Original Article

# Effect of Vanillic Acid on Nerve Conduction Velocity in Chronic Constriction Injury Model of Neuropathy

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## ABSTRACT

**Background:** Neuropathic Pain (NP) is less or symptomatically managed by presently available therapeutics. Therefore developing more effective drugs with minimum adverse effects is essential. Vanillic acid is phenolic secondary plant metabolite. Extensive research regarding phenolic acids with antioxidant, free radical scavenging and neuroprotective roles have been published. **Objectives:** The aim of this undertaken study was to evaluate the efficacy of vanillic acid (V.A.) to improve nerve conduction velocity in neuropathic pain induced by CCI (chronic constriction injury) and to evaluate its antioxidant potential. **Methods:** Rats were divided into 7 groups ($n = 6$), as negative control, positive control (CCI), sham control, CCI + gabapentin (300 mg/kg, p.o.), V.A. (25 mg/kg, p.o.), V.A. (50 mg/kg, p.o.) and V.A. (100 mg/kg, p.o.). After surgery oxytetracycline (25 mg/kg, i.m.) was administered in animals to avoid any infection. Vanillic acid and gabapentin administered post-surgery from day 4$^{th}$ till 28$^{th}$ day. Velocity of nerve conduction and antioxidant and histopathological studies were conducted on 28$^{th}$ day. **Results:** Repeated oral administration of vanillic acid (50 mg/kg, 100 mg/kg) significantly improved MNCV. V.A. showed antioxidant property by significantly elevating level of GSH and also reversed histopathological changes induced by CCI. **Conclusion:** This study has suggested antioxidant and neuroprotective effect of vanillic acid in CCI induced peripheral neuropathy.

**Key words:** CCI, MNCV, Neuropathy, Gabapentin, Vanillic acid.

## INTRODUCTION

Neuropathic Pain (NP) is initiated or caused by neuronal injury or functional disabilities in the nervous system.[1] NP is arising from damage to nerve due to tumors, diabetic neuropathy, herpes zoster, complex regional pain syndrome, AIDS, hypoxia.etc.[2] NP majorly affects quality of life of patients and has a great economic and social impact. It is reported by the institute of medicines that millions of American adults usually suffer from chronic pain and 17.9% suffer from neuropathic pain.[3] NP is multifactorial causing impairment in nerve function. The pathophysiology of pain is complex and involves central and peripheral pathways viz. neurotransmitter release, alteration in expression of ion channels and pain pathway.[4] It is known that both hyperalgesia and allodynia coexist in both, inflammatory and neuropathic pain.[5] Physiological stress caused by metabolic disorders, various inflammatory responses, viral infections, direct neuronal trauma, diseases like cancer or use of chemotherapeutic drugs and primary neurological diseases leads to neuronal functional disabilities and damage resulting into NP. Pain may be triggered by even any non-specific, small intensity stimulus, as neuronal injury changes neurophysiology to the long extent. These neuronal changes leads to over-expressions of ion channels and/or neuronal receptors generating abnormal action potentials and such synaptic transmission can result in

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neuropathic pain. This mechanism is currently explored as novel opportunity for drug discovery.[6] Impairment in the electrophysiological measurements are observed in neuropathic pain due to axonal damage and demylination.[7]

It has been well-documented that oxidative stress contributes significantly in pathogenesis of NP.[8] The mechanism of nerve dysfunctions induced by oxidative stress include generation of Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), lipid peroxidation, DNA damage and decrease in endogenous antioxidants. Increased ROS and RNS damage lipids from myelinated structures of nerves. This damage results in axonal loss and microvasculature disruption in the PNS (Peripheral Nervous System).[9-14] The role of secondary plant metabolites such as phenolic acids, in the treatment and prevention of various diseases has been extensively described. Previously, many authors focused mainly on the free radical scavenging and antioxidant activity of phenolic acids. However, in recent years, protecting role of phenolic acids on neurons and glial cells has been interestingly considered and number of research works exploring neuroprotective role of phenolic acids has been successfully carried out and published. In future, therapy of neurological disorders may be combined with phenolic acids as promising approach because of abundant availability, high stability, high oral absorption and efficient brain absorption of many phenolic acids.[15] Isolated chemical constituents from medicinal plant are documented as promising free radical scavengers playing crucial role in amelioration of neuropathy in animals.[16] Recently, research has shown the role of various phenolic acids e.g. chlorogenic acid, Caffeic Acid Phenethyl Ester (CAPE), ferulic acid, protocatechuic acid in treatment of neuropathic pain.[17]

