

EFFECT OF VATA GAJANKUSHA RASA IN RAT SCIATIC NERVE CRUSH INJURY

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ABSTRACT

Peripheral nerve injury is common and results in significant disability to patients. Aim of this rat sciatic nerve axonotmetic study is to observe the effect of Ayurvedic drug *Vata gajankusha rasa* in the neuronal regeneration after crush injury using established morphometric parameters. The 36 wistar albino rats were equally divided into 3 groups consisting of Group I: Control animals, Group II: Sciatic nerve lesioned animals and Group III: Sciatic nerve lesioned animals treated with the drug (3mg/kg orally). 3mm segment of left sciatic nerve of Group II and III animals were crushed at mid – thigh level using artery forceps for fifteen minutes. Six animals from each group were sacrificed at the end of 2nd and 4th weeks after crush injury. 5mm of left sciatic nerves distal to the crushed site were processed for histological study by postfixing them in 1% Osmium tetroxide and used for morphometric analysis. Slides of the sciatic nerves of group II and III showed degeneration and active process of regeneration in the 2nd and 4th postoperative weeks respectively. In the morphometric analysis, the fiber density and myelin thickness showed an initial decrease in the 2nd postoperative week in groups II and group III thereafter a significant increase in 4th week when compared to the control group I. High fiber density and thicker myelin of nerves indicates better regeneration and functional recovery of the group III animals when compared to group II animals showing the drug has a beneficial effect in the peripheral nerve regeneration.

Key words: *Vata gajankusha rasa*, *Strychnos nux-vomica*, sciatic nerve, nerve regeneration

INTRODUCTION

Peripheral nerves are prone to injury and are capable of regeneration after the injury. Though injured peripheral nerves regenerate, complete functional recovery is not possible always due to various biological factors.

Gridrasi is a neurological condition described in Ayurvedic texts characterized

by severe pain starting from the low back region and radiating down along the back of the leg. It resembles the clinical condition ‘Sciatica’ produced by the compression of sciatic nerve. In the treatment of *Gridrasi*, a herbo-mineral preparation called *Vata gajankusha rasa* mentioned in the text *Basavarajiyam*¹ is used.

The drug *Vata gajankusha rasa* contains the following ingredients: Purified mercury, purified sulphur, purified *Strychnos nux-vomica*, *Terminalia bellerica*, *Phyllanthus emblica*, *Terminalia chebula*, *Zingiber officinale*, *Piper nigrum* and *Piper longum*. Among these, *Strychnos nux-vomica* is a key ingredient. The drug is indicated in nervous disorders like facial paralysis¹.

Unpurified seeds of *Strychnos nux-vomica* are toxic to humans and animals and when ingested produces muscle stiffness, convulsion and leads to death. Strychnine, an alkaloid present in *Strychnos nux-vomica* prevents glycine uptake at inhibitory synapses of anterior horn cells of spinal cord. There is a net excitability effect and a minimal sensory stimulation can produce powerful muscle contraction. It was first used medically in 1540, and continued to be used in many stimulants, tonic and cathartic till 1960s. But it is used even today in the preparation of Ayurvedic medicine after purification in the following diseases: neurological disorders, neurogenic impotency². Strychnine nitrate has been shown to improve patient condition in cases of childhood paralysis, sciatic neuralgia and neurasthenia³.

The drug *Vata gajankusha rasa* containing *Strychnos nux-vomica* used in treatment of neurological diseases like sciatica lacks experimental evidence. This study was attempted to evaluate experimentally the effect of *vata gajankusha rasa* on peripheral nerve regeneration after sciatic nerve crush injury using established morphometric parameters.

MATERIALS AND METHODS

Groups: Thirty six adult wistar albino rats of either sex, weighing 200 – 250 gm were used for this study after obtaining permission from Institutional Animal Ethical Committee. They were housed in polypro-

pylene cages and were provided with rat pellets and water ad libitum. The animals were equally divided into three groups: Group I – Control animals, Group II – Sciatic nerve lesioned animals and Group III – Animals to which *vata gajankusha rasa* was administered after sciatic nerve lesion. Six animals from each group were sacrificed at the end of second and fourth week.

Nerve crush injury: The rats were anaesthetized with Pentathol Sodium (Neon Laboratories Ltd., India, 45 mg/kg body weight, I.P.). After anaesthetization the left sciatic nerve of Group II and III animals were surgically exposed and 3mm segment of the sciatic nerve was crushed at mid-thigh level using artery forceps for fifteen minutes. After crushing the wound was sutured and dressed.

Drug Administration: Drug *Vata gajankusha rasa* obtained from IMPCOPS, Chennai was administered 3mg/kg body weight/day once orally to Group III animals from the first postoperative day till their sacrifice by making a suspension with 1ml of distilled water.

