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Original Article

Prevention of Vincristine-induced Peripheral Neuropathy by Methanolic Extract of Bee Pollen and Pentoxifylline in Mice

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Abstract

Background: Anticancer drug-induced neuropathy is a common side effect that results from multiple mechanisms, such as neuroinflammation and oxidative stress. This study evaluated the potential preventive and therapeutic effects of bee pollen methanolic extract (BPE), known for its anti-inflammatory properties, and pentoxifylline, an antioxidant, on vincristine-induced neuropathy.

Methods: A total of 90 male mice weighing 20-25 g were randomly divided into nine groups. Different doses of BPE (100, 200, 400 mg/kg/ip) and pentoxifylline (25, 50, 100 mg/kg/ip) were administered 3 days before vincristine injection and neuropathy induction. On the fourth day, vincristine (0.1 mg/kg/ip) was administered as a single dose. Then, it was administered along with the drugs for 10 days. Subsequently, vincristine was discontinued, and only the drugs were injected. Finally, the hot-plate test was performed in all animals to assess neuropathy on days 4, 8, 12, 14, and 17, and sampling was performed for biochemical evaluations on the 17th day.

Results: The findings indicated that BPE and pentoxifylline prevented vincristine-induced neuropathy, with varying effects observed at different doses. Conversely, their low doses and combination proved significantly effective in behavioral tests. The low dose of BPE enhanced total antioxidant capacity (TAC) levels, while the low dose of pentoxifylline reduced malondialdehyde (MDA) levels, improving neuropathy.

Conclusion: The combination of pentoxifylline and BPE helps prevent vincristine-induced neuropathy by regulating lipid peroxidation mechanisms and enhancing antioxidant capacity.

Keywords: Vincristine, Neuropathy, Bee pollen methanolic extract, Pentoxifylline, Mice

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Introduction

Peripheral neuropathy occurs due to damage to the peripheral and autonomic nervous systems. This damage can result from various factors, including diabetes, neurotoxic chemotherapy, HIV, antiretroviral medications, alcoholism, nutritional deficiencies, and exposure to heavy metals.[1] Chemotherapy-induced peripheral neuropathy (CIPN) is a progressive, persistent, and often irreversible side effect of various antineoplastic drugs, associated with common sensory abnormalities.[2] Six major classes of anticancer drugs damage sensory, motor, and autonomic neurons, leading to CIPN. These agents include platinum-based antineoplastic drugs, vinca alkaloids (such as vincristine), epothilones, taxanes, proteasome inhibitors, and immunomodulatory drugs.[3] These drugs trigger inflammatory cascades, with several inflammatory mediators, including tumor necrosis factor (TNF-α), interleukin 1β (IL-1β), IL-6, and IL-8, closely linked to CIPN. This complication of neuropathy restricts the continuation of treatment with certain chemotherapy drugs.[4]

Vincristine is one of the most widely used anti-cancer drugs in the vinca alkaloid class that is highly toxic.[5] All vinca alkaloids can cause sensorimotor neuropathy, with symptoms typically appearing within the first three months of treatment. Common symptoms include pain, numbness, tingling in the hands and feet, constipation, and hyporeflexia.[6] Vincristine is utilized in chemotherapy regimens for leukemia, lymphoma, sarcoma, and central nervous system tumors.[7] The toxic effects of vincristine manifest at a cumulative dose of 4 mg/m^2, leading to peripheral neuropathy due to its impact on microtubules, mitochondria, and the endothelium of nerve cells.[7,8]

Pentoxifylline is a derivative of methylxanthine that enhances blood flow. It is used to treat peripheral vascular disease, cerebrovascular disease, and conditions characterized by circulatory defects. Pentoxifylline increases the deformability of red blood cells, decreases

blood viscosity, and decreases platelet aggregation and thrombosis.[9] The drug exhibits significant anti-inflammatory effects by reducing the production of TNF-α and other pro-inflammatory cytokines, including IL-1, IL-6, and IL-8.[10]

