RESEARCH PAPER

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Piperine relieves neuropathic pain induced by paclitaxel in mice

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Piperine is an amide alkaloid isolated from the black pepper plant. This study examined the pain-relieving activity of piperine against paclitaxel (PTX)-induced neuropathy. Male mice were divided into 6 groups: Sham-operated group (remained intact), PTX group (PTX-treated mice receiving normal saline), PTX+ piperine 10, 25, and 50 mg/kg groups (PTX-treated mice receiving piperine) and positive control group (PTX-treated mice receiving imipramine 10 mg/kg). Neuropathic pain was induced by PTX 2 mg/kg/day on days 1, 3, 5 and 7. On day 7, behavioral tests were conducted and serum levels of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) were assayed. PTX produced significant thermal hyperalgesia compared to the sham group. Piperine at all doses alleviated neuropathic pain, and significantly decreased IL-6, TNF-α, and MDA, but induced CAT and SOD activities compared to the control group. Piperine could confer beneficial effects against neuropathic pain, at least partially, *via* reduction of inflammatory and oxidative stress markers.

Key words: piperine, neuropathic pain, paclitaxel, neuroinflammation, cytokines

INTRODUCTION

To treat cancer as a leading cause of death worldwide (Ferlay et al., 2020), various chemotherapeutics are being used (Takanashi et al., 2021). Although their use produces favorable outcomes such as enhanced survival rate, these agents may create an array of adverse impacts (e.g., pain) that are difficult to manage by conventional treatments (Shahraki et al., 2020).

Peripheral neuropathic pain is considered one of the main side effects of paclitaxel (PTX), a member of the taxane chemotherapeutics and first-line treatment for solid tumors including breast, ovarian, and lung cancers (Duggett et al., 2016; Rezaee et al., 2019). PTX-induced neuropathic pain (PINP) is characterized by severe symptoms such as numbness, tingling, unusual sensations, weakness, and ongoing burning pain in the glove and stocking areas of the hands and feet (Zhou et al., 2020). Of note, PINP may emerge even after administration of a single dose of PTX and may persist for months or years following its cessation. PINP not only decreases psychosocial well-being and quality of life but may also result in the discontinuation of a potentially beneficial therapeutic approach (Qabazard et al., 2020; Singh et al., 2022). Unfortunately, PINP is refractory to conventional analgesics including nonste-

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roidal anti-inflammatory drugs (NSAIDs), antiepileptics, and antidepressants (Xiao et al., 2008; Zhou et al., 2020). To date, no treatment satisfactorily alleviates or prevents PINP due to its multifactorial pathophysiology. In this context, various natural compounds have been shown to relieve PINP (Hidaka et al., 2009; Gao et al., 2016; Faheem & Khan, 2022).

Natural products are a rich source of biologically active compounds and indicate desirable pharmacological profiles (Mishra et al., 2008). The antinociceptive effect of natural products has been shown in various experimental models (Ward et al., 2014; Chen et al., 2015; Singh et al., 2019). These compounds exert antinociceptive characteristics *via* several mechanisms including modulation of inflammatory mediators and reduction of oxidative stress (Hashemzaei et al., 2020; Santos et al., 2022).

Piperine (2E,4E)-5-(1,3-benzodioxol-5-yl)-1-piperidin-1--ylpenta-2,4-dien-1-one; molecular weight 285.34 g/mol), is an alkaloid that is abundantly present in the fruits and roots of *Piper nigrum* (black pepper) and *Piper longum* (long pepper) (Ziegenhagen et al., 2021). It has been indicated that piperine can regulate mesencephalic astrocyte-derived neurotrophic factor (MANF) expression and diminish endoplasmic reticulum stress involved in neuropathology in mice with spinocerebellar ataxia type 17 (Guo et al., 2018). Piperine was shown to trigger mitofusin activation at low nanomolar concentrations (Zhang et al., 2022), and interestingly, mitochondrial injury induced, at least partly *via* loss of mitofusin activity, has been reported to mediate chemotherapeutics peripheral neuropathy (Yamashita et al., 2017; Bobylev et al., 2018).

