RESEARCH ARTICLE

A Novel Approach for Evaluation of Hesperidin in Vincristrine-Induced Neuropathy in Rats

Vinod R Patil*, Chandrashekar D Upasani

Department of Pharmacology, MGV's SPH College of Pharmacy, Nashik, Maharashtra, India.

Received: 11th October, 2023; Revised: 13th January, 2024; Accepted: 22nd February, 2024; Available Online: 25th March, 2024

ABSTRACT

The present study set out to determine how hesperidin altered the neuropathic pain that vincristine-induced on rats. Rats were given vincristine to cause painful neuropathy. To measure the mechanical dynamic allodynia, cold allodynia, degree of mechanical hyperalgesia, heat hyperalgesia in addition to muscle relaxants rota rod, respectively, several pain-sensitive tests, including the von frey hair test pinprick, hot plate, and rota rod, were carried out on various weeks (00, 04 and 08 week). As indicators of inflammation and oxidative stress, the IL-1β, IL-10, and tumor necrosis factor-α, tissue parameters like Na^+/K^+ ATPase, Ca^{2+} ATPase & Mg^{2+} ATPase, and SOD, CAT level, reduced glutathione (GSH), lipid peroxidase (LPO), NO level were assessed. Gabapentin (30 mg/kg i.p.) in addition to hesperidin (50, 100, as well as 150 mg/kg orally) were given for 08 weeks. Hesperidin administration significantly reduced vincristine-induced behavioral and biochemical alterations ($p < 0.05$). Hesperidin also reduced the inflammation IL-10, and increased in IL-1ß, and TNF-α. Hesperidin also reduces oxidative stress (LPO, NO level) and increases at (GSH, SOD, CAT levels) that vincristine caused. Hesperidin can alle*via*te the painful states brought on by vincristine-induced painful neuropathy, which might also be explained by its anti-inflammatory effects and following reduction of oxidative stress.

Keywords: Vincristine neuropathy, Oxidative stress, Hesperidin, Gabapentin, Behavioral, Biochemical alteration.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.1.03

How to cite this article: Patil VR, Upasani CD. A Novel Approach for Evaluation of Hesperidin in Vincristrine-Induced Neuropathy in Rats. International Journal of Pharmaceutical Quality Assurance. 2024;15(1):20-27.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Patients with leukemia have been treated with vincristine, a medication that belongs to the vinca alkaloids class of antineoplastics.[1] One well-known adverse effect of vincristine is neurotoxicity, which can include cranial neuropathy, autonomic neuropathy, and peripheral neuropathy.[2] Allodynia, hyperalgesia, and spontaneous pain are examples of peripheral neuropathy symptoms. About 60% of individuals have peripheral neuropathy brought on by vincristine.[3] Vincristine-induced neuropathy symptoms are dose-dependent and last for a few months after stopping the drug. Painful neuropathic pain is a chief problem of unwelcome interruptions in vincristine therapies and restrictions on vincristine dose escalation. Vincristine is a plant-derived preparation with anti-neoplastic goods that is utilized for treating many different malignancies, including breast cancer, leukemia, lymphomas, as well as principal brain cancer. At therapeutic doses, it causes consistent and predictable neurotoxicity in all patients.[1-3]

Neuropathic pain can remain triggered through a lesion, toxicity of drugs, or a somatosensory system ailment.[4] Neuropathic pain is assessed to affect 7 to 8% of the overall people in Europe, as well as around 10% of the adult population in the United States. Its incidence is predicted to increase by 17% through report on market and research in 2020.[5] According to Berger *et al*., 2004 the cost of health care in neuropathic pain patients was threefold higher than in matched control subjects.[6] Despite current developments in the understanding of neuropathic pain, it is still challenging and expensive to treat it pharmaceutically.[7] Peripheral neuropathy develops as a result of inflammation. IL-1β, IL-10, tumor necrosis factor-α (TNF-α), and nitric oxide (NO) since glial cells, macrophages, and Langerhans cells have elevated levels of cytokines that are essential for the progression of neuropathic pain.[8]

Individuals with long-standing diseases and ailments, such as acquired immune deficiency syndrome (AIDS), amniotomy, diabetes, leprosy, cervical disc protrusion, cancer, and following surgery, are more likely to have peripheral neuropathic pain. Peripheral neuropathy is also associated with post-herniorrhaphy, post-mastectomy, post-sternotomy and post-thoracotomy conditions. Hyperalgesia (increased pain response to normally painful stimuli), paraesthesia (abnormal sensation to normally pleasant stimuli), dysesthesia (painful abnormal sensation), and with allodynia (pain due to a stimulus that typically doesn't induce pain) are the characteristics of neuropathic pain.[9]

**Author for Correspondence: vinodpatil28@rediffmail.com*

In the citrus species, such as lemons and hesperidin (hesperetin-7- rhamnoglucoside), sweet oranges is cheap and plentiful.[10-12] Hesperidin and curcumin, two flavonoids known for their potent anti-inflammatory, antioxidant, and cytokine-inhibiting properties, are utilized towards treatment of a variation of inflammatory & neurodegenerative diseases of the central nervous system (CNS) such as major depression, diabetic neuropathy, and Alzheimer's disease.[13] Several researchers have studied both of these kinds of flavonoids for their potential anti-nociceptive effects in inflammatory and neuropathic pain.[14]

Hesperetin, an aglycone of hesperidin, reduced pro-inflammatory cytokines like TNF-α, IL-1ß, as well as IL-6 in the same manner to relieve allodynia and hyperalgesia.[15] Researchers have observed the anti-inflammatory properties of hesperidin and hesperetin, which lower levels of COX-2, iNOS, IL-1ß, and TNF-α *via* MAPK and NF-k pathways.[16] Hesperidin's neuroprotective properties compelled are to investigate its character in vincristine-induced peripheral neuropathy in rats.

