

EVALUATION OF PROTECTIVE EFFECT OF VITAMIN -E ON VINCRIStINE INDUCED PERIPHERAL NEUROPATHY IN ALBINO RATS

L. Nagkrishna, Naveen Kumar Tamma, Naser Ashraf Tadvi, S. Mabu Shareef

1. MSc Medical. Department of Pharmacology, Kamineni Institute of Medical Sciences, Sreepuram, Narketpally Dist, Nalgonda , Andhra Pradesh, India.
2. Associate Professor. Department of Pharmacology, Apollo Institute of Medical Sciences & Reserach, Hyderabad, Andhra Pradesh, India.
3. Associate Professor. Department of Pharmacology, Kamineni Institute of Medical Sciences, Sreepuram, Narketpally Dist, Nalgonda , Andhra Pradesh, India.
4. Assistant Professor. Department of Pharmacology, Kamineni Institute of Medical Sciences, Sreepuram, Narketpally Dist, Nalgonda , Andhra Pradesh, India.

CORRESPONDING AUTHOR:

L. Nagkrishna,

H.NO: 4-2-151/69/2/A ,

Srinivasnagar, Khammam,

Pin -507003.

E-mail: nagakrishnalodangi@yahoo.com

ABSTRACT: BACK GROUND: Vitamin -E is a lipid soluble substance which ,effectively protects against neuronal toxicity by preventing free -radical damage to biological membrane. It is a major antioxidant used clinically and its study in nerve injury models has not been encountered in literature. **METHODS :**In the present study ,the neuroprotective effect of vitamin-E (graded doses of 50mg/kg,100mg/kg,200mg/kg orally) were assessed by giving vitamin -E daily for 14 days followed by vincristine(100 µg/kg I.P) on alternative days for 14 days in albino rats to induce peripheral neuropathy. The protective effect of vitamin-E were assessed by analgesic models like tail flick method, tail immersion methods and tail clip method on 0,7 & 14th day. **RESULTS AND CONCLUSION :** vitamin -E treated groups with 100mg/kg and 200mg/kg showed significant reduction in reaction time on 7th and 14th day compared to control group in all the three models of experiments. These findings indicate that vitamin-E has a promising neuroprotective action in treating hyperalgesia in models of rats.

KEYWORDS: Vitamin-E, Vincristine, Peripheral neuropathy, Analgesic models.

INTRODUCTION: Neuropathic pain has been described as the damage to the peripheral nervous system, the vast communication network that transmits information from the brain and spinal cord to every other part of the body^[1]. Neuropathic pain is generally characterized by the sensory, motor and autonomic abnormalities such as unpleasant abnormal sensation (dysesthesia).^[2] Peripheral neuropathic pain is frequently observed in patients with cancer, Aids, long standing diabetes, lumbar disc syndrome, herpes infection, traumatic spinal cord injury, multiple sclerosis and stroke ^[3]. More over post-thoracotomy, post-herniorrhaphy, post-mastectomy, post-sternotomy, nutritional deficiencies, alcoholism, vascular and metabolic disorders are some other conditions often associated with peripheral neuropathy ^[4]. Vincristine is unique among the chemotherapeutic agents that it produces predictable and uniform neurotoxicity in all the patients even at the therapeutic doses ^[5]. This peripheral neuropathy is dose-related with a marked variability in individual susceptibility. After stopping vincristine administration, partial or complete clinical recovery follows which takes several months.

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 clinical use. Moreover none of the medications, assessed in randomized controlled studies conducted, has been found to be effective in injury induced and chemotherapy-induced neuropathic pain . Therefore, there has been an urgent need of alternative medicine for managing neuropathy particularly in injury and chemotherapy-induced neuropathic pain [6].