For piperine, antinociceptive, neuroprotective, antioxidant, anti-inflammatory, immunosuppressive, anti-tumor, and antimicrobial activities have been reported (Zadorozhna et al., 2019; de Almeida et al., 2020; Jiu-Wang et al., 2021). The antinociceptive activity of piperine is likely mediated through the activation of opioid (Bukhari et al., 2013), Transient receptor potential vanilloid 1 (TRPV1), and γ-Aminobutyric acid type A ($GABA_A$) receptors (Sánchez-Trujillo et al., 2020). In this study, we evaluated the possible analgesic effects of piperine against neuropathic pain induced by PTX in mice by conducting behavioral tests and assessing oxido-inflammatory markers in sera samples.

METHODS

Animals grouping

Forty-eight male albino mice (30–35 g and four weeks old) were purchased from the Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Animals were housed in Plexiglass cages (n=8) at 22 ± 2°C with a 12:12 hours light-dark cycle with *ad libitum* access to food and water. Animals were randomly grouped into the following six groups of eight (Arifin & Zahiruddin, 2017): Sham-operated group: This group received only intraperitoneal injections of normal saline for 7 consecutive days; PTX group (negative control): PTX-treated mice that were given normal saline for 7 consecutive days; Positive control group: PTX-treated mice that received imipramine 10 mg/kg on day 7; and PTX + Piperine groups: PTX-treated mice that respectively received piperine 10, 25, and 50 mg/kg for 7 consecutive days. The piperine was first dissolved in a negligible amount of ethanol and then added to saline to obtain the various concentrations.

All animal experiments were conducted in accordance with the National Ethical Guidelines for the Use and Care of Laboratory Animals, following the approval of the ethics committee of Mashhad University of Medical Sciences, Mashhad, Iran (Approval No. IR.MUMS.MEDICAL.REC.1400.393).

Chemicals

Paclitaxel (AqVida, 30 mg/5 ml vial) was purchased from Oncotec Pharma Produktion GmbH and piperine was obtained from Golexir Pars Company (http://www.golexir.com/, Mashhad, Iran).

Induction of the mice model of PTX neuropathy and piperine administration

Based on previous studies (Toma et al., 2017; Rezaee et al., 2019), a cumulative dose of 8 mg/kg of PTX was administered to mice (i.e., PTX 2 mg/kg was intraperitoneally administered on days 1, 3, 5, and 7). As outlined in Fig. 1, the 10, 25, and 50 mg/kg doses of piperine were intraperitoneally injected for 7 consecutive days (Peng et al., 2018; Abdel-Daim et al., 2019; Mohammadi et al., 2020). Behavioral (primary outcomes) and biochemical parameters (secondary outcomes) were assessed.

Behavioral assessments

The hot plate test (Thermal hyperalgesia)

On day 7, mice were placed individually on the metal surface of a hot-plate apparatus with a preset temperature of 55 ± 1°C (Ozcan et al., 2008). The latency to the first sign of licking, lifting paws, or jumping to avoid the heat was considered the pain threshold. Animals

