Research Square

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Dose-Dependent Neuroprotective Effect of Hesperidin Against Vincristine-Induced Neuropathy

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Abstract

Background

Vincristine-induced peripheral neuropathy (VIPN) is a common, dose-limiting complication of chemotherapy for which no effective preventive treatment currently exists. Oxidative stress is a key mechanism underlying vincristine neurotoxicity, and hesperidin, a citrus-derived flavonoid with well-documented antioxidant and neuroprotective properties, represents a promising candidate for its prevention.

Objective

This study evaluated the dose-dependent preventive effects of hesperidin on VIPN in mice, assessed through thermal nociceptive behavior and serum oxidative stress biomarkers.

Methods

Fifty male mice were randomly divided into five groups (n = 10/group): vehicle + saline, vehicle + vincristine (0.1 mg/kg/day, i.p., 10 days), and vincristine co-administered with hesperidin (25, 50, or 100 mg/kg/day, i.p.). Thermal nociceptive latency was assessed using the Hot Plate test at baseline and on days 6, 10, 15, and 19. Serum malondialdehyde (MDA) and total antioxidant capacity (TAC) were measured at the end of the protocol, and body weight was monitored throughout.

Results

Vincristine administration induced significant thermal hyperalgesia from day 10 onward, accompanied by significantly elevated serum MDA and reduced TAC compared with controls ($P < 0.001$). Hesperidin at 25 and 50 mg/kg did not significantly modify these outcomes; however, hesperidin at 100 mg/kg significantly preserved nociceptive latency and normalized serum MDA and TAC levels relative to the vincristine-only group ($P < 0.001$). No significant differences in body weight gain were observed among the experimental groups.

Conclusion

Hesperidin exerts a dose-dependent protective effect against vincristine-induced peripheral neuropathy, with 100 mg/kg being the most effective dose, likely mediated through attenuation of oxidative stress. These findings support further investigation of hesperidin as a candidate adjunct for the prevention of chemotherapy-induced peripheral neuropathy.

1. Introduction

Cancer chemotherapy has substantially improved patient survival over recent decades, owing largely to advances in early diagnosis and the development of more effective cytotoxic regimens [1–4]. This progress, however, has been accompanied by a range of treatment-limiting adverse effects [5–7], among which chemotherapy-induced peripheral neuropathy (CIPN) is one of the most common and debilitating [8–10]. A systematic review and meta-analysis of 31 studies comprising over 4,000 patients estimated CIPN prevalence at 68.1% within the first month after chemotherapy, declining to 60.0% at three months and 30.0% at six months or later [11], indicating that a substantial proportion of survivors continue to experience neuropathic symptoms long after treatment has ended. CIPN not only compromises patients' quality of life but also frequently necessitates dose reduction or premature discontinuation of otherwise effective chemotherapy regimens [12, 13].

Vincristine, a vinca alkaloid derived from Catharanthus roseus (Madagascar periwinkle), is a cornerstone agent in the treatment of acute lymphoblastic leukemia, lymphomas, and several solid tumors [14, 15]. Its antineoplastic activity results from binding to tubulin, which inhibits microtubule polymerization and arrests cell division at metaphase [16, 17]. Peripheral neuropathy is among the most frequent and dose-limiting toxicities associated with vincristine, typically manifesting as a symmetric, length-dependent sensorimotor polyneuropathy, occasionally accompanied by autonomic dysfunction [18]. Vincristine-induced peripheral neuropathy (VIPN) arises through disruption of the same microtubule-dependent axonal transport processes that underlie its antitumor effect, ultimately leading to axonal degeneration [19, 20].

Beyond direct cytoskeletal disruption, accumulating evidence implicates oxidative stress, mitochondrial dysfunction, and neuroinflammation as key contributors to the pathogenesis of VIPN [21–23]. Elevated production of reactive oxygen species and impaired antioxidant defenses have been linked to axonal injury and heightened nociceptive sensitivity in vincristine-treated animals [24]. In parallel, interleukin-1β released from activated macrophages via canonical NLRP3 inflammasome signaling has been shown to drive mechanical allodynia and gait disturbances in murine models of VIPN, with IL-1 receptor antagonism attenuating neuropathy progression [25]. Metabolomic profiling of pediatric leukemia patients has further linked sphingolipid dysregulation to VIPN susceptibility, underscoring the multifactorial nature of its pathophysiology [20, 26].

