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RESEARCH ARTICLE

EXPLORING THE POTENTIAL PHARMACOLOGICAL EFFECT OF THYMOQUINONE IN AMELIORATING THE ACQUISITION AND EXPRESSION OF VINCRISTINE INDUCED NEUROPATHIC PAIN IN MICE

Mehreen Arif [1], Naeem Ur Rehman[2], Hurmat Malik[1], Muhammad Usman[1], Ahmed Tokhi[1], Syeda Sharmeen Shah[1], Khalid Rauf [1]*

[1]Department of Pharmacy, COMSATS university Islamabad, Abbottabad Campus.[2]Faculty of Pharmacy, Gomal University Dera Ismail Khan, K.P Pakistan.

***Corresponding author's email:** khalidrauf@cuiatd.edu.pk

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ABSTRACT

Background: Chemotherapy-induced neuropathy (CIPN) is the most severe consequence, causing both sensory and motor impairment with an incidence of between 19% and over 85%. Leukaemia, lymphoma, and sarcoma are just a few of the cancers that are treated using vincristine (VCR), a typical anticancer treatment used in chemotherapy. Peripheral neuropathy caused by vincristine is the primary impediment to the efficacy of ongoing anti-cancer treatment and continued use. **Objectives:** The current study's goal was to contemplate the significance of thymoquinone on the development and expression of vincristine-induced neuropathy. **Methodology:** All groups except normal saline were administered vincristine 0.1mg/kg i.p for 14 days. In selected groups gabapentin (75mg/kg) and thymoquinone were administered at 10, 20, and 30 mg/kg/day for 14 days orally along with vincristine (acquisition) and once at day 14 (expression). Thermal hyperalgesia and mechanical allodynia were quantified on days 1, 6, and 14 after 30, 60, 90, and 120 minutes. The data were processed using the Students' t-test and post hoc Dunnett's test. **Results:** The acquisition and expression of thermal hyperalgesia as well as mechanical allodynia was significantly improved by thymoquinone at all the tested doses at days 6 and 14 as well as after 30, 60, 90, and 120 minutes, significant improvement was observed. **Conclusion:** It is manifested that TQ can be an impending candidate for the management of vincristine-induced neuropathy.

Keywords: Vincristine, Thymoquinone, Neuropathic pain, Allodynia, Hyperalgesia

INTRODUCTION

Traumatic nerve injury, post-herpetic neuralgia, chemotherapy-induced and diabetic neuropathy are a few examples of nerve injuries that result in neuropathic pain (1). With an incidence of between 19% and over 85%, it is one of the most severe consequences and presents a significant challenge to both cancer patients and medical professionals (2). Common chemotherapy drugs, including vincristine, paclitaxel, and oxaliplatin are known to cause crippling neuropathic pain (3). Leukemia, lymphoma, and sarcoma are among the majority of cancers that are treated with vincristine (VCR), a common chemotherapy medicine (4).

Peripheral neuropathy brought on by vincristine is the main obstacle to the effectiveness and continued use of anti-cancer therapy (5). VCR causes a variety of distinct nociceptive pain behaviors, including dysesthesia, hyperalgesia, and allodynia, which are severe dose-limiting side effects that necessitate therapy discontinuation, endanger cancer patients' lives, and result in high medical expenses (6). Anticonvulsants, tricyclic antidepressants, and opioids are among the medicines used to treat the clinical signs and symptoms of neuropathy caused by VCR but these medications are linked to negative side effects that limit how well they work clinically to treat neuropathy (7, 8).

Natural plant chemicals are emerging as intriguing therapeutic options in the search for new medications for neuropathic pain (1). A member of the Ranunculaceae family, Nigella sativa is a flowering plant. It has been utilized in the Prophetic medical system as well as in Ayurvedic and Chinese medicine (9). Thymoquinone, an active chemical component of Nigella sativa, has also been isolated from other plants (10-16). It has been reported as an antioxidant (17), hepatoprotective (18), anti-inflammatory (19), neuroprotective (20), anti-cancerous (21), gastroprotective (20), cardioprotective (22), nephroprotective (20) and anti-microbial (23). Given the current clinical environment, further study on novel, safe, and efficient therapeutic medicines for the management of chemotherapy-induced neuropathic pain will be required. Therefore, the goal of the current investigation was to investigate the pharmacological effects of thymoquinone on vincristine-induced mechanical allodynia and thermal hyperalgesia.