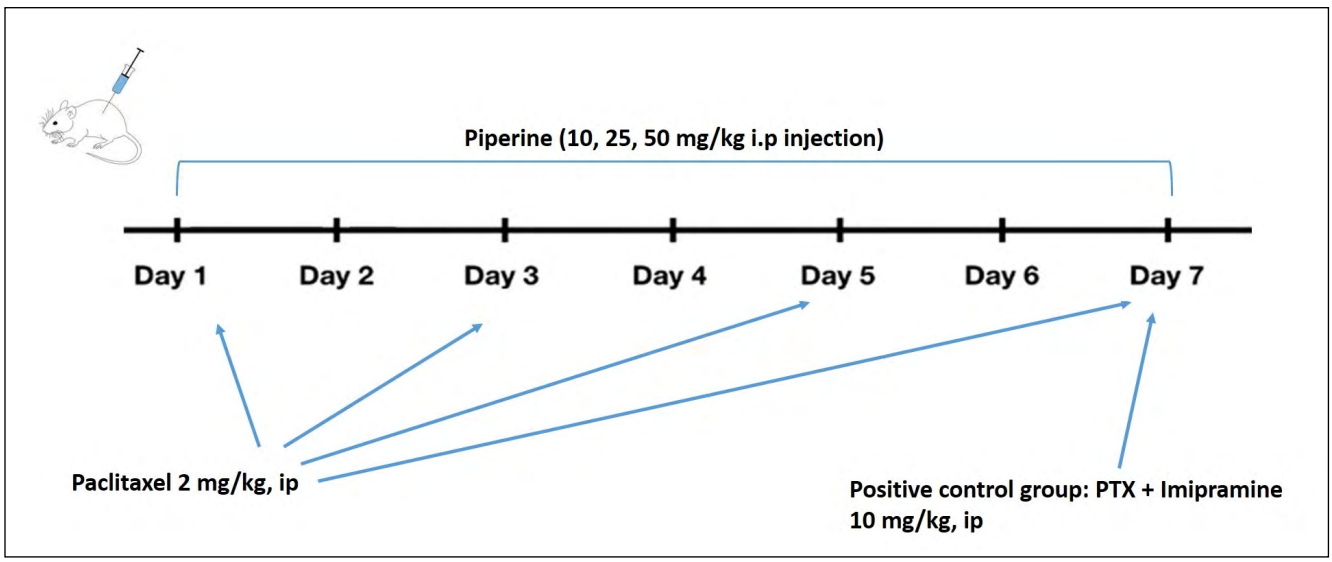

Fig. 1. A schematic presentation of animal groupings and treatments. PTX group (received PTX on days 1, 3, 5, and 7 and normal saline for 7 consecutive days); Positive control group (received PTX on days 1, 3, 5, and 7 and 10 mg/kg imipramine on day 7 as acute treatment); and three piperine-treated groups (received PTX on days 1, 3, 5, and 7 and piperine 10, 25, and 50 mg/kg for 7 consecutive days). (PTX) Paclitaxel; (ip): Intraperitoneal.

were removed 45 s after placement on the hot surface to prevent tissue damage (Reda et al., 2016). Latencies were measured 60 min after administration of the last dose of piperine.

The von Frey test (Mechanical allodynia)

The mice were placed in an elevated cage (30×30×30 cm) with a floor made of metal mesh. Each animal was individually tested using an ascending series of von Frey filaments of different stiffness (0.6-6 g) (Bioseb, USA) applied to the plantar surface (Naji-Esfahani et al., 2016; Hsiao et al., 2024). Each filament was applied five times at 2-3 sec intervals. Immediate paw withdrawal, licking, or shaking the paw upon filament removal was considered a positive response. If animals responded to at least 3 withdrawals out of 5 consecutive stimulations, that Gram force was documented as the paw withdrawal threshold (Rakhshandeh et al., 2022).

Biochemical assays

At the end of the experiments, the mice were deeply anesthetized by intraperitoneal injections of ketamine (100 mg/kg) and xylazine (10 mg/kg), and euthanized. Blood was collected *via* the left ventricle using a 23-25-gauge needle in sterile tubes with no anticoagulants and serum was separated. The serum levels of interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), and malondialdehyde (MDA), as well as superoxide dismutase (SOD) and catalase (CAT) activities, were detected using commercial ELISA kits (Karmania Pars Gene Company, Kerman, Iran).

Statistical analysis

SPSS software version 26 was used to analyze the data. Data are reported as mean ± SD. The Kolmogorov-Smirnov test was used to determine whether sample data are normally distributed. The data were compared among the experimental groups using One-way ANOVA and Tukey's *post hoc* (in a normal distribution). Kruskal-Wallis and Mann-Whitney U tests were used to detect differences among the groups when the data were not normally distributed. Independent samples t-test was used to check differences between the sham and PTX groups. P-values lower than 0.05 were considered statistically significant.