Current management of CIPN remains largely symptomatic and unsatisfactory. Pharmacological approaches such as gabapentin, pregabalin, and tricyclic antidepressants provide only partial relief and are limited by their own side-effect profiles [27, 28], and no agent has yet been established as an effective preventive therapy that can be co-administered with chemotherapy without compromising antitumor efficacy [29]. This unmet need has prompted growing interest in naturally occurring antioxidant and anti-inflammatory compounds as candidate adjuncts capable of mitigating chemotherapy-associated neurotoxicity.

Hesperidin, a flavanone glycoside abundant in the peel and pulp of citrus fruits, has attracted particular attention in this regard [30]. Despite relatively limited oral bioavailability owing to its glycosidic structure [31], hesperidin and its aglycone metabolite hesperetin exhibit well-documented antioxidant, anti-inflammatory, and neuroprotective properties [32, 33]. Mechanistically, hesperidin enhances the activity of endogenous antioxidant enzymes and scavenges free radicals [34], while suppressing NF-κB–mediated inflammatory signaling and pro-inflammatory cytokine production [35]; these properties have translated into neuroprotective effects across diverse animal models of neurological injury and neurodegenerative disease [32].

Of direct relevance to the present study, hesperidin has previously been shown to attenuate paclitaxel-induced peripheral neuropathy in rats, reducing oxidative stress within sciatic nerve tissue and improving nerve function [36]. Likewise, a micronized purified flavonoid fraction containing hesperidin and diosmin has demonstrated protective effects against vincristine-induced neuropathy in rats, partly through modulation of the nitric oxide pathway [37]. Nonetheless, the isolated, dose-dependent effect of hesperidin alone on vincristine-induced peripheral neuropathy, together with its impact on systemic oxidative stress biomarkers, has not been comprehensively characterized, representing a clear gap that the present study addresses.

Given the shared mechanistic involvement of oxidative stress in both hesperidin's protective activity and vincristine's neurotoxic action, we hypothesized that hesperidin would exert a dose-dependent preventive effect against VIPN. Accordingly, the present study aimed to evaluate the preventive effects of three doses of hesperidin (25, 50, and 100 mg/kg, i.p.) on vincristine-induced peripheral neuropathy in male mice, assessed through thermal nociceptive behavior (Hot Plate test) and serum oxidative stress biomarkers, namely malondialdehyde (MDA) and total antioxidant capacity (TAC).

2. Materials and Methods

2.1. Materials

Normal saline (0.9%; Iran Pharmaceutical and Injectable Products Co., Iran), vincristine sulfate (Cipla Ltd., India), carboxymethyl cellulose (CMC) (Merck, Germany), hesperidin (≥ 80% purity; Sigma-Aldrich, USA), ketamine hydrochloride (Exir Pharmaceutical Co., Iran), and xylazine (Alfasan, Netherlands) were used in this study.

2.2. Animals

Fifty adult male albino mice (25–35 g; Pasteur Institute, Tehran, Iran) were randomly divided to five experimental groups (n = 10 per group). Only male animals were used to eliminate the potential confounding influence of cyclical fluctuations in female sex hormones on nociceptive behavior. Animals were housed individually in standard polypropylene cages under controlled environmental conditions (temperature 24 ± 0.5°C; relative humidity 50 ± 10%; 12 h light/dark cycle), with free access to standard chow and water. All experimental procedures were carried out during the light phase of the diurnal cycle.

The studies were conducted following the guidelines outlined in the Guide for Care and Use of Laboratory Animals, (National Institutes of Health Publication No 85 – 23, revised 1985). The proposal was approved by the ethical committee of Tabriz university of medical sciences (IR.TBZMED.PHARMACY.REC.1403.055).