MATERIALS AND METHODS

Drug and Reagents

Sun Pharmaceutical Industries Ltd., located in Gujarat, India, provided a gift sample of gabapentin. Hesperidin,5,5′-dithio, bis 2-nitro benzoic acid (DTNB), glutathione (GSH), and a 1,1,3,3-tetra-methoxy propane (Sigma-Aldrich, USA) acquired from Sigma-Aldrich, Mumbai, India. Vincristine was procured from Celon Laboratories Pvt. Ltd, Telangana (Gift sample received from Manvata Clinical Research Institute, Nashik) for the current investigations. All the substances utilized in the contemporary investigations were of analytical quality.

Experimental Animals

For the purpose of this experiment, 180 to 250 g wistar albino rats of both genders were used. They were purchased from Bombay Veterinary College, Parel (India). They were kept in a cage with unrestricted access to water and fed a normal diet of laboratory pellet feed. The rats were kept in a typical light-dark cycle with temperatures between 18 and 23°C and relative humidity levels between 30 and 35%. Rats were housed in plastic cages, and to maintain the cleanliness of the cages, the bedding was replaced every other day. The investigational procedure was permitted through the Institutional Animal Ethics Committee and the care of the animals was in accordance with the standards set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (MGV/PC/CPCSEA/XXXVI/01/2019/21).

Experimental Protocol

The test drug samples, vincristine (Intraperitoneal) was administered in disease control rats, standard marketed drug gabapentin (30 mg/kg) and test 1 to 3 rats, 5 days cycle with 2 days pause for 8 weeks. Standard drug and test 1 to 3 were administered orally once daily after induction of neuropathy for 8 weeks through oral gavage. The dose of vincristine was considered as 100 µg/kg based on the previously reported dose suitable for causing neuropathy. Hesperidin was given orally at 50, 100, and 150 mg/kg dosages with dissolved pure water. We selected dosages (50, 100, and 150 mg/kg oral) of hesperidin for the current investigation since, in numerous neuroprotective results associated with hesperidin, the dosages series among 50 to 150 mg/kg (oral) was utilized in rat research.[17,18] The nociceptive signal thresholds were evaluated using a variety of behavioral measures. After that, the animals as a whole were slaughtered for biochemical testing.

The Institutional Animal Ethical Committee (IAEC) gave its authorization earlier than the study could begin, and it was carried out in accordance with CPCSEA regulations.

Six groups have been formed for the animals.

Group 1: Normal control
Group 2: Disease control- Vincristine
Group 3: Standard marketed drug- Vincristine +Gabapentin
Group 4: Test 1 (Vincristine +50 mg/kg Hesperidin)
Group 5: Test 2 (Vincristine +100 mg/kg Hesperidin)
Group 6: Test 3 (Vincristine +150 mg/kg Hesperidin)

Vincristine-induced neuropathic pain

Vincristine sulfate (100 µg/kg per day i.p.) were given towards rats to cause peripheral neuropathy for 56 days (5-day cycles with a 2-day interval in between each cycle).[19,20] According to reported data, distinct weeks, week 0 (prior to vincristine administration), week 4, and week 8 were used to quantify pain.[21,22]

Examination of the behavior

The mechanical dynamic allodynia, cold-allodynia, mechanical hyperalgesia, heat hyperalgesia, and rota rod tests were conducted every measurement week.

Mechanical allodynia (Von frey assessment)

Each rat was placed in an acrylic cage with an elevated labyrinth and subjected to a test environment for at least 15 minutes. Von Frey filament was positioned so that it was beneath the mesh floor and toward the planter surface of the hind paw. Provide enough filament force to the paw to cause a slight bending, then hold the position for a short while. The retraction of the paw is thought to be a positive response.[23] Mechanical allodynia observations were made with six consecutive applications of various Von Frey filament forces. OXXOXO was used for recording assessments, where O stood for no withdrawal response and X for withdrawing reaction. This technique for monitoring is the Dixon up-and-down technique with a 50% gm threshold determined by an equation:

$$50\%\ g\ threshold = (xf+k\delta)^{-10}/10,000$$

Where the mean difference (in log units) concerning stimuli (in this case 0.224) and Xf are the log units of the last von Frey filament utilized. The recurring sequence of positive/negative replies has a tabular value of k.

Cold allodynia using acetone solution

This section utilized the acetone drop method to evaluate the cold chemical thermal sensitivity. Rats were put in a cage through a metal mesh and given 20 minutes to get used to it. A thin layer of acetone (50 µL) was applied to the back paw's mid-plantar region. It causes paw linking, shaking, or rubbing of the hind paw together through swift foot withdrawal subsequently applying a solution of acetone, which was thought to have an aanti-nociceptiveaction.

Pin prick test used for mechanical hyperalgesia

The plantar surface of the left hind paw was briefly in contacted with the point of a bent 18 gauge needle (at a 90° angle) in order to cause a reflex withdrawal reaction in a normal rat, but none of the groups were able to puncture the skin with this pressure. Once every two weeks, the time it took for the paw to withdraw was measured in seconds.