METHODOLOGY

Animal handling

The Veterinary Research Institute Peshawar in Pakistan provided adult male BALB/c mice (n=6/group; 22-28 g). Animals were kept at a constant temperature of 22.0±2.0°C with a 12/12 light/dark cycle and had full access to food and water. The Animals Scientific Procedure Act (1986) of the UK was followed in all experimental methods at the COMSATS University Islamabad Abbottabad campus (approval number: PHM.Eth/CS-M03-015-1106).

Protocol of experiment

On experiment day, group 1:normal saline (10 ml/kg i.p), Group 2:vincristine (0.1 mg/kg i.p) for 14 days (24, 25), Group 3:Vincristine+Gabapentin (75 mg/kg p.o), Group 4, 5 and 6:Vincristine+Thymoquinone (10, 20, 30 mg/kg/day orally) for 14 days 15 minutes before vincristine (26, 27). Thermal and mechanical tests were performed on days 1, 6, and 14 following the vincristine injection to determine the nociceptive threshold (28). The test readings were recorded after 30, 60, 90, and 120 min of TQ administration (17) because TQ has a half-life of 3.6 hours (29). For acute studies same experimental procedure was used except groups 3,4,5 and 6 received single doses of gabapentin and thymoquinone 10, 20, and 30 mg/kg orally respectively at day 14 (17) (Figure 1).

Thermal method (tail Immersion)

On days 1, 6, and 14, the tail immersion method was used to gauge the onset of thermal hyperalgesia. Using a water bath, the water's temperature was maintained at 54 ± 0.5^{o}C. (30). After the tail had been submerged in water for one-third of it, the tail flick time was measured. Cut-off duration of 15 seconds has been set to prevent any tissue injury (31-33).

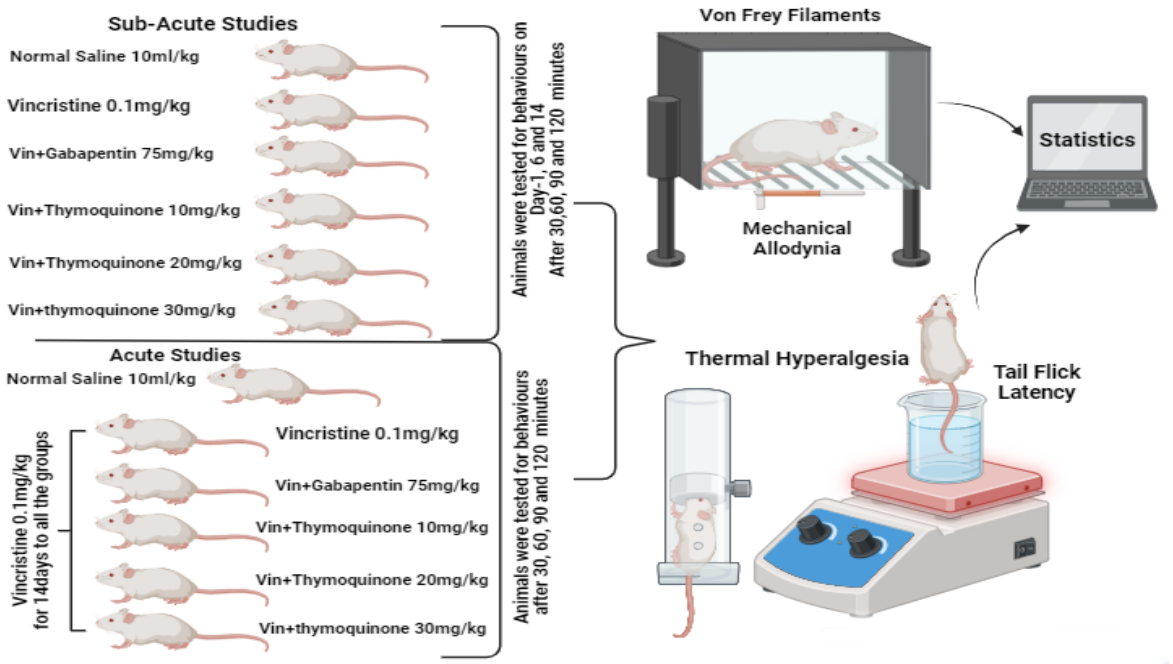


Figure 1. Graphical presentation of the experimental protocol

Mechanical method (static allodynia)