Binding of vincristine to β -tubulin with subsequent disruption of microtubules has been documented for its anti-tumor actions and the same is also assumed to produce neuro-toxicity by axonal degeneration⁽⁷⁾. Unfortunately, neither prophylactic strategies nor symptomatic treatments of this chemotherapy-induced peripheral neuropathy (CIPN) have proven useful. Although Aspirin, ibuprofen and celebrex are commonly prescribed to treat patients of CIPN, they show limited efficacy⁽⁸⁾. Furthermore, gabapentin, lamotrigine, nortriptyline and amitriptyline studies were disappointing in treating CIPN⁽⁹⁾.

Vitamin -E forms α -tocopherol an important lipid-soluble antioxidant. It performs its functions as antioxidant in the glutathione peroxidase pathway, and it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This would remove the free radical intermediates and prevent the oxidation reaction. The oxidized α -tocopheroxyl radicals produced in this process may be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, ubiquinol45 or retinol.⁽¹⁰⁾

AIMS AND OBJECTIVES: The aim of the present study is to ascertain the protective effect of Vitamin - E on Vincristine induce peripheral neuropathy in albino rats by using various analgesic models.

MATERIAL AND METHODS: All the experimental procedures used in this study were reviewed and approved by Institutional Animal Ethical Committee of Kamineni Institute of Medical Sciences, Narketpally, Andhra Pradesh, India ⁽¹¹⁾. Twenty four adult Wistar albino rats (150-200g) were obtained from National Institute of Nutrition, Hyderabad were used. Animals were acclimatized to the laboratory environment for 5 to 7 days before being used in the study. Animals were housed 6 per cage in a temperature and humidity controlled environment under a 12-hour light/dark cycle (lights on at 7 pm). Food and water was available for all animals for their access.

INSTRUMENTS AND APPARATUS : The standard methods for measuring analgesic models like tail flick method is analgesiometer, tail immersion method by hot water bath and tail clip method by arterial clip, as described by M.N.Ghosh and Gerhard Vogel.^(12,13)

DRUGS AND CHEMICALS USED IN THE EXPERIMENT :

- a) Vincristine 1mg/ml concentration (Cipla Ltd, Goa)
- b) Vitamin-E (Evion Merck Ltd)
- c) Glycerol (Sd Fine-Chem Limited, Mumbai.)
- d) Normal saline

PERIPHERAL NEUROPATHY CAUSED BY VINCRISTINE (I.P) IN RATS : In the vincristine-induced peripheral neuropathy model, Vincristine 100 μ g/kg solution of desired dosage was

ORIGINAL ARTICLE

prepared by diluting the standard solution (vincristine 1mg/ml conc obtained from cipla) in normal saline for IP injection.

TAIL FLICK LATENCY: Tail flick latency was assessed by using analgesiometer (heated nichrome wire). Antinociceptive effect was determined according to the time taken for withdrawal of the tail to thermal stimulation. Cut off period of 10sec was taken to prevent damage to the tail. The pain threshold was tested on day 0,7 and 14 day.¹

TAIL IMMERSION LATENCY: Tail immersion latency was assessed by using hot water bath (55°C). Antinociceptive effect was determined by the time taken to withdraw the tail clearly out of water as the reaction time. Cut off period of 15sec was taken to prevent damage to the tail. The pain threshold was tested on day 0,7 and 14 day.

TAIL CLIP LATENCY: Tail clip latency was assessed by applying arterial clip with thin rubber sleeves at the base of the tail. Antinociceptive effect was determined by the time taken to dislodge the clip by biting the clip as the reaction time. Cut off period of 30sec was taken to prevent damage to the tail. The pain threshold was tested on day 0, 7 and 14 day.

Briefly, baseline responses to mechanical stimulation, direct contact stimulus and heat stimulus were established on day zero (baseline) and 24 hours gap was given to each method to avoid damage to the tail of rats. Pre treatment with Vitamin -E or glycerol(control) was given daily for 14 days in albino rats and Vincristine was given on alternate day for 14 days and the readings were taken on day 0, before vincristine administration followed by readings on 7 and 14th day. The dose of vincristine is 100 µg/kg and Vitamin -E dose is 50mg/kg, 100mg/kg and 200mg/kg as incremental doses^(14,15).