Apitherapy is a highly beneficial practice that utilizes products produced by honeybees for their nutritional and medicinal purposes. Bee products include honey, bee pollen methanolic extract, beeswax, royal jelly, propolis, and bee venom.[11] Bee pollen methanolic extract (BPE) is a highly nutritious substance used as a supplement in traditional medicine. Bees collect pollen from plant anthers, mix it with a small amount of secretion from their salivary glands or nectar, and transport it to the hive.[12] Traditional medicine has long utilized BPE due to its potential medical and nutritional benefits. In modern medicine, significant therapeutic effects of BPE have also been reported, including antifungal, antimicrobial, antiviral, anti-inflammatory, hepatoprotective, anticancer, and local analgesic effects. Additionally, it has been reported to have a significant free radical scavenging effect.[13-15] Due to rich nutrient content, BPE supports nervous system health and boosts cognitive function by increasing blood flow to nerve tissues.[16] Considering the significance of neuropathy and the difficulties in quickly administering chemotherapy, this study aimed to explore the potential advantages of combining BPE with pentoxifylline to prevent vincristine-induced neuropathy.

Methods

Animals and Drugs

A total of 90 male Swiss mice weighing 20-25 g were purchased from the Laboratory Animal Breeding Center of Tabriz University of Medical Sciences. The animals were randomly divided into 9 study groups and housed in a standard polypropylene cage under controlled conditions (a temperature of 23 ± 2°C, a relative humidity of 50 ± 10%, and a light/dark cycle of 12/12 hours starting at 7 a.m.) with unrestricted access to water and food.

Vincristine and Pentoxifylline were obtained from Amin Pharmaceutical Company (Esfahan, Iran) and Sobhan Oncology Factory (Iran), respectively. According to our previous study, the bee pollen methanolic extract (BPE) was extracted using the maceration method. Briefly, powdered bee pollen (350 g) was macerated with 2 L of methanol. To prevent chemical changes, the extraction process was conducted in a dark environment. The extraction lasted 5 days at room temperature, after which the resulting liquid was filtered twice to obtain a clear extract. Then, the extract was concentrated using a rotary evaporator at 45°C. Finally, it was prepared as a suspension by mixing with normal saline in a shaker tube and placed in an ultrasonic bath [17].

Study Design

Nine groups were included in this study (Table 1). The animals in the normal saline + normal saline (NS + NS) group received normal saline (10 mL/kg, IP) for 17 days. The normal saline + vincristine (NS + Vin) group received normal saline (10 mL/kg, IP) for the first 4 days, followed by vincristine (0.1 mg/kg, IP) for 10 days, administered alongside normal saline. Finally, from days 14 to 17, only normal saline was injected. In the three groups of bee pollen methanolic extract + vincristine (BPE + Vin), animals were given 3 different doses of BPE (100, 200, 400 mg/kg, IP) three days before the initial dose of vincristine (0.1 mg/kg, IP). Then, vincristine and BPE were administered simultaneously from days 4 to 14, and BPE was injected alone from days 14 to 17. Then, in the Pen + BPE + Vincristine (Pen + Vin) group, animals received three different doses of pentoxifylline (25, 50, and 100 mg/kg, IP) three days before the first dose of vincristine (0.1 mg/kg, IP). From days 4 to 14, vincristine and pentoxifylline were injected simultaneously for 10 days, and pentoxifylline administration continued from days 14 to 17. Finally, in the pentoxifylline + BPE + Vin group, animals received BPE (100 mg/kg, IP) and pentoxifylline (25 mg/kg, IP) three days before the first dose of vincristine (0.1 mg/kg, IP), and from days 4 to 14, vincristine and BPE were injected simultaneously with pentoxifylline for 10 days. From days 14 to 17, the injections of BPE and pentoxifylline continued. Subsequently, the hot-plate test was performed in all animals on days 4, 8, 12, 14, and 17 to assess neuropathy, and the results were evaluated. Finally, sampling was performed for biochemical evaluations (Figure 1). To reduce potential bias, behavioral testing and biochemical assessments were conducted by someone blinded to the study.