Animal sacrifice: At the end of second and fourth week the animals were sacrificed by giving excess anesthesia and perfused transcardially with normal saline.

Histological preparation: 5mm of the left sciatic nerves distal to the crushed site were taken for histological processing. The nerves were fixed in 10% formal saline and postfixed in 1% osmium tetroxide. After embedding in paraffin, 6 µm transverse serial sections were taken. Every tenth section was picked up and five such sections were selected and five random fields per section were studied.

Morphometry: 1. Diameter of the myelinated nerve fibers (D) and diameter of the axons (d) were calculated by using the following formula:

$$\text{Axial Ratio} = (L + B) / 2$$

(Where L is maximum length of the fiber and B is maximum breadth of fiber, which is perpendicular to L)

2. Thickness of myelin was calculated using the following formula:

$$\text{Myelin Thickness} = (D - d) / 2$$

(Where, D is the diameter of the myelinated fiber and d is the diameter of the axon)

3. Numerical density of the myelinated fibers in sciatic nerve per unit volume was calculated using the following formula:

$$NV = \frac{NA}{A(D + T)}$$

(Where NV = Numeric density or number of fibers per cubic millimeter, NA = Average number of fibers counted, A = Area of reticule in square millimeter, T = Thickness of section in millimeter, D = Mean diameter of the fiber in millimeter)

Statistical analysis: The results were analyzed by ANOVA test ($p < 0.05$) using SPSS software (student version 7.5) to determine the difference between the groups.

RESULTS

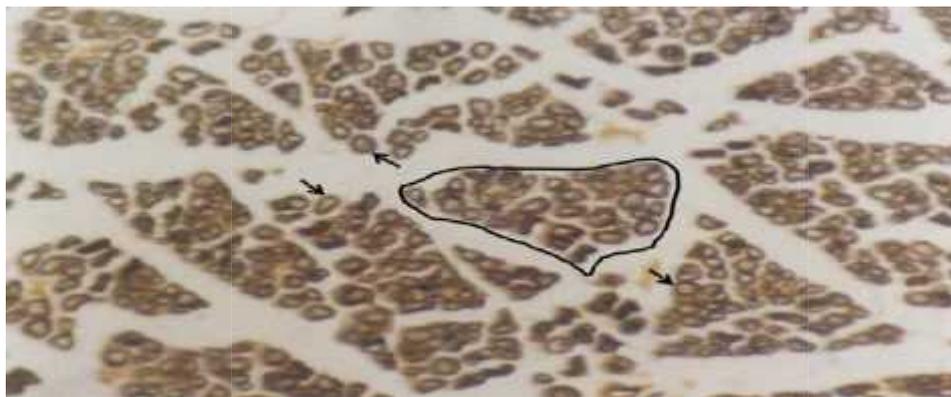
Immediately after creating the nerve lesion, the crushed areas of sciatic nerve was

flattened, but its continuity was not interrupted. Complete flaccid paralysis of the left foot was observed. All rats survived without wound infection and autotomy was noticed in only one rat.

Histological changes: The photomicrograph of a normal sciatic nerve taken from group I (Fig.1) shows the orderly arrangement of neuronal fascicles with less epineurial tissue. It also shows the presence of more number of myelinated fibers.

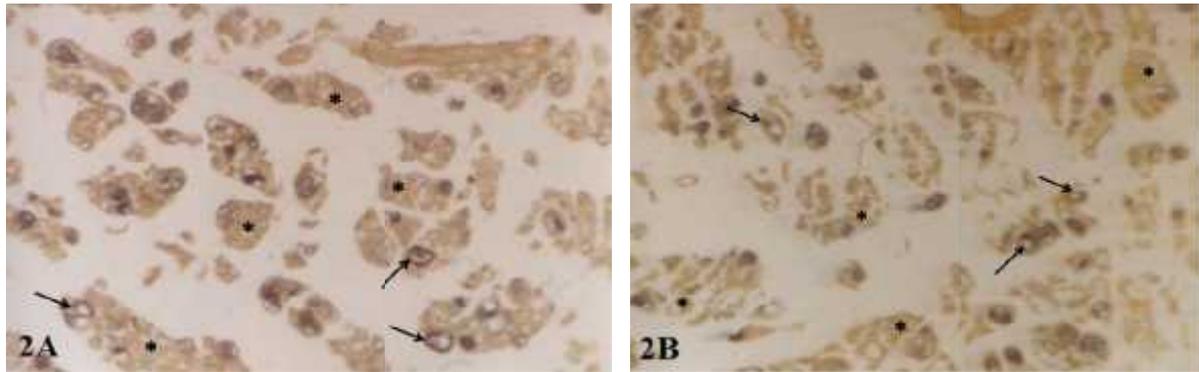
The slides of the sciatic nerves of groups II and III (Fig. 2A and 2B), taken 2 weeks after crush injury, show disruption in the arrangement of neuronal fascicles and very few myelinated fibers. There are many bundles of unmyelinated nerve fibers seen in 2A and 2B. Figures 3A and 3B show regenerated nerves 4 weeks after crush injury from group II and III respectively. Regenerated nerves showed the presence of myelinated fibers with smaller caliber and thinner myelin sheath in comparison to normal (Fig.1).

