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AMELIORATIVE EFFECTS OF *VANDA TESTACEA* IN SCIATIC NERVE TRANSECTION-INDUCED NEUROPATHY IN RATS.

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ABSTRACT

The present study was aimed at investigating the ameliorative effects of *Vanda testacea (VT)* in sciatic nerve transection (axotomy)-induced peripheral neuropathy in rats. Adult male albino rats weighing 130-150gm were used for the study, and were divided into five groups and axotomy was performed on left sciatic nerve in group II to group V. Tail cold-hyperalgesia, motor co-ordination, foot deformation tests, and total calcium levels were estimated to assess the extent of neuropathy. Superoxide dismutase (SOD), catalase (CAT), lipid peroxides (LPO), calcium, prostaglandin E_2 levels were estimated. Hydro-alcoholic extract of *VT* was administered at a dose of 175 and 350 mg/kg/p.o for 15 days. *VT* attenuated sciatic nerve transection-induced motor in-coordination, reversed axotomy-induced alterations in lipid peroxides, total calcium, superoxide dismutase, catalase levels in a dose-dependent manner. Ameliorative effects of *VT* in axotomy-induced neuropathy may be due to its antioxidant, calcium and prostaglandin attenuating actions.

KEYWORDS: **Axotomy, Complete sciatic nerve transection, Neuropathic pain, Neuropathy, *Vanda testacea*.**

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INTRODUCTION

Neuropathic pain is associated with peripheral nerve injury and is characterized by unpleasant abnormal sensation (dysesthesia), an increased response to painful stimuli (hyperalgesia), that does not normally provoke pain (allodynia) [1]. The sciatic nerve transection axotomy is a good model to study both the degeneration and the neuropathic pain [2] and is widely accepted as an experimental model for inducing peripheral neuropathy in rats. Even though some drugs have been found to be effective in managing the symptoms of neuropathy, yet their full clinical exploitation is limited due to wide spectrum of adverse effects associated with their use. So there is a need to explore better treatment options for neuropathic pain. Postherpetic neuralgia (PHN) and painful diabetic neuropathy (PDN) are two types of neuropathic pains (NP) commonly seen in the elderly population. It is estimated that 1 million people have PHN [3] and 3 million have PDN [4]. This disease commonly affects the quality of the life and lasts for months to years. Generally NP is insensitive to opiates and is usually treated empirically with tricyclic antidepressants and anticonvulsants, which have limited efficacy and undesirable side effects[5]. *Vanda testacea (VT)or (V. parviflora Lindl.)* is an indigenous plant belongs to Orchidaceae family; commonly found in India especially in Kailasakona, Tirupati. *Vanda testacea* commonly known as "Banda" or "Rasna", is an alkaloid rich epiphytic species of vandaceous orchids, is widely known for its medicinal properties, all plant parts (roots, leaves, flowers) in powder form or as an extract are used as herbal medicine to cure cuts, wounds, ear aches, rheumatism, bronchitis, nervous disorders, piles, and inflammation as well as cancer. Traditionally, it is used as a nerve tonic to alleviate the problems related to nerves. However, its potential role in neuropathic pain is unexplored. The present study was designed to investigate the possible ameliorative effect of *Vanda testacea* in sciatic nerve transection-induced painful neuropathy in rats.

MATERIALS AND METHODS

Plant material

The plant material was collected from the Kailasakona, Tirupati, Andhra Pradesh, India. The plant was authenticated by Dr.Madhava Shetty, Sri Venkateswara University (SVU), Tirupati. The fresh leaves of *Vanda testacea* were collected and shade dried. Plant sample was kept (voucher specimen number 985) at Botany Department of SVU, Tirupati for future reference.

Extraction

The fresh leaves of *Vanda testacea* were shade dried at room temperature and reduced to coarse powder. The powder was extracted with ethanol and water (3:1) using soxhelt extractor for about 12 hrs. The solvent was removed under reduced pressure to yield a dark green residue. The extract was stored in a desiccator. The extract was subjected to preliminary phytochemical analysis.

Animals

Male albino rats weighing 150-180 g were chosen to avoid fluctuations due to estrous cycle. The rats were housed in polypropylene cages under 12 hrs light/dark cycle, fed with standard laboratory chow (Hindustan Lever Limited, Mumbai) and water ad libitum. Animals were acclimatized to the laboratory conditions prior to experimentation and all the experiments were carried out according to the study protocol approved by the Institutional Animal Ethical Committee.