2.3. Preparation of the vehicle solution

Given the limited injection volume tolerated by mice and the need for a suitable carrier for the poorly water-soluble hesperidin, a 0.5% CMC suspension was used as vehicle. The suspension was prepared by dispersing 0.5 g of CMC in chilled deionized water (25 mL), followed by the gradual addition of boiling deionized water (75 mL) with continuous magnetic stirring, and final homogenization at 65°C to obtain a uniform 100 mL suspension.

2.4. Experimental design and drug administration

Animals were randomly assigned to one of five groups (n = 10/group):

Control: 0.5% CMC (10 mL/kg, i.p.) + normal saline (10 mL/kg, i.p.)

Vincristine (VIPN) group: 0.5% CMC (10 mL/kg, i.p.) + vincristine (0.1 mg/kg, i.p.)

3–5. Hesperidin groups: hesperidin at 25, 50, or 100 mg/kg (i.p.) + vincristine (0.1 mg/kg, i.p.)

Hesperidin (or its CMC vehicle in the control and vincristine-only groups) was administered intraperitoneally twice daily for 15 consecutive days. To induce peripheral neuropathy, vincristine (0.1 mg/kg, i.p.) was administered once daily for 10 consecutive days, beginning on day 6 of the protocol. In the hesperidin-treated groups, each daily hesperidin dose was administered 30 min prior to the vincristine injection throughout the 10-day induction period. Nociceptive testing (Hot Plate test) was performed in all groups up to day 19 of the protocol, as detailed below.

2.5. Assessment of thermal nociception (Hot Plate test)

Thermal nociceptive thresholds were assessed using a Hot Plate apparatus (Pars Teb Novin, Iran), consisting of a metal plate (23 × 23 cm) enclosed by a transparent glass cylinder, with surface temperature maintained at 55 ± 1°C by a digital thermostatic controller. This method, originally described in our previous work [38] and widely adopted as a standard assay for assessing supraspinally mediated thermal nociception in rodents, measures the latency from placement of the animal on the heated surface to the onset of a nocifensive response (typically hind-paw licking). Each animal was placed on the heated surface, and the response latency was recorded using a stopwatch; a cut-off time of 30 s was imposed to prevent thermal injury to the paw. Testing was performed on days 6, 10, 15, and 19 of the protocol. A reduction in latency relative to baseline was interpreted as increased pain sensitivity and functional impairment of sensory nerve fibers, whereas an increase in latency following hesperidin

treatment was interpreted as attenuation of neuropathic pain and preservation of peripheral nerve function.

2.6. Blood sampling and euthanasia

On day 19 of the experiment, the animals were deeply anesthetized via intraperitoneal administration of ketamine (100 mg/kg) to ensure complete loss of consciousness before sample collection. Blood was then obtained by cardiac puncture, which also served as the terminal euthanasia procedure. This method induces rapid death through hypovolemia while adhering to ethical guidelines, since the animals remained fully anesthetized and unresponsive to noxious stimuli throughout the procedure. The collected blood was allowed to clot for 15 min at room temperature and subsequently centrifuged at 3500 rpm for 10 min to separate the serum [39]. Serum aliquots were stored at − 70°C until assayed for MDA and TAC. Following blood collection, animals were euthanized under deep anesthesia, and carcasses were disposed of according to the institution's standard biosafety protocol. All laboratory safety procedures relevant to handling of chemicals and laboratory animals were observed throughout.

2.7. Determination of serum malondialdehyde (MDA)

Serum MDA, a well-established end product of lipid peroxidation and marker of oxidative stress [40], was quantified using a commercial thiobarbituric acid (TBA)-based colorimetric assay kit, following the manufacturer's protocol. Briefly, 500 µL of serum was mixed with 3 mL of 1% orthophosphoric acid, followed by the addition of 1 mL of 0.67% thiobarbituric acid solution. Samples were incubated in a boiling water bath for 45 min, cooled, and extracted with 3 mL of n-butanol. After centrifugation (3000 rpm, 10 min), the absorbance of the organic phase was measured spectrophotometrically at 532 nm against an n-butanol blank. Serum MDA concentrations were calculated from a standard curve and expressed as nmol/mL.

