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Curcumin enhances nerve regeneration and functional recovery of peripheral sciatic nerve in rats with sciatic nerve cut and crush injury

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Abstract

Peripheral nerve injury can lead to sensory loss, motor loss, chronic pain, oxidative stress and histological alterations. The present study was designed in order to explore the possible neuroprotective role of curcumin against sciatic nerve cut and crush injury induced behavioural impairment, oxidative stress marker dysfunction and histological changes in rat. Rats were divided into four groups. The first group served as control. The second served as Sciatic Nerve cut/Degenerate (ND) and third group was Sciatic Nerve crushing (NC). The fourth and fifth groups were injured sciatic nerve through cut and crushing along with treatment of curcumin (20 mg/kg bw i.p.) for a period of 10 days. After the treatment period, behavioural studies were conducted and then sciatic nerves were collected used for oxidative stress markers and histopathological studies. The results showed that the curcumin treatment significantly reversed the Sciatic Nerve cut/Degenerate (ND) and Sciatic Nerve crushing (NC) induced increased lipid peroxidation (LPO) and decreased superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) levels. Curcumin also improved the cut/Degenerate (ND) and Sciatic Nerve crushing (NC) made motor impairment, hot plate latency and histological alterations in sciatic nerve. The findings of the present study conclude neuroprotective role of curcumin against Sciatic Nerve cut/Degenerate and Sciatic Nerve crushing induced oxidative stress, neural damage of brain and behavioural alterations in rat.

Keywords: Curcumin, Sciatic Nerve, Oxidative stress, Behavioural alterations, Peripheral nerve injury.

INTRODUCTION:

The Peripheral nervous system (PNS) consists of the nerves and ganglia that lies outside the Central nervous system (CNS) (Benjamin et al., 2009). The peripheral nerves provide sensory and motor innervation and serves as a relay between the brain, spinal cord and the rest of the body hence carry out various sensory, motor and autonomic nerve functions (Geuna et al., 2009). Unlike CNS, the PNS is not protected by the vertebral column and skull or by the blood brain barrier which leaves it exposed to toxins and mechanical injuries. The injury to a peripheral nerve is a partial or complete damage to the nerve caused by transection or laceration, lesion, crushing or compression (Siwei et al., 2022). The limited regeneration rate and repair ability of PNS often lead to partial or complete loss of sensory, motor and autonomic nerve function and damage the function of the organs associated (Silver et al., 2014 and Reier et al., 2017). Martins et al., (2013) reported traction or laceration (cut) and compression or stretch (crush) related injuries caused by sharp object or motor vehicle accidents is found to be the most common cause of peripheral nerve injuries (PNI). The increased morbidity from PNIs not only results in restricted activity or long term physical disability but also brings psychological pressure to patients that subsequently add a severe economic and social burden on society (Taylor et al., 2008, Roseberg et al., 2013).

Sciatic Nerve Injury (SNI) model is the widely used experimental model for the pre-clinical research of peripheral nerve regeneration (Liu et al., 2019; Ogut et al., 2018 and Zeng et al., 2020). The sciatic nerve is the largest nerve in mammals, innervating the posterior compartment of the thigh and all compartments of the lower leg and foot (Schmalbruch, 1986), SNI model thus provides the easy surgical access convenient for various surgical procedures (Geuna et al., 2015). These are the most common type of peripheral axon injuries (Burnett, 2004), depending on the method and degree of damage induced on the nerve, their physiological and functional processes (Dagum, 1998). The transection or cut injury induces the permanent disconnection between the distal nerve targets and CNS thus resulting in complete loss of sensory and motor function (Geuna et al., 2015). Crush injury interrupts the continuity of all axons (axonotmesis) without interruption of the connective tissue, epineurium (Geuna et al., 2009). After sciatic PNI, a process called wallerian degeneration takes place, characterized by the cellular changes in nerve stumps located at distal site and limited breakdown at proximal stumps (Feng et al., 2015). The cellular and molecular responses of the non neuronal cells, namely Schwann cells create a protective environment for injured axons (Sulaiman et al., 2013). However, after transect PNI the damaged axons and myelin portion rejected by the Schwann cells downregulates the expression of several proteins and inhibits the repair and regeneration of peripheral nerves (Menorca et al., 2013). The scarce extracellular signals trigger apoptotic cascades leading to neuronal death. Oxidative stress is one of the main causes of neural damage. Subsequent decrease in pressure on nerve tissue leads to a high pressure disposition of oxygen, increasing the formation of free radicals (Benga et al., 2017). Antioxidant enzymes like superoxide dismutase, catalase and glutathione acts as the ROS scavengers. After Injury the activity of these enzymes is reduced while the activity of lipid peroxidation is increased leading to oxidative stress (Ogut et al., 2018).