Induction of peripheral neuropathy by axotomy

Peripheral neuropathy was induced in rats by complete sciatic nerve transection (axotomy) as described by Wall [6]. Briefly, rats were anesthetized with thiopental sodium (35mg/kg *i.p.*). The hair of the rat's lower back in the thigh region of left paw was shaved, and the skin was sterilized with povidone-iodine (Betadine TM). The skin of the lateral surface of the left thigh was incised and a cut was made directly through the biceps femoris muscle to expose the sciatic nerve. It was tightly ligated with silk at two locations before trifurcation of the terminal branches (the sural, common peroneal and tibial nerves) of approximately 5mm of length. The sciatic nerve was transected between the ligatures, in the left paw. After performing nerve transection, muscular and skin layer was immediately sutured with thread and topical antibiotic was applied. No surgery was performed on the right thigh and uninjured right paw served as control. Nociceptive thresholds were assessed before and after performing surgery on 5 day intervals up to 15th day.

Study protocol

Rats (150-180g) were randomly divided into five groups of 6 rats each, fed with drug or vehicle for 14 days prior to experiment. The first group served as sham control and received 1% Tween 80 in water orally. The second group served as axotomy control, received 1% Tween 80 in water orally. The third group served as standard, received Diclofenac (10mg/kg) in water orally. The fourth and fifth groups were treated with hydro-alcoholic extract of *VT* 175 and 350 mg/kg/p.o., respectively for 15 days. Neurological examinations were performed in all the groups at 5 day intervals up to 15th day. Then all the six rats in each group were decapitated to obtain brain tissue samples for biochemical analysis.

Neurological deficit

Neurological deficit in the vehicle and drug treated groups were determined after 24 hrs of induction according to Etsuko [7] in a blinded manner.

Behavioral studies and pharmacological studies

(i) Assessment of foot deformation

The foot deformation in axotomised and drug treated groups were assessed by foot deformation score. The rat was placed on a plate with a neutral temperature and the posture of the foot was observed. The foot deformation was scored as follows[7] in a blinded manner.

- score 0 = if the paw is in normal position with fanned toes,
- score 1 = if the toe is ventroflexed,
- score 2 = if the paw is everted so that only the internal edge of the paw touches the floor.

(ii) Cold-hyperalgesia test (tail immersion test)

Tail cold-hyperalgesia was noted by immersing the terminal part of the tail (1 cm) in the water, maintained at a temperature of 0–4^{0}C. The tail withdrawal latency was recorded and a cut-off time of 20 sec was maintained[8].

(iii) Motor Co-ordination Test (Rota Rod Test)

Motor Co-ordination test was conducted according to Kulakarni[9] using a Rota rod apparatus (Inco Ambala, India). The animals were placed on the moving rod prior to the treatment and the rats that stayed on the rod without falling for 120 seconds were choosen for the study. The fall of time of animals in different groups was noted.

(iv) Assessment of nociception by Tail Flick method

Tail flick latencies were recorded (Basal reaction time) according to the method of

Battachary by using Techno Analgesiometer. A cut off period of 10sec was observed to prevent the damage to the tail[13]. 3-5 basal reaction times for each rat were taken at a gap of 5 min to confirm normal behavior of the animal.

(v) Assessment of Memory:

Cognitive performance was measured according to the method of Sharma [10], notably to evaluate the spatial long term memory in rats.

(vi) Exploratory behavior (Hole Board Test)

This test was done using Hole Board according to the method of File and Wardril, [11]. The Hole Board consisted of a $0.5m^3$ wooden board with 16 holes (3cm in diameter). The rat was placed at the corner of the board and allowed to move freely. First two minutes were allowed for adaptation and the number of head dippings in next four minutes was counted.

(vii) Assessment of inflammation

Oedema volume was assessed according to the method of Otterness [12] by using Digital Plethysmograph (UGO Basile, Italy). Average edema volume in the VT and Diclofenac treated rats was measured and expressed as percent edema inhibition.

Biochemical estimation of markers of oxidative stress

After 15 days of surgery, animals were sacrificed by cervical dislocation and sciatic nerve was immediately isolated. Tissue homogenate was prepared with 50mM phosphate buffer (p^H 7.0) containing 0.1mM EDTA. The homogenate was centrifuged at 10,000rpm for 10min at -4^0C in cold centrifuge. The separated supernatant part was employed to estimate total protein, superoxide dismutase, lipid peroxide, catalase, total calcium and prostaglandin E_2 content.