Vanillic acid (4-hydroxy-3-methoxybenzoic acid), a phenolic derivatives of natural source, has interesting pharmacological profile. Experimental studies have provided evidences of its effectiveness on cardiovascular,[18] gastrointestinal[19] and liver diseases.[20] Its beneficial activity on acute inflammatory processes has also been described.[21] Furthermore, vanillic acid has been demonstrated to inhibit the synthesis or release of tumor necrosis factor (TNF)-α, interleukin (IL)-6, cyclooxygenase-2 (COX-2) and nitric oxide (NO), which are mediators released during inflammatory processes.[22] Based on this literature review, present study is undertaken to explore undiscovered benefits of vanillic acid to improve nerve conduction velocity in neuropathic pain by using Chronic Constriction Injury (CCI) induced animal model.

## MATERIALS AND METHODS

**Animals:** Wistar rats (either sex, 150-200 g) were housed under laboratory conditions as per CPCSEA guidelines. Study protocol was approved by the Institutional Animal Ethics Committee (Project Approval number: MGV/PC/CPCSEA/XXXIV/01/2018/05), of MGV's Pharmacy College, Nashik.

**Experimental design:** Animals were divided into 6 groups (*n=6*) and treated for 4 weeks.

Group I: Normal control received vehicle as saline only.

Group II: Rats with CCI without any treatment (positive control).

Group III: CCI surgery and treatment with gabapentin (300 mg/kg, p.o.)

Group IV**:** CCI surgery and treatment with vanillic acid (25 mg/kg, p.o.)

Group V: CCI surgery and treatment with vanillic acid (50 mg/kg, p.o.)

Group VI: CCI surgery and treatment with vanillic acid (100 mg/kg, p.o.)

Group VII: Sham surgery operated animals.

On 28$^{th}$ day of treatment schedule Motor Nerve Conduction Velocity (MNCV) was measured by using 8 channel Powerlab (AD Instrument). After scarification, antioxidant assay of sciatic nerve to determine level of GSH in tissue homogenate was performed and sciatic nerve sample was sent for histopatholgical studies.

### Induction of neuropathy Chronic Constriction Injury

Animal were anesthetized by using mixture of ketamine and xylazine (90 mg/kg, i.p and 10 mg/kg, i.p, respectively). After induction of anaesthesia, the hair around the mid-thigh were shaved. The common sciatic nerve of the right hind was exposed at the level of the middle of thigh by blunt dissection through biceps femoris. The sciatic nerve exposed and tied with ligature (4.0 silk chromic gut ligature) around it with about 1mm spacing long. After ligation, muscles and skin were sutured separately. After suturing the skin, povidone iodine solution was applied externally by cotton swab and prophylactically oxytetracycline (Terramycin, Pfizer, India) was injected I.M at dose of 25 mg/kg for next 3 days. The operated animals were caged individually and feed and water provided ad-libitum.[23,24]

### Antioxidant study: Preparation of and tissue homogenate

After scarification of animals, isolated sciatic nerve quickly transferred to ice-cold tris HCl buffer (10mM, pH 7.4). 10% w/v of cross-chopped fine pieces of sci-

atic nerve were minced and homogenized in ice-cold tris HCl buffer (pH 7.4). The homogenate was centrifuged (Remi C-24 high speed cooling centrifuge) at 10,000 rpm for 15 min. The clear supernatant was used for antioxidant determinations.[25]