Curcumin is the principal curcuminoid of the popular Indian and southeast Asian spice Turmeric, extract of

powdered rhizome part of a perennial medicinal herb Curcuma longa linn, which belongs to the ginger family Zingiberaceae. Curcumin is a polyphenol compound with powerful antioxidant and anti-inflammatory properties**.** The Epidemiological and experimental evidences have demonstrated that Curcumin can preserve cognitive functions and provide protection against neurodegeneration. Nabavi et al., (2011), reported curcumin is shown to inhibit ROS formation and scavenge free radicals in pathological conditions, resulting in the protection of vital cellular components such as lipids, proteins and DNA. Curcumin has been shown to enhance the activities of antioxidant enzymes such as GSH, GPX, SOD and Catalase (Mohammad et al., 2014). A previous study by (Morsy et al., 2012) shown that curcumin protects cells from oxidative stress and autophagy. In vitro studies suggest that Curcumin can scavenge and neutralize hydroxyl, superoxide, and metal-induced free radicals (Noha et al., 2017). Curcumin can provide potentially more efficacious treatment in prevention of multiple neurological conditions (Elmajeed et al., 2015)**.** Commonly, microsurgical repair is required for the architectural reconstruction of the injured nerve however its functional recovery is poor and slow (Silver at al., 2014), so searching for effective neuroprotective drugs for promoting nerve regeneration, especially the naturally occurring ones, has gained extensive attention over the years. In the present study we have administered this natural flavonoid, curcumin and demonstrate its role in promoting peripheral nerve regeneration by increasing antioxidant level and decreasing level of free radicals. This study reports the protective effect of Curcumin on functional and structural recovery after sciatic nerve crush and cut/degenerate injury induced oxidative stress, behavioural perturbation and histological alterations in rat.

MATERIAL AND METHOD

Animals

The albino Wistar rats Rattus norvegicus were obtained from Jeeva Life science Laboratory, Hyderabad, Telangana, India. The protocols of the experiments were approved by the Departmental ethical committee. The rats were maintained at standard laboratory conditions with 12/12hr: day/night cycle at 22-24°C room temperature. The rats were divided into five groups, each group maintained in a separate polypropylene cage bedded with 2 to 3 cm paddy husk. The cage top was covered with a stainless steel grill. Distilled water and food was provided with standard pellet diet.

Experimental groups

Animals were divided into five groups.

Group-I: Served as control.

Group-II: Sciatic Nerve cut/Degenerate (ND).

Group-III: Sciatic Nerve crushing (NC).

Group-IV: Sciatic Nerve cut/Degenerate group of rats received Curcumin (20mg/kg BW) by oral gavage.

Group-V: Sciatic Nerve Crush rat provided with Curcumin (20mg/kg BW) by oral gavage. The study period was for 10 days. After 10 days behavioural studies (Rotarod and hotplate) test were conducted. The rats were sacrificed later and sciatic nerve were dissected out to perform biochemical and histological (H&E stain) studies.

Sciatic nerve injury model:

Albino wistar rats weighing 250-300g were utilized for sciatic nerve cut/degenerate and crush injury model.

Behavioural Functional Evaluation

Rotarod Test:

The Rotarod test is a widely used performance test to measure the fore and hind limb coordination, motor skills in rodents. The rotarod test measures the riding time (In seconds) for motor coordination or endurance of the animals being placed on a horizontally oriented, rotating rod rotarod (Hutter-Saunders et al., 2012).

Hot plate test:

Hot plate test was performed according to the method (Gunn et al., 2011). Rats were placed on a hot plate (Analgesiometer - Eddy's Hot Plate), maintained at constant temperature 53°C. The response latency to either a hind-paw lick or a jump on a hot plate was recorded. In the absence of a response, animals were removed from the hot plate at 60 seconds (cut-off time) and 60 seconds latency was assigned as the passive response. The results were expressed as time in seconds.

Biochemical Assay

Lipid peroxidation:

Lipid peroxidation was estimated by the method of (Garcia et al., 2005). The Malondialdehyde (MDA) is the oxidative stress marker and the end product of polyunsaturated fatty acids peroxidation. MDA was estimated by utilizing its reactivity with Thiobarbituric acid (TBA).

Superoxide dismutase:

Superoxide dismutase enzyme activity was assayed according to the method of (Marklund and Marklund, 1974). A simple and rapid method for the assay of superoxide is based on the ability of the enzyme to inhibit the autoxidation of pyrogallol.

