves multiple events such as ionic imbalance, release of free radicals, glutamate excitotoxicity, blood-spinal cord barrier damage and inflammation. These events are mediated and potentiated by glial cells, macrophages and T-lymphocytes (Anwar et al., 2016). It is well established that reduction of secondary tissue damage is a prime goal in treating spinal cord injury (Dumont et al., 2001).

The main focus of our study was to study the effects of Curcumin on two of the main hallmarks of secondary SCI; the inflammatory process and the oxidative damage. Our second goal was to find if dietary supplement of Curcumin would have a similar role to direct local application.

Curcumin is a naturally occurring nonsteroidal that manifests an array of beneficial pharmacological effects as anti-inflammatory, antioxidant, anticarcinogenic and anti-bacterial functions (Shishodia 2013). Thus detecting a potential role for Curcumin in spinal cord injury might add a potential tool in ameliorating the damaging cascade of events in SCI, as well as other CNS conditions thought to be caused by inflammation and oxidative damages. It has been proposed in several studies that curcumin administration enhances neuroprotection by decreasing inflammatory response, oxidative stress damage, glial scar formation and enhancing motor recovery as will be discussed below.

This study focuses on detecting the hypothesized role of Curcumin as a neuroprotective agent through its anti-inflammatory and an antioxidant effect. The main findings of this

study were as follows: 1) a significant increase in inflammatory and oxidative markers was noticed following injury, MDA, TNF α and IL-6 respectively. 2) Elevation in TAC levels following treatment specifically by 7 days. 3) Curcumin local treatment regimen showed an anti-inflammatory and antioxidant potential 4) Dietary curcumin supplement showed a beneficial effect; however, we conclude that both regimens need further modification.

Following SCI, a milieu is developed that shifts the balance pro-oxidant/ antioxidant towards oxidative stress (Bains and Hall, 2012). It results from failure of free radicals scavenging capacity. Free radicals cause lipid peroxidation and protein damage within spinal neurons, glia and other cells (Fatima et al., 2015). In SCI rat models, Malondialdehyde (MDA) elevation has been documented following injury (Varija et al., 2009). MDA is an aldehyde end product of lipid peroxidation in SCI and is regarded as an evidence of oxidative stress in rat SCI models (Liu et al., 2013, Kim et al., 2014).

In the present study Curcumin showed a noticeable antioxidant effect; however, varied depending on the treatment regimen applied. After injury, MDA levels were significantly elevated by 1 day compared to the control group, this is consistent with other studies which show that ROS generation in rat model is characterized by an elevation in MDA accompanied with reduction of antioxidant markers as superoxide dismutase (Paterniti et al., 2009; Liu et al., 2013; Guo et al., 2014). Xu et al. showed that 6 h after spinal cord compression injury, MDA levels were elevated in a modest; however, a significant manner, this elevation was also detected by immunohistochemical staining in motor neurons (Xu et al., 2005)

We noticed that in Curcumin local treatment groups (SCI +CUR 200 mg/kg), MDA levels were significantly lowered compared to injury groups either at 1 day or 7 days. This is consistent with other studies where Kavakli et al. reported a decrease in MDA level 24 h in serum post Curcumin treatment in a weight drop SCI model where Curcumin was given

200 mg/ kg orally (Kavakli et al., 2011). Sanli et al. reported similar findings following Curcumin administration 300 mg/kg intraperitoneally (Sanli et al., 2011).

At 7 days we noticed a significant decline in MDA levels following local treatment compared to injury and control groups. This is not consistent with the findings of Kim and colleagues, who report a significant elevation in MDA level by 1 week that only lowered significantly at 2 weeks when compared to vehicle group (Kim et al., 2014). However, their findings were based on plasma levels and not in cord tissue as our current study. Another possibility is the difference in the model used where Kim et al used an aneurysm clip compression SCI model, while we used a hemisection model.

Curcumin Dietary supplement given 7 days before and 7 days after injury, did not affect MDA levels significantly when compared to 7 days injury group. We assume that the dietary regimen that we applied was not capable enough of affecting MDA values post injury. Moreover, Dietary supplement with crude Curcumin was introduced to the animals ad libitum, thus a variation between the amounts of Curcumin between animals might have affected the overall effect. Although our goal was to test the potential neuroprotective effects of Curcumin when used as a dietary supplement, we recommend to avoid such variability by introducing Curcumin in a consistent and reproducible manner by gavage as previously described (Reeta et al., 2009; CONEAC et al., 2017).

Total antioxidant capacity (TAC) represents the synergistic effect between different antioxidants (Valkonen and Kunsu, 1997). At 1 and 7 days, we noticed a significant decrease in TAC values in all injury and treatment groups when compared to control group except in the dietary supplement group where higher TAC values were detected. However, when comparing to injury group 7 days after SCI, both local treatment (200 mg/kg) and Dietary supplement induced a significant elevation in TAC values compared to 7 days injury group. Moreover TAC levels in Diet supplement groups were more significantly elevated when compared to local treatment group.

We assume that this effect by the Curcumin dose we used (200 mg/kg) was masked at 1 day by the surge of oxidative damage that follows injury; however, by 7 days this surge has subsided due to the initial treatment and Curcumin treatment possibly had a delayed effect.

Al Rubaei and colleagues detected higher TAC and lower MDA levels in Curcumin treated groups when compared with H₂O₂ experimentally induced oxidative stress group in liver tissue homogenate (Al Rubaei et al., 2014). In a rat inflammation model, oral Curcumin in carboxy-methylcellulose (150 mg/kg), liver Total antioxidant capacity (TAC) was increased significantly in Curcumin treatment groups where samples were collected 15 min to 3h after Curcumin treatment, while serum TAC levels in Curcumin/carboxy-methylcellulose group and inflammation were lower than Curcumin only group (CONEAC et al., 2017).

In this context we have to highlight three main points. First, at 1 day local treatment the significant decrease in MDA was not accompanied by significant TAC elevation when compared to injury group. Second, at 7 days local treatment (200 mg/kg) we noticed that TAC was significantly elevated and MDA was significantly lowered as compared to 1 week injury group. Third, dietary Curcumin supplement elevated TAC significantly; however, MDA levels were not significantly lowered when compared to SCI group

Regarding the first point, MDA lowering might be attributed to an increased activity of a certain antioxidant enzyme such as SOD or Catalase which were reported to be elevated by Curcumin treatment in other SCI models, (Cemil et al., 2010; Kavakli et al., 2011; Kim et al., 2014). Hence, MDA might have been affected by a certain enzyme (s) rather than the total antioxidant capacity in the spinal cord tissue

Following dietary Curcumin supplementation, although TAC levels were significantly elevated, MDA values were not significantly lowered compared to 1 week injury group.

We assume that in the dietary supplement group the elevation of TAC was still not enough to cause MDA lowering, or possibly this elevation in TAC levels was not enough to induce the expression of antioxidant enzymes that affects MDA levels. Another possibility is that crude forms of Curcumin have a mix of active ingredients that induce various biochemical effects, therefore we recommend using a pure form of Curcumin extract, or other Curcumin derivatives such as (CNB-001; 500 ppm) should be considered. This derivative is more stable and enhances neuroprotection as well (Liu et al., 2008; Wu et al., 2011).

A proposed mechanism of Curcumin is that it enhances the activation of nuclear factor erythroid 2-related factor (Nrf2). Elevation in the level of antioxidant enzymes is regulated through antioxidant response element (ARE) that is present in the regulatory region of genes of antioxidant enzymes (Nguyen et al., 2003). ARE is an enhancer of a number of phase II antioxidant enzyme genes as Hemeoxygenase, reduced nicotinamide adenine dinucleotide phosphate quinone oxidoreductase and glutamate cysteine ligase (Wilson, 1997). When Nrf2 dissociates from kelch-like ECH associated protein 1 (keap1), it is translocated to the nucleus and binds to ARE enhancing the transcription of downstream target genes (Motohashi and Yamamoto 2004).

A study by Jiang and colleagues was the first to report the effect of Curcumin on Nrf2 in astrocytes, demonstrating that Curcumin enhanced phase II enzymes induction in Nrf2 +/+ astrocytes where this effect was not evident in Nrf2 -/- astrocytes suggesting that Curcumin enhances Nrf2 cytoprotective role in astrocytes. Moreover, Curcumin inhibited reactive oxygen species (ROS) in Nrf +/+ Astrocytes and not in Nrf -/- Astrocytes. They propose that this effect can add a lot to Curcumin therapeutic value especially as it can be a part of human diet or daily supplement with low toxicity (Jiang et al., 2011).

Jin et al. also demonstrated that Curcumin (IP injections 100mg/kg) enhanced of Nrf2 activity in SCI vascular clip compression model reduced locomotor impairment, apoptosis and spinal cord edema (Jin et al., 2014).

Other markers may also be used to evaluate oxidative stress following SCI and treatment efficacy, such as superoxide dismutase (SOD) (Kavakli et al., 2011) and hemeoxygenase (HO-1) (Diaz-Ruiz., 2013). The main identified free radicals are: superoxides ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), Peroxynitrite ($ONOO^-$) and nitric oxide ($^{\bullet}NO$) (Yang et al., 2013) where mainly the superoxide (Liu et al., 1998) and hydroxyl radical (Bao and Liu, 2004) are the most damaging and play the main role within the other ROS following SCI. They are produced by a complex system of enzymes in both microglia and immune cells as: nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase and cyclooxygenase (Bains and Hall, 2012).

Levels of $TNF\alpha$ are elevated following SCI, reaching its peak within one hour after primary injury (Dinomais et al., 2009). It is released faster than other inflammatory cytokines (Feldman, 2008) and it activates several other cytokines and growth factors release as well as immune cells recruitment. This chief role made $TNF\alpha$ an attractive target in inflammatory conditions including SCI (Esposito and Cuzzocrea, 2011). Studies suggest a dual role of $TNF\alpha$. For instance, it is suggested that it might exert deleterious effects in acute phase versus beneficial effect in chronic phase after SCI (Chi et al., 2010) or exerting anti-apoptotic effect through $TNFR-NF\kappa B$ signal transduction pathway (Kim et al., 2001).

$TNF\alpha$ is secreted by microglia and it stimulates glutamate release which in turn through metabotropic glutamate receptors enhances more $TNF\alpha$ release. Consequently, it acts on astrocytes inducing glutamate release and upregulating its levels consequently. Due to this excitatory elevation, calcium entry is elevated causing excitotoxicity and neuronal death, thus $TNF\alpha$ links neuro-inflammation and excitotoxic effects (Olmos and Lladó, 2014).

Initially $TNF\alpha$ is synthesized as transmembrane protein where its extracellular domain is cleaved by matrix metalloprotease $TNF\alpha$ converting enzyme into the soluble form where $TNFR1$ signaling activates a number of transduction pathways as: JNK, $NF-\kappa B$, ERK and

p38 MAPK (Byun et al., 2012; Olmos and Lladó, 2014) which makes it somehow ambiguous to clearly define which of them impacts TNF α expression (Olmos and Lladó, 2014). For instance, a study reported that TNF α expression was prevented through inhibition of MEK/ERK pathway. They also showed that NF- κ B binding to DNA which is initially activated by interferon gamma (IFN γ) is completely dependent on TNF α release via MEK/ERK pathway in BV-2 microglial cell line (Mir et al., 2009). NF- κ B pathway regulates promoters of cytokines producing genes as TNF α , IL-6 and IL-1 β (Hang et al., 2004). This cascade amplifies initial inflammation and aggravates consequences in SCI following the initial trauma (Ni et al., 2015).

The production of IL-6 is in part modulated by many factors as IL-1 β and TNF α . IL-6 production was correlated with glial scar formation (Brunello et al., 2000). IL-6 knockout caused astrogliosis suppression (Klein et al., 1997). Besides, following SCI noticeable impedance of axonal growth was detected in mice with increased IL-6 signals (Lacroix et al., 2002).

In the present study, TNF α levels were measured at 7 days in all groups (control, SCI, SCI+ local treatment and SCI+ Dietary supplement). TNF α levels were significantly elevated at 7 days injury in SCI group compared to control group. Further analysis is planned to evaluate 1 day expression across different groups.

IL-6 expression was significantly elevated in all groups either SCI (1 day and 7 days) or SCI +local treatment 200 mg/kg at 1 day and 7 days when compared to control group. However, data regarding Dietary supplement is not represented for IL-6 and yet to be completed.