Hot-plate Test

Based on the protocols of previous studies, this experiment was performed to measure pain threshold and neuropathy in animals, and the latency time was recorded [18]. First, the thermostat of the hot plate device was set to the target temperature of 65°C. Once the device plate was heated, the animal was placed on it, and the device timer was started. When the animal responded to the heat-induced pain, such as licking its hind paw, as observed through the glass walls, the timer was stopped. The recorded time, reflecting the resistance of the animal to pain caused by the set temperature, was then analyzed.

Sampling

After behavioral assessment, animals were deeply anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Then, blood samples were collected from the heart and centrifuged at 3500 rpm for 10 minutes to separate the serum. Finally, serum levels of MDA and TAC were measured.

Assessment of Malondialdehyde (MDA)

To study the oxidative stress pathway, serum levels of malondialdehyde (MDA) were measured using the Kia

Table 1. The Study Groups and Related Treatments

Groups	Treatment drugs	Dosage and injection methods
NS + NS (Normal Saline + Normal Saline)	Normal saline Normal saline	10 mL/kg/ip (daily/for 17 days) 10m1/kg/ip (daily/for 17 days)
NS + Vin (Normal Saline + Vincristine)	Normal saline Vincristine	10 mL/kg/ip (daily/for 17 days) 0.1 mg/kg/ip (daily/for 10 days)
Pen 25 + Vin (Pentoxifylline 25 mg + Vincristine)	Pentoxifylline Vincristine	25 mg/kg/ip (daily/for 17days) 0.1 mg/kg/ip (daily/for 10 days)
Pen 50 + Vin (Pentoxifylline 50 mg + Vincristine)	Pentoxifylline Vincristine	50 mg/kg/ip (daily/for 17days) 0.1 mg/kg/ip (daily/for 10 days)
Pen 100 + Vin (Pentoxifylline 100 mg + Vincristine)	Pentoxifylline Vincristine	100 mg/kg/ip (daily/for 17days) 0.1 mg/kg/ip (daily/for 10 days)
BPE 100 + Vin (Bee pollen 100 mg + Vincristine)	Bee pollen Vincristine	100 mg/kg/ip (daily/for 17 days) 0.1 mg/kg/ip (daily/for 10 days)
BPE 200 + Vin (Bee pollen 200 mg + Vincristine)	Bee pollen Vincristine	200 mg/kg/ip (daily/for 17 days) 0.1 mg/kg/ip (daily/for 10 days)
BPE 400 + Vin (Bee pollen 400 mg + Vincristine)	Bee pollen Vincristine	400 mg/kg/ip (daily/for 17days) 0.1 mg/kg/ip (daily/for 10 days)
Pen 25 + BPE 100 + Vin (Pentoxifylline 25 mg + bee pollen 100 mg + Vincristine)	Pentoxifylline Bee pollen Vincristine	25 mg/kg/ip (daily/for 17days) 100 mg/kg/ip (daily/for 17 days) 0.1 mg/kg/ip (daily/for 10 days)

Changes in Serum TAC Concentration Following Vincristine-induced Neuropathy. (A) The TAC Levels in the Pentoxifylline Groups (***$P<0.001$ vs. NS + NS Group, ###$P<0.001$, and ###$P<0.001$ vs. NS + Vin Group, @@@$P<0.001$ vs. Pen 50 + Vin Group). (B) The TAC Levels in the BPE Groups (***$P<0.001$ vs. NS + NS Group, ##$P<0.01$, and ###$P<0.001$ vs. NS + Vin Group, $$P<0.05$, and $$$$P<0.001$ vs. BPE 100 + Vin Group). (C) The TAC Levels in the Pentoxifylline + BPE Groups (***$P<0.001$ vs. NS + NS Group, ##$P<0.01$, and ###$P<0.001$ vs. NS + Vin). Data are represented as the mean ± SEM (n = 10).