(a) Estimation of superoxide dismutase

SOD activity was estimated according to the method of Misra and Fridovich [14] at room temperature. 100µl of tissue extract was added to 880µl of carbonate buffer (0.05M, P_H -10.2, containing 0.1mM EDTA) and 20µl of 30 mM epinephrine (in 0.05% acetic acid) was added to the mixture and the optical density values were measured at 480nM for 4 min on a UV-Vis Spectrophotometer. Activity is expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50% is equal to 1 unit.

(b) Estimation of lipid peroxidation

MDA levels were measured according to the method of Okhawa [15]. 200µl of the tissue extract was added to 50µl of 8.1% sodium dodecyl sulphate, vortexed and incubated for 10min atroom temperature. 375µl of thiobarbituric acid (0.6%) was added and placed in a boiling water bath for 60min and then the samples were allowed to cool at room temperature. A mixture of 1.25ml of butanol: pyridine (1.5: 1), was added, vortexed and centrifuged at 1000rpm for 5mins. The coloured layer(500µl) was measured at 532nm on a Systronics UV Spectrophotometer, using 1, 1, 3, 3-tetraethoxypropane as standard. The values were expressed as mmoles of MDA formed/mg protein/hr

(c) Estimation of Catalase

Catalase activity was measured by a slightly modified version of Aebi[16] at room temperature. Catalase activity was expressed in moles of hydrogen peroxide degraded/mg protein/min.

(d) Estimation of total calcium

Total calcium levels were estimated according to the method of Lorentz[17]. To 4.5ml of deproteinated buffer in a glass centrifuge tube, 0.5ml of the sample was added and was placed in water bath for 3minutes. Tubes were centrifuged while they

were still hot, 0.5ml of supernatant was transferred into clean test tube, and 0.5ml of standard was pipetted into test tubes. 0.5ml of blank solution was prepared by mixing 9 volumes of deproteination buffer with one volume of water. 5ml of working colouring reagent was added to each tube, mixed well and then read at 570nm.

(e) Estimation of PGE_2

The sciatic nerve was isolated and was stored in buffer containing normal saline for 60mins. Then the buffer was centrifuged at 3000rpm, for 10min and 0.1ml supernatant was carefully transferred into a fresh tube, after adding 2ml of 0.5 mol/L KOH- methanol. Then the mixture was incubated at 50^0C for 20min and then diluted to 20ml with methanol. The PGE_2 level in the mixture was determined with UV Spectrophotometer at 278nm[18].

(f) Estimation of tissue protein

Protein concentration was estimated according to the method of Lowry[19] using BSA (bovine serum albumin) as a standard.

Statistical analysis

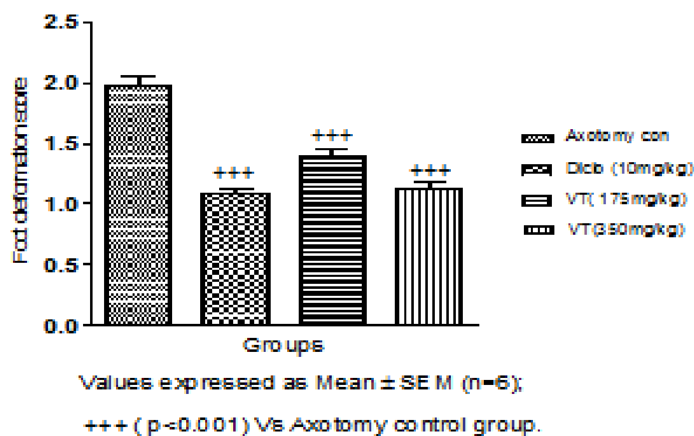
All the results were expressed as mean ± S.E.M. Data was analyzed using one-way ANOVA, followed by Dunnet's T test using Graph pad Insta5. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

(i) Foot deformation

Upon axotomy, foot deformation occurred in all animals. The rats with induced neuropathy developed abnormal gait and posture. The foot was ventroflexed, with the toes held tightly together and rats were unwilling to place weight on the foot of the injured side. Foot positioning and toe spread rating was significantly different between control and experimental groups. The foot deformation score is given in Fig1. Treatment with *VT* (175, 350mg/kg) and Diclofenac significantly reduced the foot deformation (p<0.001).