The present data is consistent with previous reports that demonstrated alteration in the levels of inflammatory cytokines following SCI. In an SCI clamp model, serum levels of TNF α , IL-6, IL-10 and IL-1 α gradually increased at 24h reaching peak at 3 days. Pro-

inflammatory cytokine expression started lowering at day 7, although shown to remain significantly higher than control group, and stabilizing at day 28 (Geng et al., 2015). In an SCI weight drop contusion model, IL-6 receptor expression quantified by western blot was increased 8 fold within 12 hours following injury when compared to before injury (Okada et al., 2004). In an aneurysm clip compression model showed that inflammatory cytokines including TNF α , NF- κ B and IL-1 β increased at 1 hour after injury and peaking at 12 hours SCI (Yuan et al., 2015). Guo and colleagues also showed an elevation in TNF α and IL-6 mRNA levels at 2h and 6 h respectively following spinal cord contusion, as well as, a significant increase in the TNF α and IL-6 protein levels at 24 hours following SCI (Guo et al., 2014).

This documented variability in peak times may be due to differences in severity of injury between different SCI models. The first study to compare severity dependent expression of IL-1 β , IL-6 and TNF α following SCI, used a graded SCI contusion model. On the gene level, IL-6 and TNF α mRNA levels were increased at 1, 3 h and peaked at 6 h post injury returning to baseline levels at 1 d following severe injury, while these levels were significantly lower in the mild injury group. On the protein level, the cytokines including TNF α and IL-6 showed a marked but transient increase following severe SCI while in the mild injury group no cytokines were detected by western blotting (Yang et al., 2005).

The effects of Curcumin have been well documented; Chen et al. demonstrated that Curcumin treatment reduced IL-1 β mRNA expression in astrocyte culture with no change in TNF α expression, while in microglia pretreatment with Curcumin decreased the elevated IL-1 β and TNF α significantly (Chen et al., 2015). Another study showed that following contusion SCI TNF α is localized in the cytoplasm of the anterior horn neurons of the spinal cord and that the number of TNF α positive cells in gray matter was significantly elevated 24 h and at 3 d post injury. TNF α expression was significantly elevated at 3d and 5d (Zhang et al., 2015).

In our study we detected changes in TNF α and IL-6 in spinal cord tissue samples as an indicator for the inflammatory process following SCI hemisection model. After Curcumin administration, TNF α levels were significantly decreased in SCI + local (200 mg/kg) and SCI + diet groups when compared to 7 days SCI group. Furthermore at 7 days, IL-6 levels were significantly decreased Curcumin local treatment when compared to SCI group; however, at 1 day local treatment did not show a significant decrease. This might be due the strong recruitment of inflammatory cytokines by immune cells that might mask Curcumin effect. Nonetheless, Curcumin significantly lowered IL-6 level by 7 days, this might be explained by a delayed effect of curcumin that was not manifested at 1 day.

Machova et al used a single local dose of Curcumin followed by daily IP administration of Curcumin daily for 28 days following SCI using a balloon compression model TNF α levels were only significantly decreased at days 1 and 14. They demonstrated a transient elevation of TNF α levels between day 3 and 14; however, they assumed that it might be due to activation of the immune response cells which when stabilized TNF α levels decreased. They also proposed that Curcumin dose might have not been effective (Machova et al., 2015). They also demonstrated that IL-6 levels were significantly elevated upon treatment with curcumin when compared to vehicle control at days 14 and 28 (Machova et al., 2015). It is worth mentioning that Machova et al. study used a different SCI model and a different Curcumin treatment regimen than the regimens we used in the present study.

Machova et al. 2015 also showed that following treatment, no alteration was found in IL-6 levels at days 1, 3, 7 and 10; however, its level was increased by days 14 and 28. They proposed that Curcumin might act more effectively in early stages or that its effect is for a distinct period of time, but their detection window was till 28 days so the early phase in their study was 7 days which is regarded as the delayed phase in our current study. However, we believe that further investigation is needed to validate IL 6 changes at different time points.

Yuan et al. demonstrated that TNF α besides other inflammatory cytokines were elevated significantly at the gene and protein levels following SCI through aneurysm clip compression where Curcumin was given as 300 mg /kg once daily for 7 days. The elevation at the protein level was documented as soon as 1 hour following injury with a peak elevation at 12 hours post injury followed by a decline; however, they reported that the expression remained high in their therapeutic detection window which was 72 hours. In their study they correlated these high cytokine levels by a strong inflammatory response in acute SCI. Moreover, the authors suggested a correlation between the higher cytokine levels including TNF α , IL-1 β and NF- κ B in the simple injury group and a larger glial scar area aggravating secondary damage and tissue necrosis (Yuan et al., 2015).

Another study using vascular clip compression SCI model, showed that TNF α , IL-1 β and IL-6 were elevated by 257.9%, 229.0% and 296.3% increase respectively compared to sham group. The authors suggested a mechanism behind this elevation, they showed that following SCI, an increased expression of toll like receptor 4 (TLR4) on the gene and protein level followed by upregulation of NF- κ B was documented. In turn, this upregulation potentiates the activation of the inflammatory cytokines as TNF α which in turn activates NF- κ B. Thus a positive feedback mechanism is initiated amplifying inflammation and exacerbating damage following SCI (Ni et al., 2015). Hence, they proposed that Curcumin therapeutic effect is due to modulation of TLR4/NF- κ B inflammatory pathway and (Ni et al., 2015).

In a vascular clip compression model, Jin et al. showed that at 72 hours following injury, TNF α , IL-1 β and IL-6 concentrations were significantly lower in the Curcumin treatment group when compared to the SCI group. Curcumin was administered in a similar dose to Ni et al. (intraperitoneally as 100 mg/kg in PBS including 1% DMSO). Jin and Colleagues demonstrated that curcumin administration up-regulated Nrf2 activity while NF- κ B activity was down regulated. They proposed that Curcumin beneficial effect was due to enhancing

the activity of Nrf2, decreasing NF- κ B and hence decreasing the activity of the downstream inflammatory cytokines (Jin et al., 2014).

Cytokines specific mechanism of action in neurodegeneration is considered complex and still unclear. They can either act directly on neurons or indirectly through affecting blood flow, glia or brain vasculature. Cytokines can act on many levels exerting multiple actions on different cells eventually causing excess calcium release, apoptosis, enhancing calcium uptake and free radical production (Allan and Rothwell, 2001).

In our study, neurological assessment has shown that Curcumin treatment groups exhibit a trend towards improved functional activity in treatment groups relative to injury groups. However, our results didn't evaluate the functional improvements on longer term, yet an improvement upon injury was noticed when treatment groups were compared to injury group 7 days after SCI

A complete behavioral assessment was not presented as well. However, this is a long term study and since our main goal is to compare between dietary regimen and local regimen we assume that motor function test starting at 1 week would reflect more reliable data especially regarding diet supplement regimen. For instance another study performed the behavioral assessment at postoperative days 21 and 42 (Holly et al., 2012).

Several studies show functional improvement following Curcumin treatment in various SCI models. Liu et al. performed a neurological assessment test on ischemia/ reperfusion (I/R) SCI model 48 hours following reperfusion. They showed that after Curcumin treatment, although neurological score was lower than sham group yet it was higher than that of the I/R group significantly (Liu et al., 2013). Ni et al. used Basso, Beattie and Bresnahan (BBB) locomotor scale to assess forelimb-hindlimb coordination, hind limb movements and whole body movements as well. By 72 h following SC compression injury, BBB score was nearly zero; however, after Curcumin treatment 100 mg/kg IP the score was significantly increased (Ni et al., 2015). In a left hemisection SCI model, daily Curcumin

treatment (40 mg/kg) starting 1 day before surgery and continued until 6 days, showed that Curcumin treatment was accompanied by improved BBB score of the affected hind limb on days 3 and 7 (Lin et al., 2011). Yuan et al also reported a dose effect correlation where higher Curcumin concentrations caused more improvement of functional recovery on the BBB score when compared with methylprednisolone (MP) or with the simple injury group starting from week one till week 8 (Yuan et al., 2015).

Regarding the scope of our study, which is comparing the dietary regimen with local treatment regimen, a long term behavioral assessment should be considered to reliably evaluate if the regimens used would give a sustained motor function improvement or not. For instance Yuan et al. evaluated the effect of the given curcumin treatment from week 1 till week 8 (Yuan et al., 2015). Besides, Holly et al. compared different groups for behavioral assessment on postoperative day 21 and 42 where they were detecting the effect of docosahexaenoic acid-curcumin (DHA-Cur) diet on enhancing neuroprotection in cervical spondylotic myelopathy (CSM) rat model (Holly et al., 2012).

Potential limitations of our study could be the dietary regimen using crude turmeric which only contains 3-5 % Curcuminoids 60% of which is Curcumin (Gupta et al., 2013). Thus using a more stable and efficacious form as CNB-001 used by Wu et al. study might have represented clearer results. Another limitation was not looking at time points between 1 day and 7 days as some markers in our study have shown a change by 7 days thus their fluctuation pattern before 7 days should be investigated. Moreover, time points beyond 7 days should be considered as well for investigating the sustainability of Curcumin either biologically or regarding on motor function assessment following treatment.

In another study, Curcumin was applied locally then systemically throughout the experiment (Machova et al., 2015). We should consider daily dosing or a sustained release formulation especially that Curcumin is rapidly degraded. However, we were trying to monitor if this initial dose would affect the oxidative and inflammatory processes

especially that it was applied locally and immediately post injury thus we assumed a local treatment regimen would be more effective than the oral or the systemic regimen.

Thus, we believe that Curcumin has a great potential in CNS injury due its potential as an anti-inflammatory and anti-oxidant, although longer term investigation are necessary to evaluate the sustainability of Curcumin effects in CNS injury.

CONCLUSION

The results presented here reflect a role of Curcumin in alleviating oxidative damage and inflammation following SCI via lowering MDA, TNF α and IL-6 and elevating TAC. At 7 days following treatment we noticed that Curcumin exerted better effect; however, we believe that further modifications to our studies should be implemented in future investigation. Overall in our current model, local treatment regimen (200 mg / kg) immediately after injury gave better results than Curcumin as a dietary supplement, suggesting that although Curcumin as a dietary supplement may have beneficial effects in general by increasing TAC; however, these benefits are not enough to reverse or reduce the inflammatory process and parenteral administration of Curcumin is needed.

FUTURE RECOMMENDATIONS

Cumulative studies demonstrate a beneficial therapeutic potential of Curcumin following SCI (Cemil et al., 2010; Kavakli et al., 2011; Machova et al., 2015; Yuan et al., 2015). Our study has shown that curcumin might alleviate the consequences of oxidative and inflammatory processes which are two main hallmarks of secondary spinal cord injury.

Many mechanisms have been proposed for its beneficial role yet a verification of the mechanism through which it affects its targets has not been fully described or validated where some recent reports have described Curcuminoids as pan assay interference compounds (PAINS) (Baell and Walters 2014). Thus understanding Curcumin and its mechanism of action towards its targets should be a priority in the ongoing research evaluating its efficacy and potential.

To get results that are more descriptive for the role of Curcumin in SCI we recommend that a new Curcumin delivery system as liposomal curcumin should be used for local treatment regimen. Liposomal Curcumin formulation demonstrated a remarkable enhancement of curcumin bioavailability in cells.

For the dietary regimen we recommend the use of CNB-001 as used by Wu et al. (2011) that would enhance the stability and absorption of curcumin in the biological system. In the present study we measured the total antioxidant capacity; in future investigation we would recommend measuring antioxidant enzymes and investigate which of them is affected by Curcumin treatment following injury as SOD which was found to be elevated by Curcumin (Kavakli et al., 2011; Liu et al., 2013).

Long term behavioral assessment using motor (inclined plane test), sensory (withdrawal reflex test) and sensorimotor tests (Grid walk test) is a future direction regarding our study

to detect if Curcumin dietary supplement or local treatment regimen would manifest sustained motor improvement.

Measuring markers for astrogliosis and axonal sprouting as GFAP and GAP 43 (Growth Associated Protein 43) respectively is an important future investigation for the effect of Curcumin on the environment surrounding the injury site and whether Curcumin will contribute to enhancement of neurological recovery.

Detecting time points before 1 day and between 1 day and 7 days would reflect a clearer idea on Curcumin's effect in SCI. However, in this study our main scope was comparing between the dietary and local regimens so 7 days and beyond, in our view was suitable for the dietary supplement effect to be investigated.

One of the major obstacles impeding Curcumin's progress is its low bioavailability, rapid metabolism and poor absorption as well (Kong 2013). For this purpose Curcumin nano-formulations have been developed to enhance its bioavailability and efficacy. This included liposomes, micelles, nanocrystals and others (Rachmawati et al., 2013).