The Von Frey cage with a wire mesh floor was used which allowed complete access to the mouse paw. A translucent cup was used to block out any visual stimulus. After 15 minutes of acclimatization, initial exploration, and grooming, tests were conducted (34). The up-down technique indicated by the Chaplain was used when applying Von Frey filaments. Until the paw withdrawal reaction buckled, filaments were placed perpendicularly on the plantar surface of the hind paws. Once the animal's paw withdrawal threshold was determined and known, the animal was tested using the following descending filament utilizing the up-down method. For five readings, a cut-off value of 20 seconds was utilized to prevent any tissue damage to an animal (31-33, 35).

Statistical analysis

The data, which were presented as mean standard error, were processed using Graph Pad Prism-8. Students' t-test and *post hoc* Dunnett's test were performed after one-way ANOVA. The data were estimated significant if ***p<0.001, **p<0.01, and *p<0.05.

RESULTS

Development of vincristine induce neuropathy-effects of thymoquinone on acquisition and expression of vincristine-induced thermal hyperalgesia and mechanical allodynia

Development of thermal hyperalgesia with vincristine

Treatment with vincristine has shown significant development of thermal hyperalgesia as tail-flick latency time was notably lowered at days 6 and 14 (###p<0.001) in relevance to the normal saline group (Figure 2A).

Effects of Thymoquinone on the acquisition of vincristine-induced thermal hyperalgesia

Thymoquinone has shown no significant results in improving the tail flick latency time in relevance to the vincristine control group on day 1 (Figure 2B). At day 6 (Figure 2C) significant results were observed after 30 minutes (***p<0.001) with all three doses as well as gabapentin. At 60 minutes with gabapentin and 30mg/kg (***p<0.001), 10mg/kg (*p<0.05), with 20mg/kg TQ (**p<0.01). After 90 minutes significant results were observed with gabapentin, 20mg/kg and 30mg/kg TQ

(***p<0.001) and 10mg/kg (**p<0.01). After 120 minutes gabapentin has shown significant improvement in tail-flick latency time (**p<0.01). All three doses of thymoquinone have also shown significant results with 10mg/kg and 20mg/kg TQ (*p<0.05) and 30mg/kg TQ (***p<0.001) in relevance with the vincristine control group in increasing the tail flick latency time. Thymoquinone has significantly improved the acquisition of thermal hyperalgesia as tail-flick latency time was significantly improved by all the tested doses of thymoquinone at day 14 (Figure 2D) after 30, 60, 90, and 120 minutes (***p<0.001) in relevance to vincristine group.

Effects of Thymoquinone on the expression of vincristine-induced thermal hyperalgesia

All three doses of thymoquinone have significantly improved the expression of thermal hyperalgesia as tail-flick latency time was enhanced by all three doses at 30, 60, 90, and 120 minutes (***p<0.001) in relevance to the vincristine group (Figure 2E).