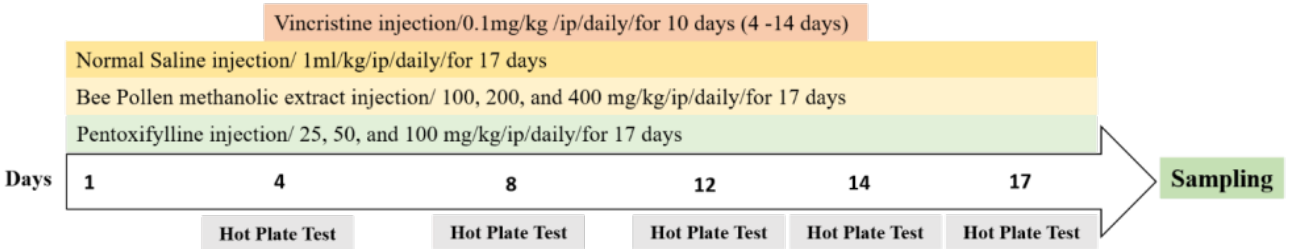

Figure 1. Experimental Design and Timeline of the Study

Zist kit (Tehran, Iran). MDA is the main product of the peroxidation of unsaturated fatty acids and indicates the level of oxidative stress in the body. To measure the MDA level, 0.5 mL of serum was mixed with 3 mL of 1% phosphoric acid and vortexed. Then, 1 mL of 675% thiobarbituric acid was added to the test tube. After complete vortexing, the sample was placed in a boiling water bath for 45 minutes. Subsequently, the test tubes were cooled under cold water, and 3 mL of n-butanol was added. Then, the mixture was vortexed for 1 to 2 minutes and centrifuged for 10 minutes at 3000 rpm. After separation of the supernatant, the absorbance was measured at 532 nm against a blank n-butanol sample, and the results were plotted on a standard curve to determine the serum MDA concentration of the samples [19].

Assessment of Total Antioxidant Capacity (TAC)

To examine the antioxidant effect, serum TAC was measured using a Kia Zist kit (Tehran, Iran). Briefly, for TAC measurement, 20 µL of serum sample and 1 mL of chromogen were placed in a sample cuvette, while in a separate cuvette, 20 µL of distilled water was mixed with 1 mL of chromogen. Additionally, 1 mL of chromogen was mixed with 20 µL of standard solution in another cuvette. First, the absorbance of the cuvettes was measured at 600 nm at 37°C, compared to air (A1). Then, 200 µL of substrate (hydrogen peroxide) was added to each cuvette, and the absorbance was re-evaluated 3 minutes later (A2). TAC chromogenic reagents was calculated as µmol/mL; the substrate and Standard solution were used according to the previous study protocol with the designated concentrations. Finally, the amount of TAC was calculated according to the formula [20].

Statistical Analysis

The results of this experimental study are presented as mean ± SEM. One-way ANOVA tests were used to evaluate the significance of differences between group means, followed by Tukey's post-hoc test depending on the groups examined, with significant results at $P<0.05$.

Results

Investigating the Effect of Pentoxifylline on Vincristine-induced Neuropathy

The results indicate the effect of vincristine injection in inducing neuropathy in mice. A significant difference was observed in the reaction time to pain after vincristine injection, with increased pain sensitivity compared to the NS + NS group, leading to the development of neuropathy in the mice.

The findings showed that pentoxifylline administered on day 8 at a dose of 100 mg ($P<0.05$) increased the duration of the pain response. Injections of different doses (Pen 25 + Vin, $P<0.01$, Pen 50 + Vin, $P<0.001$, and Pen 100 + Vin, $P<0.001$) significantly improved neuropathy in animals treated with pentoxifylline compared to the NS + Vin group on day 12. Additionally, all three doses of pentoxifylline (Pen 25 + Vin, $P<0.001$; Pen 50 + Vin, $P<0.001$; Pen 100 + Vin, $P<0.001$) significantly improved neuropathy in these animals compared to the NS + Vin group on days 14 and 17 (Table 2).

Investigating the Effect of BPE on Vincristine-induced Neuropathy

As shown in Table 2, the hot-plate test revealed that three different doses of BPE (BPE 100 + Vin, $P<0.01$; BPE 200 + Vin, $P<0.001$; and BPE 400 + Vin, $P<0.001$) significantly increased the pain duration on day 8 compared with the NS + Vin group. From day 12 onward, the doses of BPE (BPE 100 + Vin, $P<0.001$; BPE 200 + Vin, $P<0.001$; and BPE 400 + Vin, $P<0.001$) had significant effects compared to the NS + Vin group, preventing vincristine-induced neuropathy.