GROUPING OF ANIMALS : Animals were divided into 4 groups each containing 6 animals (n=6 in each group, total 4 groups, 24 rats required)

Division of groups for evaluation of antinociceptive activity (Table - 1)

Groups	Drugs	Dose
Group I (control)	Glycerol (ORAL) + Vincristine (I.P)**	0.2ml + 100 µg/kg
Group II	Vitamin-E (ORAL)* + Vincristine (I.P)**	50mg/kg + 100 µg/kg
Group III	Vitamin-E (ORAL) + Vincristine (I.P)	100mg/kg + 100 µg/kg
Group IV	Vitamin-E (ORAL) + Vincristine (I.P)**	200mg/kg + 100 µg/kg

I.P: intraperitoneal

Vitamin-E at various doses was administered orally for 14 days daily dissolved in glycerine before the administration of vincristine on alternate days for 14 days .

ORIGINAL ARTICLE

Group I (control group) is given glycerin 0.25 ml orally. Group II, III & IV (n=6) each were given vitamin-E 50mg/kg/day, 100mg/kg/day, 200 mg/kg/day respectively orally for 15 days.

RESULTS : The effect of untreated group showed a significant increase in tail flick latency (day 0, 7 and 14th day) and vitamin -E pretreatment reduced the tail flick latency, ie day 0, 7 and 14.

Comparison of tail flick latency in seconds (mean±sd) (control vs. vitamin- E pretreatment) in vincristine treated rats (Table -2) :

	Group 1(control)	Group II	Group III	Group IV	One way Anova
Day / Treatment	Glycerol + vincristine	Vit. E (50mg/kg) + Vincristine	Vit. E (100mg/kg) + Vincristine	Vit. E(200mg/kg) + Vincristine	P value
Day 0	4.6 ± 0.5	4.5 ± 1.3	4 ± 1.0	3.5 ± 1.0	0.2
Day 7	8.1 ± 1.3	5.6 ± 0.5	3.8 ± 0.9	4.3 ± 0.8	0.001
Day 14	9.5 ± 0.5	8.1 ± 0.7	2.8 ± 0.5	3.3 ± 1.5	0.001

Vincristine treatment increased tail flick latency to the radiant heat on day 7 & day 14 (8.1±1.3, 9.5±0.5 respectively) compared to day 0 (4.6±0.5 sec).

Comparison of tail immersion withdrawal latency in second (mean ± sd) (control vs. vitamin -E pretreatment) in vincristine treated rats (Table - 3) :

	Group 1(control)	Group II	Group III	Group IV	One way Anova
Day / Treatment	Glycerol + vincristine	Vit. E (50mg/kg) + Vincristine	Vit. E (100mg/kg) + Vincristine	Vit. E(200mg/kg) + Vincristine	P value
Day 0	4 ± 0.8	3.8 ± 0.7	3.8 ± 0.7	3.8 ± 0.7	0.97
Day 7	9.3 ± 0.8	7.5 ± 1.0	6.8 ± 1.4	7 ± 1.6	0.012
Day 14	12.6 ± 1.5	11.1 ± 1.2	8.8 ± 1.4	9.6 ± 2.1	0.003

vincristine treated animals increased tail withdrawal latency after immersion in hot water of 50±5°C on day 7 and day 14 was also more (9.3±0.8, 12.6±1.5 sec respectively) in comparison to day 0 (4±0.8 sec). Gurpreet Kaur et al 2010 also observed increased paw withdrawal duration in vincristine treated rats in the hot plate test.