Figure1: Photomicrograph of sciatic nerve of group I, 40X



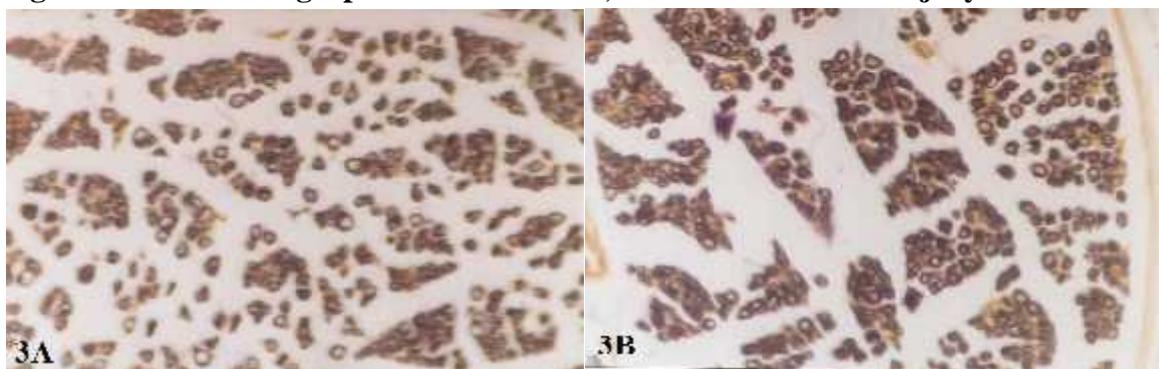
One neuronal fascicle is shown by a black outline drawn in the centre of the photomicrograph. The arrows point to some of the myelinated nerve fibers.

Figure 2: Photomicrograph of sciatic nerves, 2 weeks after crush injury



2A – Sciatic nerve of Group II and 2B – Sciatic nerve of Group III, 40X. Arrows point to myelinated fibers and asterisks are the bundles of unmyelinated nerve fibers.

Figure 3: Photomicrograph of sciatic nerves, 4 weeks after crush injury



3A – Sciatic nerve of Group II and 3B – Sciatic nerve of Group III, 40X.

Histomorphometry: Results of the morphometric analysis of sciatic nerves of Group I, II and III animals are shown in table1. Increase in the mean diameter of the myelinated fibers and axons was noticed in the 2nd and 4th weeks after crush injury in both group II and III sciatic nerves when compared with group I. The diameters of the myelinated fibers and their axons were significantly larger in group II when compared with group III.

Thickness of the myelin was significantly lower in group II and III animals when compared to group I. When compared to group II, group III had thicker myelin in the postoperative weeks.

The mean density of the myelinated fibers was markedly reduced in group II and III in the 2nd postoperative week. During 4th week, there was an increase in the density of myelinated fibers in both group II and III. Group III showed higher fiber density when compared to group II.

Table 1: Histomorphometric Measurements Of Sciatic Nervesof Group I, II& III Animals At 2nd And 4th Weeks After Crush Injury

	Diameter of Myelinated fibers (in microns)		Diameter of the axons (in microns)		Thickness of the myelin (in microns)		Myelinated Fiber density N/cu.mm.	
	2 nd week	4 th week	2 nd week	4 th week	2 nd week	4 th week	2 nd week	4 th week
Group I	9.77	8.12	5.55	4.62	2.27	2.25	22.71	21.43
Group II	13.19 ^{a,b}	10.37 ^{a,b}	9.77 ^{a,b}	5.91 ^{a,b}	1.70 ^{a,b}	2.21 ^{a,b}	3.98 ^{a,b}	22.57 ^{a,b}
Group III	10.74 ^{a,c}	9.29 ^{a,c}	7.16 ^{a,c}	4.86 ^{a,c}	1.79 ^{a,c}	2.23 ^{a,c}	6.01 ^{a,c}	22.85 ^{a,c}

N/sq.mm.– Neuronal fibers per cubic millimeter, **a-** $p < 0.05$ with respect to Group I, **b-** $p < 0.05$ with respect to Group III and **c-** $p < 0.05$ with respect to Group II

DISCUSSION AND CONCLUSION

Peripheral nerve can regenerate after an injury. In order to study the peripheral nerve injury and its regeneration various experimental models are available. Among the models, nerve crush injury is an established axonotmetic (Sunderland second-degree injury) model. It is used to evaluate the effect of pharmacological interventions on neuronal regeneration^{4,5}.