2.8. Determination of serum total antioxidant capacity (TAC)

Serum TAC, reflecting the cumulative antioxidant defense capacity of the sample [41], was measured using a commercial colorimetric kit based on the inhibition of ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] radical cation formation by antioxidants present in serum. The assay employed a phosphate-buffered chromogen (metmyoglobin/ABTS) and a hydrogen peroxide substrate solution, calibrated against a Trolox-equivalent standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Absorbance was recorded at 600 nm before (A_1) and three minutes after (A_2) substrate addition using a spectrophotometer, and TAC values were calculated from the change in absorbance (ΔA) relative to the standard, per the manufacturer's formula, and expressed in standard TAC units (mmol/L).

2.9. Statistical analysis

Data were analyzed using InStat statistical software and are expressed as mean ± SEM. Differences between groups were assessed using independent-samples t-tests and one-way analysis of variance

(ANOVA) followed by Tukey's post hoc test, as appropriate. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Vincristine induces peripheral neuropathy

Figure 1 illustrates the effect of daily vincristine injection (0.1 mg/kg, i.p.) for 10 consecutive days on thermal nociceptive threshold, as assessed by the Hot Plate test. While latency remained stable in the vehicle-treated control group throughout the study, a marked and statistically significant reduction in latency was observed in the vincristine-treated group from day 10 onward ($P < 0.001$), indicating successful induction of thermal hyperalgesia consistent with peripheral neuropathy.

3.2. Dose-dependent preventive effect of hesperidin on vincristine-induced hyperalgesia

As shown in Fig. 2, co-administration of hesperidin at 25 and 50 mg/kg failed to significantly modify nociceptive latency relative to the vincristine-only group at any assessed time point. In contrast, the highest dose of hesperidin (100 mg/kg) significantly preserved thermal latency compared with the vincristine-only group from day 10 onward ($P < 0.001$), demonstrating a clear dose-dependent protective effect against vincristine-induced hyperalgesia, evident only at the highest dose tested.

3.3. Vincristine increases oxidative stress and depletes antioxidant capacity

Consistent with the behavioral findings, vincristine administration was associated with marked derangement of serum oxidative stress biomarkers. Serum MDA concentration was significantly elevated in the vincristine group compared with vehicle controls (4.6 ± 0.05 vs. 2.2 ± 0.05 nmol/mL; $P < 0.001$), while serum TAC was significantly reduced (1.09 ± 0.03 vs. 1.96 ± 0.03 mmol/L; $P < 0.001$; Table 1), confirming a state of heightened lipid peroxidation and diminished antioxidant reserve in vincristine-treated animals.

Table 1
Effect of vincristine on serum MDA and TAC levels

Group	MDA (nmol/mL)	TAC (mmol/L)
Vehicle + Saline	2.2 ± 0.05	1.96 ± 0.04
Vehicle + Vincristine	4.6 ± 0.04***	1.09 ± 0.03***

Values are mean ± SEM (n = 10/group). ***$P < 0.001$ vs. vehicle + saline.

3.4. Hesperidin (100 mg/kg) attenuates vincristine-induced oxidative stress

In line with its behavioral efficacy, hesperidin at 100 mg/kg significantly reversed the oxidative imbalance induced by vincristine. Serum MDA was significantly lower in the hesperidin (100 mg/kg) + vincristine group compared with the vincristine-only group (3.12 ± 0.04 vs. 4.6 ± 0.05 nmol/mL; $P < 0.001$), while serum TAC was significantly higher (1.50 ± 0.03 vs. 1.09 ± 0.03 mmol/L; $P < 0.001$; Table 2).

Table 2
Effect of hesperidin (100 mg/kg) on serum MDA and TAC levels in vincristine-treated mice.

Group	MDA (nmol/mL)	TAC (mmol/L)
Vehicle + Vincristine	4.6 ± 0.05	1.09 ± 0.03
Hesperidin (100 mg/kg) + Vincristine	3.12 ± 0.04***	1.50 ± 0.03***

Values are mean ± SEM (n = 10/group). ***$P < 0.001$ vs. vehicle + vincristine.