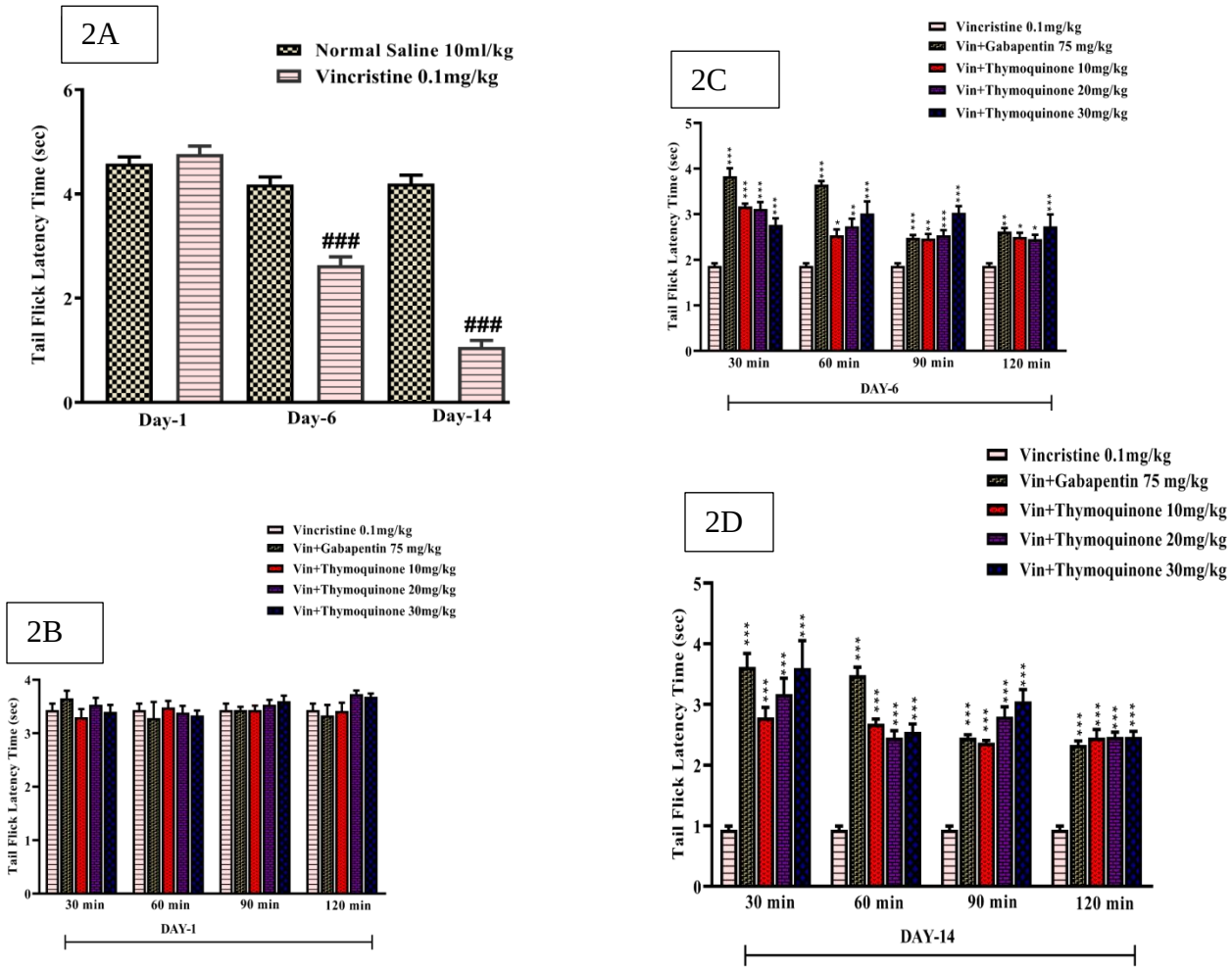


Figure 2. 2A. Induction of Vincristine-induced thermal hyperalgesia. Data significance was ###p<0.001. Figures 2B, 2C, 2D. Effect of thymoquinone on the acquisition of VCR-induced thermal hyperalgesia. Data significance was *p<0.05, **p<0.01, ***p<0.001. Figure 2E. Effect of thymoquinone on the expression of VCR-induced thermal hyperalgesia. Data significance was ***p<0.001

Development of mechanical allodynia with vincristine

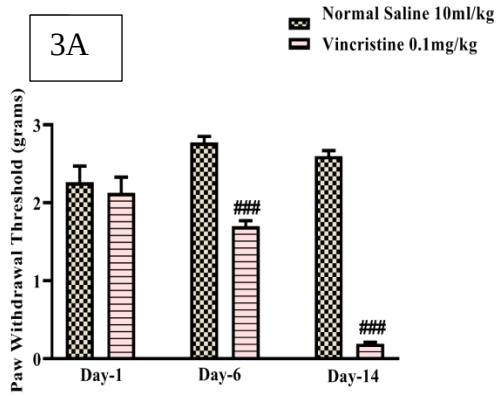
Treatment with vincristine has shown significant development of mechanical allodynia as the paw withdrawal threshold was remarkably lowered at days 6 and 14 (###p<0.001) in relevance to the normal saline group (Figure 3A).