Axotomy or crush injury causes discontinuity in the neuronal axons and leads to degeneration of distal nerve stumps referred to as Wallerian degeneration. In this injury the continuity of the endoneurial sheath is preserved. During degeneration Schwann cells extrude their myelin sheaths and hematogenous macrophages remove the myelin debris from the distal stump. Following degeneration a conducive microenvironment is created for regeneration by the continuous basal lamina. It provides guidance for the regenerating axons from the proximal stump to its targets. Once axons establish contact with its targets, Schwann cells remyelinate the nerve fibers.

Among the nerve crush injury models, sciatic nerve model is widely used. This model is inexpensive and easy to create and can simultaneously evaluate the sensory and motor nerve functions⁶. The regeneration capacity is same in rats and subhuman primates⁷. In rats, myelinated axons of uninjured nerves measure 1 – 16 μm in diameter⁸. In all peripheral nerves unmyelinated axons are more when compared to myelinated axons and in rat sciatic nerve also the ratio between myelinated and unmyelinated axons is 1: 1.9 – 2.5⁹.

The figures 2A and 2B with altered fascicular architecture and few myelinated fibers demonstrate the demyelination during Wallerian degeneration distal to site of crush injury. The presence of large number of unmyelinated fibers might be due to regenerated unmyelinated fibers that are to begin the process of myelination.

At 4th week after crush injury sciatic nerves of both group II and III showed a marked improvement in myelination of nerve fibers and reestablishment of fascicular architecture (Fig 3A and 3B). They also show the myelinated fibers were small in caliber with thinner myelin sheath. This phenomenon is called microfasciculation, a typical feature of a nerve fiber undergoing regeneration. It is clearly evident that group III (Fig. 3B) has a better fascicular arrangement and more myelinated axons when compared to the group II (Fig. 3A).

The morphometric analysis provides more quantitative information on regeneration process. The myelin thickness 2.23 μm and fiber density 22.85/cu.mm. of group III animals at 4th week were significantly higher than the group II animals (Table 1). This indicates the regeneration was better in group III.

The density of myelinated fibers initially decreases in the 2nd postoperative week in both groups II and III when compared to group I confirms the Wallerian degeneration distal to the site of injury. Thereafter in the 4th postoperative week there was significant increase in the density of myelinated fibers in group II and group III (Table 1). This was due to active regeneration and remyelination of the peripheral nerves. Higher fiber density of group III than group II suggests better regeneration and functional recovery than untreated group.

The observation that fiber density increased when compared to control values is in accordance with previous studies on rat sciatic nerve regeneration. Previous study also shows significant increase in fiber density in the first three months after nerve repair and then a slow decrease¹⁰. This observation was due to the sprouting of more than one growth cone from each damaged axon leading to more regenerating axons distal to the lesion site¹⁰. The decrease in fiber density and number following the increase was due to the progressive death of collateral fibers which did not reconnect with the appropriate distal target¹⁰.

Group III animals showed better results in all the morphometric parameters when compared to the group II (Table 1). This indicates that the drug treated group III shows faster recovery from the injury and earlier regeneration. This shows the drug, *Vata gajankusha rasa* is definitely having a beneficial effect in the peripheral nerve regeneration.

Strychnine contained in the seeds of *Strychnos nux-vomica* acts on the anterior horn cells of spinal cord. Based on this fact we hypothesize that purified seeds of *Strychnos nux-vomica* may stimulate the anterior horn cells of spinal cord in the regeneration of sciatic nerve after crush injury. This hypothesis lacks experimental evidence and further studies are needed to substantiate it.

The ingredients *Piper longum* and *Piper nigrum* contain an alkaloid called piperine. Piperine enhances the bioavailability of structurally and therapeutically diverse drugs¹¹.

In addition, the *Terminalia bellerica*, *Phyllanthus emblica*, *Terminalia chebula*, *Zingiber officinale*, *Piper nigrum* and *Piper longum* have antioxidant, anti-

inflammatory and immunomodulatory properties^{2,12}.

So, the probable mechanism of action of this drug can be summarized as follows:

- The ingredient *Strychnos nux-vomica* might influence the nerve cells in earlier regeneration.
- The anti-inflammatory, immunomodulatory and antioxidant properties of other ingredients might help in the faster recovery from the degenerative process and in early regeneration.

This is a preliminary study to show that drug *Vata gajankusha rasa* is effective in peripheral nerve regeneration. Further studies are necessary to find the exact mechanism of action it plays in the process of regeneration.

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