3.5. Body weight changes

Body weight gain (day 19 minus day 1) did not differ significantly between the vehicle + saline and vehicle + vincristine groups (0.88 ± 0.26 g vs. 0.66 ± 0.20 g), indicating that at the dose and duration used, vincristine did not produce overt systemic toxicity affecting body weight (Table 3). Similarly, none of the hesperidin doses (25, 50, or 100 mg/kg) significantly altered body weight gain relative to the vincristine-only group (0.80 ± 0.30, 1.00 ± 0.30, and 0.80 ± 0.30 g, respectively, vs. 0.66 ± 0.20 g), suggesting that the observed protective effects of hesperidin were not attributable to differences in nutritional status or general well-being between groups.

Table 3
Effect of vincristine and hesperidin on body weight gain.

Group	Body weight gain (g)
Vehicle + Saline	0.88 ± 0.26
Vehicle + Vincristine	0.66 ± 0.20
Hesperidin (25 mg/kg) + Vincristine	0.80 ± 0.32
Hesperidin (50 mg/kg) + Vincristine	1.00 ± 0.29
Hesperidin (100 mg/kg) + Vincristine	0.80 ± 0.30

4. Discussion

Chemotherapy-induced peripheral neuropathy remains one of the most significant dose-limiting toxicities in oncology, substantially impairing patients' quality of life [23, 42, 43]. Among chemotherapeutic agents, vincristine is particularly associated with peripheral neurotoxicity, mediated through a combination of oxidative stress, neuroinflammation, mitochondrial dysfunction, and disruption of microtubule-dependent axonal transport [18, 44].

In the present study, three doses of hesperidin (25, 50, and 100 mg/kg, i.p.) were evaluated for their preventive potential against vincristine-induced peripheral neuropathy in mice. Only the highest dose (100 mg/kg) produced a significant protective effect, preserving thermal nociceptive latency and indicating attenuated hyperalgesia relative to the vincristine-only group. This finding is consistent with previous reports describing hesperidin's antioxidant, anti-inflammatory, and neuroprotective properties [32, 33, 45–48].

A central mechanism implicated in vincristine-induced neurotoxicity is excessive production of reactive oxygen species coupled with depletion of cellular antioxidant defenses, which promotes membrane damage, mitochondrial dysfunction, and ultimately neuronal injury [49, 50]. Hesperidin, a flavanone abundant in the white albedo of citrus fruits, exerts potent antioxidant activity by scavenging free radicals and enhancing the activity of endogenous antioxidant enzymes such as superoxide dismutase and glutathione peroxidase [45, 51, 52]. In line with this mechanism, hesperidin (100 mg/kg) significantly increased serum TAC and decreased serum MDA relative to the vincristine-only group, supporting a role for restored antioxidant capacity and reduced lipid peroxidation in its observed neuroprotective effect.

Neuroinflammation constitutes a second major pathway implicated in vincristine neurotoxicity: vincristine has been shown to activate the NLRP3 inflammasome and promote IL-1β release from macrophages, driving neuropathic pain behavior [25, 53]. Hesperidin has been reported to attenuate NF-κB–mediated inflammatory signaling and reduce pro-inflammatory cytokine production [45, 54], suggesting a plausible complementary anti-inflammatory contribution to its protective effect; however, as inflammatory markers were not directly measured in the present study, this remains an inference to be confirmed by future work rather than a direct finding.

A further mechanism relevant to VIPN is impaired axonal transport resulting from vincristine's binding to tubulin and consequent inhibition of microtubule polymerization [24, 55]. Hesperidin does not act directly on microtubule structure; its protective effect is therefore more plausibly attributable to mitigation of the secondary oxidative and inflammatory injury that compounds axonal damage, rather than to any direct effect on the cytoskeletal target of vincristine.