Our results shows that Curcumin might exhibit an antioxidant and anti-inflammatory potential in SCI. Local treatment regimen demonstrated better results than diet supplement; however, we believe that further investigation is needed to unravel the mechanism by which Curcumin acts towards its targets.

REFERENCES

- Adjan, V. V., Hauser, K. F., Bakalkin, G., Yakovleva, T., Gharibyan, A., Scheff, S. W., & Knapp, P. E. (2007). Caspase-3 activity is reduced after spinal cord injury in mice lacking dynorphin: differential effects on glia and neurons. *Neuroscience*, 148(3), 724-736
- Aggarwal, B. B., Kumar, A., & Bharti, A. C. (2003). Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer res*, 23(1A), 363-398
- Aggarwal, B. B., Takada, Y., & Oommen, O. V. (2004). From chemoprevention to chemotherapy: common targets and common goals. *Expert opinion on investigational drugs*, 13(10), 1327-1338.
- Aggarwal, S., Takada, Y., Singh, S., Myers, J. N., & Aggarwal, B. B. (2004). Inhibition of growth and survival of human head and neck squamous cell carcinoma cells by curcumin via modulation of nuclear factor- κ B signaling. *International Journal of Cancer*, 111(5), 679-692.
- Aggarwal, B. B., & Harikumar, K. B. (2009). Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *The international journal of biochemistry & cell biology*, 41(1), 40-59.
- Ahuja, S., Kohli, S., Krishnan, S., Dogra, D., Sharma, D., & Rani, V. (2011). Curcumin: a potential therapeutic polyphenol, prevents noradrenaline-induced hypertrophy in rat cardiac myocytes. *Journal of Pharmacy and Pharmacology*, 63(12), 1604-1612.
- Alexandrow, M. G., Song, L. J., Altioek, S., Gray, J., Haura, E. B., & Kumar, N. B. (2012). Curcumin: a novel stat 3 pathway inhibitor for chemoprevention of lung cancer. *European Journal of Cancer Prevention*, 21(5), 407.
- Ali, H., & Bahbahani, H. (2010). Umbilical cord blood stem cells—potential therapeutic tool for neural injuries and disorders. *Acta Neurobiol Exp (Wars)*, 70(3), 316-324.
- Allan, S. M., & Rothwell, N. J. (2001). Cytokines and acute neurodegeneration. *Nature Reviews Neuroscience*, 2(10), 734-744.
- Allen, A. R. (1911). Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column: a preliminary report. *Journal of the American Medical Association*, 57(11), 878-880.
- Al-Rubaei, Z. M., Mohammad, T. U., & Ali, L. K. (2014). Effects of local curcumin on oxidative stress and total antioxidant capacity in vivo study. *Pakistan journal of biological sciences: PJBS*, 17(12), 1237-1241.

- Ankeny, D. P., Lucin, K. M., Sanders, V. M., McGaughy, V. M., & Popovich, P. G. (2006). Spinal cord injury triggers systemic autoimmunity: evidence for chronic B lymphocyte activation and lupus-like autoantibody synthesis. *Journal of neurochemistry*, 99(4), 1073-1087.
- Anwar, M. A., Al Shehabi, T. S., & Eid, A. H. (2016). Inflammogenesis of Secondary Spinal Cord Injury. *Frontiers in cellular neuroscience*, 10.
- Araujo, C. A. C., & Leon, L. L. (2001). Biological activities of *Curcuma longa* L. *Memorias do Instituto Oswaldo Cruz*, 96(5), 723-728.
- Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative medicine and cellular longevity*, 2014.
- Baell, J., & Walters, M. A. (2014). Chemical con artists foil drug discovery. *Nature*, 513(7519), 481.
- Baeuerle, P. A., & Henkel, T. (1994). Function and activation of NF-kappaB in the immune system. *Annual review of immunology*, 12(1), 141-179.
- Bains, M., & Hall, E. D. (2012). Antioxidant therapies in traumatic brain and spinal cord injury. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 1822(5), 675-684.
- Banovac, K., Sherman, A. L., Estores, I. M., & Banovac, F. (2004). Prevention and treatment of heterotopic ossification after spinal cord injury. *The journal of spinal cord medicine*, 27(4), 376-382.
- Bao, F., DeWitt, D. S., Prough, D. S., & Liu, D. (2003). Peroxynitrite generated in the rat spinal cord induces oxidation and nitration of proteins: Reduction by Mn (III) tetrakis (4-benzoic acid) porphyrin. *Journal of neuroscience research*, 71(2), 220-227.
- Bao, F., & Liu, D. (2004). Hydroxyl radicals generated in the rat spinal cord at the level produced by impact injury induce cell death by necrosis and apoptosis: protection by a metalloporphyrin. *Neuroscience*, 126(2), 285-295.
- Barger, S. W., Goodwin, M. E., Porter, M. M., & Beggs, M. L. (2007). Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. *Journal of neurochemistry*, 101(5), 1205-1213.
- Berney, S., Bragge, P., Granger, C., Opdam, H., & Denehy, L. (2011). The acute respiratory management of cervical spinal cord injury in the first 6 weeks after injury: a systematic review. *Spinal cord*, 49(1), 17-29.
- Bisht, S., & Maitra, A. (2009). Systemic delivery of curcumin: 21st century solutions for an ancient conundrum. *Current drug discovery technologies*, 6(3), 192-199.

- Bomstein, Y., Marder, J. B., Vitner, K., Smirnov, I., Lisaey, G., Butovsky, O., ...& Yoles, E. (2003). Features of skin-coincubated macrophages that promote recovery from spinal cord injury. *Journal of neuroimmunology*, 142(1), 10-16.
- Bracken, M. B. (2012). Steroids for acute spinal cord injury. *The Cochrane Library*.
- Brunello, A. G., Weissenberger, J., Kappeler, A., Vallan, C., Peters, M., Rose-John, S., & Weis, J. (2000). Astrocytic alterations in interleukin-6/soluble interleukin-6 receptor α double-transgenic mice. *The American journal of pathology*, 157(5), 1485-1493.
- Bunge, R. P., Puckett, W. R., & Hiester, E. D. (1997). Observations on the pathology of several types of human spinal cord injury, with emphasis on the astrocyte response to penetrating injuries. *Advances in neurology*, 72, 305-315.
- Byun, K., Young Kim, J., Bayarsaikhan, E., Kim, D., Jeong, G. B., Yun, K. N., ... & Lee, B. (2012). Quantitative proteomic analysis reveals that lipopolysaccharide induces mitogen-activated protein kinase-dependent activation in human microglial cells. *Electrophoresis*, 33(24), 3756-3763.
- Cemil, B., Topuz, K., Demircan, M. N., Kurt, G., Tun, K., Kutlay, M., ...& Kucukodaci, Z. (2010). Curcumin improves early functional results after experimental spinal cord injury. *Acta neurochirurgica*, 152(9), 1583-1590.
- Chavarria, A., & Alcocer-Varela, J. (2004). Is damage in central nervous system due to inflammation?. *Autoimmunity reviews*, 3(4), 251-260.
- Chen, A., & Xu, J. (2005). Activation of PPAR γ by curcumin inhibits Moser cell growth and mediates suppression of gene expression of cyclin D1 and EGFR. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 288(3), G447-G456.
- Chen, D., Nie, M., Fan, M. W., & Bian, Z. (2008). Anti-inflammatory activity of curcumin in macrophages stimulated by lipopolysaccharides from *Porphyromonas gingivalis*. *Pharmacology*, 82(4), 264-269.
- Chen, J. J., Dai, L., Zhao, L. X., Zhu, X., Cao, S., & Gao, Y. J. (2015). Intrathecal curcumin attenuates pain hypersensitivity and decreases spinal neuroinflammation in rat model of monoarthritis. *Scientific reports*, 5.
- Cheng, A. L., Hsu, C. H., Lin, J. K., Hsu, M. M., Ho, Y. F., Shen, T. S., ... & Yu, H. S. (2001). Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res*, 21(4B), 2895-2900.
- Cheriyian, T., Ryan, D. J., Weinreb, J. H., Cheriyian, J., Paul, J. C., Lafage, V., ...& Errico, T. J. (2014). Spinal cord injury models: a review. *Spinal Cord*, 52(8), 588-595.

- Chi, L., Yu, J., Zhu, H., Li, X., Zhu, S., Li, Z. & Kindy, M. S. (2010). Dual neuronal response to tumor necrosis factor- α following spinal cord injury. *Neural Regeneration Research*, 5(12), 917-926.
- Chinnock, P., & Roberts, I. (2005). Gangliosides for acute spinal cord injury. *The Cochrane Library*.
- Choo, A. M., Liu, J., Lam, C. K., Dvorak, M., Tetzlaff, W., & Oxland, T. R. (2007). Contusion, dislocation, and distraction: primary hemorrhage and membrane permeability in distinct mechanisms of spinal cord injury. *Journal of Neurosurgery: Spine*, 6(3), 255-266.
- Chuengsamarn, S., Rattanamongkolgul, S., Luechapudiporn, R., Phisalaphong, C., & Jirawatnotai, S. (2012). Curcumin extract for prevention of type 2 diabetes. *Diabetes care*, 35(11), 2121-2127.
- Coneac, A., Meda, S. O., Leucuta, D. C., Decea, N., Filip, M., Miha, C. M., & Moldovan, m. (2017). Effect of Curcumin on Oxidative Stress in a Model of Turpentine Induced Acute Experimental Inflammation. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 45(1).
- Correale, J., & Villa, A. (2004). The neuroprotective role of inflammation in nervous system injuries. *Journal of neurology*, 251(11), 1304-1316.
- Das, A., Smith, J. A., Gibson, C., Varma, A. K., Ray, S. K., & Banik, N. L. (2011). Estrogen receptor agonists and estrogen attenuate TNF- α -induced apoptosis in VSC4. 1 motoneurons. *Journal of Endocrinology*, 208(2), 171-182.
- Dcodhar, S. D., Sethi, R., & Srimal, R. C. (2013). Preliminary study on antirheumatic activity of curcumin (diferuloyl methane). *Indian journal of medical research*, 138(1).
- Dergham, P., Ellezam, B., Essagian, C., Avedissian, H., Lubell, W. D., & McKerracher, L. (2002). Rho signaling pathway targeted to promote spinal cord repair. *Journal of Neuroscience*, 22(15), 6570-6577.
- Diaz-Ruiz, A., Maldonado, P. D., Mendez-Armenta, M., Jiménez-García, K., Salgado-Ceballos, H., Santander, I., & Ríos, C. (2013). Activation of heme oxygenase recovers motor function after spinal cord injury in rats. *Neuroscience letters*, 556, 26-31.
- Dinareello, C. A. (2006). The paradox of pro-inflammatory cytokines in cancer. *Cancer and Metastasis Reviews*, 25(3), 307-313.
- Dinomais, M., Stana, L., Egon, G., Richard, I., & Menei, P. (2009). Significant recovery of motor function in a patient with complete T7 paraplegia receiving etanercept. *Journal of rehabilitation medicine*, 41(4), 286-288.
- Donnelly, D. J., & Popovich, P. G. (2008). Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. *Experimental neurology*, 209(2), 378-388.