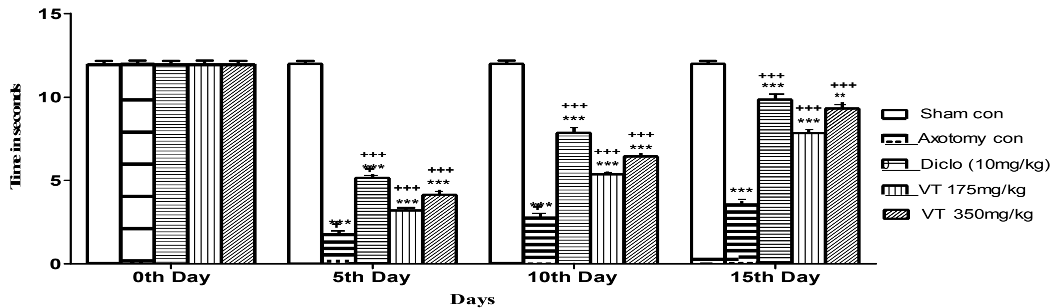
Figure 1
Effect of hydro-alcoholic extract of VT in Neuropathic pain-induced Foot deformation in the hind paw.



(ii) Cold-hyperalgesia in tail

Administration of *VT* significantly attenuated sciatic nerve transection-induced increase in spinal cold sensitivity, assessed by tail withdrawal latency time, in a dose-dependent manner (Fig 2). Tail withdrawal latency time was prolonged significantly in rats treated with *VT* (175 and 350mg/kg) when compared to axotomy control (p<0.001) and decreased when compared to sham control (p< 0.01), (p<0.001). Comparable significant increase was also observed in Diclofenac (10 mg/kg) treated rats (p<0.001).

Figure 2
Effect of hydro-alcoholic extract of VT on cold-allodynia in Neuropathic pain-induced rats.



Values expressed as Mean ± SEM (n=6);

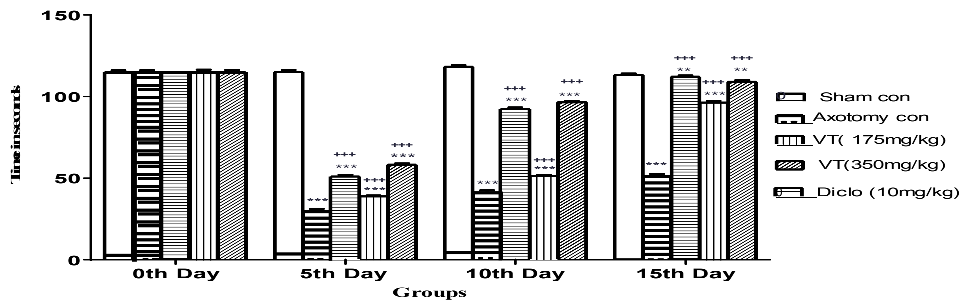
, * (p<0.01), (p<0.001) Vs Sham control group,

+++ (p<0.001) Vs Axotomy control group.

(iii) Motor coordination

Administration of *VT* significantly attenuated sciatic nerve transection-induced decrease in motor performance as assessed by time spent on Rota rod in a dose-dependent manner (Fig 3). The rats treated with *VT* (175, 350mg/kg) and Diclofenac significantly increased (p<0.001) time spent on Rota rod when compared to axotomy control and significantly decreased when compared to sham control rats (p<0.001) on 5th, 10th day and (p<0.01) 15th day.

Figure 3
Effect of hydro-alcoholic extract of VT on Motor co-ordination in Neuropathic pain induced rats.



Values expressed as Mean ± SEM (n=6);

, * (p<0.01), (p<0.001) Vs Sham control group;

+++ (p<0.001) Vs Axotomy control group.