Investigating the Effect of Co-injection of Pentoxifylline and BPE on Vincristine-induced Neuropathy

The results revealed that co-injection of pentoxifylline 25 and BPE 100 (low dose of each drug) during the study phases significantly ($P<0.001$) increased pain response time compared to the NS + Vin group (Table 2).

Investigating the Effect of Pentoxifylline and Vincristine on Changes in Serum MDA and TAC

The results demonstrated that the injection of pentoxifylline had a significant influence on MDA levels compared to the vincristine group. Conversely, intergroup analysis revealed that MDA levels were significantly decreased in the Pen 25 + Vin ($P<0.05$), Pen 50 + Vin ($P<0.001$), and Pen 100 + Vin ($P<0.05$) groups compared to the NS + Vin group (Figure 2A). Additionally, injection of pentoxifylline significantly impacted TAC levels compared to the vincristine group. Intergroup analysis revealed that TAC levels were significantly elevated in the Pen 25 + Vin ($P<0.01$) and Pen 100 + Vin ($P<0.001$) groups compared to the vincristine group. It was noted that Pen 100 ($P<0.001$) exhibited a more pronounced antioxidant effect than Pen 50 (Figure 3A).

Investigating the Effect of BPE on Serum Levels of MDA and TAC

The results in Figure 2 B and Figure 3 B indicated that administering various doses of BPE significantly affected changes in serum MDA and TAC levels, respectively. Between-group evaluations revealed that in the BPE 200 + Vin ($P<0.001$) and BPE 400 + Vin ($P<0.001$) groups, serum MDA levels were significantly lower compared to the vincristine group. Conversely, it was observed that BPE 200 and BPE 400 were more effective in reducing MDA levels than BPE 100.

In contrast, serum TAC levels were significantly increased in the BPE 100 + Vin ($P<0.001$) and BPE 200 + Vin ($P<0.01$) groups compared to the vincristine group. However, the BPE 400 did not demonstrate any significant effect. Furthermore, it was observed that BPE 200 + Vin ($P<0.05$) and BPE 400 + Vin ($P<0.001$) exhibited a lower antioxidant effect than the BPE 100 + Vin, resulting in increased TAC levels to a lesser extent.

Investigating the Effect of Co-injection of Pentoxifylline and BPE on Serum MDA and TAC

The results confirmed that co-injection of pentoxifylline and BPE at low doses ($P<0.001$) significantly changed serum MDA levels, decreasing them compared to the vincristine group. BPE 100 also showed a significant effect on lowering MDA levels compared to the Pen 25 group (Figure 2C). Examination of changes in TAC levels indicated that the combined injection of these two drugs also significantly increased TAC levels. Additionally, no significant difference was observed between the combined

Table 2. Effects of Pentoxifylline and Bee Pollen Methanolic Extract on Latency Time in the Hot-plate Test on Vincristine-induced Neuropathy

Groups	Latency times (Sec)				
	Day 4	Day 8	Day 12	Day 14	Day 17
NS + NS	7.5 ± 0.34	6.2 ± 0.32	7.0 ± .44	6.5 ± 0.26	6.7 ± 0.42
NS + Vin	7.6 ± 0.33	5.9 ± 0.23	6.0 ± 0.49	4.6 ± 0.26***	4.3 ± 0.21***
Pen 25 + Vin	8.0 ± 0.25	7.1 ± 0.23	8.8 ± 0.46##	9.3 ± 0.68###	10.6 ± 0.83###
Pen 50 + Vin	7.4 ± 0.42	7.5 ± 0.22	9.3 ± 0.47###	9.4 ± 0.65###	11.2 ± 0.75###
Pen 100 + Vin	7.8 ± 0.59	9.3 ± 0.77#	11.9 ± 0.73###	14.1 ± 0.70###	13.3 ± 0.59###
BPE 100 + Vin	8.0 ± 0.59	8.6 ± 0.87##	10.1 ± 0.65###	10.9 ± 0.78###	11.3 ± 0.74###
BPE 200 + Vin	8.2 ± 0.57	9.4 ± 0.58###	9.8 ± 0.44###	11.1 ± 0.54###	10.0 ± 0.49###
BPE 400 + Vin	7.2 ± 0.48	10.20 ± 0.66###	11.1 ± 0.50###	11.5 ± 0.52###	13.4 ± 0.68###
Pen 25 + BPE 100 + Vin	8.0 ± 0.42	8.9 ± 0.95###	10.4 ± 0.63###	10.2 ± 0.74###	13.0 ± 0.56###