Catalase:

Catalase activity was measured according to the method of (Aebi et al., 1984). In the ultraviolet range, H_2O_2 shows a continued increase in absorption with decreasing wavelength. However the decomposition of H_2O_2 can be followed directly by the decrease in absorbance.

Glutathione peroxidase:

The glutathione peroxidase activity was measured by the NADPH oxidation by glutathione reductase using hydrogen peroxide (H_2O_2) as substrate as per the method of (Rotruck et al., 1973).

Histopathology

Haematoxylin and Eosin stain (H&E Stain):

The samples collected were stored in 10 % formalin to evaluate the microstructural changes. The formalin fixed nerve samples were processed by paraffin embedding and cut into 4-5µ thick sections and stained with haematoxylin and eosin (Lillie and Fullner., 1976).

Statistical Analysis

The results are expressed as the mean± standard error of the mean (SEM). Comparison of means was conducted

using one-way analysis of variance (ANOVA), followed by least significant difference post hoc test to compare means between the different groups. The significant level was considered at P<0.05.

RESULTS

Behavioural Test: The rotarod and hotplate tests were used which are based on the hind and fore limb coordination and function in response to the given stimulus and the result was evaluated against the control group.

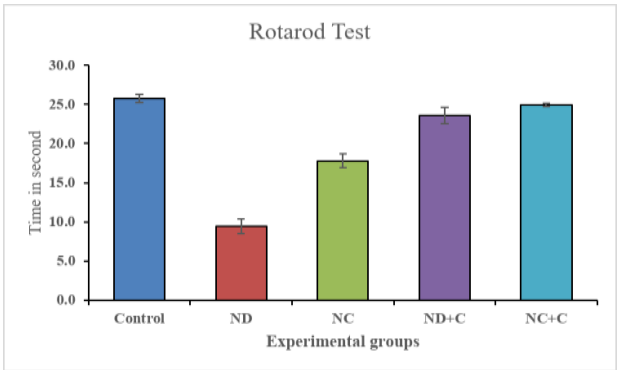

Figure-1: Effect of curcumin treatment on motor coordination (Rotarod test) in rats subjected to sciatic nerve cut/degeneration and crushing for 10 days. Data expressed as the mean±S.E.M (n=5) and results shown in time in seconds.

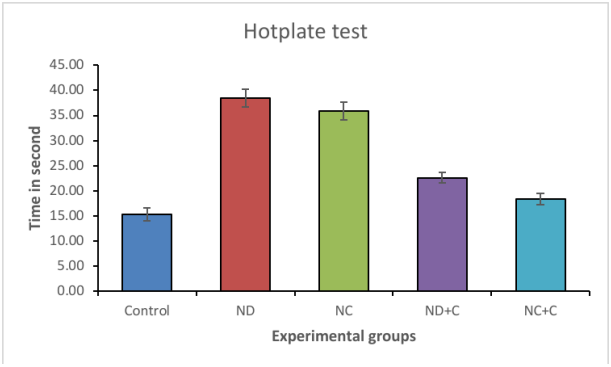

Figure 2: Effect of curcumin treatment on latency period (Hotplate test) in rats subjected to sciatic nerve cut-degenerate and crushing for 10 days. Data expressed as the mean±S.E.M (n=5) and results shown in time in seconds.

Oxidative stress markers

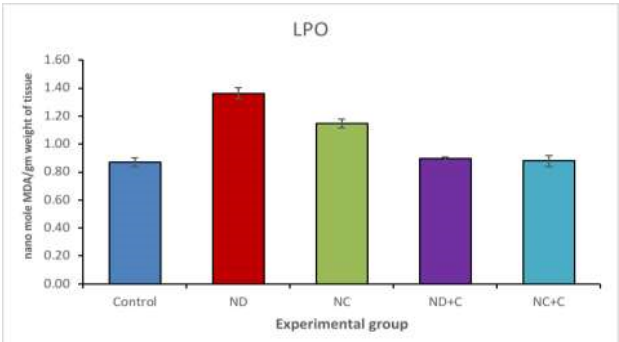

Figure-3: Effect of curcumin treatment on LPO content in rats subjected to sciatic nerve cut degenerate and crushing for 10 days. Data expressed as the mean±S.E.M (n=5) and results expressed in Nano mole MDA/gm weight of tissue (LPO).