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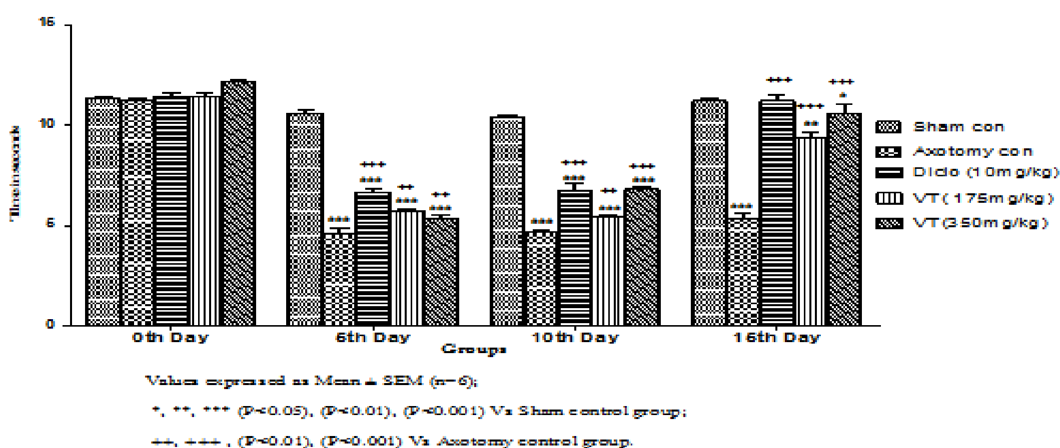
(iv) Tail flick method

Administration of *VT* significantly attenuated sciatic nerve transection-induced increase in spinal cold sensitivity, assessed by tail withdrawal latency time in a dose-dependent manner (Fig 4). Tail withdrawal latency time was decreased in axotomy control group on 5th, 10th and 15th day ($p<0.001$) when compared to sham control group. Rats treated with Diclofenac (10 mg/kg) significantly increased the tail withdrawal latency time on 5th, 10th and 15th day ($p<0.001$) when compared to axotomy control ($p<0.001$) and significantly decreased when compared to sham control ($p<0.001$). Comparable significant increase ($p<0.001$) in tail withdrawal latency time was also observed in *VT* (175 and 350mg/kg) treated rats on 10th and 15th day as compared to axotomy control.

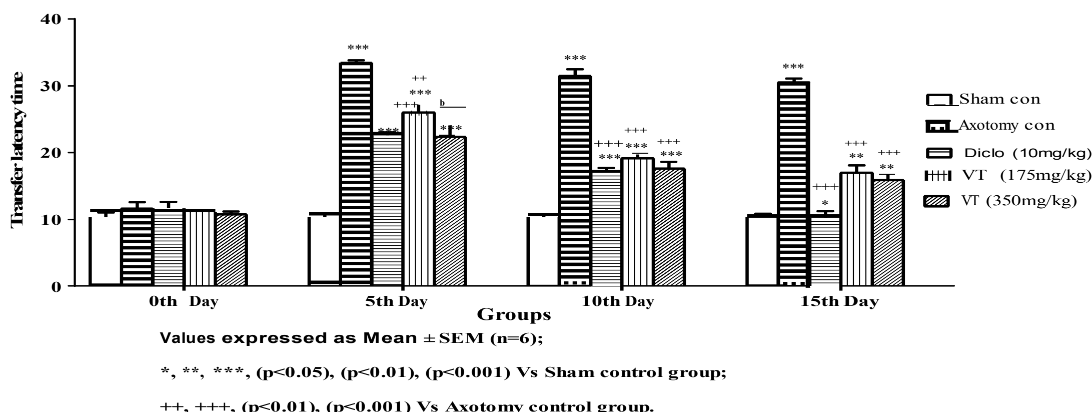
Figure 4
Effect of hydro- alcoholic extract of VT on Tail flick in Neuropathic pain-induced rats.



(v) Assessment of memory

Administration of VT significantly attenuated sciatic nerve transection-induced increase in transfer latency time, in a dose-dependent manner (Fig 5). Transfer latency time was decreased in axotomy control group on 5th, 10th and 15th day ($p<0.001$) when compared to sham control group. Rats treated with hydro-alcoholic extract of VT (175, 350 mg/kg) increased the transfer latency time on 5th, 10th and 15th day ($p<0.001$) when compared to axotomy control ($p<0.001$) and significantly decreased when compared to sham control ($p<0.001$). Comparable significant increase was also observed in Diclofenac (10 mg/kg) treated rats ($p<0.001$).