- Dumont, R. J., Okonkwo, D. O., Verma, S., Hurlbert, R. J., Boulos, P. T., Ellegala, D. B., & Dumont, A. S. (2001). Acute spinal cord injury, part I: pathophysiologic mechanisms. *Clinical neuropharmacology*, 24(5), 254-264.
- Durafourt, B. A., Moore, C. S., Zammit, D. A., Johnson, T. A., Zaguia, F., Guiot, M. C., ...& Antel, J. P. (2012). Comparison of polarization properties of human adult microglia and blood-derived macrophages. *Glia*, 60(5), 717-727
- Element, A. R. (2004). An important role of Nrf2-ARE pathway in the cellular defense mechanism. *J Biochem Mol Biol*, 37(2), 139-143.
- Esposito, E., & Cuzzocrea, S. (2011). Anti-TNF therapy in the injured spinal cord. *Trends in pharmacological sciences*, 32(2), 107-115.
- Faissner, A., & Steindler, D. (1995). Boundaries and inhibitory molecules in developing neural tissues. *Glia*, 13(4), 233-254.
- Farooqui, A. A. (2010). *Neurochemical aspects of neurotraumatic and neurodegenerative diseases*. Springer Science & Business Media.
- Farooqui, A. A. (2013). Beneficial Effects of Curcumin on Neurological Disorders. In *Phytochemicals, Signal Transduction, and Neurological Disorders* (pp. 151-197). Springer New York.
- Fatima, G., Sharma, V. P., Das, S. K., & Mahdi, A. A. (2015). Oxidative stress and antioxidative parameters in patients with spinal cord injury: implications in the pathogenesis of disease. *Spinal Cord*, 53(1), 3-6.
- Faulkner, J. R., Herrmann, J. E., Woo, M. J., Tansey, K. E., Doan, N. B., & Sofroniew, M. V. (2004). Reactive astrocytes protect tissue and preserve function after spinal cord injury. *Journal of Neuroscience*, 24(9), 2143-2155.
- Fawcett, J. W., & Asher, R. A. (1999). The glial scar and central nervous system repair. *Brain research bulletin*, 49(6), 377-391.
- Fayed, A. H. A. (2010). Brain trace element concentration of rats treated with the plant alkaloid, vincamine. *Biological trace element research*, 136(3), 314-319.
- Fehlings, M. G., & Nguyen, D. H. (2010). Immunoglobulin G: a potential treatment to attenuate neuroinflammation following spinal cord injury. *Journal of clinical immunology*, 30(1), 109-112.
- Feldman, A. M. (2008). TNF alpha—still a therapeutic target. *Clinical and translational science*, 1(2), 145-145.

- Freund, P., Schmidlin, E., Wannier, T., Bloch, J., Mir, A., Schwab, M. E., & Rouiller, E. M. (2006). Nogo-A-specific antibody treatment enhances sprouting and functional recovery after cervical lesion in adult primates. *Nature medicine*, 12(7), 790-792.
- Fukuda, T., Kawano, H., Ohyama, K., Li, H. P., Takeda, Y., Oohira, A., & Kawamura, K. (1997). Immunohistochemical localization of neurocan and L1 in the formation of thalamocortical pathway of developing rats. *Journal of Comparative Neurology*, 382(2), 141-152.
- Galhardi, F., Mesquita, K., Monserrat, J. M., & Barros, D. M. (2009). Effect of silymarin on biochemical parameters of oxidative stress in aged and young rat brain. *Food and Chemical Toxicology*, 47(10), 2655-2660.
- Geisler, F. H., Coleman, W. P., Grieco, G., Poonian, D., & Sygen Study Group. (2001). The Sygen® multicenter acute spinal cord injury study. *Spine*, 26(24S), S87-S98.
- Geng, X., Sun, T., Li, J. H., Zhao, N., Wang, Y., & Yu, H. L. (2015). Electroacupuncture in the repair of spinal cord injury: inhibiting the Notch signaling pathway and promoting neural stem cell proliferation. *Neural regeneration research*, 10(3), 394.
- Genovese, T., Melani, A., Esposito, E., Paterniti, I., Mazzon, E., Di Paola, R., ... & Cuzzocrea, S. (2009). Selective adenosine A (2a) receptor agonists reduce the apoptosis in an experimental model of spinal cord trauma. *Journal of biological regulators and homeostatic agents*, 24(1), 73-86.
- Goel, A., Boland, C. R., & Chauhan, D. P. (2001). Specific inhibition of cyclooxygenase-2 (COX-2) expression by dietary curcumin in HT-29 human colon cancer cells. *Cancer letters*, 172(2), 111-118.
- Goel, A., Kunnumakkara, A. B., & Aggarwal, B. B. (2008). Curcumin as “Curecumin”: from kitchen to clinic. *Biochemical pharmacology*, 75(4), 787-809.
- Gokce, E. C., Kahveci, R., Gokce, A., Sargon, M. F., Kisa, U., Aksoy, N., ... & Erdogan, B. (2016). Curcumin Attenuates Inflammation, Oxidative Stress, and Ultrastructural Damage Induced by Spinal Cord Ischemia-Reperfusion Injury in Rats. *Journal of Stroke and Cerebrovascular Diseases*, 25(5), 1196-1207.
- Gota, V. S., Maru, G. B., Soni, T. G., Gandhi, T. R., Kochar, N., & Agarwal, M. G. (2010). Safety and pharmacokinetics of a solid lipid curcumin particle formulation in osteosarcoma patients and healthy volunteers. *Journal of agricultural and food chemistry*, 58(4), 2095-2099.
- Green, D. R. (1998). Apoptotic pathways: the roads to ruin. *Cell*, 94(6), 695-698.

- Grossman, S. D., Rosenberg, L. J., & Wrathall, J. R. (2001). Temporal-spatial pattern of acute neuronal and glial loss after spinal cord contusion. *Experimental neurology*, 168(2), 273-282.
- Guest, J. D., Hiester, E. D., & Bunge, R. P. (2005). Demyelination and Schwann cell responses adjacent to injury epicenter cavities following chronic human spinal cord injury. *Experimental neurology*, 192(2), 384-393.
- Guo, J., Li, Y., He, Z., Zhang, B., Li, Y., Hu, J., ... & Dai, B. (2014). Targeting endothelin receptors A and B attenuates the inflammatory response and improves locomotor function following spinal cord injury in mice. *International journal of molecular medicine*, 34(1), 74-82.
- Guo, Y., Zhang, H., Yang, J., Liu, S., Bing, L., Gao, J., & Hao, A. (2013). Granulocyte colony-stimulating factor improves alternative activation of microglia under microenvironment of spinal cord injury. *Neuroscience*, 238, 1-10.
- Gupta, K. K., Bharne, S. S., Rathinasamy, K., Naik, N. R., & Panda, D. (2006). Dietary antioxidant curcumin inhibits microtubule assembly through tubulin binding. *The FEBS journal*, 273(23), 5320-5332.
- Gupta, S. C., Patchva, S., & Aggarwal, B. B. (2013). Therapeutic roles of curcumin: lessons learned from clinical trials. *The AAPS journal*, 15(1), 195-218.
- Gupta, S. C., Prasad, S., Kim, J. H., Patchva, S., Webb, L. J., Priyadarsini, I. K., & Aggarwal, B. B. (2011). Multitargeting by curcumin as revealed by molecular interaction studies. *Natural product reports*, 28(12), 1937-1955.
- Hagen, E. M. (2015). Acute complications of spinal cord injuries. *World journal of orthopedics*, 6(1), 17.
- Hamada, Y., Ikata, T., Katoh, S., Nakauchi, K., Niwa, M., Kawai, Y., & Fukuzawa, K. (1996). Involvement of an Intercellular Adhesion Molecule 1-Dependent Pathway in the Pathogenesis of Secondary Changes After Spinal Cord Injury in Rats. *Journal of neurochemistry*, 66(4), 1525-1531.
- Hang, C. H., Shi, J. X., Tian, J., Li, J. S., Wu, W., & Yin, H. X. (2004). Effect of systemic LPS injection on cortical NF- κ B activity and inflammatory response following traumatic brain injury in rats. *Brain research*, 1026(1), 23-32.
- Hatcher, H., Planalp, R., Cho, J., Torti, F. M., & Torti, S. V. (2008). Curcumin: from ancient medicine to current clinical trials. *Cellular and Molecular Life Sciences*, 65(11), 1631-1652.
- Hayes, K. C., Hull, T. C. L., Delaney, G. A., Potter, P. J., Sequeira, K. A. J., Campbell, K., & Popovich, P. G. (2002). Elevated serum titers of proinflammatory cytokines and CNS

- autoantibodies in patients with chronic spinal cord injury. *Journal of neurotrauma*, 19(6), 753-761.
- Heiman, A., Pallottie, A., Heary, R. F., & Elkabes, S. (2014). Toll-like receptors in central nervous system injury and disease: a focus on the spinal cord. *Brain, behavior, and immunity*, 42, 232-245.
- Hollier, L. H., Money, S. R., Naslund, T. C., Procter, C. D., Buhrman, W. C., Marino, R. J., ... & Kazmier, F. J. (1992). Risk of spinal cord dysfunction in patients undergoing thoracoabdominal aortic replacement. *The American journal of surgery*, 164(3), 210-214.
- Holly, L. T., Blaskiewicz, D., Wu, A., Feng, C., Ying, Z., & Gomez-Pinilla, F. (2012). Dietary therapy to promote neuroprotection in chronic spinal cord injury. *Journal of Neurosurgery: Spine*, 17(2), 134-140.
- Huang, S. S., Tsai, M. C., Chih, C. L., Hung, L. M., & Tsai, S. K. (2001). Resveratrol reduction of infarct size in Long-Evans rats subjected to focal cerebral ischemia. *Life sciences*, 69(9), 1057-1065.
- Indira Priyadarsini, K., MAITY, D. K., Naik, G. H., Sudheer Kumar, M., Unnikrishnan, M. K., Satav, J. G., & MOHAN, H. (2003). Role of phenolic OH and methylene hydrogen on the free radical reactions and antioxidant activity of curcumin. *Free radical biology & medicine*, 35(5), 475-484.
- Ireson, C., Orr, S., Jones, D. J., Verschoyle, R., Lim, C. K., Luo, J. L., ... & Steward, W. P. (2001). Characterization of metabolites of the chemopreventive agent curcumin in human and rat hepatocytes and in the rat in vivo, and evaluation of their ability to inhibit phorbol ester-induced prostaglandin E2 production. *Cancer Research*, 61(3), 1058-1064.
- ISHIHARA, M., & SAKAGAMI, H. (2005). Re-evaluation of Cytotoxicity and Iron Chelation Activity of Three β -Diketones by Semiempirical Molecular Orbital Method. *In Vivo*, 19(1), 119-123.
- Jablonska, A., Kozłowska, H., Markiewicz, I., Domanska-Janik, K., & Lukomska, B. (2010). Transplantation of neural stem cells derived from human cord blood to the brain of adult and neonatal rats. *Acta Neurobiol Exp (Wars)*, 70(4), 337-350.
- Jiang, H., Tian, X., Guo, Y., Duan, W., Bu, H., & Li, C. (2011). Activation of nuclear factor erythroid 2-related factor 2 cytoprotective signaling by curcumin protect primary spinal cord astrocytes against oxidative toxicity. *Biological and Pharmaceutical Bulletin*, 34(8), 1194-1197.
- Jiao, Y., Wilkinson, J., Di, X., Wang, W., Hatcher, H., Kock, N. D., ... & Torti, S. V. (2009). Curcumin, a cancer chemopreventive and chemotherapeutic agent, is a biologically active iron chelator. *Blood*, 113(2), 462-469.

- Jin, W., Wang, J., Zhu, T., Yuan, B., Ni, H., Jiang, J., ... & Liang, W. (2014). Anti-inflammatory effects of curcumin in experimental spinal cord injury in rats. *Inflammation Research*, 63(5), 381-387.
- Johnson, R. T., Joy, J. E., Altevogt, B. M., & Liverman, C. T. (Eds.). (2005). *Spinal cord injury: progress, promise, and priorities*. National Academies Press.
- Johnson-Green, P. C., Dow, K. E., & Riopelle, R. J. (1991). Characterization of glycosaminoglycans produced by primary astrocytes in vitro. *Glia*, 4(3), 314-321.
- Jones, T. B., Hart, R. P., & Popovich, P. G. (2005). Molecular control of physiological and pathological T-cell recruitment after mouse spinal cord injury. *Journal of Neuroscience*, 25(28), 6576-6583.
- Jung, G. Y., Lee, J. Y., Rhim, H., Oh, T. H., & Yune, T. Y. (2013). An increase in voltage-gated sodium channel current elicits microglial activation followed inflammatory responses in vitro and in vivo after spinal cord injury. *Glia*, 61(11), 1807-1821.
- Kavakli, H. S., Koca, C., & Alici, O. (2011). Antioxidant effects of curcumin in spinal cord injury in rats. *Ulus Travma Acil Cerrahi Derg*, 17(1), 14-18.
- Kawasaki, Y., Zhang, L., Cheng, J. K., & Ji, R. R. (2008). Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1 β , interleukin-6, and tumor necrosis factor- α in regulating synaptic and neuronal activity in the superficial spinal cord. *Journal of Neuroscience*, 28(20), 5189-5194.
- Keirstead, H. S., Nistor, G., Bernal, G., Totoiu, M., Cloutier, F., Sharp, K., & Steward, O. (2005). Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. *Journal of Neuroscience*, 25(19), 4694-4705.
- Kigerl, K. A., McGAUGHY, V. M., & Popovich, P. G. (2006). Comparative analysis of lesion development and intraspinal inflammation in four strains of mice following spinal contusion injury. *Journal of Comparative Neurology*, 494(4), 578-594.
- Kim, H. Y., Park, E. J., Joe, E. H., & Jou, I. (2003). Curcumin suppresses Janus kinase-STAT inflammatory signaling through activation of Src homology 2 domain-containing tyrosine phosphatase 2 in brain microglia. *The Journal of Immunology*, 171(11), 6072-6079.
- Kim, G. M., Xu, J., Xu, J., Song, S. K., Yan, P., Ku, G., ...& Hsu, C. Y. (2001). Tumor necrosis factor receptor deletion reduces nuclear factor- κ B activation, cellular inhibitor of apoptosis protein 2 expression, and functional recovery after traumatic spinal cord injury. *Journal of Neuroscience*, 21(17), 6617-6625.
- Kim, S. J., Son, T. G., Park, H. R., Park, M., Kim, M. S., Kim, H. S., ...& Lee, J. (2008). Curcumin stimulates proliferation of embryonic neural progenitor cells and neurogenesis in the adult hippocampus. *Journal of Biological Chemistry*, 283(21), 14497-14505.