### RGSH (Reduced Glutathione)

Reduced glutathione was determined as follows, equal volumes of tissue homogenate (supernatant) and 20% TCA were mixed. The precipitate was centrifuged and to 0.25ml of supernatant, 2ml of DTNB reagent was added. The final volume was made up to 3ml with phosphate buffer. The colour developed was read at 412nm against reagent blank.[26] and results were expressed % inhibition RGSH activity.[27]

### Motor Nerve Conduction Velocity Evaluation (MNCV)

MNCV recording were carried out 4 weeks after induction of diabetes on day 28th. Animals were anesthetized by ketamine (90 mg/kg, i.p) and xylazine (10 mg/kg, i.p). Assessment was done by using 8 channel Powerlab (AD Instruments) using animal nerve stimulating electrode (MLA0320) and needle electrodes (MLA1204) of AD Instruments. Stimulating electrodes applied on proximal end to generate action potential which is measured by recording electrodes from distal end.[28] Conduction velocity is calculated by formula

Here latent period is the time elapsed between the applications of stimulus until the peak of the maximum compound action potential (CAP).[29]

$$\text{Conduction velocity (m/sec)} = \frac{\text{Distance between the stimulating and recording electrodes}}{\text{Latent period}}$$

### Histopathological analysis of sciatic nerve

Histopathological study was done at histopathological lab (Dr. Vasantarao Pawar Medical College and Research Center, Nashik). After scarification, isolated sciatic nerve portion was kept in the 10% formalin (fixation solution). Staining was done by using hematoxyline and eosin. Then cross sections were observed using light microscope (40×) for axonal degeneration and vascular defects.[30,31]

## RESULTS

**Reduced glutathione:** RGSH is primary antioxidant in the cell. Significant (*$p<0.05$*) increase in % Inhibition was observed in positive control group compared with normal control group, while in neuropathic rats treated with gabapentin(300 mg/kg, p.o) and vanillic acid (100 mg/kg, p.o ) showed significant decrease in % inhibition levels as compared with positive control group (Figure 1).

### Motor nerve conduction velocity

Because of nerve damage there was impairment into the nerve conduction velocity. Nerve damage was observed in positive control rats after 28th day. Nerve damage indicated by shortening of nerve conduction velocity as compare to negative control rats. Neuropathic rats which are treated with the gabapentin (300 mg/kg, p.o) and vanillic acid (50 and 100 mg/kg, p.o) showed significant (*$p<0.05$*) Improvement in MNCV as compared to positive control rats (Figure 2).

### Histopathology

Section of H and E stained sciatic nerve of CCI positive control rats showed epineuronal oedema and infiltration of neutrophils around the blood vessels and swelling of nerve fibers (40x) compared to negative control group. Whereas, few infiltration of neutrophils around blood vessels and minor swelling of nerve fibres were observed in gabapentin (300 mg/kg, p.o) treated group. Treatment with vanillic acid (25 mg/kg, p.o) rats showed several area of edema degraded myelin sheets

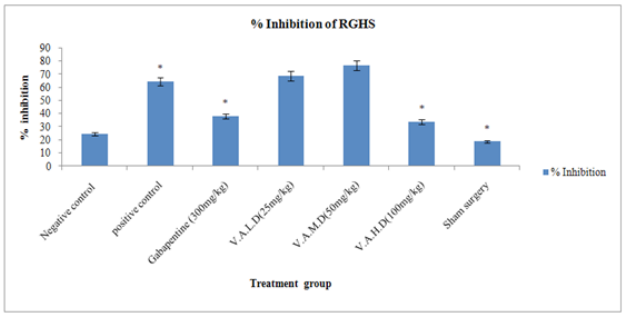


**Figure 1: Effect of vanillic acid (25, 50 and100 mg/kg, p.o) and gabapentin (300 mg/kg, p.o) on reduced glutathione in sciatic nerve tissue homogenate of CCI-induced peripheral neuropathy model of rats.**