RESULTS

Anti-neuropathic effects of piperine measured by behavioral tests

Hot plate test

As shown in Fig. 2A, four doses of PTX (2 mg/kg/day) induced hyperalgesia as reflected by a remarkable difference between the sham and the PTX group (t_{14}=-2.302, P<0.05). According to the Kruskal-Wallis

test, there were significant differences in hyperalgesia values among the studied groups (χ^2_4=30.573, *P*<0.001). The hyperalgesia was significantly decreased after treatment with piperine 10 mg/kg (*U*=10.000, *P*<0.05), 25 and 50 mg/kg (*U*=0.00, *P*<0.001 for both cases) compared to the PTX group. In addition, the piperine 25 and 50 mg/kg effect was significantly greater than that of piperine 10 mg/kg (*U*=0.00, *P*<0.001 for both cases, Fig. 2B).

Mechanical allodynia

Results of the Kruskal-Wallis test indicated significant differences in the paw withdrawal threshold from the different animal groups (χ^2_4=13.400, *P*<0.01). Findings obtained from the von Frey test indicated that 25 and 50 mg/kg piperine showed statistically significant enhancements in paw withdrawal thresholds in PTX-induced neuropathic mice on day 7 compared to the PTX group (*U*=7.000, *P*<0.05 and *U*=9.500, *P*<0.05, respectively). However, treatment with low-dose piperine (10 mg/kg) did not significantly affect the paw withdrawal threshold (Fig. 3).

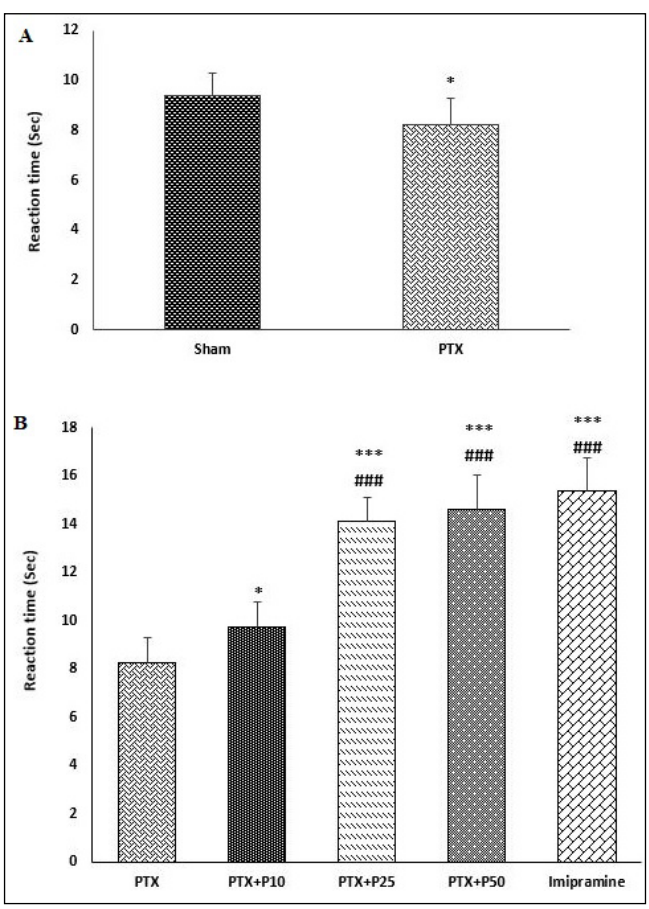

Fig. 2. Effects of piperine on PTX-induced neuropathic pain in mice. Values are mean ± SD. Section A confirms the induction of neuropathy following injection of PTX, with a significant difference in latency observed between the PTX and the sham groups. (B) **P*<0.05 and ***<0.001 indicate significant differences compared to the PTX group and ###*P*<0.001 indicates differences compared to the P10 group. PTX: PTX-treated mice that were injected with normal saline for 7 consecutive days; *PTX+P* 10, 25, and 50: PTX-treated mice respectively treated with piperine 10, 25, and 50 mg/kg per day for 7 consecutive days; Imipramine: PTX-treated mice treated with a single dose of imipramine 10 mg/kg on day 7 as acute treatment.