The dose-dependency observed in this study, with only the 100 mg/kg dose conferring significant protection, is consistent with the comparatively limited oral/systemic bioavailability of hesperidin due to its glycosidic structure [31], which may necessitate higher doses to achieve tissue concentrations sufficient for a measurable neuroprotective effect. These findings are consistent with those of Semis et al., who reported that hesperidin reduced pain and improved nerve function in a rat model of paclitaxel-induced peripheral neuropathy [36], and with Abharian et al. (2024), who demonstrated protective effects

of a hesperidin–diosmin flavonoid fraction against vincristine-induced neuropathy in rats [37]. The present findings extend this literature by characterizing the isolated, dose-dependent effect of hesperidin alone specifically in a vincristine-induced neuropathy model. More broadly, hesperidin has been reported to exert direct neuroprotective actions, including reduction of neuronal apoptosis and preservation of mitochondrial function, in other models of neurological injury [32, 56], and flavonoids in general are recognized for their capacity to cross the blood–brain barrier and modulate neuroinflammatory and oxidative signaling pathways relevant to neurodegenerative disease [57, 58], supporting the biological plausibility of hesperidin's protective effect in the peripheral nervous system as well.

With regard to body weight, vincristine administration did not significantly affect body weight gain over the 19-day protocol compared with vehicle controls, nor did any hesperidin dose significantly alter weight gain relative to the vincristine-only group. This finding suggests that, at the dose and duration used in this study, vincristine did not produce overt systemic toxicity, and that the neuroprotective effects of hesperidin observed here are unlikely to be confounded by differences in nutritional status or general health between groups.

Several limitations of the present study should be acknowledged. Oxidative status was assessed using only two systemic biomarkers (MDA and TAC), without direct measurement of specific antioxidant enzymes (e.g., superoxide dismutase, glutathione peroxidase) or inflammatory mediators (e.g., IL-1β, TNF-α, NF-κB activity). In addition, nociceptive assessment was limited to thermal stimuli (Hot Plate test), without mechanical sensitivity testing, and no histopathological evaluation of sciatic nerve tissue was performed to directly confirm structural nerve protection.

5. Limitations and Future Directions

Future studies should address the limitations noted above by evaluating additional biochemical markers of oxidative stress (e.g., superoxide dismutase, glutathione), performing histopathological assessment of sciatic nerve tissue for direct confirmation of structural protection, and examining the expression of genes and proteins involved in inflammation and apoptosis. Comparative studies with other antioxidant compounds, assessment of long-term efficacy in chronic CIPN models, and ultimately clinical studies in patients undergoing vincristine-based chemotherapy would help establish the translational relevance of these findings.

6. Conclusion

The present findings indicate that hesperidin exerts a dose-dependent protective effect against vincristine-induced peripheral neuropathy in mice. Among the doses tested, hesperidin at 100 mg/kg was the most effective, significantly increasing nociceptive latency on days 10, 15, and 19 ($P < 0.001$) and significantly improving serum oxidative stress markers (MDA and TAC) relative to vincristine-treated controls, indicating reduced pain sensitivity and improved biochemical indices of nerve protection.

Declarations

Ethics approval and consent to participate

The proposal was approved by the ethical committee of the Faculty of Pharmacy at Tabriz University of Medical Science (IR.TBZMED.PHARMACY.REC.1403.055).

Consent for publication

Not applicable

Consent to Participate

Not applicable

Competing interests

The authors declare there is no conflict of interest.

Declaration of generative AI and AI-assisted technologies:

The authors used ChatGPT (GPT-5, OpenAI) to assist in refining the language and grammar of the manuscript. Following the use of this tool, the author(s) reviewed and edited the text to ensure accuracy and appropriateness. The authors take full responsibility for the content of this publication.