Hot plate test (Thermal hyperalgesia)

Eddy's hotplate was used to investigate the thermal nociceptive threshold, which was kept at a temperature of 52 ± 2°C. Animals were tested individually by being placed on a hot plate and having their paw-licking latency measured (in seconds). The 20-second test cut-off period was kept.[24]

Rota rod test for evaluating motor impairment (Rota rod test)

Rats were tested utilizing a rota rod apparatus while being rotated at a speed of 15 rpm. Each rat's fall-off time from the rotating spindle was timed over a five-minute period.[24]

Estimation of cytokines level

According to Krishgen Biosystems' ELISA cytokine kit, which was created for the simulated flow, cytometric cytokine detection is at the Pune-based APT Research laboratory.[24]

Biochemical Estimations

In the eighth week, ketamine (140 mg/kg by i.p.) was utilized as a high-dose anesthetic during all of the animal sacrifices. Both the sciatic nerv and the tissue beneath it were promptly separated. Sciatic nerve tissue that was excised was located between the point of transection and the point at which it emerged since the spinal cord as well as terminated. The sciatic nerve transection site was precisely beneath the centre of a portion of tissue that was 1-cm in diameter. The sciatic nerve segments were subsequently separated in addition to testingover SOD, CAT, GSH, LPO, and NO. The samples were afterward frozen and examined simultaneously. The 10% w/v sciatic nerve homogenate (pH 7.4) was prepared by using 0.1 M tris-HCl buffers. After being immersed in ice water for 30 minutes, the homogenate-encompassing tubes were centrifuged for 10 minutes at 4°C at 2000 rpm. Calculations for SOD, CAT, GSH, LPO, and NO were done using the homogenate supernatant, which was separated.[24]

Assay of superoxide dismutase

In accordance with the process as per reported outlined, SOD was measured in the nerve homogenate. At 560 nm, superoxide anions were used to reduce NBT to blue formazan beneath aerobic situations. The amount of inhibition experienced when superoxide dismutase (SOD) is added is used to determine the degree of enzyme activity. The enzyme's activity was expressed as units/mg protein, where a unit of enzyme is defined as a quantity that reduces the rate of reaction by approximately 50%.[25]

Catalase analysis

Catalase (CAT) action was measured using the Beers and Sizer technique in the nerve homogenate. The spectrophotometric (at 240 nm) monitoring of hydrogen peroxide (H_2O_2) breakdown in CAT followed the decrease in absorbance. The enzyme activity was measured in mmoles of H_2O_2 putrefied min/mg of protein.[26]

Assessment of glutathione

GPxaction was assessed using Paglia and Valentine's technique in the nerve homogenate. The process gauged how quickly reduced glutathione (GSH) was being oxidized through H_2O_2 and initiated *via* the GPx. By including exogenous glutathione reductase and NADPH, that instantly converts whichever developed oxidized glutathione disulfide (GSSG) to GSH, GSH is kept at a consistent concentration. Then, for 5 minutes, the absorbance of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm is monitored to determine the rate of GSSG production. The action of the enzyme was measured in moles of NADPH oxidized mM/mg protein units.[27]

Measurement of tissue nitrite level

As per the methodology as per reported outlined, the Griess reagent (0.1% naphthyl ethylenediamine hydrochloric acid in water and 1% sulfanilamide in 5% phosphoric acid in a 1:1 ratio) was used to measure the amount of nitrate/nitrite in the nerve homogenate. At 540 nm, the color saturation of the chromogen was determined. Outcomes were given in units of mM/mg protein.[28]

Statistical Analysis

Graph Pad Prism (version 5.0) was employed to compute the Mean + SEM from the dataset, which were then subjected to a one-way ANOVA to determine their statistical significance for them.

RESULT

Effect of Hesperidin on Vincristine-Induced Allodynia

Now, in comparison to the control group, vincristine administration led to a considerable improvement of mechanical dynamic allodynia (Figure 1) in addition to cold allodynia (Figure 2). Vincristine that has been Hesperidinattenuated is administered, and this significantly causes allodynia ($p < 0.05$). Additionally, in mechanical dynamic allodynia, hesperidin administered had a better effect on vincristine-induced neuropathy than when compared with standard vincristine after 8 weeks ($p < 0.05$) (Figure 1). Additionally, in cold allodynia, hesperidin administered had an increased effect on vincristine-induced neuropathy than

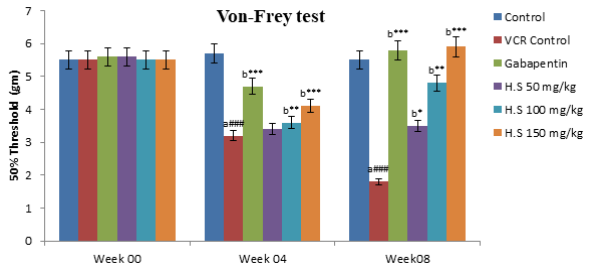


Figure 1: In vincristine-induced neuropathy, hesperidin affects mechanical dynamic allodynia

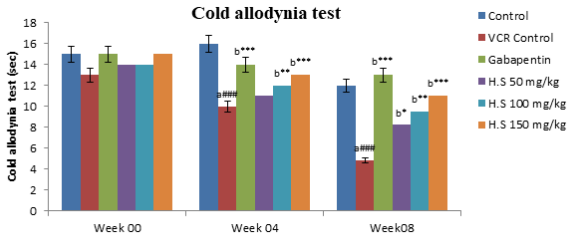


Figure 2: In vincristine-induced neuropathy, the impact of hesperidin on cold allodynia