Effects of piperine on serum inflammatory markers in PTX-treated mice

According to one-way ANOVA, treatment had significant effects on the IL-6 levels in the different groups of animals induced with PTX ($F_{3,28}$=28.337, *P*<0.001). In this study, IL-6 levels in the animals' sera decreased significantly following 7-day treatment with piperine 10, 25, and 50 mg/kg compared to the PTX group (*P*<0.01 for piperine 10 mg/kg and *P*<0.001 for both piperine 25 and 50 mg/kg, according to Tukey's *post-hoc* test). Furthermore, treatment with piperine 25 and 50 mg/kg significantly decreased serum IL-6 levels compared to low-dose (10 mg/kg) piperine (*P*<0.05 and *P*<0.01, respectively; according to Tukey's *post-hoc* test, Fig. 4A). As displayed in Fig. 4B, the serum TNF-α level was significantly different across all groups based on ANOVA test ($F_{3,27}$=21.355, *P*<0.001). Administration of 10, 25, and 50 mg/kg of piperine significantly decreased the serum TNF-α levels compared to the PTX group (*P*<0.01 for piperine 10 mg/kg and *P*<0.001 for both piperine 25

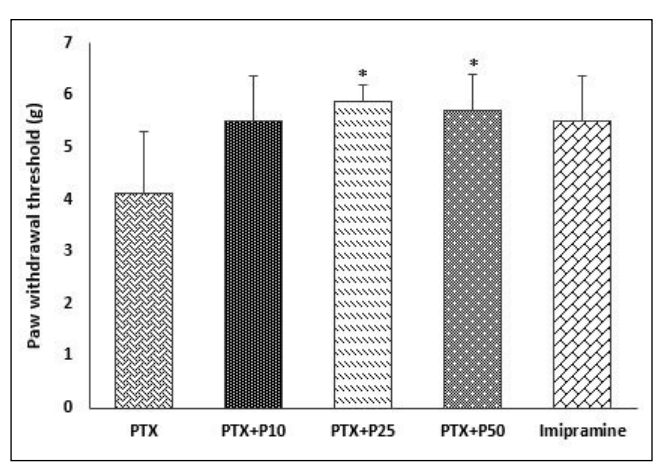

Fig. 3. Effect of piperine on paw withdrawal threshold. Values are mean ± SD. **P*<0.05 indicates significant differences compared to the PTX group. PTX: paclitaxel-treated mice that were injected with normal saline for 7 consecutive days; *PTX+P* 10, 25, and 50: PTX-treated mice respectively treated with piperine 10, 25, and 50 mg/kg per day for 7 consecutive days; Imipramine: PTX-treated mice treated with a single dose of imipramine 10 mg/kg on day 7 as acute treatment.