- Kim, K. T., Kim, M. J., Cho, D. C., Park, S. H., Hwang, J. H., Sung, J. K., ... & Jeon, Y. (2014). The neuroprotective effect of treatment with curcumin in acute spinal cord injury. *Neurologia medico-chirurgica*, 54(5), 387-394.
- Klein, M. A., Möller, J. C., Jones, L. L., Bluethmann, H., Kreutzberg, G. W., & Raivich, G. (1997). Impaired neuroglial activation in interleukin-6 deficient mice. *Glia*, 19(3), 227-233.
- Kohta, M., Kohmura, E., & Yamashita, T. (2009). Inhibition of TGF- β 1 promotes functional recovery after spinal cord injury. *Neuroscience research*, 65(4), 393-401.
- Kong, A. N. T. (2013). *Inflammation, oxidative stress, and cancer: dietary approaches for cancer prevention*. CRC Press.
- Kraft, A. D., Johnson, D. A., & Johnson, J. A. (2004). Nuclear factor E2-related factor 2-dependent antioxidant response element activation by tert-butylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult. *The Journal of neuroscience*, 24(5), 1101-1112.
- Kunwar, A., Narang, H., Priyadarsini, K. I., Krishna, M., Pandey, R., & Sainis, K. B. (2007). Delayed activation of PKC δ and NF κ B and higher radioprotection in splenic lymphocytes by copper (II)–Curcumin (1: 1) complex as compared to curcumin. *Journal of cellular biochemistry*, 102(5), 1214-1224.
- Kwon, B. K., Tetzlaff, W., Grauer, J. N., Beiner, J., & Vaccaro, A. R. (2004). Pathophysiology and pharmacologic treatment of acute spinal cord injury. *The Spine Journal*, 14(4), 451-464.
- Lacroix, S., Chang, L., Rose-John, S., & Tuszynski, M. H. (2002). Delivery of hyper-interleukin-6 to the injured spinal cord increases neutrophil and macrophage infiltration and inhibits axonal growth. *Journal of Comparative Neurology*, 454(3), 213-228.
- Lantz, R. C., Chen, G. J., Sarihan, M., Solyom, A. M., Jolad, S. D., & Timmermann, B. N. (2007). The effect of extracts from ginger rhizome on inflammatory mediator production. *Phytomedicine*, 14(2), 123-128.
- Lao, C. D., Ruffin, M. T., Normolle, D., Heath, D. D., Murray, S. I., Bailey, J. M., ... & Brenner, D. E. (2006). Dose escalation of a curcuminoid formulation. *BMC complementary and alternative medicine*, 6(1), 10.
- Lee, J., & Thumbikat, P. (2015). Pathophysiology, presentation and management of spinal cord injury. *Surgery (Oxford)*, 33(6), 238-247.
- Lee, Y. B., Du, S., Rhim, H., Lee, E. B., Markelonis, G. J., & Oh, T. H. (2000). Rapid increase in immunoreactivity to GFAP in astrocytes in vitro induced by acidic pH is mediated by calcium influx and calpain I. *Brain research*, 864(2), 220-229.

- Lee, S., Lee, J., Kim, S., Park, J. Y., Lee, W. H., Mori, K., ...& Suk, K. (2007). A dual role of lipocalin 2 in the apoptosis and deramification of activated microglia. *The Journal of Immunology*, 179(5), 3231-3241.
- Li, Y., Walker, C. L., Zhang, Y. P., Shields, C. B., & Xu, X. M. (2014). Surgical decompression in acute spinal cord injury: A review of clinical evidence, animal model studies, and potential future directions of investigation. *Frontiers in biology*, 9(2), 127
- Li, Z. W., Li, J. J., Wang, L., Zhang, J. P., Wu, J. J., Mao, X. Q., & Zou, J. (2014). Epidermal growth factor receptor inhibitor ameliorates excessive astrogliosis and improves the regeneration microenvironment and functional recovery in adult rats following spinal cord injury. *Journal of neuroinflammation*, 11(1), 71.
- Lin, M. S., Lee, Y. H., Chiu, W. T., & Hung, K. S. (2011). Curcumin provides neuroprotection after spinal cord injury. *Journal of Surgical Research*, 166(2), 280-289.
- Liu, D., Sybert, T. E., Qian, H., & Liu, J. (1998). Superoxide production after spinal injury detected by microperfusion of cytochrome c. *Free Radical Biology and Medicine*, 25(3), 298-304.
- Liu, N. K., Zhang, Y. P., Titsworth, W. L., Jiang, X., Han, S., Lu, P. H., ... & Xu, X. M. (2006). A novel role of phospholipase A2 in mediating spinal cord secondary injury. *Annals of neurology*, 59(4), 606-619.
- Liu, Y., Dargusch, R., Maher, P., & Schubert, D. (2008). A broadly neuroprotective derivative of curcumin. *Journal of neurochemistry*, 105(4), 1336-1345
- Liu, Z. Q., Xing, S. S., & Zhang, W. (2013). Neuroprotective effect of curcumin on spinal cord in rabbit model with ischemia/reperfusion. *The journal of spinal cord medicine*, 36(2), 147-152.
- Machova Urdzikova, L., Karova, K., Ruzicka, J., Kloudova, A., Shannon, C., Dubisova, J., ... & Jendelova, P. (2015). The anti-inflammatory compound curcumin enhances locomotor and sensory recovery after spinal cord injury in rats by immunomodulation. *International journal of molecular sciences*, 17(1), 49.
- Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., & Locati, M. (2004). The chemokine system in diverse forms of macrophage activation and polarization. *Trends in immunology*, 25(12), 677-686.
- Marczylo, T. H., Verschoyle, R. D., Cooke, D. N., Morazzoni, P., Steward, W. P., & Gescher, A. J. (2007). Comparison of systemic availability of curcumin with that of curcumin formulated with phosphatidylcholine. *Cancer chemotherapy and pharmacology*, 60(2), 171-177.
- Mazzone, G. L., & Nistri, A. (2011). Delayed neuroprotection by riluzole against excitotoxic damage evoked by kainate on rat organotypic spinal cord cultures. *Neuroscience*, 190, 318-327.

- Menet, V., Prieto, M., Privat, A., & y Ribotta, M. G. (2003). Axonal plasticity and functional recovery after spinal cord injury in mice deficient in both glial fibrillary acidic protein and vimentin genes. *Proceedings of the National Academy of Sciences*, 100(15), 8999-9004.
- Menon, V. P., & Sudheer, A. R. (2007). Antioxidant and anti-inflammatory properties of curcumin. In *The molecular targets and therapeutic uses of curcumin in health and disease* (pp. 105-125). Springer US.
- Merrill, J. E., Ignarro, L. J., Sherman, M. P., Melinek, J., & Lane, T. E. (1993). Microglial cell cytotoxicity of oligodendrocytes is mediated through nitric oxide. *The Journal of Immunology*, 151(4), 2132-2141.
- Mir, M., Asensio, V. J., Tolosa, L., Gou-Fabregas, M., Soler, R. M., Llado, J., & Olmos, G. (2009). Tumor necrosis factor alpha and interferon gamma cooperatively induce oxidative stress and motoneuron death in rat spinal cord embryonic explants. *Neuroscience*, 162(4), 959-971.
- Motohashi, H., & Yamamoto, M. (2004). Nrf2–Keap1 defines a physiologically important stress response mechanism. *Trends in molecular medicine*, 10(11), 549-557.
- Munford, R. S., & Pugin, J. (2001). Normal responses to injury prevent systemic inflammation and can be immunosuppressive. *American journal of respiratory and critical care medicine*, 163(2), 316-321.
- Nafisi, S., Adelzadeh, M., Norouzi, Z., & Sarbolouki, M. N. (2009). Curcumin binding to DNA and RNA. *DNA and cell biology*, 28(4), 201-208.
- Nair, A. B., & Jacob, S. (2016). A simple practice guide for dose conversion between animals and human. *Journal of basic and clinical pharmacy*, 7(2), 27.
- National Research Council. (2010). *Guide for the care and use of laboratory animals*. National Academies Press.
- Nguyen, D. H., Cho, N., Satkunendrarajah, K., Austin, J. W., Wang, J., & Fehlings, M. G. (2012). Immunoglobulin G (IgG) attenuates neuroinflammation and improves neurobehavioral recovery after cervical spinal cord injury. *Journal of neuroinflammation*, 9(1), 1.
- Nguyen, T., Sherratt, P. J., & Pickett, C. B. (2003). Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. *Annual review of pharmacology and toxicology*, 43(1), 233-260.
- Ni, H., Jin, W., Zhu, T., Wang, J., Yuan, B., Jiang, J., ...& Ma, Z. (2015). Curcumin modulates TLR4/NF-κB inflammatory signaling pathway following traumatic spinal cord injury in rats. *The journal of spinal cord medicine*, 38(2), 199-206.
- Nimse, S. B., & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Advances*, 5(35), 27986-28006.

- Noble, L. J., Donovan, F., Igarashi, T., Goussev, S., & Werb, Z. (2002). Matrix metalloproteinases limit functional recovery after spinal cord injury by modulation of early vascular events. *Journal of Neuroscience*, 22(17), 7526-7535.
- Okada, S., Nakamura, M., Mikami, Y., Shimazaki, T., Mihara, M., Ohsugi, Y., ... & Okano, H. (2004). Blockade of interleukin-6 receptor suppresses reactive astrogliosis and ameliorates functional recovery in experimental spinal cord injury. *Journal of neuroscience research*, 76(2), 265-276.
- Olivera, A., Moore, T. W., Hu, F., Brown, A. P., Sun, A., Liotta, D. C., ... & Miller, A. H. (2012). Inhibition of the NF- κ B signaling pathway by the curcumin analog, 3, 5-Bis (2-pyridinylmethylidene)-4-piperidone (EF31): anti-inflammatory and anti-cancer properties. *International immunopharmacology*, 12(2), 368-377.
- Olmos, G., & Lladó, J. (2014). Tumor necrosis factor alpha: a link between neuroinflammation and excitotoxicity. *Mediators of inflammation*, 2014.
- Oppenheimer, A. (1937). Turmeric (curcumin) in biliary diseases. *The Lancet*, 229(5924), 619-621.
- Ormond, D. R., Shannon, C., Oppenheim, J., Zeman, R., Das, K., Murali, R., & Jhanwar-Uniyal, M. (2014). Stem cell therapy and curcumin synergistically enhance recovery from spinal cord injury. *PLoS One*, 9(2), e88916.
- Oyinbo, C. A. (2011). Secondary injury mechanisms in traumatic spinal cord injury: a nugget of this multiply cascade. *Acta Neurobiol Exp (Wars)*, 71(2), 281-299.
- Palipoch, S., Punsawad, C., Koomhin, P., & Suwannalert, P. (2014). Hepatoprotective effect of curcumin and alpha-tocopherol against cisplatin-induced oxidative stress. *BMC complementary and alternative medicine*, 14(1), 111.
- Pan, M. H., Huang, T. M., & Lin, J. K. (1999). Biotransformation of curcumin through reduction and glucuronidation in mice. *Drug metabolism and disposition*, 27(4), 486-494.
- Pan, W., Zhang, L., Liao, J., Csernus, B., & Kastin, A. J. (2003). Selective increase in TNF α permeation across the blood-spinal cord barrier after SCI. *Journal of neuroimmunology*, 134(1), 111-117.
- Park, C. (2007). Curcumin attenuates the release of pro-inflammatory cytokines in lipopolysaccharide-stimulated BV2 microglia. *Acta Pharmacologica Sinica*, 28(10), 1645-1651.
- Paterniti, I., Genovese, T., Crisafulli, C., Mazzon, E., Di Paola, R., Galuppo, M., ... & Cuzzocrea, S. (2009). Treatment with green tea extract attenuates secondary inflammatory response in an experimental model of spinal cord trauma. *Naunyn-Schmiedeberg's archives of pharmacology*, 380(2), 179-192.