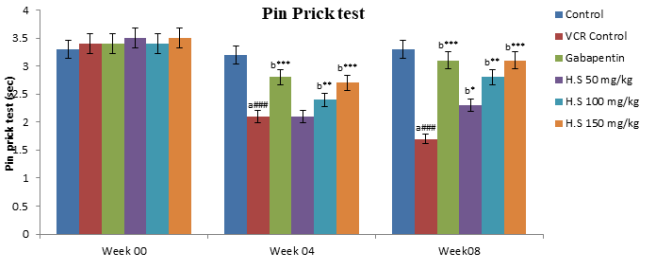


Figure 3: Vincristine induced neuropathy and the impact of hesperidin on mechanical hyperalgesia

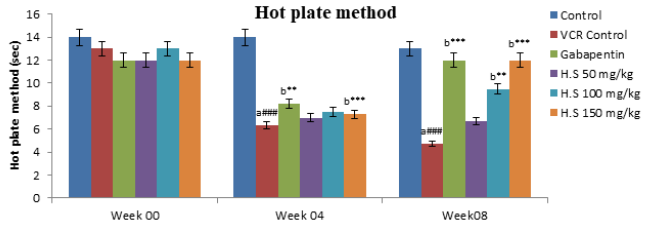


Figure 4: Vincristine induced neuropathy and the impact of hesperidin on heat hyperalgesia

when compared with standard GBA after 8 weeks ($p < 0.05$) (Figure 2), when all hesperidin atteunted results show similar results to standard GBA.

Outcome of Hesperidin on Vincristine-Induced Hyperalgesia

Vincristine therapy induced both mechanical and thermal hyperalgesia relative to the control group (Figures 3 and 4). Hesperidin (50, 100, and 150 mg/kg) substantially increased the beginning of hyperalgesia while reducing vincristine treatment ($p < 0.05$). Additionally, in mechanical hyperalgesia, hesperidin administered had a better effect on vincristine-induced neuropathy than when compared with standard vincristine after 8 weeks ($p < 0.05$) (Figure 3). Additionally, in thermal hyperalgesia, hesperidin administered had an increased effect on vincristine-induced neuropathy than when compared with standard GBA after 8 weeks ($p < 0.05$) (Figure 4), when all hesperidin atteunted results show similar results to standard GBA.

Effect of Hesperidin on Motor Impairment in Vincristine-Induced Neuropathy

In comparison to the control group, when vincristine was administered the motor impairment improved (Figure 5). Hesperidin (150 mg/kg) attenuated vincristine administration suggestively ($p < 0.05$) enlarged the induction of motor impairment. Additionally, the administration of hesperidin had a better outcome alone in treating vincristine-induced neuropathy (Figure 5). When compared to GBA, it also shows similar results as hesperidin showed.

Effect of Hesperidin on Level of Cytokines in Vincristine-Induced Neuropathy

In comparison to control, after administering of vincristine the levels of IL-1ß & TNF-α are significantly increased. However, in comparison to the control, it markedly reduced the amounts of IL-10. Hesperidin administrations for 8 weeks rapidly ($p < 0.05$) reduced the vincristine-caused increase in IL-1β and TNF-α levels. Hesperidin, however, accelerates the decline in IL-10 levels brought on by vincristine. Although, as shown in (Figures 6 and 7), the administration of hesperidin significantly alters the level of IL-1β, TNF-α and IL-10 in vincristine-induced neuropathic pain.

Impact of Hesperidin on Tissue Parameters in Neuropathy Produced by Vincristine

When compared to the control, vincristine administration significantly kept the levels of Na+/K+ ATPase constant. However, compared with the control, it significantly raised the Ca^{2+} & Mg^{2+} ATPase level. Hesperidin administrations for 8 weeks significantly ($p < 0.05$) increased the vincristine-induced Na^{+}/K^{+}ATPase levels in rats. Hesperidin does, however, increase in the Ca^{2+} and Mg^{2+}ATPase levels brought on by vincristine. As indicated in (Figure 8), the injection of GBA significantly show the level of all ATPase as hesperidin showed in vincristine-induced neuropathic pain.

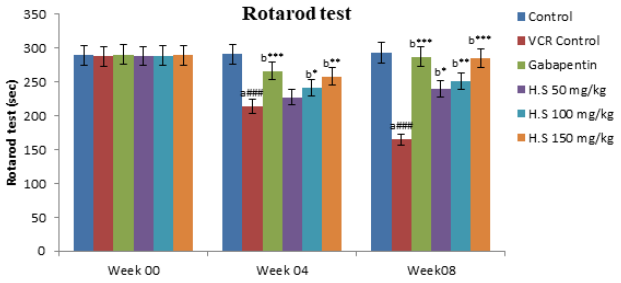


Figure 5: Vincristine induced neuropathy and the impact of hesperidin on motor dysfunction

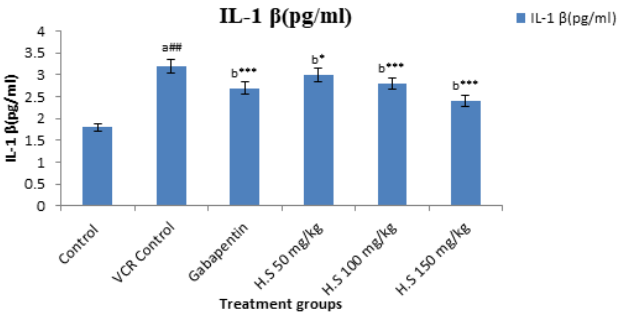


Figure 6: Vincristine-induced neuropathy and cytokines (IL-1ß)