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Author Contribution

Sana amjadi: Performed the study. Javad Khalili Fard: Conceptualization, Supervision, review & editing. Adel Mahmoudi Gharehbaba: Writing – review & editing. Samar. Bohloul Habibi Asl: Conceptualization, Supervision, review & editing.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request

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Figures

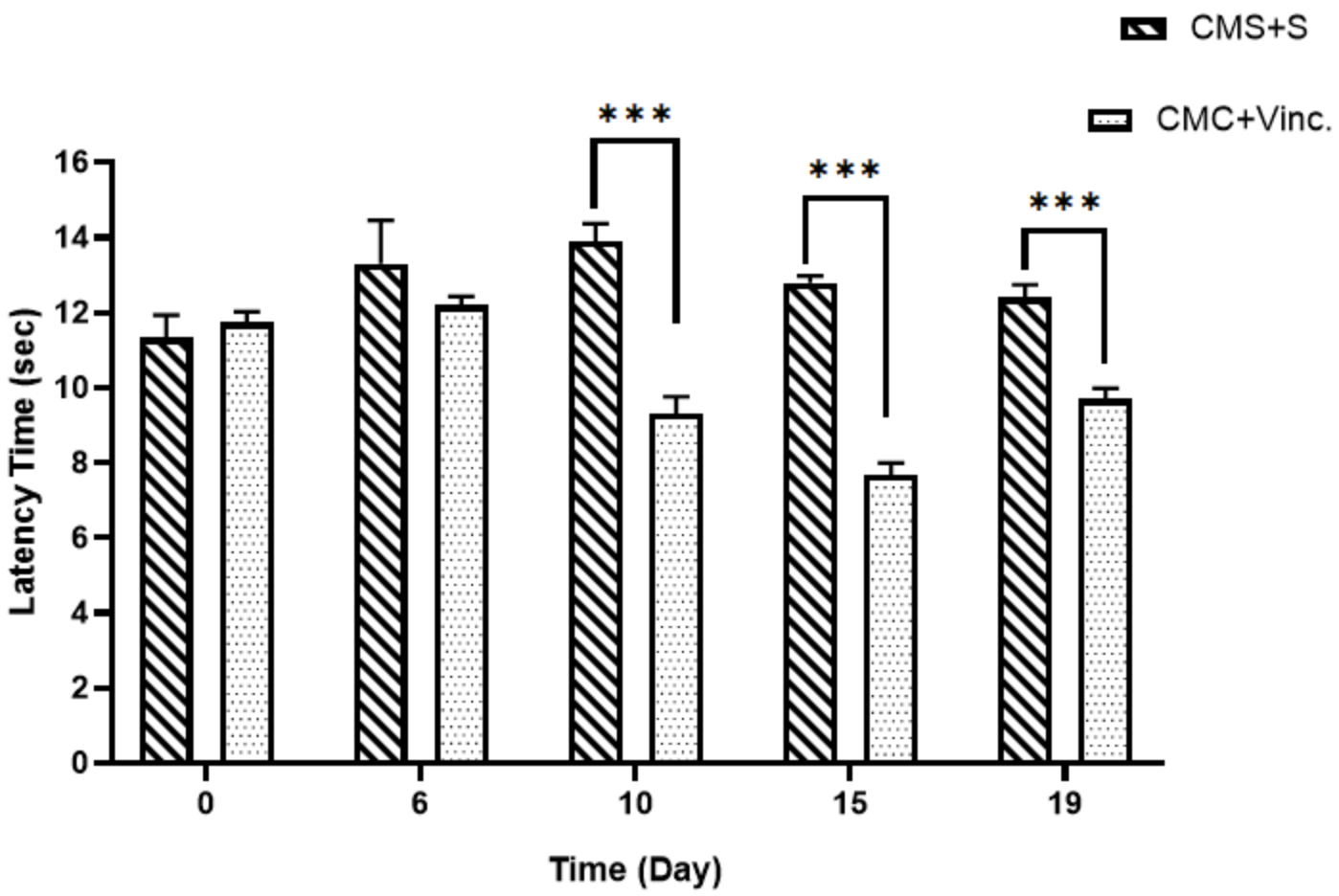


Figure 1

Effect of vincristine on the induction of peripheral neuropathy. Thermal nociceptive latency was assessed using the Hot Plate test. Data are expressed as mean ± SEM (n = 10 mice/group, male). ***P < 0.001 vs. vehicle (CMC) + saline group. CMC: carboxymethyl cellulose; Vinc: vincristine.