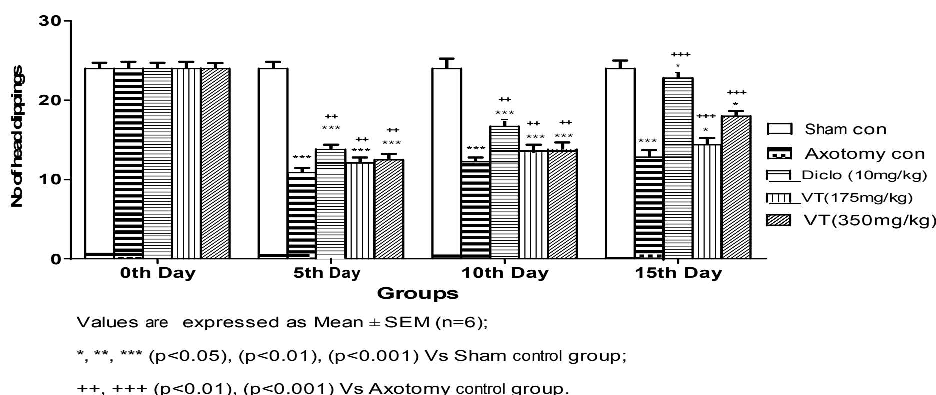
Figure 5
Effect of hydro-alcoholic extract of VT on memory impairment in Neuropathic pain-induced rats.



(vi) Exploratory behaviour

Administration of hydro-alcoholic extract of VT significantly attenuated sciatic nerve transection-induced abnormality in exploratory behavior in a dose dependent manner as assessed by number of head dippings (Fig 6). The number of exploratory movements was decreased in axotomy control group on 5th (p<0.01), 10th and 15th day (p<0.001) when compared to sham control group. Rats treated with hydro-alcoholic extract of VT (175, 350 mg/kg) showed increased number of exploratory movements on 5th, 10th and 15th day (p<0.001) when compared to axotomy control (p<0.001) and significantly decreased when compared to sham control group (p<0.001). Comparable significant increase was also observed in Diclofenac (10 mg/kg) treated rats (p<0.001).

Figure 6
Effect of hydro-alcoholic extract of VT on exploratory behavior in Neuropathic pain induced rats.



(vii) Assessment of Inflammation

Administration of the *VT* significantly attenuated sciatic nerve transection-induced inflammation as assessed by the paw edema volume by Digital Plethysmograph in a dose-dependent manner. The percentage inhibition of paw edema volume was given in Table 1. The percentage inhibition of paw edema in *VT*

(175($p<0.05$), 350mg/kg) treated rats showed 52%, 63% respectively and is significant when compared to sham control ($p<0.001$) and axotomy control ($p<0.001$). Whereas Diclofenac treated rats showed 89% inhibition ($p<0.001$).

Table 1
Effect of hydro-alcoholic extract of VT on % Reduction in oedema, in Neuropathic pain induced rats.

GROUPS	% Reduction in Oedema
Sham Control	17.06± 1.20
Axotomy Control	45.70±2.41***
Diclofenac (10mg/kg)	89.37± 1.31***,$^{+++}$
VT (175mg/kg)	52.75±1.97***,$^{+}$
VT (350 mg/kg)	63.47±1.32***,$^{+++}$

Values are expressed as Mean ± SEM (n=6); ***, (p< 0.001) Vs Sham control group; +, +++, (p<0.05), (p< 0.001) Vs Axotomy control group.

Table 2
Effect of hydro-alcoholic extract of VT on SOD, Catalase, Lipid peroxide, Calcium, PGE_2 levels, in Neuropathic pain induced rats.

GROUPS	SOD (Units/mg of protein)	Catalase (µm of H_2O_2degraded/mg protein/min)	MDA (nm/mg of protein)	Calcium (µg/ mg of protein)	PGE_2 (µg/ mg of protein)
Sham Con	18.35± 1.05	45.31± 1.80	14.37± 0.69	14.37± 0.69	0.059± 0.006
Axotomy Con	3.68 ± 0.66***	6.90 ± 0.62***	20.79±1.20***	20.79±1.205***	0.599± 0.005***
Diclo (10mg/kg)	14.71±1.43^{+++}	44.98±3.15^{+++}	16.06± 0.68**,$^{++}$	18.25± 0.83**	0.138±0.01**,$^{+++}$
VT (175mg/kg)	8.78±0.79***,$^{+}$	21.26±1.53***,$^{+++}$	19.78±1.55***,$^{+++}$	17.78±0.60*,$^{+}$	0.497±0.01***, $^{+++}$
VT (350 mg/kg)	15.3±1.57^{+++}	36.90±2.37***,$^{+++}$	12.10±0.49^{+++}	12.10±0.49^{+++}	0.150±0.01**,$^{+++}$

Values are expressed as Mean ± SEM (n=6); *, **, ***, (p<0.05), (p< 0.01), (p< 0.001) Vs Sham control group; +, ++, +++, (p<0.05), (p< 0.01), (p< 0.001) Vs Axotomy control group.