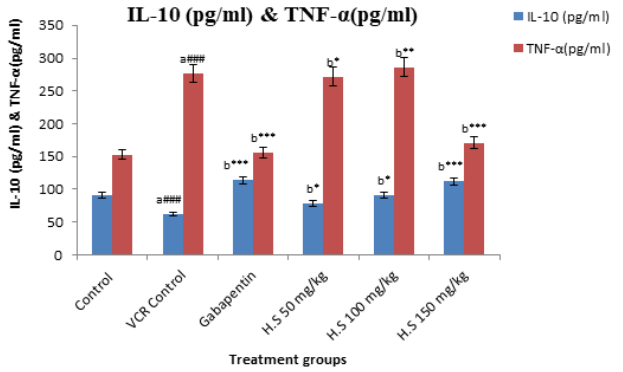


Figure 7: Cytokines (IL-10 and TNF-α) in vincristine-induced neuropathy

Impact of Hesperidin on Oxidative Stress Indicators in Vincristine-Induced Neuropathy

When compared to the control, vincristine caused a sizable drop in GSH levels. Administration of the hesperidin expressively (p <0.05) mitigated the increase in the sciatic nerve GSH level caused by vincristine. Additionally, treatment of hesperidin compared through standard GBA had aim proved outcome (p <0.05) (Figure 9 (C)).

Impact of Hesperidin on an Inflammatory Marker in Neuropathy Caued by Vincristine

Compared to the control, administering vincristine substantially improved the stages of LPO. Hesperidin consumption considerably lowers the tissue LPO elevation caused by vincristine (p <0.05). Additionally, when hesperidin was administered, the results were similar (p <0.05) to standard GBA(Figure 9 (D)). Vincristine administration considerably raised the levels of NO ((Figure 9 (E)) compared to the control. When compared to the control, vincristine administration significantly declined the levels of SOD (Figure 9 (A)). When compared to the control, the administration of vincristine significantly raised the decline of CAT (Figure 9 (B)). As indicated in all shown figures, overall from all inflammatory marker SOD, GSH, CAT levels raised and NO, LPO level declined in vincristine-induced neuropathy.

DISCUSSION

The nociceptive threshold for both painful and non-painful response was significantly reduced by vincristine injection in the current investigation, indicating the development

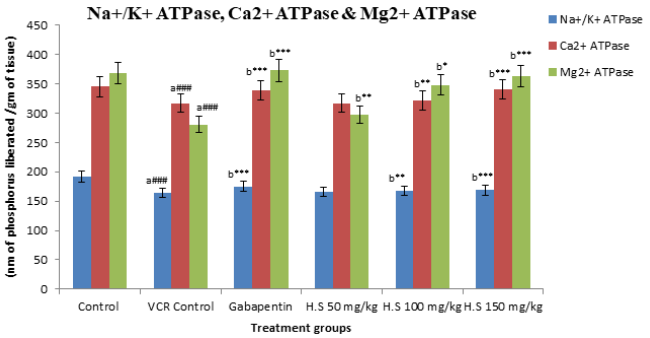
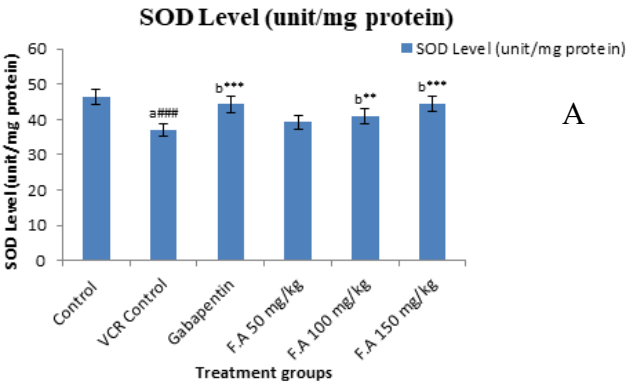
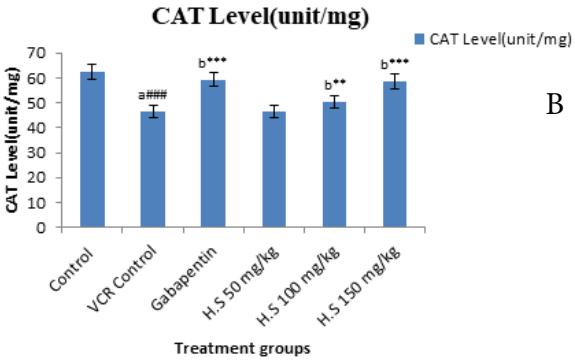