Comparison of tail clip latency in seconds (mean±sd) (control vs. vitamin- E pretreatment) in vincristine treated rats (Table - 4) :

	Group 1(control)	Group II	Group III	Group IV	One way Anova
Day / Treatment	Glycerol + vincristine	Vit. E (50mg/kg) + Vincristine	Vit. E (100mg/kg) + Vincristine	Vit. E(200mg/kg) + Vincristine	P value
Day 0	7.8 ± 1.4	8.1 ± 0.9	8.6 ± 0.5	8.6 ± 0.8	0.420
Day 7	9.5 ± 1.3	8.1 ± 1.4	8.8 ± 0.9	9.1 ± 1.1	0.330
Day 14	18.6 ± 1.2	17.3 ± 1.8	8.6 ± 0.5	9.1 ± 1.1	0.028

The latency of bite of the clip by the rat applied to the tail is also increased on day 7 and day 14 (9.5±1.3, 18.6±1.2 sec respectively) in comparison to day 0 (7.8±1.4 sec). All these three observations suggest vincristine treatment produced neuronal damage in the albino rats by intra peritoneal administration for 14 days (alternate day administration).

DISCUSSION: Vitamin-E is considered as one of the principle protective vitamin against oxidative damage in neuronal tissue and is the major lipid soluble chain breaking antioxidant in the body tissues which effectively protects against neuronal damage. Vitamin-E is capable of indirectly participating in the reduction of oxidative stress in diabetic patients by its antioxidant activity. Experimental studies have shown that the use of vitamin-E after ischemia/ reperfusion injury in animals not only attenuated the oxidative injury of the muscle cells but also reduced the formation of edema in these cells, which means that they have partial protective action. Supplementation of patients receiving cisplatin chemotherapy with vitamin-E decreased the incidence and severity of peripheral neurotoxicity. Hence the present study is under taken to evaluate the protective effect of vitamin-E against the neuropathy produced by vincristine in albino rats.

In the present study Vitamin -E could produced significant antagonism of vincristine induced increase in the tail flick latency, tail clip biting latency and tail immersion with drawl latency in two doses of 100mg/kg and 200mg/kg .

Vitamin-E (table -2) pretreatment in graded doses of 50, 100, 200 mg/kg orally for 14days significantly reduced the tail flick latency on day 7 (5.6±0.5, 3.8±0.9, 4.3±0.8 sec respectively) in comparison to glycerine (control) (8.1±1.3 sec) pretreated vincristine received animals, and on day 14 (8.1±0.7, 2.8±0.5, 3.3±1.5 sec respectively) in comparison to glycerine (control) (9.5±0.5 sec) pretreated vincristine received animals.

Inter group comparison by one way anova in tail flick latency method (table -2) on day 7 and day 14(P<0.001) showed significant decrease in tail flick latency comparison to day 0 (P<0.2) showing significant effect .

Vitamin-E pretreatment (table-3) in graded doses of 50,100,200 mg/kg orally for 14 days significantly reduced the tail immersion withdrawal latency on day 7 (7.5±1.0, 6.8±1.4, 7.0±1.6 sec respectively) in comparison to glycerine (control) (9.3±0.8 sec) pretreated vincristine received animals and on day 14 (11.1±1.2, 8.8±1.4, 9.6±2.1 sec respectively) in comparison to glycerine (control) (12.6±1.5 sec) pretreated vincristine received animals in the present study.

Inter group comparison by one way anova in tail immersion withdrawal latency method (table -3) on day 7($P<0.012$) and day 14($P<0.003$) showed significant decrease in tail withdrawal latency comparison to day 0 ($P<0.97$) showing significant effect.

Vitamin-E pretreatment(table -4) in doses of 100 & 200 mg/kg orally for 14 days significantly reduced the tail clip latency on day 7 (8.8 ± 0.9 , 9.1 ± 1.1 sec respectively) in comparison to glycerin (control) (9.5 ± 1.3 sec) pretreated vincristine received animals and on day 14 (8.6 ± 0.5 , 9.1 ± 1.1 sec respectively) in comparison to glycerin (control) (18.6 ± 1.2 sec) pretreated vincristine received animals in our present study.