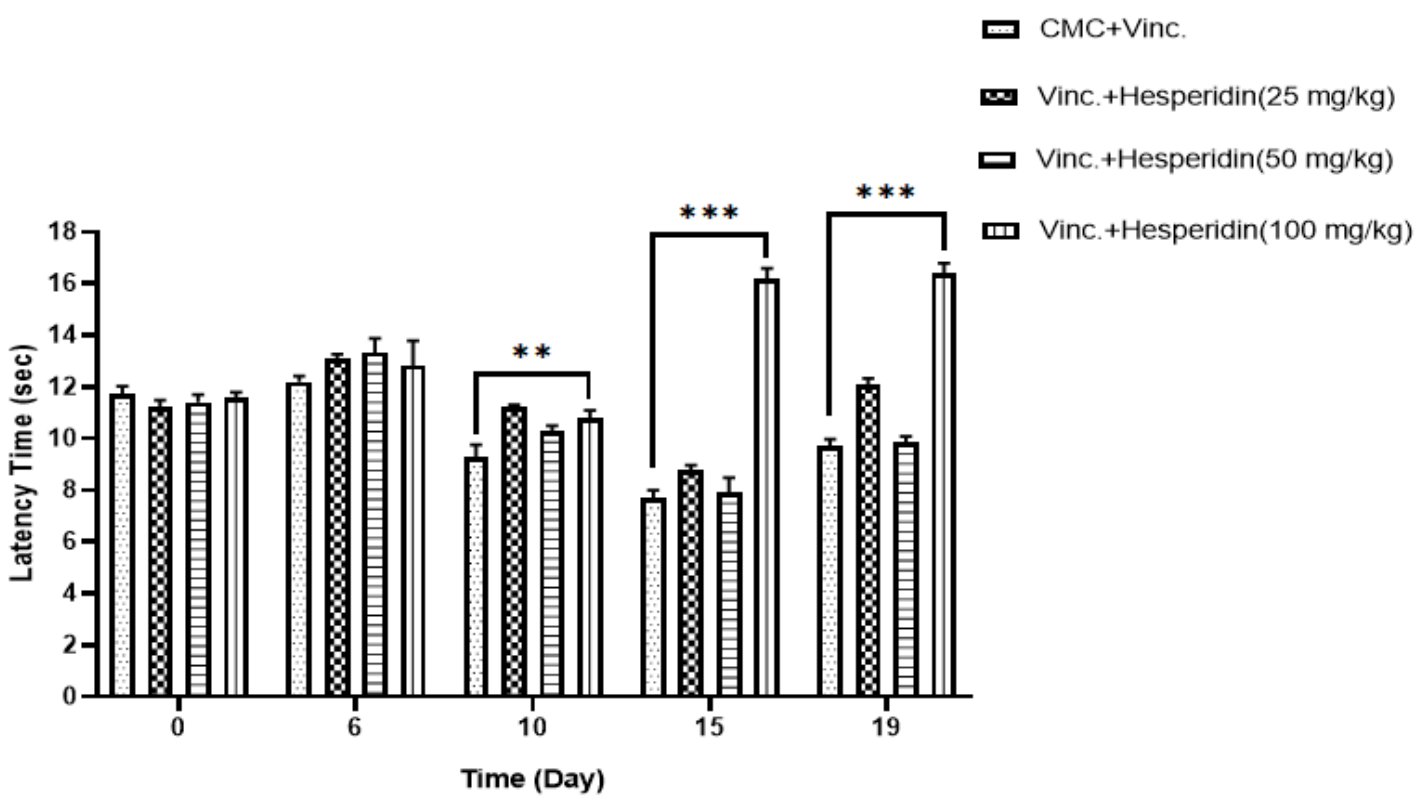


Figure 2

Dose-dependent preventive effect of hesperidin on vincristine-induced peripheral neuropathy. Mice were co-administered hesperidin (25, 50, or 100 mg/kg, i.p.) with vincristine, and thermal nociceptive latency was assessed using the Hot Plate test. Data are expressed as mean ± SEM (n = 10 mice/group, male). ***P < 0.001 vs. vehicle (CMC) + vincristine group. CMC: carboxymethyl cellulose; Vinc: vincristine.