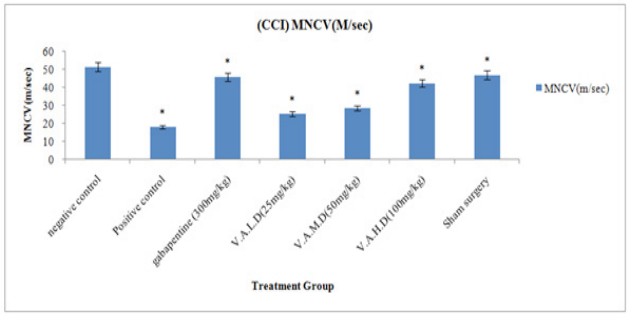


**Figure 2: The comparative increase in MNCV (m/sec) by vanillic acid (25, 50 and100 mg/kg, p.o) and gabapentin (300 mg/kg, p.o) on after 28th day.**

The observations are Mean±SEM and subjected statistical analysis by ANOVA followed by Dunnett's test, * p<0.05 as compared to positive control group.

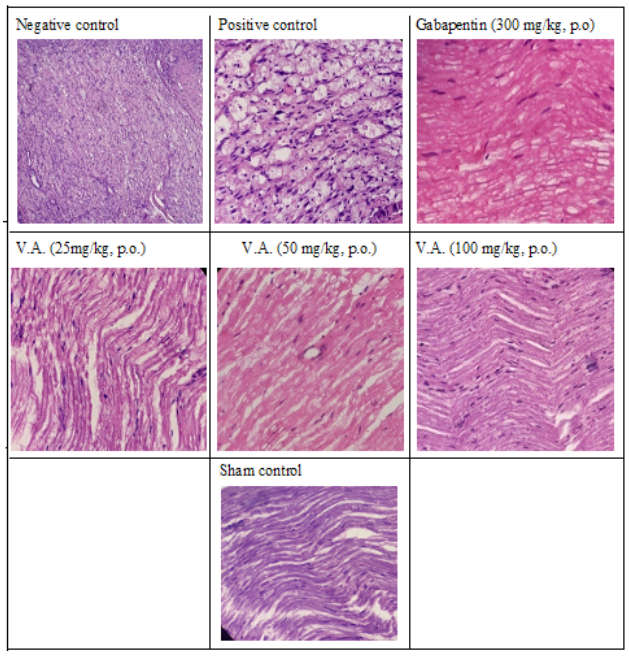


**Figure 3: Reversal of histopathological changes by vanillic acid and gabapentin after CCI induced neuropathy.**

and several infiltrating mononuclear cells, vanillic acid (50 mg/kg, p.o) showed mild edema and infiltration and vanillic acid (100 mg/kg, p.o) showed mild edema few infiltration and showed well organized myelin sheets around axons (Figure 3).

## DISCUSSION

Chronic constriction injury model is valid model used to induce neuropathic pain. The experimental model of neuropathy produced by sciatic nerve ligation in animals mimic symptoms observed in human beings with nerve injury. In this model, significant alterations in neurotransmitters and expressions of receptors, results into central sensitization in response to release many mediators of inflammation and pain, which further increases the sensitivity of peripheral sensory nerves at the site of injury and also in the CNS.[31] Pain mechanism may be due to immune response initiated by suture thread. It may cause nerve oedema and compression in addition to cellular degeneration. Electrophysiological studies have shown decreased conduction velocity and histopathological studies shows major injury in myelinated fibers, A-delta fibers and unmylinated C-fibers which are responsible for pain.[32]

Glutathione (GSH) is highly abundant in all cell compartments and is the major soluble antioxidant. Reduced GSH/Oxidized GSH ratio is a significant indicator of oxidative stress. Antioxidant effects GSH may be achieved by nemerous ways. It removes $H_2O_2$ and lipid peroxides via action of GSH-Peroxidase.[30] In present study, significant decrease in the amount of RGHS was observed in positive control group as compared with negative control group.