Results of Biochemical estimations

(i) Superoxide dismutase

The levels of SOD in the sciatic nerve were significantly decreased ($p<0.001$) in axotomy control group as compared to the sham control group. Treatment with *VT* (175 ($p<0.05$), 350 mg/kg/p.o) significantly increased the SOD levels ($p<0.001$)as compared to the axotomy control group (Table 2). Diclofenac treatment significantly increased SOD levels as compared to the axotomy control group ($p<0.001$).

(ii) Catalase

The levels of Catalase in the sciatic nerve was significantly decreased ($p<0.001$) in axotomy control group as compared to the sham control group. Treatment with *VT* (175, 350mg/kg/p.o) significantly increased the

Catalase levels ($p<0.001$), as compared to the axotomy control group (Table 2). Treatment with Diclofenac also significantly increased Catalase levels as compared to the axotomy control group ($p<0.001$).

(iii) Lipid peroxidation

The levels of MDA in the sciatic nerve was significantly increased ($p<0.001$) in axotomy control group as compared to the sham control group. Treatment with *VT* (175, mg/kg/p.o) decreased the MDA levels ($p<0.001$), higher dose of VT (350mg/kg/p.o) decreased the MDA levels non significantly and Diclofenac significantly decreased the MDA levels ($p<0.01$), as compared to the axotomy control group (Table 2).

(iv) Calcium

The levels of calcium in the sciatic nerve was significantly increased ($p<0.001$) in axotomy control group as compared to the sham control group. Treatment with *VT* (175($p<0.05$), 350mg/kg/p.o) and Diclofenac significantly decreased the calcium levels ($p<0.001$), as compared to the axotomy control group (Table 2).

(v) Prostaglandin E_2

The levels of PGE_2 in the sciatic nerve was significantly increased ($p<0.001$) in axotomy control group as compared to the sham control group. Treatment with *VT* (175, 350mg/kg/p.o) and Diclofenac significantly decreased the calcium levels ($p<0.001$), as compared to the axotomy control group (Table 2).

DISCUSSION

Axotomy, complete transection of peripheral nerve, is widely employed as an experimental model for inducing peripheral neuropathy in rats. Axotomy-induced neuropathy in experimental animals refers to Complex Regional Pain Syndrome (CRPS) in humans [20]. Complete sciatic nerve transection induced neuropathic pain is a useful model to assess the contribution of inflammatory mediators that are involved in neuropathic pain. Peripheral nerve contains resident macrophages, which constitute 1–4% of the cell population in the rat sciatic nerve [21]. When peripheral nerves are injured, macrophages are recruited to damage nerve [22, 23] where they contribute to the removal of degenerating axons and myelin sheath [24]. After an injury in the peripheral nervous system, chemicals are released from damaged cells and inflammatory cells (e.g., mast cells, lymphocytes) [25]. Chemicals include noradrenaline, bradykinin, histamine, prostaglandins, potassium, cytokines, 5HT and neuropeptides. These mediators act on nociceptors to sensitise for further neural input [26]. It cause changes in the number and location of ion channels especially sodium ion channels in injured nerve fibers and their dorsal ganglia [27]. As a result, the threshold for depolarization is lowered and a spontaneous discharge known as ectopic discharge occur in abnormal locations. Thus, nociceptors response to thermal and mechanical stimuli is increased, a phenomenon known as peripheral sensitization [26, 27]. At the cell body of primary afferent neurons within the dorsal root ganglia (DRG), sympathetic neuronal sprouting occurs and may account for sympathetically maintained pain. Sometimes nerve demyelination from diminished blood supply may also contribute to the production of ectopic discharges along the nerve fiber [25].