Inter group comparison by one way anova in tail clip latency method (table -4) on day 7($P<0.330$) and day 14($P<0.028$) showed significant decrease in tail clip biting comparison to day 0 ($P<0.420$) showing significant effect.

Ashish. Morani 2008 studied the effect of vitamin-E on the thermal hyperalgesia produced by sciatic nerve ligation and sciatic nerve crush injury on day 7 and day 8 respectively in albino rats and observed an improvement by vitamin-E in these models of nerve injuries .

SUMMARY AND CONCLUSION : The present study showed that Vitamin-E has a significant protective role on vincristine induced peripheral neuropathy in albino rats against all the three models (tail flick test, tail immersion & tail clip test).The results conclude that vitamin-E(doses 100, 200mg/kg) can be used in improving the peripheral neuropathy, but further studies and needed to explore the role of vitamin-E for definitive conclusion

REFERENCES:

1. Gurpreet Kaur, Amteshwar Singh Jaggi, Nirmal Singh.Exploring the potential effect of Ocimum sanctum in vincristine-induced neuropathic pain in rats. Kaur et al. Journal of Brachial Plexus and Peripheral Nerve Injury 2010;5:3
2. Woolf CJ, Mannion, RJ. Neuropathic pain, aetiology, symptoms, mechanisms and management, Lancet 1999;353:1959-64.
3. Werhagen L, Budh CN, Hultling C, Molander C.Neuropathic pain after traumatic spinal cord injury relations to gender, spinal level, completeness, and age at the time of injury. Spinal cord 2004;42:665-73.
4. Alston RP, Pechon P.Dysesthesia associated with sternotomy for heart surgery, Br J Anaesth 2005;95:153-58.
5. Pal PK: clinical and electrophysiological studies in vincristine induced neuropathy. Electromyograph Clinical Neurophysiology 1999;39:323-30.
6. Kalita J, Vajpayee A, Misra UK. Comparison of prednisolone with piroxicam in complex regional pain syndrome following stroke : a randomized controlled trail. QJM 2006; 99(2) : 89-95.
7. Tripathi KD,Anticancer drugs.In:Tripathi.Essentials Of Medical Pharmacology,6th ed.New Delhi:Jaypee Brothers Medical Publishers;2010.p.825.
8. Lynch JJ, Wade CL, Zhong CM, Mikusa JP, Honore P (2004).Attenuation of mechanical allodynia by clinically utilized drugs in a rat chemotherapy-induced neuropathic pain model.2004;110:56-63.
9. Kaley TJ, Deangelis LM . Therapy of chemotherapy-induced peripheral neuropathy. Br. J. Haematol.,2009;145:3-14.

ORIGINAL ARTICLE

10. Wefers H, Sics. The protection of ascorbate and glutathione against microsomal lipid peroxidation is dependent on Vitamin E. *European Journal of Biochemistry* 1988; **174** (2): 353-357.
11. M. Ethical guidelines for investigators of experimental pain in conscious animals. *pain*. 1983; **16**: 109-110.
12. Ghosh MN. Some common evaluation techniques. In: *Fundamentals Of Experimental Pharmacology*, 5th ed. Kolkata: S.K. Ghosh and others; 2011. p. 151-52.
13. Gerhard Vogel H. *Drug Discovery and Evaluation, Pharmacological assays*, 2nd ed., Radiant heat method 2002; p. 694-97.
14. Weng HR, Cordella JV, Dougherty PM. Changes in sensory processing in the spinal dorsal horn accompany vincristine-induced hyperalgesia and allodynia 2003; **103**: 131-38
15. Aashish S. Morani, Subhash L. Bodhankar. Neuroprotective effect of vitamin E acetate in models of mononeuropathy in rats. *Neuroanatomy* 2008; **7**: 33-37