Figure 8: Tissue level parameter in vincristine Induced neuropathy



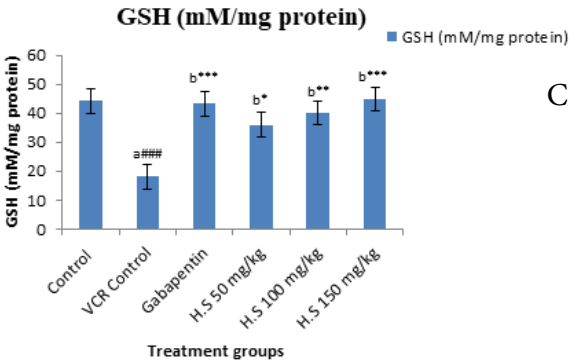
A

Figure 9: (A). SOD level



B

Figure 9: (B). CAT level



C

Figure 9: (C). GSH level

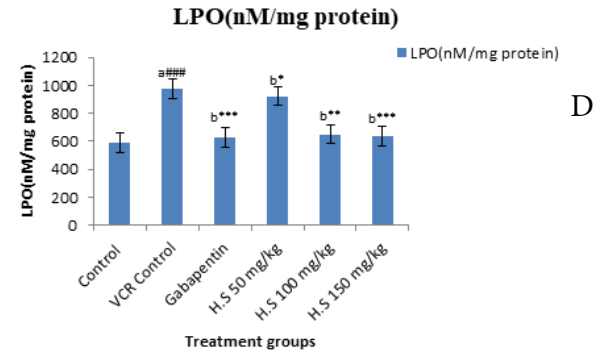


Figure 9: (D). LPO level

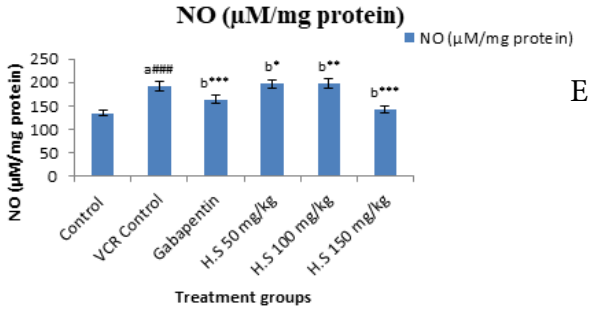


Figure 9: (E). NO level

Figure 9: Vincristine-induced neuropathy: indicators of oxidative stress and inflammation

of mechanical, cold allodynia, mechanical, and thermal hyperalgesia. Following vincristine administration, these behavioral changes began in the first week and peaked in the subsequent weeks. The behavioral changes brought on by vincristine that were seen in this investigation are consistent with other observations.[27,28] Vincristine additionally caused biochemical alterations in the form of a drop in IL-10 and an upsurge in the levels of GSH, SOD, CAT, NO, TNF-alpha, IL-1ß.[29]

Furthermore, at the 2nd and/or 4th weeks of treatment, the increased regulation of p-p38, tumor necrosis factor-α, stromal cell-derived hesperidinctor 1-α (SDF1-α), and CXCR4 prompted through gp120 in the lumbar spinal dorsal horn and/or the dorsal root ganglion (DRG) was reversed by the expression of IL-10 through herpes simplex virus vectors.[31] It was advised to use IL-10 for its anti-inflammatory properties. Additionally, research using various models of spinal injury showed that IL-10 hinders the making of pro-inflammatory species like IL1 beta, IL2 and IL6, TNF-α, ROS, LPO, NO synthase, matrix metalloproteinase-9, and interferon-gamma.[32] IL-10 increased the anti-apoptotic proteins Bcl2 and Bcl2-linked X, B-cell lymphoma extra-large (Bcl-xl), but it down-regulated a number of causing apoptosis hesperidinctors, such as cytochrome-C, caspase-3, and Bax.[33]

Hesperidin treatment also reduced the oxidative stress brought on by vincristine. This suggests that hesperidin-mediated oxidative stress reduction also contributes to vincristine-induced neuropathy's anti-nociceptive effect. Treatment with hesperidin in the current investigation reduced vincristine-induced changes in heat hyperalgesia, the threshold of nociceptive (hyperalgesia, dynamic allodynia, in addition to cold allodynia), pointing to its potential to be anti-nociceptivein vincristine-induced neuropathy.[34]

TNF-alpha, IL-1ß, and LPO levels increased after the administration of hesperidin-attenuated vincristine, indicating a decrease in neuropathy-related inflammation. The neuroprotective effects of hesperidin in ischemia-reperfusion injury were demonstrated through to be mediated *via* lowering TNF-α, oxidative stress, in addition to JNK activation levels. The increase in oxidative stress and elevation in TNF-α and IL-1ß levels brought on by vincristine.[35]

Gabapentin (30 mg/kg i.p.) and hesperidin (50, 100 & 150 mg per kg orally) were administered for 08 weeks. Hesperidin administration markedly decreased vincristine-induced changes in behavior and metabolism alterations ($p < 0.05$). Hesperidin also reduced the inflammation IL-10 and increased in IL-1ß and TNF-α. Hesperidin also reduce oxidative stress (LPO, NO level) and increase at (GSH, SOD, CAT level) that vincristine caused. Hesperidin can alle*via*te the painful states brought on by vincristine-induced painful neuropathy, which also could explained by its anti-inflammatory effects and consequent decrease of oxidative stress.[33-35]

However, despite the hesperidinct that high doses of hesperidin reduced pain, these groups still experienced significant pain. Therefore, hesperidin-high dose was used to achieve complete pain relief. Treatment with hesperidin had a stronger anti-nociceptiveeffect than treatment with either drug alone. However, hesperidin works through the mechanisms mentioned above, which encourages the use of therapy for more effective neuropathic pain relief.

CONCLUSION

Increasing of TNF-alpha IL-1ß, levels, as well as an inhibiting in IL-10, is all indicators of hesperidin's considerable anti-inflammatory effect, according to our study. Its antioxidant potential is supported by a decrease in NO, LPO and a rise in GSH, SOD, CAT levels caused by hesperidin. Additionally, hesperidin administration may be able to balance calcium homeostasis and reduce the oxidative stress and inflammation that vincristine causes. The protection of cytokine levels through hesperidin turn out to be a crucial element in nerve regeneration, in addition to reducing electrophysiological imbalance. Furthermore, hesperidin could develop a novel nutritional strategy for treating chemotoxic neuropathy. According to evidence, hesperidin's benefits, such as anti-inflammatory, anti-oxidative, and calcium homeostasis, in addition to level of cytokine regulation, In vincristine-induced neuropathy caused neuroprotective action. Hesperidin may eventually prove to be cutting-edge target for the successful treatment of painful neuropathy.