- Pérez-Hernández, J., Zaldívar-Machorro, V. J., Villanueva-Porras, D., Vega-Ávila, E., & Chavarría, A. (2016). A potential alternative against neurodegenerative diseases: Phytodrugs. *Oxidative medicine and cellular longevity*, 2016.
- Pineau, I., & Lacroix, S. (2007). Proinflammatory cytokine synthesis in the injured mouse spinal cord: multiphasic expression pattern and identification of the cell types involved. *Journal of Comparative Neurology*, 500(2), 267-285
- Plemel, J. R., Duncan, G., Chen, K. W. K., Shannon, C., Park, S., Sparling, J. S., & Tetzlaff, W. (2008). A graded forceps crush spinal cord injury model in mice. *Journal of neurotrauma*, 25(4), 350-370.
- Popovich, P. G., Stuckman, S., Gienapp, I. E., & Whitacre, C. C. (2001). Alterations in immune cell phenotype and function after experimental spinal cord injury. *Journal of neurotrauma*, 18(9), 957-966.
- Porter, R. (2001). *The Cambridge illustrated history of medicine*. Cambridge University Press.
- Probert, L., & Selmaj, K. (1997). TNF and related molecules: trends in neuroscience and clinical applications. *Journal of neuroimmunology*, 72(2), 113-117.
- Purves, D., Augustine, G. J., Fitzpatrick, D., Hall, W. C., JO, M., & Neuroscience, W. S. (2004). *Sinauer associates. Inc.*, 3(5), 24.
- Qureshi, S., Shah, A. H., & Ageel, A. M. (1992). Toxicity studies on *Alpinia galanga* and *Curcuma longa*. *Planta medica*, 58(2), 124-127.
- Rachmawati, H., Shaal, L. A., Müller, R. H., & Keck, C. M. (2013). Development of curcumin nanocrystal: physical aspects. *Journal of pharmaceutical sciences*, 102(1), 204-214.
- Rajasekaran, S. A. (2011). Therapeutic potential of curcumin in gastrointestinal diseases. *World J Gastrointest Pathophysiol*, 2(1), 1-14.
- Rao, C. V., Rivenson, A., Simi, B., & Reddy, B. S. (1995). Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. *Cancer research*, 55(2), 259-266
- Reeta, K. H., Mehla, J., & Gupta, Y. K. (2009). Curcumin is protective against phenytoin-induced cognitive impairment and oxidative stress in rats. *Brain research*, 1301, 52-60.
- Renault-Mihara, F., Okada, S., Shibata, S., Nakamura, M., Toyama, Y., & Okano, H. (2008). Spinal cord injury: emerging beneficial role of reactive astrocytes' migration. *The international journal of biochemistry & cell biology*, 40(9), 1649-1653.
- Rezvanpour, A., Santamaria-Kisiel, L., & Shaw, G. S. (2011). The S100A10-annexin A2 complex provides a novel asymmetric platform for membrane repair. *Journal of Biological Chemistry*, 286(46), 40174-40183.

- Rhee, J. M., Shamji, M. F., Erwin, W. M., Bransford, R. J., Yoon, S. T., Smith, J. S., ...& Kalsi-Ryan, S. (2013). Nonoperative management of cervical myelopathy: a systematic review. *Spine*, 38(22S), S55-S67.
- Rhoney, D. H., Luer, M. S., Hughes, M., & Hatton, J. (1996). New pharmacologic approaches to acute spinal cord injury. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, 16(3), 382-392.
- Ricote, M., Li, A. C., Willson, T. M., Kelly, C. J., & Glass, C. K. (1998). The peroxisome proliferator-activated receptor- γ is a negative regulator of macrophage activation. *Nature*, 391(6662), 79-82.
- Rimaniol, A. C., Haïk, S., Martin, M., Le Grand, R., Boussin, F. D., Dereuddre-Bosquet, N., ...& Dormont, D. (2000). Na⁺-dependent high-affinity glutamate transport in macrophages. *The Journal of Immunology*, 164(10), 5430-5438.
- Ritz, M. F., Graumann, U., Gutierrez, B., & Hausmann, O. (2010). Traumatic spinal cord injury alters angiogenic factors and TGF- β 1 that may affect vascular recovery. *Current neurovascular research*, 7(4), 301-310.
- Rivlin, A. S., & Tator, C. H. (1978). Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. *Surgical neurology*, 10(1), 38-43.
- Sallusto, F., Mackay, C. R., & Lanzavecchia, A. (2000). The role of chemokine receptors in primary, effector, and memory immune responses. *Annual review of immunology*, 18(1), 593-620.
- Sanli, A. M., Turkoglu, E., Serbes, G., Sargon, M. F., Besalti, O., Kilinc, K., & Sekerci, Z. (2011). Effect of curcumin on lipid peroxidation, early ultrastructural findings and neurological recovery after experimental spinal cord contusion injury in rats. *Turkish neurosurgery*, 22(2), 189-195.
- Satzer, D., Miller, C., Maxon, J., Voth, J., DiBartolomeo, C., Mahoney, R., & Parr, A. M. (2015). T cell deficiency in spinal cord injury: altered locomotor recovery and whole-genome transcriptional analysis. *BMC neuroscience*, 16(1), 74.
- Scaltriti, M., & Baselga, J. (2006). The epidermal growth factor receptor pathway: a model for targeted therapy. *Clinical Cancer Research*, 12(18), 5268-5272.
- Scapagnini, G., Sonya, V., Nader, A. G., Calogero, C., Zella, D., & Fabio, G. (2011). Modulation of Nrf2/ARE pathway by food polyphenols: a nutritional neuroprotective strategy for cognitive and neurodegenerative disorders. *Molecular neurobiology*, 44(2), 192-201.
- Schwab, M. E. (2004). Nogo and axon regeneration. *Current opinion in neurobiology*, 14(1), 118-124.

- Schwab, J. M., Brechtel, K., Mueller, C. A., Failli, V., Kaps, H. P., Tuli, S. K., & Schluesener, H. J. (2006). Experimental strategies to promote spinal cord regeneration—an integrative perspective. *Progress in neurobiology*, 78(2), 91-116
- Schwartz, M., & Kipnis, J. (2001). Protective autoimmunity: regulation and prospects for vaccination after brain and spinal cord injuries. *Trends in molecular medicine*, 7(6), 252-258.
- Schwartz, M., & Yoles, E. (2006). Immune-based therapy for spinal cord repair: autologous macrophages and beyond. *Journal of neurotrauma*, 23(3-4), 360-370.
- Sebastian, J., & Schroeter, M. (2003). Detrimental and beneficial effects of injury-induced inflammation and cytokine expression in the nervous system. In *Molecular and Cellular Biology of Neuroprotection in the CNS* (pp. 87-113). Springer US
- Shamash, S., Reichert, F., & Rotshenker, S. (2002). The cytokine network of Wallerian degeneration: tumor necrosis factor- α , interleukin-1 α , and interleukin-1 β . *The Journal of Neuroscience*, 22(8), 3052-3060.
- Shan, L. Q., Ma, S., Qiu, X. C., Zhou, Y., Zhang, Y., Zheng, L. H., ... & Ma, B. A. (2010). Hydroxysafflor Yellow A protects spinal cords from ischemia/reperfusion injury in rabbits. *BMC neuroscience*, 11(1), 98.
- Sharma, H. S., Winkler, T., Stålberg, E., Gordh, T., Alm, P., & Westman, J. (2003). Topical application of TNF- α antiserum attenuates spinal cord trauma induced edema formation, microvascular permeability disturbances and cell injury in the rat. In *Brain Edema XII* (pp. 407-413). Springer, Vienna
- Sharma, H. S. (2007). A select combination of neurotrophins enhances neuroprotection and functional recovery following spinal cord injury. *Annals of the New York Academy of Sciences*, 1122(1), 95-111
- Shen, L. R., Parnell, L. D., Ordovas, J. M., & Lai, C. Q. (2013). Curcumin and aging. *Biofactors*, 39(1), 133-140.
- Shi, E., Jiang, X., Wang, L., Akuzawa, S., Nakajima, Y., & Kazui, T. (2010). Intrathecal injection of hepatocyte growth factor gene-modified marrow stromal cells attenuates neurologic injury induced by transient spinal cord ischemia in rabbits. *Anesthesiology: The Journal of the American Society of Anesthesiologists*, 113(5), 1109-1117.
- Shimizu, H., & Yozu, R. (2011). Current strategies for spinal cord protection during thoracic and thoracoabdominal aortic aneurysm repair. *General thoracic and cardiovascular surgery*, 59(3), 155.
- Shin, H. J., Lee, J. Y., Son, E., Lee, D. H., Kim, H. J., Kang, S. S., & Roh, G. S. (2007). Curcumin attenuates the kainic acid-induced hippocampal cell death in the mice. *Neuroscience letters*, 416(1), 49-54.

- Shishodia, S. (2013). Molecular mechanisms of curcumin action: gene expression. *Biofactors*, 39(1), 37-55.
- Silberstein, M., & McLean, K. (1994). Non-contiguous spinal injury: clinical and imaging features, and postulated mechanism. *Spinal Cord*, 32(12), 817-823.
- Silva, N. A., Sousa, N., Reis, R. L., & Salgado, A. J. (2014). From basics to clinical: a comprehensive review on spinal cord injury. *Progress in neurobiology*, 114, 25-57.
- Simon, C. M., Sharif, S., Tan, R. P., & LaPlaca, M. C. (2009). Spinal cord contusion causes acute plasma membrane damage. *Journal of neurotrauma*, 26(4), 563-574.
- Singh, A., Tetreault, L., Kalsi-Ryan, S., Nouri, A., & Fehlings, M. G. (2014). Global prevalence and incidence of traumatic spinal cord injury. *Clin Epidemiol*, 6, 309-331.
- Skrzypczak-Jankun, E., Zhou, K., McCabe, N. P., Selman, S. H., & Jankun, J. (2003). Structure of curcumin in complex with lipoxygenase and its significance in cancer. *International journal of molecular medicine*, 12(1), 17-24.
- Somparn, P., Phisalaphong, C., Nakornchai, S., Unchern, S., & Morales, N. P. (2007). Comparative antioxidant activities of curcumin and its demethoxy and hydrogenated derivatives. *Biological and Pharmaceutical Bulletin*, 30(1), 74-78.
- Sriram, S., & Rodriguez, M. (1997). Indictment of the microglia as the villain in multiple sclerosis. *Neurology*, 48(2), 464-470.
- Stellwagen, D., Beattie, E. C., Seo, J. Y., & Malenka, R. C. (2005). Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor- α . *The Journal of Neuroscience*, 25(12), 3219-3228.
- Sullivan, P. G., Krishnamurthy, S., Patel, S. P., Pandya, J. D., & Rabchevsky, A. G. (2007). Temporal characterization of mitochondrial bioenergetics after spinal cord injury. *Journal of neurotrauma*, 24(6), 991-999.
- Sumanont, Y., Murakami, Y., Tohda, M., Vajragupta, O., Matsumoto, K., & Watanabe, H. (2004). Evaluation of the nitric oxide radical scavenging activity of manganese complexes of curcumin and its derivative. *Biological and Pharmaceutical Bulletin*, 27(2), 170-173.
- Takahashi, J. L., Giuliani, F., Power, C., Imai, Y., & Yong, V. W. (2003). Interleukin-1 β promotes oligodendrocyte death through glutamate excitotoxicity. *Annals of neurology*, 53(5), 588-595.
- Takeuchi, T., Ishidoh, T., Iijima, H., Kuriyama, I., Shimazaki, N., Koiwai, O., ...& Yoshida, H. (2006). Structural relationship of curcumin derivatives binding to the BRCT domain of human DNA polymerase λ . *Genes to Cells*, 11(3), 223-235.