Data are represented as the mean ± SEM(n = 10). NS: Normal Saline, Vin: Vincristine, Pen: Pentoxifylline, BPE: Bee Pollen Methanolic Extract. ***$P<0.01$ compared to the NS + NS group; # $P<0.05$, ## $P<0.01$, and ### $P<0.001$, compared to NS + Vin group using one-way ANOVA, followed by Tukey's test

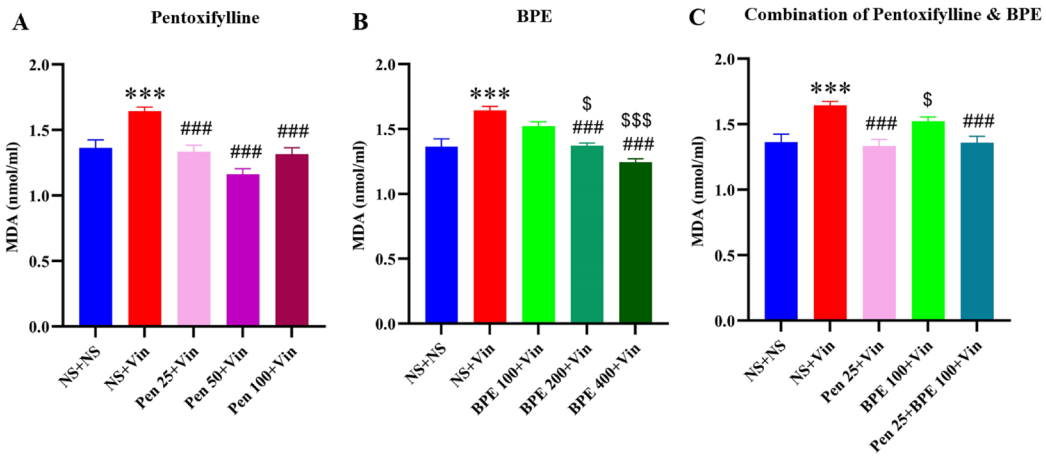

Figure 2. Investigating the Effect of Different Doses of Pentoxifylline, BPE, and Pentoxifylline+Bee Pollen Methanolic Extract on Changes in Serum MDA Concentration Following Vincristine-induced Neuropathy. (A) The MDA Levels in the Pentoxifylline Groups (***$P<0.001$ vs. NS+NS Group, ###$P<0.001$ vs. NS+Vin Group). (B) The MDA Levels in the BPE Groups (***$P<0.001$ vs. NS+NS Group, ###$P<0.001$ vs. NS+Vin Group, $$P<0.05$, and $$$P<0.001$ vs. Pen 25+Vin Group). (C) The MDA Levels in the Pentoxifylline+BPE Groups (***$P<0.001$ vs. NS+NS Group, ###P<0.001 vs. NS+Vin Group, $$P<0.05$ vs. Pen 25+Vin Group). Data are represented as the mean±SEM (n=10).