After a peripheral nerve injury, a neuroma develops at the proximal nerve stump. It consists of unmyelinated sprouts (C-fibres) growing out from the transected axons [28]. The peripheral sensitization, mediated through C-fiber (primary afferent neurons), is the mechanism responsible for hyperalgesia [29, 30]. A sensation of burning pain is due to continuous discharge in C-fibers, whereas dysesthesias (unpleasant abnormal sensations) and paresthesias (abnormal sensations) may arise from intermittent spontaneous discharges in A- delta or A-beta fibers [31]. In the present study, axotomised

rats showed a decrease in the levels of superoxide dismutase and catalase and increase in the lipid peroxide levels indicating increased production of free radicals. The possible mechanism for pathophysiological phenomenon associated with nerve injury is oxidative stress [32, 33]. This enzyme is implicated as an essential defense against the potential toxicity of oxygen [34]. The decreased SOD levels found in the present study are in accordance with Varija[35]. Catalase is a heam containing enzyme that catalyzes the dismutation of hydrogen peroxide to water and oxygen [36].

VT, in the present study restored the levels of SOD and Catalase due to its anti-oxidant potential against free radicals. Therefore, the administration of *VT* in axotomised rats showed an increase in the anti-oxidant enzyme levels. The antioxidant property of VT decreased the oxidative stress induced by axotomy. Cytoplasmic calcium activates enzymes and second messenger cascades that contribute to cell death. Activated proteolytic enzymes break down elements of the cytoskeleton, leading to protein aggregation. Calcium-mediated lipolysis damages membranes and along with nitric oxide synthase activation provides nitric oxide and fattyacid substrates for free radical production. Glutamate release is stimulated by calcium-dependent exocytosis and the released glutamate in turn causes Ca^{2+} channels to open, leading to further Ca^{2+} overload [37]. In the present study axotomy increased in the levels of calcium, indicating the key role of calcium in sciatic nerve transection induced neuropathy. Increase in calcium levels are demonstrated to induce series of biochemical changes leading to degradation of the axonal cytoskeleton and thus, axonal degeneration. Therefore, the administration of *VT* in axotomised rats showed a decrease in the levels of calcium significantly. In the present study, axotomised rats showed an increase in the levels of PGE_2. The administration of *VT* and Diclofenac treatment showed significantly decreased levels of PGE_2. It may be either due to inhibition of cycloxygenase or phospholipase A_2. Rats treated with hydro-alcoholic extract of *VT* showed significant protective action against foot deformation. Rats treated with Diclofenac, higher dose of *VT* both showed least score for foot deformation, indicating its protective action against foot deformation. Foot positioning and toe spread are useful in assessing not only the locomotory and behavioral movements but also the degree of injury. Rats treated with Diclofenac showed significant decrease in the paw edema volume (89%). Rats treated with lower and high doses of *VT* showed significant decrease in the paw edema volume (52% and 63%) comparable to the standard drug, due to its anti-inflammatory effect. The inflammation plays a critical role in the chronic constriction injury, the partial sciatic nerve ligation and the diabetes-induced neuropathic pain [38]. The decrease in inflammation may be due to decrease in PGE_2 levels and improvement in foot deformation may be due to decrease in oxidative stress, Calcium channels. Inflammation, contributes for pain in the initial days of neuropathic pain in rats. In the present study NSAID is used as standard drug, it acts by inhibiting the cycloxygenase enzyme. Neither Diclofenac nor *VT* higher and lower doses reversed the pain or inflammation to normal as they contribute in part only. The phytochemical analysis of *VT* showed the presence of flavanoids, terpenoids, tannins and reducing sugars but the results were not included. Flavanoids and tannins of *VT* may be responsible for antioxidant activity. Further, neuropathic pain mainly involves persistent activation of sodium channels, even though we have not studied the effect on sodium channels as the *VT* did not reversed the foot deformation to normal, may be it is not inactivating the sodium channels. Nevertheless, more elaborative studies are required to identify the active components and precise site of action of *Vanda testacea* in

ameliorating sciatic nerve transection-induced neuropathy.

CONCLUSION

Neuropathic pain is one of the most common and debilitating clinical sensory disorders that can result from injury to primary afferent neurons. Pain is the most common reason that needs medical care. Treatment of NP needs multi-drug therapy. VT is claimed to be used in nervous disorders. Neurological score, PGE_2 and calcium levels were increased indicating the developmentof neuropathic pain. Further tail flick latency, grip strength were decreased confirming the pain induced behavioural alterations. Treatment with VT reversed the neuropathic pain induced alterations such as neurological score, PGE2 levels, calcium levels, cold allodynia and tail flick latency time, but none of the values reached normal. It's anti-oxidant, PGE_2 inhibition and calcium attenuating actions may be in part responsible for the observed ameliorative effects.

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