- Tator, C. H. (1998). Biology of neurological recovery and functional restoration after spinal cord injury. *Neurosurgery*, 42(4), 696-707.
- Tator, C. H. (2006). Review of treatment trials in human spinal cord injury: Issues, difficulties, and recommendations. *Neurosurgery*, 59(5), 957-987.
- Tayyem, R. F., Heath, D. D., Al-Delaimy, W. K., & Rock, C. L. (2006). Curcumin content of turmeric and curry powders. *Nutrition and cancer*, 55(2), 126-131.
- Thuret, S., Moon, L. D., & Gage, F. H. (2006). Therapeutic interventions after spinal cord injury. *Nature Reviews Neuroscience*, 7(8), 628-643.
- Tian, J. W., Fu, F. H., Jiang, W. L., Wang, C. Y., Sun, F., & Zhang, T. P. (2004). Protective effect of hydroxysafflor yellow A against rat cortex mitochondrial injuries induced by cerebral ischemia. *Yao xue xue bao = Acta pharmaceutica Sinica*, 39(10), 774-777.
- Trinchese, F., Liu, S., Zhang, H., Hidalgo, A., Schmidt, S. D., Yamaguchi, H., ... & Arancio, O. (2008). Inhibition of calpains improves memory and synaptic transmission in a mouse model of Alzheimer disease. *The Journal of clinical investigation*, 118(8), 2796-2807.
- Trivedi, A., Olivas, A. D., & Noble-Haeusslein, L. J. (2006). Inflammation and spinal cord injury: infiltrating leukocytes as determinants of injury and repair processes. *Clinical neuroscience research*, 6(5), 283-292.
- Ueki, M., Ueno, M., Morishita, J., & Maekawa, N. (2013). Curcumin ameliorates cisplatin-induced nephrotoxicity by inhibiting renal inflammation in mice. *Journal of bioscience and bioengineering*, 115(5), 547-551.
- US Department of Health and Human Services. (2015). Office of Laboratory Animal Welfare—Public Health Service policy on humane care and use of laboratory animals. Bethesda (MD): National Institutes of Health
- Valkonen, M., & Kuusi, T. (1997). Spectrophotometric assay for total peroxyl radical-trapping antioxidant potential in human serum. *Journal of Lipid Research*, 38(4), 823-833.
- Vander, A. J., Luciano, D., Sherman, J. H., 2001. *Human Physiology: The Mechanisms of Body Function*. McGraw-Hill
- Vanický, I., Urdžíková, L., Saganová, K., Čížková, D., & Gálik, J. (2001). A simple and reproducible model of spinal cord injury induced by epidural balloon inflation in the rat. *Journal of neurotrauma*, 18(12), 1399-1407
- Varija, D., Kumar, K. P., Reddy, K. P., & Reddy, V. K. (2009). Prolonged constriction of sciatic nerve affecting oxidative stressors & antioxidant enzymes in rat.

- Varma, A. K., Das, A., Wallace, G., Barry, J., Vertegel, A. A., Ray, S. K., & Banik, N. L. (2013). Spinal cord injury: a review of current therapy, future treatments, and basic science frontiers. *Neurochemical research*, 38(5), 895-905
- Von Boxberg, Y., Salim, C., Soares, S., Baloui, H., Alterio, J., Ravaille-Veron, M., & Nothias, F. (2006). Spinal cord injury-induced up-regulation of AHNK, expressed in cells delineating cystic cavities, and associated with neoangiogenesis. *European Journal of Neuroscience*, 24(4), 1031-1041.
- Von Büdingen, H. C., Tanuma, N., Villoslada, P., Ouallet, J. C., Hauser, S. L., & Genain, C. P. (2001). Immune responses against the myelin/oligodendrocyte glycoprotein in experimental autoimmune demyelination. *Journal of clinical immunology*, 21(3), 155-170
- Wagner, J. A. (1996). Is IL-6 both a cytokine and a neurotrophic factor?. *Journal of Experimental Medicine*, 183(6), 2417-2419.
- Wang, J., Wang, H., Zhu, R., Liu, Q., Fei, J., & Wang, S. (2015). Anti-inflammatory activity of curcumin-loaded solid lipid nanoparticles in IL-1 β transgenic mice subjected to the lipopolysaccharide-induced sepsis. *Biomaterials*, 53, 475-483.
- Wang, Y. J., Pan, M. H., Cheng, A. L., Lin, L. I., Ho, Y. S., Hsieh, C. Y., & Lin, J. K. (1997). Stability of curcumin in buffer solutions and characterization of its degradation products. *Journal of pharmaceutical and biomedical analysis*, 15(12), 1867-1876.
- Webb, A. A., Ngan, S., & Fowler, J. D. (2010). Spinal cord injury I: A synopsis of the basic science. *The Canadian Veterinary Journal*, 51(5), 485.
- White-Schenk, D., Shi, R., & Leary, J. F. (2015). Nanomedicine strategies for treatment of secondary spinal cord injury. *International journal of nanomedicine*, 10, 923.
- Wilhelmsson, U., Li, L., Pekna, M., Berthold, C. H., Blom, S., Eliasson, C., ... & Pekny, M. (2004). Absence of glial fibrillary acidic protein and vimentin prevents hypertrophy of astrocytic processes and improves post-traumatic regeneration. *Journal of Neuroscience*, 24(21), 5016-5021.
- Wilson, J. R., Forgione, N., & Fehlings, M. G. (2013). Emerging therapies for acute traumatic spinal cord injury. *Canadian Medical Association Journal*, 185(6), 485-492.
- Wilson, J. X. (1997). Antioxidant defense of the brain: a role for astrocytes. *Canadian journal of physiology and pharmacology*, 75(10-11), 1149-1163.
- Witcher, K. G., Eiferman, D. S., & Godbout, J. P. (2015). Priming the inflammatory pump of the CNS after traumatic brain injury. *Trends in neurosciences*, 38(10), 609-620.
- Witsch, E., Sela, M., & Yarden, Y. (2010). Roles for growth factors in cancer progression. *Physiology*, 25(2), 85-101.

- Woo, M. S., Jung, S. H., Kim, S. Y., Hyun, J. W., Ko, K. H., Kim, W. K., & Kim, H. S. (2005). Curcumin suppresses phorbol ester-induced matrix metalloproteinase-9 expression by inhibiting the PKC to MAPK signaling pathways in human astrogloma cells. *Biochemical and biophysical research communications*, 335(4), 1017-1025.
- Wu, K. L., Chan, S. H., Chao, Y. M., & Chan, J. Y. (2003). Expression of pro-inflammatory cytokine and caspase genes promotes neuronal apoptosis in pontine reticular formation after spinal cord transection. *Neurobiology of disease*, 14(1), 19-31.
- Wu, A., Ying, Z., Schubert, D., & Gomez-Pinilla, F. (2011). Brain and spinal cord interaction: a dietary curcumin derivative counteracts locomotor and cognitive deficits after brain trauma. *Neurorehabilitation and neural repair*, 25(4), 332-342.
- Wynn, T. A. (2004). Fibrotic disease and the TH1/TH2 paradigm. *Nature Reviews Immunology*, 4(8), 583-594.
- Xiong, Y., Rabchevsky, A. G., & Hall, E. D. (2007). Role of peroxynitrite in secondary oxidative damage after spinal cord injury. *Journal of neurochemistry*, 100(3), 639-649.
- Xu, J., Fu, Y., & Chen, A. (2003). Activation of peroxisome proliferator-activated receptor- γ contributes to the inhibitory effects of curcumin on rat hepatic stellate cell growth. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 285(1), G20-G30.
- Xu, W., Chi, L., Xu, R., Ke, Y., Luo, C., Cai, J., ... & Liu, R. (2005). Increased production of reactive oxygen species contributes to motor neuron death in a compression mouse model of spinal cord injury. *Spinal cord*, 43(4), 204-213.
- Yakovlev, A. G., & Faden, A. I. (1994). Sequential expression of c-fos protooncogene, TNF- α , and dynorphin genes in spinal cord following experimental traumatic injury. *Molecular and Chemical Neuropathology*, 23(2-3), 179-190.
- Yamashita, T., Fujitani, M., Yamagishi, S., Hata, K., & Mimura, F. (2005). Multiple signals regulate axon regeneration through the Nogo receptor complex. *Molecular neurobiology*, 32(2), 105-111.
- Yang, L., Jones, N. R., Blumbergs, P. C., Van Den Heuvel, C., Moore, E. J., Manavis, J., ... & Ghabriel, M. N. (2005). Severity-dependent expression of pro-inflammatory cytokines in traumatic spinal cord injury in the rat. *Journal of clinical neuroscience*, 12(3), 276-284.
- Yang, Y., Bazhin, A. V., Werner, J., & Karakhanova, S. (2013). Reactive oxygen species in the immune system. *International reviews of immunology*, 32(3), 249-270.
- Yiu, G., & He, Z. (2006). Glial inhibition of CNS axon regeneration. *Nature Reviews Neuroscience*, 7(8), 617-627.

- Yuan, J., Zou, M., Xiang, X., Zhu, H., Chu, W., Liu, W., ...& Lin, J. (2015). Curcumin improves neural function after spinal cord injury by the joint inhibition of the intracellular and extracellular components of glial scar. *Journal of Surgical Research*, 195(1), 235-245.
- Zamanian, J. L., Xu, L., Foo, L. C., Nouri, N., Zhou, L., Giffard, R. G., & Barres, B. A. (2012). Genomic analysis of reactive astrogliosis. *Journal of Neuroscience*, 32(18), 6391-6410.
- Zhang, N., Yin, Y., Xu, S. J., Wu, Y. P., & Chen, W. S. (2012). Inflammation & apoptosis in spinal cord injury. *Indian journal of medical research*, 135(3), 287.
- Zhang, X., Shi, L. L., Gao, X., Jiang, D., Zhong, Z. Q., Zeng, X., ...& Li, L. (2015). Lentivirus-mediated Inhibition of Tumour Necrosis Factor- α improves motor function associated with PRDX6 in spinal cord contusion rats. *Scientific reports*, 5.
- Zheng, S., & Chen, A. (2006). Curcumin suppresses the expression of extracellular matrix genes in activated hepatic stellate cells by inhibiting gene expression of connective tissue growth factor. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 290(5), G883-G893.
- Zhou, H., Beevers, C. S., & Huang, S. (2011). Targets of curcumin. *Current drug targets*, 12(3), 332.

APPENDIX

1) MDA 1 day:

Results are expressed as mean \pm standard deviation

Sig. = Significant , Sign.= Significance, N.S.= Not significant

Table 1 : One way ANOVA

Marker	1 Day				
	Control	SCI	SCI+CUR	p-value	Sig.
MDA	414.83 \pm 126.8	835.67 \pm 61.5	493.60 \pm 75.2	0.004	Significant
Statistical test used: One-way ANOVA					
p-value \leq 0.05 considered statistically significant (95% confidence interval).					

Table 2: Dunnett Post Hoc to identify variables that made significant change

Marker	1 Day						
	Control	SCI	p-value	Sig.	SCI+CUR	p-value	Sig.
MDA	414.83 \pm 126.8	835.67 \pm 61.5	0.003	Significant	493.60 \pm 75.2	0.611	N.S
Statistical test used: Dunnett post Hoc test							
p-value \leq 0.05 considered statistically significant different from control (95% confidence interval).							

Table 3: Independent t-test

Marker	1 Day			
	SCI	SCI+CUR	p-value	Sig.
MDA	835.67 \pm 61.5	493.60 \pm 75.2	0.011	Significant
Statistical test used: Independent t-test				
p-value \leq 0.05 considered statistically significant different (95% confidence interval).				

2) MDA 7 days

Table 4: One way ANOVA

Marker	7 Days					
	Control	SCI	SCI+CUR	SCI+DIET	p-value	Sig.
MDA	414.83±126.78	480.73±121.14	245.38±36.88	406.32±145.71	0.047	Significant
Statistical test used: One-way ANOVA						
p-value ≤ 0.05 considered statistically significant (95% confidence interval).						

Table 5: Dunnett Post Hoc to identify variables that made significant change

Marker	7 Days									
	Control	SCI	p-value	Sig.	SCI+CUR	p-value	Sig.	SCI+DIET	p-value	Sig.
MDA	414.83±126.78	480.73±121.14	0.702	N.S	245.38±36.88	0.131	N.S	406.32±145.71	0.999	N.S
Statistical test used: Dunnett post Hoc test										
p-value ≤ 0.05 considered statistically significant different from control (95% confidence interval).										