REFERENCES

1. Di Cataldo A, Lanteri R, Rapisarda C, Di Raimondo F, Licata A. Lymphoma of the cecum: a case report. International

surgery. 2002 Jan 1;87(1):12-4. https://europepmc.org/article/med/12144183
2. Ito TO, Mochida AK, Saito K, Nishi KI, Sasaki S, Hisada T, Morinari H, Nakahara K, Tahara M, Masuda S, Yakumaru K. An autopsy case of pulmonary and central nervous system metastatic osteosarcoma treated with thirty-six courses of chemotherapy over four years. Nihon KokyukiGakkaiZasshi= the Journal of the Japanese Respiratory Society. 2002 Jan 1;40(1):71-6. https://europepmc.org/article/med/11925923
3. Pal PK. Clinical and electrophysiological studies in vincristine induced neuropathy. Electromyography and clinical neurophysiology. 1999 Sep 1;39(6):323-30. https://europepmc.org/article/med/10499201
4. Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms, and management. The lancet. 1999 Jun 5;353(9168):1959-64. https://doi.org/10.1016/S0140-6736(99)01307-0
5. Research and Market Report. Epidemiology: peripheral neuropathic pain – a common comorbidity for HIV, diabetes, and herpes zoster. Available from: http://www.researchandmarkets.com/reports/2014030/epidemiology_peripheral_neuropathic_pain_a [accessed 2022 Dec 21].
6. Berger A, Dukes EM, Oster G. Clinical characteristics and economic costs of patients with painful neuropathic disorders. The Journal of Pain. 2004 Apr 1;5(3):143-9. https://doi.org/10.1016/j.jpain.2003.12.004
7. Attal N, Cruccu G, Baron RA, Haanpää M, Hansson P, Jensen TS, Nurmikko T. EFNS guidelines on the pharmacological treatment of neuropathic pain: 2010 revision. European journal of neurology. 2010 Sep;17(9):1113-e88. https://doi.org/10.1111/j.1468-1331.2010.02999.x
8. Clark AK, Old EA, Malcangio M. Neuropathic pain and cytokines: current perspectives. Journal of pain research. 2013 Nov 21:803-14. https://www.tandfonline.com/doi/full/10.2147/JPR.S53660
9. Rana AC, Gulliya B, Rana S. Analgesic and Anti-allodynic Effects of Two Flavonoids in Partial Sciatic Nerve Ligation in Rat Model. Indian Journal of Pharmaceutical Education and Research. 2019 Oct 1;53(4):S684-90. https://ijper.org/sites/default/files/IndJPhaEdRes_53_4s_684.pdf
10. Ghosh S, Banerjee S, Sil PC. The beneficial role of curcumin on inflammation, diabetes and neurodegenerative disease: A recent update. Food and Chemical Toxicology. 2015 Sep 1;83:111-24. https://doi.org/10.1016/j.fct.2015.05.022
11. Visnagri A, Kandhare AD, Chakravarty S, Ghosh P, Bodhankar SL. Hesperidin, a flavanoglycone attenuates experimental diabetic neuropathy *via* modulation of cellular and biochemical marker to improve nerve functions. Pharmaceutical biology. 2014 Jul 1;52(7):814-28. https://doi.org/10.3109/13880209.2013.870584
12. Carballo-Villalobos AI, González-Trujano ME, Alvarado-Vázquez N, López-Muñoz FJ. Pro-inflammatory cytokines involvement in the hesperidin antihyperalgesic effects at peripheral and central levels in a neuropathic pain model. Inflammopharmacology. 2017 Apr;25(2):265-9. https://doi.org/10.1007/s10787-017-0326-3
13. Benavente-Garcia O, Castillo J. Update on uses and properties of citrus flavonoids: new findings in anticancer, cardiovascular, and anti-inflammatory activity. Journal of agricultural and food chemistry. 2008 Aug 13;56(15):6185-205. https://doi.org/10.1021/jf8006568
14. Zhao X, Xu Y, Zhao Q, Chen CR, Liu AM, Huang ZL. Curcumin exerts anti-nociceptive effects in a mouse model of neuropathic pain: descending monoamine system and opioid receptors are differentially involved. Neuropharmacology. 2012 Feb 1;62(2):843-54. https://doi.org/10.1016/j.neuropharm.2011.08.050
15. Aswar M, Kute P, Mahajan S, Mahajan U, Nerurkar G, Aswar U. Protective effect of hesperetin in rat model of partial sciatic nerve ligation induced painful neuropathic pain: an evidence of anti-inflammatory and anti-oxidative activity. Pharmacology Biochemistry and Behavior. 2014 Sep 1;124:101-7. https://doi.org/10.1016/j.pbb.2014.05.013
16. Raza SS, Khan MM, Ahmad A, Ashafaq M, Khuwaja G, Tabassum R, Javed H, Siddiqui MS, Safhi MM, Islam F. Hesperidin ameliorates functional and histological outcome and reduces neuroinflammation in experimental stroke. Brain Research. 2011 Oct 28;1420:93-105. https://doi.org/10.1016/j.brainres.2011.08.047
17. Siau C, Bennett GJ. Dysregulation of cellular calcium homeostasis in chemotherapy-evoked painful peripheral neuropathy. Anesthesia and analgesia. 2006 May;102(5):1485. https://doi.org/10.1213%2F01.ane.0000204318.35194.ed
18. Erichsen HK, Blackburn-Munro G. Pharmacological characterisation of the spared nerve injury model of neuropathic pain. Pain. 2002 Jul 1;98(1-2):151-61.https://doi.org/10.1016/S0304-3959(02)00039-8
19. Borta A, Schwarting RK. Inhibitory avoidance, pain reactivity, and plus-maze behavior in Wistar rats with high versus low rearing activity. Physiology & behavior. 2005 Mar 16;84(3):387-96. https://doi.org/10.1016/j.physbeh.2005.01.009
20. Guevara I, Iwanejko J, Dembińska-Kieć A, Pankiewicz J, Wanat A, Anna P, Gołąbek I, Bartuś S, Malczewska-Malec M, Szczudlik A. Determination of nitrite/nitrate in human biological material by the simple Griess reaction. Clinicachimicaacta. 1998 Jun 22;274(2):177-88. https://doi.org/10.1016/S0009-8981(98)00060-6
21. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological chemistry. 1972 May 25;247(10):3170-5. https://doi.org/10.1016/S0021-9258(19)45228-9
22. Lowry OH, Rosenbrough NJ, Hesperidinrr AL, Randall RJ. Protein measurement with Folin phenol reagent. The Journal of biological chemistry. 1951;193(1):265-275. https://pubmed.ncbi.nlm.nih.gov/14907713/
23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry. 1979 Jun 1;95(2):351-8.https://doi.org/10.1016/0003-2697(79)90738-3
24. Beutler E, Duron O and Kelly BM. Improved method for the determination of blood glutathione. The Journal of laboratory and clinical medicine. 1963;61:882-888. https://pubmed.ncbi.nlm.nih.gov/13967893/
25. Severenghaus JW, Ferrebee JW. Calcium determination by flame photometry; methods for serum, urine, and other fluids. Journal of Biological Chemistry. 1950;187:621-30. https://www.cabdirect.org/cabdirect/abstract/19501404640
26. Martucci C, Trovato AE, Costa B, Borsani E, Franchi S, Magnaghi V, Panerai AE, Rodella LF, Valsecchi AE, Sacerdote P, Colleoni M. The purinergic antagonist PPADS reduces pain related behaviours and interleukin-1β, interleukin-6, iNOS and nNOS overproduction in central and peripheral nervous system after peripheral neuropathy in mice. Pain®. 2008 Jun 30;137(1):81-95. https://doi.org/10.1016/j.pain.2007.08.017