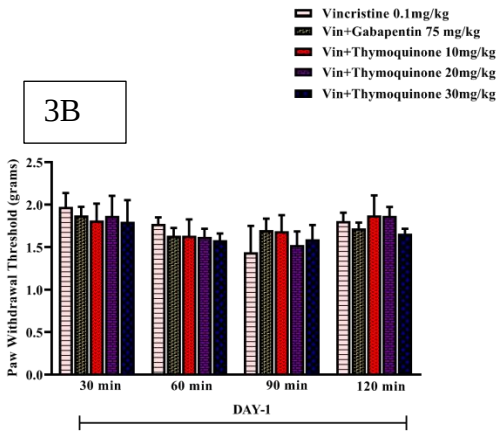
Effects of thymoquinone on the acquisition of vincristine-induced mechanical allodynia

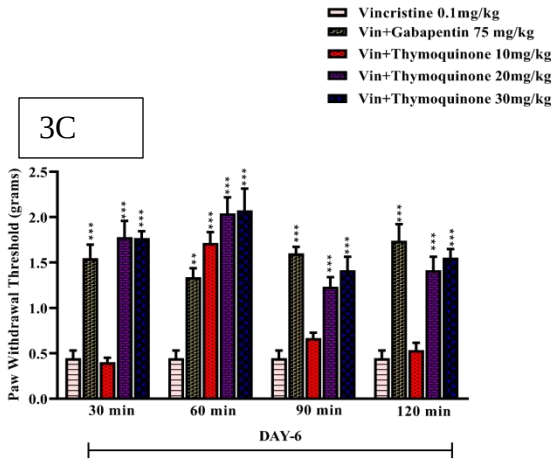
Thymoquinone has shown no significant results in improving paw withdrawal threshold time in relevance to the vincristine control group at day 0 (Figure 3B). Thymoquinone has significantly reversed the acquisition of mechanical allodynia at day 6 with 10mg/kg after 30 and 120 min (*****p<0.05) (******p<0.01) respectively, however at day 14 thymoquinone significantly improved paw withdrawal threshold at 30,120 and 180 min (*****p<0.05), (*******p<0.001) respectively. The paw withdrawal threshold was also increased by 20mg/kg after 30 and 60 min (**p<0.01) on day 6 and also after 120 and 180 min (*******p<0.001) respectively. At day 14 20mg/kg showed significant results after 60,120 and 180 min (*****p<0.05), (**p<0.01) and (*******p<0.001) respectively (Fig 1c).30mg/kg thymoquinone showed a significant increase in paw withdrawal threshold after 30 and 180 min at day 6 (*****p<0.05). Significant results were also observed on day 14 after 30, 60,120, and 180 min (*******p<0.001) (Figures 2C and 2D).

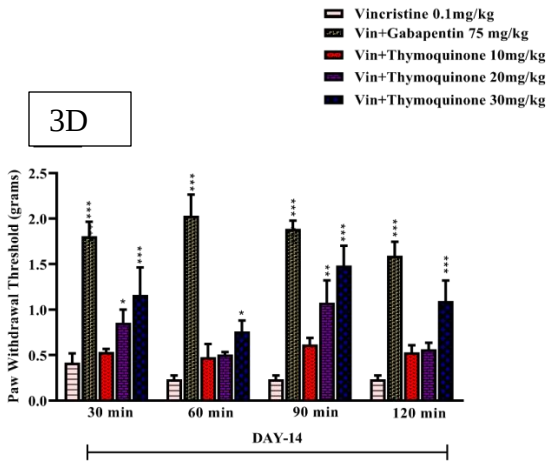
Effects of thymoquinone on the expression of vincristine-induced mechanical allodynia

Thymoquinone has improved the expression of mechanical allodynia with 10, 20, and 30mg/kg after 30, 60,120, and 180 min (***p<0.001) in relevance to the vincristine group (Figure 3E