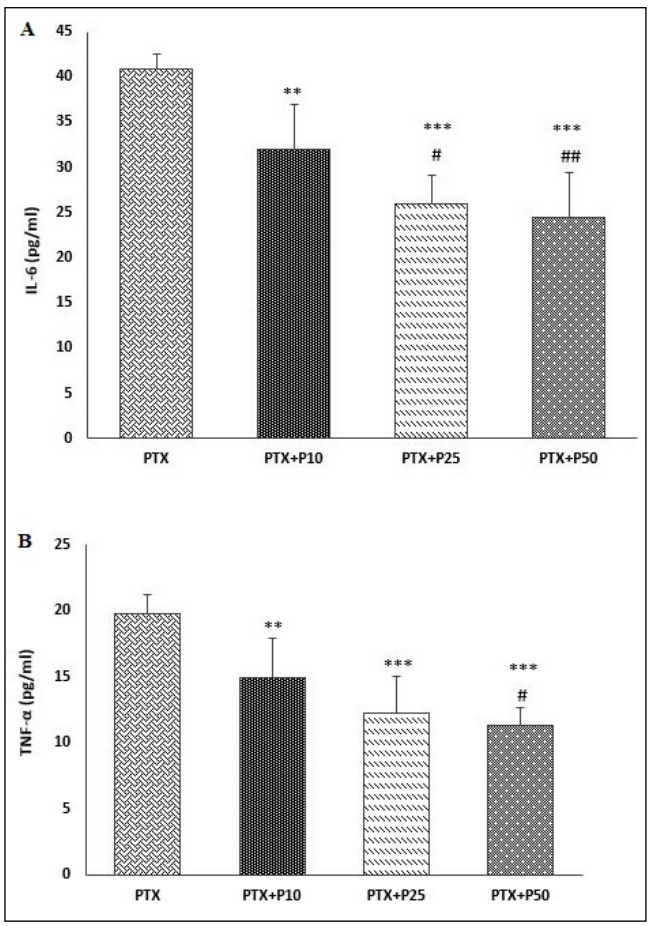

Fig. 4. Effect of piperine on (A) IL-6 and (B) TNF-α concentration. Values are mean ± SD. **P<0.01 and ***P<0.001 indicate significant differences compared to the PTX group. $^{\#}P$<0.05 and $^{\#\#}P$<0.01 indicates significant differences compared to the P10 group. PTX: paclitaxel-treated mice that were injected with normal saline for 7 consecutive days; *PTX+P* 10, 25, and 50: PTX-treated mice respectively treated with piperine 10, 25, and 50 mg/kg per day for 7 consecutive days.

and 50 mg/kg, according to Tukey's *post-hoc* test). The group treated with piperine 50 mg/kg had significantly lower levels of TNF-α compared to the group treated with piperine 10 mg/kg (P<0.05, according to Tukey's *post-hoc* test).

Effects of piperine on serum oxidative stress status in PTX-treated mice

Lipid peroxidation was evaluated in terms of serum MDA concentration; the current study showed that MDA levels significantly decreased in all piperine-treated groups compared to the PTX group (U=4.500, P<0.01 for piperine 10 mg/kg and U=0.00, P<0.001 for both piperine 25 and 50 mg/kg). As displayed in Fig. 5A, piperine 25 and 50 mg/kg caused significantly greater decreases in serum MDA levels than piperine 10 mg/kg (U=8.500, P<0.05 and U=1.000, P<0.001, respectively).

Moreover, values for CAT activity were significantly different among the studied groups ($F_{3,28}$=111.303, P<0.001). Piperine at all doses significantly increased CAT activity compared to the PTX group (P<0.001 for all cases, according to Tukey's *post-hoc* test, Fig. 5B).

The serum levels of SOD were significantly changed in all studied groups ($F_{3,27}$=15.461, P<0.001). Injection of piperine also increased SOD levels relative to the PTX group (P<0.01 for piperine 10 mg/kg and P<0.001 for both piperine 25 and 50 mg/kg, according to Tukey's *post-hoc* test). As shown in Fig. 5C, interestingly, the piperine 25 mg/kg-treated group had the highest level of SOD activity.

DISCUSSION

Side effects of cancer chemotherapy are a major issue for clinicians (Emery et al., 2022). Neuropathic pain induced by chemotherapeutics extensively affects the quality of life of patients (Dhawan et al., 2020). Investigating the potential of natural products to manage neuropathic pain in cancer patients has recently attracted the attention of the research community (Rezaee et al., 2019; Shahraki et al., 2020; Hashemzaei & Rezaee, 2021; Faheem et al., 2022; Alkholifi et al., 2023). In the current study, piperine administration alleviated hyperalgesia and mechanical hypersensitivity, and improved oxido-inflammatory status in PTX-treated mice, as reflected by reduced serum levels of IL-6, TNF-α, MDA, but induced activities of CAT and SOD.

In this study, we administered imipramine which is a tricyclic antidepressant with analgesic effects to the positive control group, consistent with previous research (Zarrindast et al., 2000; Shahraki et al., 2020). In our study, imipramine decreased PTX-induced thermal hyperalgesia and mechanical allodynia in mice. The analgesic effects of single-dose imipramine have been previously established in mice (Miri et al., 2015; Cong et al., 2024). Given that our aim in this study was not to investigate the effects of multiple-dose imipramine *versus* multiple-dose piperine, but to compare the potential pain-relieving effect of piperine *versus* a standard of care drug, imipramine (10 mg/kg) was injected only on day 7.