27. Kiguchi N, Maeda T, Kobayashi Y, Saika F, Kishioka S. Involvement of inflammatory mediators in neuropathic pain caused by vincristine. International review of neurobiology. 2009 Jan 1;85:179-90. https://doi.org/10.1016/S0074-7742(09)85014-9
28. Muthuraman A, Singh N. Attenuating effect of hydroalcoholic extract of Acorus calamus in vincristine-induced painful neuropathy in rats. Journal of natural medicines. 2011 Jul;65:480-7. https://link.springer.com/article/10.1007/s11418-011-0525-y
29. Jaggi AS, Singh N. Mechanisms in cancer-chemotherapeutic drugs-induced peripheral neuropathy. Toxicology. 2012 Jan 27;291(1-3):1-9.https://doi.org/10.1016/j.tox.2011.10.019
30. Milligan ED, Langer SJ, Sloane EM, He L, Wieseler-Frank J, O'Connor K, Martin D, Forsayeth JR, Maier SF, Johnson K, Chavez RA. Controlling pathological pain by adenovirally driven spinal production of the anti-inflammatory cytokine, interleukin-10. European Journal of Neuroscience. 2005 Apr;21(8):2136-48. https://doi.org/10.1111/j.1460-9568.2005.04057.x
31. Milligan ED, Sloane EM, Langer SJ, Cruz PE, Chacur M, Spataro L, Wieseler-Frank J, Hammack SE, Maier SF, Flotte TR, Forsayeth JR. Controlling neuropathic pain by adeno-associated virus driven production of the anti-inflammatory cytokine, interleukin-10. Molecular pain. 2005 Feb 25;1:1744-8069. https://journals.sagepub.com/doi/pdf/10.1186/1744-8069-1-9
32. Zheng W, Huang W, Liu S, Levitt RC, Candiotti K.A, Lubarsky DA, Hao S. IL-10 mediated by herpes simplex virus vector reduces neuropathic pain induced by HIV gp120 combined with ddC in rats. Molecular pain. 2014;10:1744-8069. https://journals.sagepub.com/doi/pdf/10.1186/1744-8069-10-49
33. Thompson CD, Zurko JC, Hanna BF, Hellenbrand DJ, Hanna A. The therapeutic role of interleukin-10 after spinal cord injury. Journal of neurotrauma. 2013 Aug 1;30(15):1311-24. https://doi.org/10.1089/neu.2012.2651
34. Sung JH, Gim SA, Koh PO. Ferulic acid attenuates the cerebral ischemic injury-induced decrease in peroxiredoxin-2 and thioredoxin expression. Neuroscience Letters. 2014 Apr 30;566:88-92. https://doi.org/10.1016/j.neulet.2014.02.040
35. Kim HY and Le, SM. Hesperidin attenuates ischemia/reperfusion-induced hepatocyte apoptosis *via* inhibition of JNK activation. European Journal of Pharmaceutical Sciences. 2012;45(5):708-715. https://doi.org/10.1016/j.ejps.2012.01.010