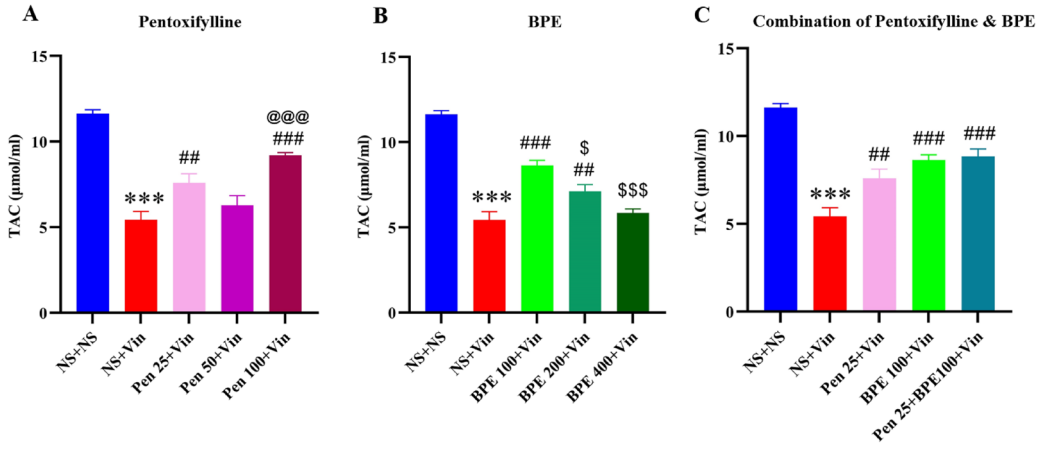

Figure 3. Investigating the Effect of Different Doses of Pentoxifylline, BPE, and Pentoxifylline+BPE on Changes in Serum TAC Concentration Following Vincristine-induced Neuropathy. (A) The TAC Levels in the Pentoxifylline Groups (***$P<0.001$ vs. NS+NS Group, ###$P<0.001$, and ###$P<0.001$ vs. NS+Vin Group, @@@$P<0.001$ vs. Pen 50+Vin Group). (B) The TAC Levels in the BPE Groups (***$P<0.001$ vs. NS+NS Group, ##$P<0.01$, and ###$P<0.001$ vs. NS+Vin Group, $$P<0.05$, and $$$P<0.001$ vs. BPE 100+Vin Group). (C) The TAC Levels in the Pentoxifylline+BPE Groups (***$P<0.001$ vs. NS+NS Group, ##$P<0.01$, and ###$P<0.001$ vs. NS+Vin). Data are represented as the mean±SEM (n=10).

and individual doses (Figure 3C).

Discussion

Therapeutic approaches that focus on the upregulation of the antioxidant system and modulation of the oxidative stress pathway can be used to fight neuropathies caused by chemotherapy drugs, including vincristine. This study showed that injection of vincristine caused neuropathy and lowered pain thresholds. Administering various doses of BPE three days before the first vincristine dose reduced pain sensitivity. Similarly, different doses of pentoxifylline administered three days before the first vincristine injection effectively lowered pain sensitivity and improved neuropathic symptoms. In contrast, combining low doses of pentoxifylline with BPE extended the time before pain started and reduced vincristine-induced neuropathy.

Vincristine-induced neuropathy results from various factors that affect neuroinflammation, triggering the activation of leukocytes and microglia while notably increasing inflammatory gene expression in the dorsal root ganglia.[21] This process recruits and activates pro-inflammatory cytokines, such as TNF-α and IL-1β, leading to significant neuroinflammation.[22] Moreover, hindered microtubule polymerization disrupts axonal transport, contributing to axonal neuropathy and the activation of oxidative stress pathways.[23] Oxidative stress and lipid peroxidation may serve as potential pathways for neuropathy caused by vincristine; the levels of MDA, TAC, GSH, 8-OHdG, TNF-α, and IL-1β in the sciatic nerve rise, correlating with heightened neuropathic inflammation.[24,25]

Pentoxifylline functions as a phosphodiesterase inhibitor and a nonspecific cytokine inhibitor, improving

blood circulation and reducing inflammation by suppressing the production of TNF-α, IL-1, IL-6, and IL-8. Additionally, it decreases blood viscosity and enhances the flexibility of red blood cells.[26,27] Our research indicated that varying doses of pentoxifylline may enhance vincristine-induced neuropathy by influencing the oxidative stress pathway and lowering serum MDA levels. Conversely, the serum TAC level, which reflects antioxidant capacity, significantly increased, highlighting the antioxidant effects of pentoxifylline injection.