Although antinociceptive effects of piperine have been previously shown (Sabina et al., 2013; Tasleem et al., 2014; Sánchez-Trujillo et al., 2020), this is the first study to identify its antiallodynic effect in mice with neuropathic pain. It was shown that piperine (3.1–100 mg/kg) induces the activation of $GABA_A$,

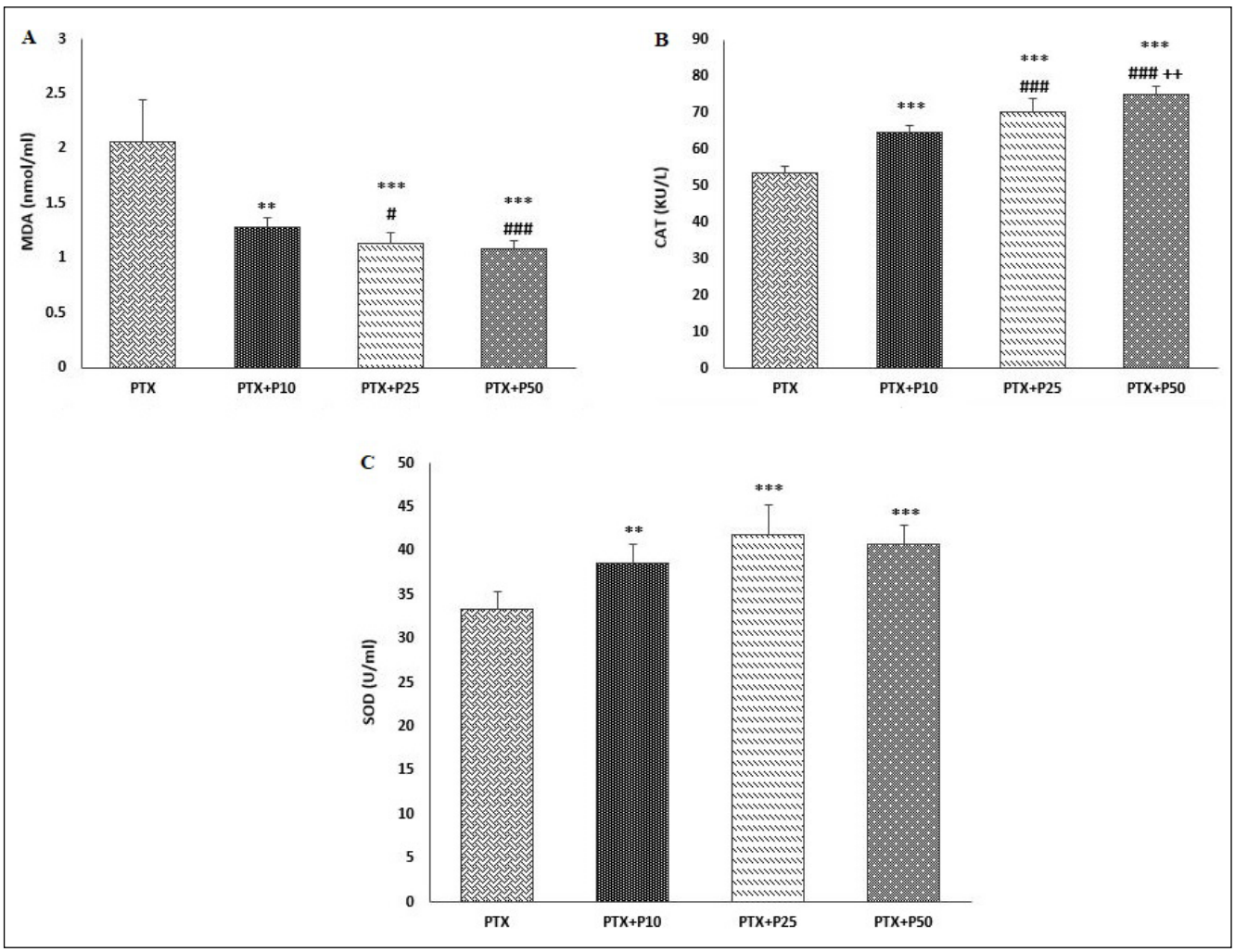

Fig. 5. Effect of piperine on (A) MDA concentration, (B) SOD activity, and (C) CAT activity. Values are mean ± SD. ***P*<0.01 and ****P*<0.001 indicate significant differences compared to the PTX group. #*P*<0.05 and ###*P*<0.001 indicate significant differences compared to the P10 group. ++*P*<0.01 shows significant differences compared to the P25 group. PTX: paclitaxel-treated mice that were injected with normal saline for 7 consecutive days; *PTX+P* 10, 25, and 50: PTX-treated mice respectively treated with piperine 10, 25, and 50 mg/kg per day for 7 consecutive days.