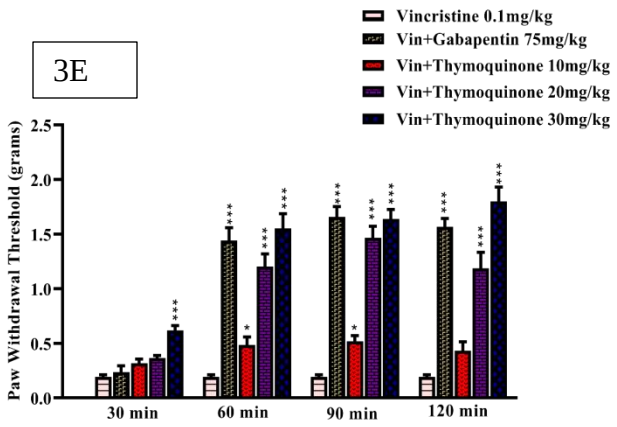


Figure 3. 3A. Induction of Vincristine-induced thermal hyperalgesia. Data significance was ###p<0.001. 3B, C, D. Effect of thymoquinone on the acquisition of VCR-induced mechanical allodynia. Data significance was *p<0.05, **p<0.01, ***p<0.001. Figure 3E. Effect of thymoquinone on the expression of VCR-induced mechanical allodynia. Data significance was *p<0.05, **p<0.01, ***p<0.001

DISCUSSION

As a chemotherapeutic agent, vincristine was first introduced in the early 1960s. Since then, it has been used successfully to treat a variety of adult and pediatric neoplasms, including breast cancer, non-Hodgkin's lymphomas, and leukemia (36). Microtubule dynamics are disrupted, which is a hallmark of its antineoplastic action. Vincristine, in particular, delays mitosis and subsequently triggers apoptosis by reducing tubulin polymerization and microtubule incorporation, which hinder the assembly of the mitotic spindle (37). In neuropathic pain, both central and peripheral mechanisms are involved (38, 39). Myelin sheath is a structure rich in lipoproteins that cover nerve fibers and are responsible for the insulation and quick conduction velocity in nerves. Compared to unmyelinated nerve C-fibers, myelinated nerve A-fibers have better insulation and conduction velocity (40). Chemotherapeutic drugs can additionally damage myelinated nerve fibers (41) But it's unclear how much demyelination manifests with CIPN (42). The conduction has been impeded because of injury to the myelin sheath and peripheral nerves (40).In our study there was a noteworthy development of thermal hyperalgesia (Fig 2A) and mechanical allodynia (Fig 3A) by treatment with vincristine. Numerous investigations have highlighted the crucial part that NF-κB signalling plays in triggering the development of peripheral neuropathy. (2, 43). VCR stimulates NF-κB signalling, which causes inflammatory and immune responses that might cause cellular damage and the release of cytokines and chemokines (25). Nitric oxide, cyclo-oxygenase-2 (COX-2), and inflammatory cytokines including TNF-α, and IL-1β are all stimulated by the NF-κB axis. These inflammatory mediators directly trigger pain hypersensitivity in the nerves (19).

Sub-acute thymoquinone treatment inhibited the acquisition of thermal hyperalgesia (Figs. 2B, IC, and ID) as well as expression of thermal hyperalgesia was also moderated by thymoquinone (Fig. 2E). Studies have shown that the nitric oxide (NO)-cGMP pathway has been documented to be involved in the development and expression of thermal hyperplasia in vincristine therapy (44). The nitric oxide (NO)-cGMP pathway is inhibited by thymoquinone, and this mechanism may play a role in the reduction of thermal hyperalgesia

(45). The plausible molecular cause of neuropathic pain caused by VCRs is oxidative stress (26). Amplified oxidative stress, augmented superoxide anion generation, and reduced GSH have been linked to vincristine use and are significant factors in neuropathic pain (46). It has been discovered that thymoquinone has strong antioxidant action. It removes reactive oxygen from the environment, including singlet molecular oxygen, superoxide anion, and hydroxyl radical. Additionally, it has been discovered that thymoquinone raises GSH expression and activity (20). The antioxidant function of thymoquinone has also been demonstrated in radiation-induced oxidative and nitrosamine stress models, where it reduced NO and peroxynitrite levels (18, 47). Therefore, it was successful in alleviating NOS brought on by stress. It proved advantageous in reducing oxidative stress linked to diabetic neuropathy when combined with proanthocyanidin (17). Thymoquinone has a strong GABAergic action in animal models and has been shown to increase GABA levels (48) it has been shown that vincristine-induced allodynia is suppressed by GABAergic medications. Neuropathic pain has a very strong emotional component, and this anxiety- and depressive-like behavior hastens the onset of hyperalgesia and allodynia (49). Strong anxiolytic effects of thymoquinone have been documented, and it is anticipated that these effects may have helped in some way to regulate the emotional aspect of neuropathic pain (48). Thymoquinone has also been shown to reduce oxidative stress and levels of inflammatory mediators and cytokines in a sciatic nerve damage model, which hastens thermal hyperalgesia, cold, and mechanical allodynia development (44, 50, 51).