Table 6: Dunnett Post Hoc to identify variables that made significant change

Marker	7 Days								
	SCI			SCI+CUR			SCI+DIET		
	Mean ±SD	p-value	Sig.	Mean ±SD	p-value	Sig.	Mean ±SD	p-value	Sig.
MDA	480.73±121.14			245.38±36.88			406.32±145.71		
SCI	480.73±121.14	-----	-----	480.73±121.14	0.014	Sign.	480.73±121.14	0.563	N.S
SCI+CUR	245.38±36.88	0.014	Sign.	245.38±36.88	-----	-----	245.38±36.88	0.137	N.S
SCI+DIET	406.32±145.71	0.563	N.S	406.32±145.71	0.137	N.S	406.32±145.71	-----	-----
Statistical test used: Dunnett post Hoc test									
p-value ≤ 0.05 considered statistically significant different (95% confidence interval).									

3) TAC 1 day

Table 7: One way ANOVA

Marker	1 Day				
	Control	SCI	SCI+CUR	p-value	Sig.
TAC	5.66±0.94	0.46±0.21	0.54±0.13	0.0001	Significant
Statistical test used: One-way ANOVA					
p-value ≤ 0.05 considered statistically significant (95% confidence interval).					

Table 8: Dunnett Post Hoc to identify variables that made significant change

Marker	1 Day						
	Control	SCI	p-value	Sig.	SCI+CUR	p-value	Sig.
TAC	5.66±0.94	0.46±0.21	0.0001	Significant	0.54±0.13	0.0001	Significant
Statistical test used: Dunnett post Hoc test							
p-value ≤ 0.05 considered statistically significant different from control (95% confidence interval).							

Table 9: Independent t-test

Marker	1 Day			
	SCI	SCI+CUR	p-value	Sig.
TAC	0.46±0.21	0.54±0.13	0.577	Not significant
Statistical test used: Independent t-test				
p-value ≤ 0.05 considered statistically significant different (95% confidence interval).				

4) TAC 7 days

Table 10: One way ANOVA

Marker	7 Days					
	Control	SCI	SCI+CUR	SCI+DIET	p-value	Sig.
TAC	5.66±0.94	1.30±0.51	3.06±1.40	5.81±0.83	0.0001	Significant
Statistical test used: One-way ANOVA						
p-value ≤ 0.05 considered statistically significant (95% confidence interval).						

Table 11: Dunnett Post Hoc to identify variables that made significant change

Marker	7 Days									
	Control	SCI	p-value	Sig.	SCI+CUR	p-value	Sig.	SCI+DIET	p-value	Sig.
TAC	5.66±0.94	1.30±0.51	0.0001	Sign.	3.06±1.40	0.002	Sign.	5.81±0.83	0.992	N.S
Statistical test used: Dunnett post Hoc test										
p-value ≤ 0.05 considered statistically significant different from control (95% confidence interval).										

Table 12: Dunnett Post Hoc to identify variables that made significant change

Marker	7 Days								
	SCI			SCI+CUR			SCI+DIET		
	Mean ±SD	p-value	Sig.	Mean ±SD	p-value	Sig.	Mean ±SD	p-value	Sig.
TAC	1.30±0.51			3.06±1.40			5.81±0.83		
SCI	1.30±0.51	-----	-----	1.30±0.51	0.024	Sign.	1.30±0.51	0.0001	Sign.
SCI+CUR	3.06±1.40	0.024	Sign.	3.06±1.40	-----	-----	3.06±1.40	0.003	Sign.
SCI+DIET	5.81±0.83	0.0001	Sign.	5.81±0.83	0.003	Sign.	5.81±0.83	-----	-----
Statistical test used: Dunnett post Hoc test									
p-value ≤ 0.05 considered statistically significant different (95% confidence interval).									

5) TNF α 7 days

Table 13: One way ANOVA

Marker	7 Days					
	Control	SCI	SCI+CUR	SCI+DIET	p-value	Sig.
TNF alpha	17.52±1.94	56.09±7.53	18.29±3.76	27.17±5.50	0.0001	Significant
Statistical test used: One-way ANOVA						
p-value ≤ 0.05 considered statistically significant (95% confidence interval).						

Table 14: Dunnett Post Hoc to identify variables that made significant change

Marker	7 Days									
	Control	SCI	p-value	Sig.	SCI+CUR	p-value	Sig.	SCI+DIET	p-value	Sig.
TNF alpha	17.52±1.94	56.09±7.53	0.0001	Sign.	18.29±3.76	0.994	N.S	27.17±5.50	0.067	N.S
Statistical test used: Dunnett post Hoc test										
p-value ≤ 0.05 considered statistically significant different from control (95% confidence interval).										

Table 15: Dunnett Post Hoc to identify variables that made significant change

Marker	7 Days								
	SCI			SCI+CUR			SCI+DIET		
	Mean ±SD	p-value	Sig.	Mean ±SD	p-value	Sig.	Mean ±SD	p-value	Sig.
<i>TNF alpha</i>	56.09±7.53			18.29±3.76			27.17±5.50		
SCI	56.09±7.53	-----	-----	56.09±7.53	0.0001	Sign.	56.09±7.53	0.0001	Sign.
SCI+CUR	18.29±3.76	0.0001	Sign.	18.29±3.76	-----	-----	18.29±3.76	0.114	N.S
SCI+DIET	27.17±5.50	0.0001	Sign.	27.17±5.50	0.114	N.S	27.17±5.50	-----	-----
Statistical test used: Dunnett post Hoc test									
p-value ≤ 0.05 considered statistically significant different (95% confidence interval).									

6) IL-6 1 day

Table 16: One way ANOVA

Marker	1 Day				
	Control	SCI	SCI+CUR	p-value	Sig.
IL-6	53.23±5.55	327.99±58.93	244.81±90.51	0.009	Significant
Statistical test used: One-way ANOVA					
p-value ≤ 0.05 considered statistically significant (95% confidence interval).					

Table 17: Dunnett Post Hoc to identify variables that made significant change

Marker	1 Day						
	Control	SCI	p-value	Sig.	SCI+CUR	p-value	Sig.
IL-6	53.23±5.55	327.99±58.93	0.008	Significant	244.81±90.51	0.019	Significant
Statistical test used: Dunnett post Hoc test							
p-value ≤ 0.05 considered statistically significant different from control (95% confidence interval).							

Table 18: Independent t-test

Marker	1 Day			
	SCI	SCI+CUR	p-value	Sig.
IL-6	327.99±58.93	244.80±90.51	0.315	Not significant
Statistical test used: Independent t-test				
p-value ≤ 0.05 considered statistically significant different (95% confidence interval).				

7) IL-6 7 days

Table 19: One way ANOVA

Marker	7 Days				
	Control	SCI	SCI+CUR	p-value	Sig.
IL-6	53.23±5.55	349.56±43.17	214.18±82.07	0.0001	Significant
Statistical test used: One-way ANOVA					
p-value ≤ 0.05 considered statistically significant (95% confidence interval).					

Table 20: Dunnett Post Hoc to identify variables that made significant change

Marker	7 Days						
	Control	SCI	p-value	Sig.	SCI+CUR	p-value	Sig.
IL-6	53.23±5.55	349.56±43.17	0.0001	Significant	214.18±82.07	0.009	Significant
Statistical test used: Dunnett post Hoc test							
p-value ≤ 0.05 considered statistically significant different from control (95% confidence interval).							

Table 21: Independent t-test

Marker	7 Days			
	SCI	SCI+CUR	p-value	Sig.
IL-6	349.56±43.17	214.18±82.07	0.020	Significant
Statistical test used: Independent t-test				
p-value ≤ 0.05 considered statistically significant different (95% confidence interval).				

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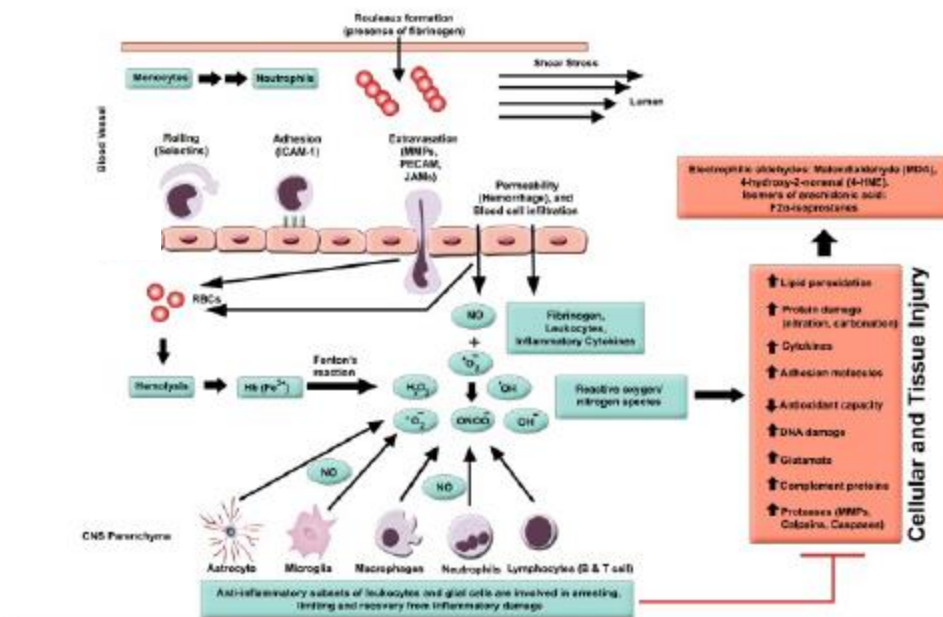
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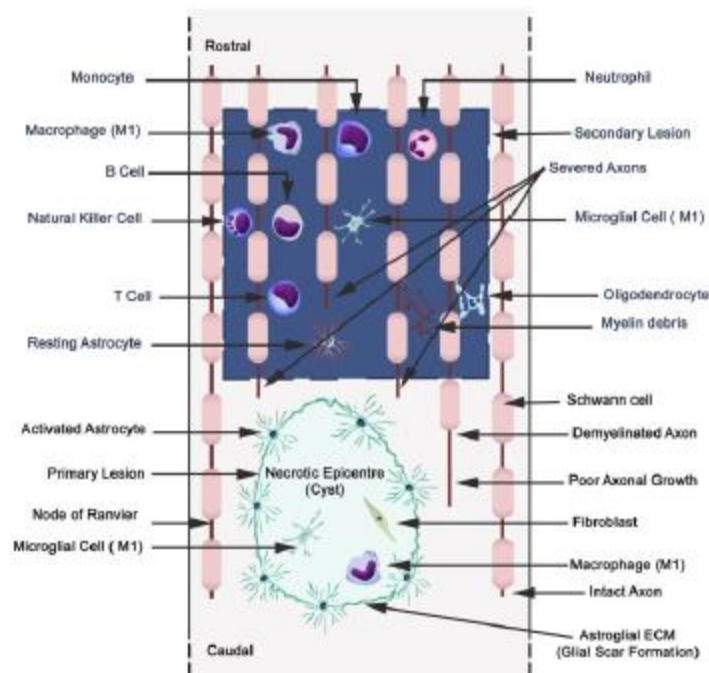
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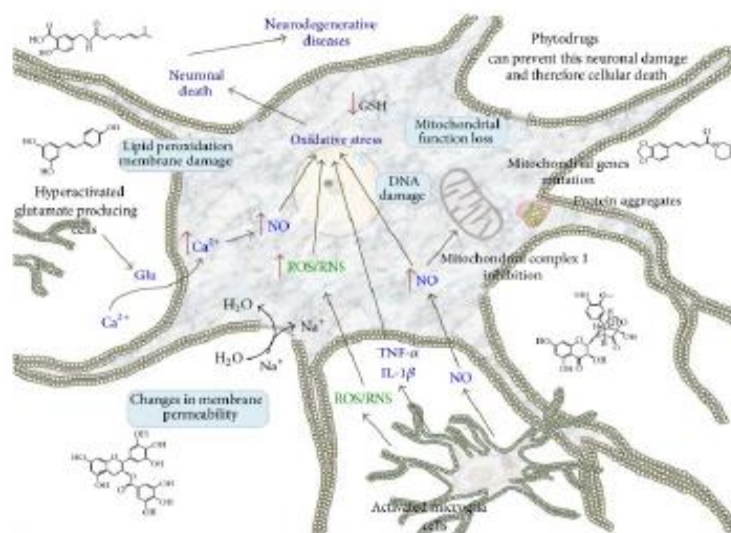
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Spinal Cord Injury

Distraction: Stretching of cord

Compression of cord: Burst fractures

Dislocation: Displacement of vertebra

Contusion: Displacement and damage of cord

Ischemia-reperfusion injury: aortic cross-clamp

Transection of the T4 spinal cord: Partial or complete separation

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