TRPV1, and TRPA1 receptors in rats with ligation-induced neuropathic pain in spinal nerves L5/L6 (Sánchez-Trujillo et al., 2020). In addition, antinociceptive and anti-inflammatory effects of piperine were reported to be mediated *via* activation of GABAergic and opioidergic systems (Bukhari et al., 2013). However, in another study, oral piperine at doses of 2.5, 5, and 10 mg/kg showed no analgesic properties in male ICR Balb/c mice (Sudjarwo, 2005). This disparity might be due to the different administration routes and doses of piperine considered by these studies. Our data also indicated that piperine attenuated the inflammatory and oxidative stress conditions in a dose-dependent manner.

TNF-α has a predominant role in cell activation and recruitment, and it is identified as the primary "messenger" in triggering the generation of pro-inflammatory factors such as IL-6 and IL-1β. IL-6 mediates the acute inflammatory response in the early stage of inflammation. When IL-6 transduces inflammatory signals, it provides grounds for the switch from an acute transformation response to a chronic inflammatory response (Duan et al., 2022).

Previous evidence showed that neuropathic pain is associated with excessive inflammation in both the central and peripheral nervous systems (Ellis & Bennett, 2013; Sommer et al., 2018). It has been found that piperine improves the functional recovery of spinal cord injury through suppression of inflammation mediated by autophagy activation (Zhang et al., 2023). Autophagy is an evolutionarily conserved lysosomal pathway that involves the degradation of cytoplasmic

contents and is an important factor in nonapoptotic cellular death (Levine & Yuan, 2005). A previous study indicated that piperine protected against neuro-inflammation by inhibiting lipopolysaccharide-induced TNF-α, IL-1β, IL-6, and prostaglandin E2 (PGE2) production in BV2 microglia cells (Wang-Sheng et al., 2017). Based on the literature, PGE2 suppression has a crucial role in relieving pain (Ricciotti & Fitz-Gerald, 2011). Of note, piperine has been reported as a potent suppressor of nuclear factor-kappa B (NF-κB) (Pradeep & Kuttan, 2004; Kumar et al., 2007; Ran et al., 2024; Duan et al., 2022), an element that plays a critical part in neuropathic pain (Huang et al., 2019; Cao et al., 2021; Zhao et al., 2024). This may at least in part, contribute to the effects observed in the present study.

Another crucial aspect of neuropathic pain pathogenesis that needs to be addressed is oxidative stress (Carrasco et al., 2018). It has been reported that enhanced reactive oxygen species production, *via* induction of lipid peroxidation (reflected by higher levels of MDA), changes the mitochondrial genome and proteome (Ilari & Giancotti, 2020). Several studies, similar to the present work, indicated that piperine can protect against oxidative damage by boosting the activities of SOD and CAT and the levels of glutathione (Vurmaz et al., 2019; Zhouwei et al., 2022), as well as reducing the levels of nitric oxide and MDA (Vurmaz et al., 2019; Adeyemo et al., 2021; Amin et al., 2023).

Recently, a few clinical trials evaluated the effects of piperine, alone and in combination with other natural or synthetic compounds, for disease prevention and management (Tripathi & Ray, 2022; Yadav et al., 2023). The present results may encourage researchers to conduct clinical trials on the potential antinociceptive effects of piperine. Also, since the cytotoxic effects of piperine have been reported (Tawani et al., 2016; Turrini et al., 2020; de Almeida et al., 2020; Wojtowicz et al., 2021), its anti-neuropathic effects in patients with PINP are worth investigating.

As a limitation, the present study lacked mechanistic investigations; future studies should investigate the molecular mechanisms behind the anti-neuropathic properties observed in the present work.

CONCLUSION

Evidence provided by this study demonstrates, for the first time, that piperine induces an antiallodynic effect in a mouse model of PTX-induced neuropathic pain. Piperine was also effective in alleviating thermal hyperalgesia and could protect against inflammatory and oxidative stress conditions.

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