Vincristine causes neuronal inflammation in the spinal cords' dorsal horn, activating astrocytes, microglia, and satellite glial cells. As a result, pro-algesic chemical mediators tumor necrosis factor and interleukin are produced and released (52). The p38 cascade in the DRG and spinal cord is reported to be activated by TNF. The expression of inflammatory cytokines is enhanced when P38 is activated. The development of mechanical allodynia after nerve damage has reportedly been linked to the TNF-p38 cascade (53). In our study vincristine treatment has shown significant development of mechanical allodynia (Fig 3A). However, the acquisition (Fig 3B, C, and D) and expression (Fig 3E) of mechanical allodynia were improved by thymoquinone. The phosphorylation of the p38 cascade results in lipopolysaccharide peroxidation, which has been observed to be inhibited by thymoquinone. TQ significantly lowers serum levels of IL-1β and TNF-α in rheumatoid arthritis experimental mouse models at a dose of 5 mg/kg/day (54). The anti-allodynic effect of thymoquinone in vincristine-induced neuropathy may be produced by a similar mechanism. Vincristine has also been discovered to boost calcium levels in the cytoplasm from both extracellular stores, such as ion channels, and from intracellular stores, such as mitochondria (55). Augmented intracellular levels have been associated with tissue and neural damage (56). The release of pronociceptive mediators and lowered action potential threshold in afferent nociceptors are two factors that contribute to primary afferent neurons' enhanced feeling of pain (57). It has been reported that thymoquinone blocks calcium channels, causing intestinal spasmolysis and having relaxant effects on the heart muscle. (58). The modulatory effect of thymoquinone on calcium channels may be responsible for its ameliorative effects on allodynia. Vincristine affects axonal transport, increases the responsiveness of C fibers, stabilizes microtubules, and may give rise to axonal degeneration and axonopathy (59). An oral administration of *Nigella sativa* seed to an animal model of autoimmune encephalomyelitis caused the cerebral cortex and hippocampus, which were largely demyelinated, to remyelinate (60, 61). The same process might be partially or entirely responsible for the suppression of peripheral nerve growth and the subsequent development of hyperalgesia and allodynia. Thymoquinone has been reported to exert its anti-nociceptive effect via supra-spinal opioid receptors and it has been discovered that naloxone, an opioid receptor antagonist, reverses this analgesic effect (62). Morphine has been reported to mitigate the allodynia produced by vincristine treatment when administered

systemically (63). In animal models of neuropathic pain, thymoquinone has been shown to suppress the manifestation of diabetic allodynia (64). Thymoquinone has been shown to reduce excessive levels of free cytosolic calcium via reducing cytosolic oxidative stress (65).Vincristine elevates reactive oxygen species and reduces mitochondrial transmembrane potential in neurons, each of which are frequently correspond to neuropathies (66). Thymoquinone has been shown to improve neuronal protection from vincristine-induced oxidative stress and subsequent neurotoxicity by preventing an overall reduction of mitochondrial transmembrane potential (65). In behavioral tests on vincristine-induced neuropathy, thymoquinone mitigated the development and expression of neuropathic pain.

CONCLUSION

It is a behavioral investigation that aims to determine how thymoquinone affects the development and manifestation of vincristine-induced neuropathic pain. The TQ needs to be further investigated at both the neurotransmitter level and the receptor level.

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None

DECLARATIONS

Authors' Contributions

KR supervised and designed the study concept; MA contributed to the study design, data collection, and manuscript write-up. NU, HM, MU, AK, and SS contributed to data analysis, and interpretation and critically reviewed the manuscript. All authors reviewed the results and approved the final version of the manuscript.

Ethical Approval

The Animals Scientific Procedure Act (1986) of the UK was followed in all experimental methods at the COMSATS University Islamabad Abbottabad campus (approval number: PHM.Eth/CS-M03-015-1106).

Conflict of Interest

The authors declared no conflict of interest among them.

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