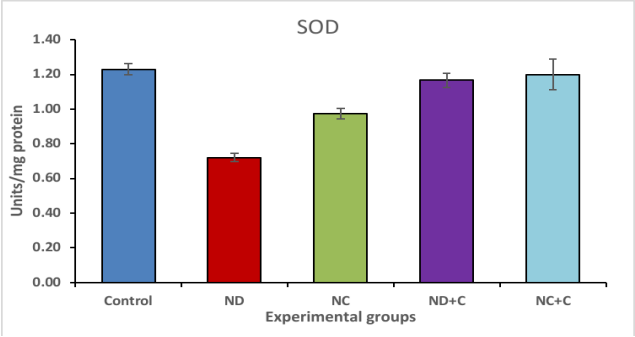

Figure-4. Effect of curcumin treatment on SOD activity in rats subjected to sciatic nerve cut degenerate and crushing for 10 days. Data expressed as the mean±S.E.M (n=5) and results expressed in Units/mg protein (SOD).

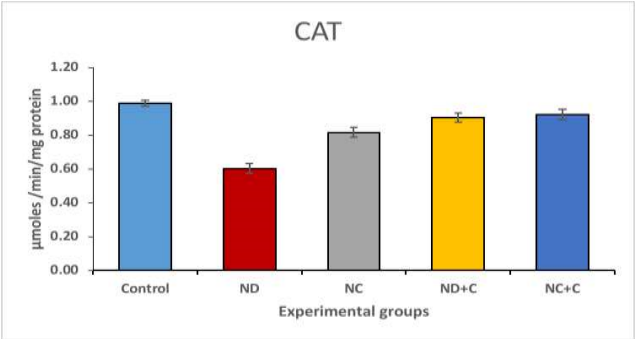

Figure-5: Effect of curcumin treatment on Catalase activity in rats subjected to sciatic nerve cut/degenerate and crushing for 10 days. Data expressed as the mean±S.E.M (n=5) and results expressed in µmoles /min/mg protein (CAT).

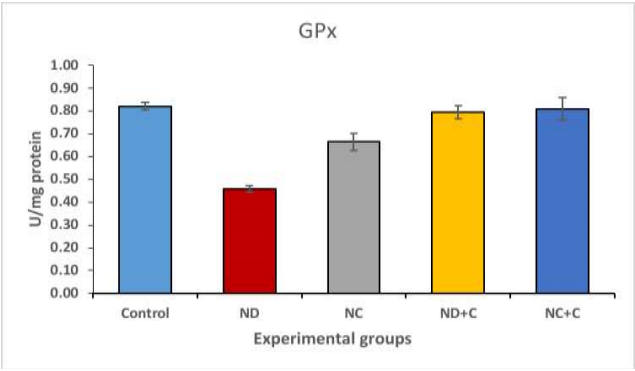

Figure-6: Effect of curcumin treatment on GPx activity in rats subjected to sciatic nerve degenerate/cut and crushing for 10 days. Data expressed as the mean±S.E.M (n=5) and results expressed in U/mg protein (GPx).

The control group of rats in **(figure.1)** Showed normal motor coordination, while the experimental (sciatic nerve cut/degenerate ND and crush NC) animal group showed significantly (P<0.05) decreased motor coordination and normal activities. Sciatic nerve cut/degenerate and crushing along with curcumin (ND+C) and (NC+C) respectively, administered group showed significantly (P<0.05) improved motor coordination and behavioural activities. The paw withdrawal in hot plate test, **(figure.2)** latency period significantly (P<0.05) increased in sciatic nerve cut/degenerate ND and crushing NC group of rats compared to control rats. Whereas, sciatic nerve cut/degenerate (ND+C) and crushing (NC+C) curcumin

treated rats showed significantly (P<0.05) decreased paw withdrawal latency period.

The values of the LPO content and SOD, CAT, and GPx activities are shown in **(figures 3, 4, 5 and 6)** respectively. The sciatic nerve cut/ degenerate (ND) and crushing (NC) group of rat showed significantly (P<0.05) increased MDA levels and decreased levels of SOD, CAT and GPx activities when compared with control. The sciatic nerve cut or degenerate and crushing (ND+C and NC+C) respectively along with curcumin has shown protective effect via decreasing significantly (P<0.05) the elevated LPO content nearly to the level of normal/control and also significantly increased the reduced antioxidant enzyme activities (SOD, CAT and GPx) when compared sciatic ND and crushing NC injured rat to that of control.