While the rats treated with gabapentin (300 mg/kg, p.o), vanillic acid (50 and 100 mg/kg, p.o) showed significant increase in RGHS level as compared to positive control, indicated by decrease in % inhibition of RGHS. This suggests antioxidant activity of vanillic acid.

Nerve impairment measurement by determining electrophysiological changes in nerve after neuronal injury is considered as 'gold biomarker' in the study of neuropathic pain. A NVC is an electrical test used to determine nerve impulse conduction down to nerve. Also it is helpful to detect signs of nerve injury.[7] CCI may give axonal damage and demylination to alter NCV. Motor nerve conduction velocity assessed by using non-invasive procedure by using AD instruments Powerlab.

The defect was associated with a decrease in neuronal blood flow of sciatic nerve which causes slowing of MNCV and in neuropathic rats, the change in endoneural blood flow and nerve conduction is also observed.

In present study, CCI significant decreased MNCV in positive control rats compared to negative control rats. While significant improvement in MNCV was observed in gabapentin (300 mg/kg, p.o) and vanillic acid (50 and 100 mg/kg, p.o) treated rats.

It is reported that sciatic nerve of CCI surgery produces several histopathological changes in rats. In present study, positive control rats showed edema around epineurium and infiltration of neutrophils around the blood vessels and showed swelling of nerve fibers and demylination of nerve fibers. Treatment with vanillic acid (25 mg/kg, p.o) showed swelling of nerve fibres and accumulation of monocytes and macropages around schwan cells.

While neuropathic rats which are treated with vanillic acid (50 and 100 mg/kg, p.o.) showed reversal of changes with mild epineuronal oedema, few infiltrating neutrophils around the blood vessels and only minor swelling of nerve fibers.

Thus vanillic acid treated rats showed significant antioxidant and neuroprotective activity in CCI-induced nerve injury from the above experimental data.

## CONCLUSION

The present findings support that vanillic acid reversed electrophysiological alteration after nerve injury and histopathological neuronal damage. This possibly may be because of its antioxidant effect. Thus, vanillic acid can

be used as neuroprotective to treat peripheral neuropathy.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**NP:** Neuropathic pain; **V.A:** Vanillic acid; **CCI:** Chronic constriction injury; **MNCV:** Motor nerve conduction velocity; **GSH**: Glutathione; **ROS:** Reactive oxygen species.

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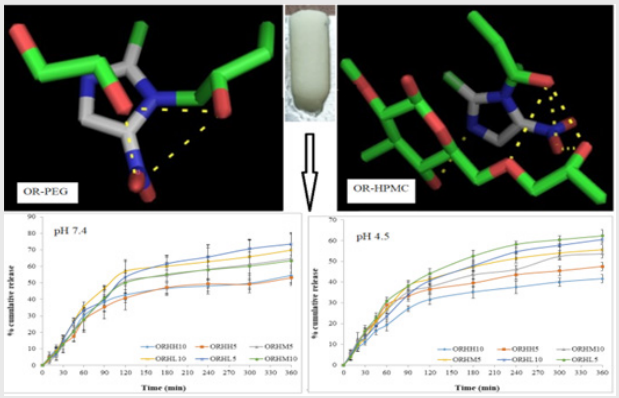
## PICTORIAL ABSTRACT




## SUMMARY

Vanillic acid is polyphenol compound having antioxidant, anti-inflammatory, antihypertensive, hepatoprotective activity. As other some phenolic acids have been proven as neuroprotective, this work was undertaken to evaluate vanillic acid in chronic constriction injury induced neuropathic pain. NP was induced by sciatic nerve ligation and MNCV was measured to assess the neuronal damage. Also *in-vivo* antioxidant GSH was determined and isolated sciatic nerve was studies histopathologically. 4 week treatment by vanillic acid has shown to improve CCI induced injury indicated by improvement in MNCV and GSH levels. Histopathological damage is also found to be reversed by V.A. treatment. Thus, this study suggests antioxidant and neuroprotective effect of vanillic acid which can be used in treatment of neuropathic pain.

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