BPE contains approximately 250 different substances, including amino acids, lipids such as triglycerides and phospholipids, vitamins like provitamin A, vitamin E, niacin, thiamine, biotin, and folic acid, as well as minerals such as zinc, copper, and iron. It also contains macro- and micronutrients, as well as flavonoids.[28] Additionally, BPE increases the number of lymphocytes and antibodies, enhancing the resistance of the body to infections and accelerating disease recovery due to its antibiotic and anti-inflammatory properties.[29] This natural product has antioxidant, anti-inflammatory, anti-fungal, hepatoprotective, anti-atherosclerotic, and immune system-enhancing properties. Its anti-inflammatory mechanism inhibits cyclooxygenase and lipoxygenase activity, thus reducing the levels of products such as prostaglandins and leukotrienes.[30]

This study demonstrated a significant effect of two doses of BPE (200 and 400) on reducing vincristine-induced neuropathy. Influencing the oxidative stress pathway decreased the serum level of MDA; however, the low dose of BPE (100) was ineffective in lowering MDA levels. In contrast, BPE 100 and BPE 200 effectively modulated neuropathy by exhibiting an antioxidant effect and increasing serum TAC levels, aligning with the results of the behavioral experiments. It was intriguing to observe that combining BPE and pentoxifylline at the minimum dose could significantly alleviate neuropathy symptoms during the behavioral test. Molecular studies indicated that the primary effect of BPE 100 was to increase TCA levels and enhance the antioxidant response; meanwhile, Pen 25 primarily reduced MDA levels and modulated the oxidative stress pathway.

Based on the results of this study, BPE 400 did not increase TAC, despite significantly reducing MDA levels. This could be due to a nonlinear dose-response relationship, where higher doses might saturate or impair the antioxidant system of the body, limiting the rise in TAC. The reduction in MDA, which indicates lipid peroxidation, may suggest that specific enzymes or local protective pathways directly prevent oxidative damage to lipids without significantly altering serum total antioxidant capacity.[31] Similar results have been found in earlier studies, where reducing MDA levels through certain medications or dietary changes improved, while TAC levels remained stable or changed less.[32] Based on our findings, the optimal dose of BPE for boosting antioxidants and reducing neuropathy appears to be 100-200 mg, and higher doses may actually reduce antioxidant effects.

Vincristine triggers hypersensitivity in A-δ and C-fiber nociceptive neurons, leading to the sensitization of dorsal horn neurons and central sensitization, resulting in hyperalgesia and allodynia.[33] Furthermore, research indicates that IL-6 might promote macrophage invasion in the peripheral nervous system and contribute to VCR-induced mechanical allodynia.[34] On the other hand, the oxidative stress pathway plays a significant role in the pathogenesis of this neuropathy, with markers such as MDA and GSH-Px showing alterations.[35,36] Aligned with these results, our study also revealed that vincristine induced neuropathy by engaging the oxidative stress pathway, as evidenced by increased MDA level and decreased TAC level, as demonstrated by the hot-plate test.

Conclusion

Our study demonstrated that both pentoxifylline and BPE, either alone or in combination, significantly reduced vincristine-induced neuropathy. Furthermore, the effects of the combined use of these drugs with the minimum dose were both convincing and promising. It was also found that one protective mechanism for nerve fibers involves modulating oxidative stress mediators and balancing antioxidant factors. Additional research is required to identify the minimum dose and combined doses as the preferred treatment for reducing vincristine-induced neuropathy.

Ethics statement

This study was ethically approved by Tabriz University of Medical Sciences (IR.TBZMED.VCR.REC.1400.460).

Disclosure of funding source

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Conflict of interests declaration

The authors declare no conflict of interests.

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Data availability statement

The data related to the study are available from the corresponding author upon reasonable request.

Author contributions

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Supervision: Tahereh Eteraf-Oskouei.

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Writing–review & editing: Tahereh Eteraf-Oskouei, Bohloul Habibi Asl, Solmaz Fallahi.

Consent for publication

Not applicable.

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