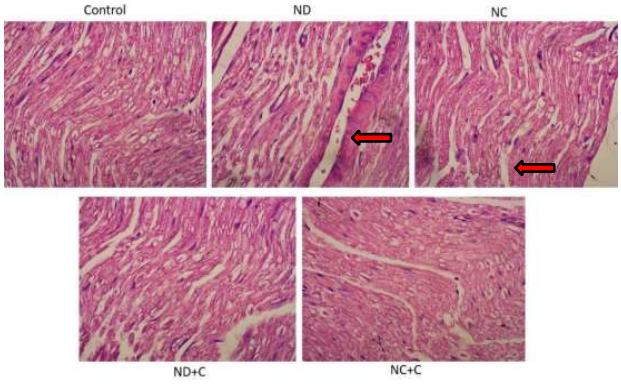

Figure-7. Haematoxylin and Eosin (H&E) staining was performed on sciatic nerve tissue sections and representative images of the results from each group are presented. Normal tissue components can be seen in control group, clear myelinated nerve fibres and other components are present. In cut or degenerate (ND) and crush (NC) group, there is visible disorganization and degeneration of myelin sheath and nerve fibres (Arrow marks). Comparatively less disorganization of myelin sheath and nerve cells/fibres in cut/degenerate and curcumin treated (ND+C) and crush and curcumin treated (NC+C) groups is seen.

DISCUSSION

The present study examined the possibility of formulating curcumin as a potential neuroprotective agent for the treatment of peripheral nerve injuries. We noticed that curcumin was capable of promoting nerve regeneration and motor functional recovery after crush nerve injury in rats. Peripheral nerve injury is the most common form of trauma. The axonal transport is necessary to maintain the integrity of the nervous system (Sta et al., 2014). In PNI, the axons are seriously damaged causing disruption in neuro conduction and axonal transport consequently inhibiting repair and regeneration of peripheral nerves. Initially unwanted proteins, lipids, and organelle accumulate at the damage site, generating stress that impedes the ability of Schwann cells to repair injured nerves (Sulaiman et al., 2013). The functional and biochemical changes take place generating large number of reactive oxygen species which inflict direct damage to lipids causing lipid peroxidation correlates with the poor activity of the antioxidant system. The two most prevalent reactive oxygen species that adversely affect the lipids are hydroxyl radical and hydroperoxyl. These ROS elicit severe damage on lipids and proteins, attack biomolecules producing multiple breakdown molecules. One such molecule is Malondialdehyde that cause oxidative damage (Antonio et al., 2014). These hydroxyl radicals are thus responsible for cellular disorders like neurodegeneration (Halliwell et al., 1984).

Antioxidants are necessary for removal of reactive oxygen species, produced due to oxidative stress generated in the body. Various antioxidants have been shown to inhibit the formation of oxidative compounds associated with many diseases (Marrocco et al., 2017). The finding in the present study reported the increase lipid peroxidation level and decline the antioxidant superoxide dismutase, catalase and GPx levels after sciatic peripheral cut and crush nerve injury in rat, indicating elevated free radical generation causing lipid peroxidation recovered by curcumin treatment. The results in present study was consistent with previous experimental studies (Kiran kumar et al., 2015), demonstrated increased lipid peroxidation because of depletion of SOD and CAT and (Liu et al., 2019), reported poor functioning of other antioxidant enzyme such as GPx. The previous antioxidant associated reports demonstrated that curcumin declined lipid peroxidation levels in the rodent models and has played a principal role in cellular redox control

(Dharmendra et al., 2016 and Hamid et al., 2017). The beneficial effects of curcumin have been attributed mainly to their strong antioxidant and anti-inflammatory activity, including their direct free radical scavenger activity (Nabavi et al., 2011).

The behavioural alterations in sciatic nerve cut/degenerate ND and crush NC, showed decreased motor coordination, sense recognition abilities and histological changes such as disorganisation, degradation of nerve fibers and myelin nerve sheaths of different diameters and thickness were observed in sciatic nerve cut and crush group. The administration of curcumin retrieved the activities of oxidative stress enzymes, behavioural abilities and alterations in sciatic nerve of rat. Present study concordance to previous studies (Nabavi et al., 2011; Noha et al., 2017 and Kiran Kumar et al., 2018). In many previous studies, the neuroprotective effect of curcumin has been gradually recognized in the central nervous system (Junxiong et al., 2013). Therefore, confirming that curcumin ameliorates oxidative damage to lipids preventing LPO that may be correlated to the improved antioxidant system suggesting its antioxidant action.

CONCLUSION

In conclusion, the present study reported that curcumin was capable of promoting nerve regeneration, regulate oxidative stress, accelerating motor and sensory functional recovery. This study described that curcumin is more potent neuroprotectant against the sciatic nerve cut and sciatic nerve crush injury. It provides efficacious treatment against peripheral nerve injury however, further study is needed in deciphering its mechanism of action. All these evidences suggest that curcumin has the potential to be an effective neuroprotective agent and can play a vital role in nerve regeneration in sciatic nerve injury repair applications.

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