6,7,8-Trimethoxycoumarin attenuates vincristine induced peripheral neuropathic pain, potential role of $5HT_3$ and opioid receptors and monoamines

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Abstract

Vincristine is the drug of choice for Hodgkin's lymphoma, acute lymphoblastic leukemia, and non-Hodgkin lymphoma. Despite its significant anticancer effects, it causes dose-dependent neuropathy, leading to compulsive dose reduction. The suggested mechanisms of vincristine-induced peripheral neuropathic pain (VIPNP) are complex and include an increased release of tumor necrosis factor α centrally and peripherally, increased 5-hydroxytryptamine (5-HT2A) receptors on dorsal horn and dorsal root ganglion (DRG) neurons, increased reactive oxygen species (ROS) and an altered intrinsic antioxidant mechanism. The available drugs used for VIPNP include antidepressants, antiepileptics and opiates, which have a range of safety, efficacy, and tolerability issues prompting a search for new therapies.6,7,8-Trimethoxycoumarin (678TMC) is a natural coumarin found in *Cryptocarya bracteolate*, *Sapium discolor*, *Platypodium elegans, Sapium sebiferum, Zanthoxylum lemairie* and *Zanthoxylum rugosum*, and it possesses anti-inflammatory, antioxidant, and carbonic anhydrase-I-inhibitory effects. This study was designed to investigate the possible analgesic and antiallodynic effects of 678TMC in a murine model of VIPNP. Vincristine was administered to groups of BALB/c male mice (0.1 mg/kg intraperitoneally) once daily for 14 days to induce VIPNP. The writhing test was performed after injection of acetic acid intraperitoneally. Thermal hyperalgesia and static mechanical allodynia were tested using tail immersion and Von Frey pressure methods. Cold and dynamic allodynia were quantified using acetone and cotton buds. 678TMC significantly repressed the number of abdominal constrictions and clearly reversed VIPNP thermal hyperalgesia, mechanical static allodynia, mechanical dynamic allodynia, and cold

allodynia. Hyperalgesia and allodynia were reversed by pre-treatment with Ondansetron and Naloxone. Vincristine induced Alterations in Vitamin C and most of the neurotransmitters concentrations in the striatum, frontal cortex and hippocampus were reversed by 678TMC. 678TMC reversed the VIPNP increased plasma level of TNF-α. In reversing the alterations in the concentrations of some neurotransmitters in the brain caused by vincristine, 678TMC showed stronger effects than gabapentin. 678TMC attenuates VIPNP by reducing neuroinflammation and targeting the opioidergic and serotonergic systems.

Key Words: Vincristine Induced Peripheral Neuropathic Pain, 6,7,8 Trimethoxycoumarin, $5HT_3$ receptors, Opioid receptors, TNF-α, Vitamin C

1 Introduction

Neuropathic pain has been defined as pain caused by a lesion or disease of the somatosensory nervous system either centrally or peripherally (IASP, 2023). The symptoms may be linked with abnormal sensations termed dysesthesia (burning, coldness, paresthesia, numbness and itching), hyperalgesia (heightened pain sensitivity) or allodynia (perception of non-nociceptive stimuli as painful) and they may be spontaneous or evoked (Kaur et al., 2019).

The causes of neuropathic pain include diabetes, alcoholism, central nervous system damage, amputation, spinal nerve compression, nerve damage during surgery, nerve compression or infiltration by tumors, radiation therapy and chemotherapy with agents such as paclitaxel, cisplatin and vincristine (Suh, 2021). In regard to this, vincristine anticancer drug, effective in the treatment of acute lymphoblastic leukemia, Wilms tumor, Hodgkin's and non-Hodgkin's lymphoma, neuroblastoma and rhabdomyosarcoma (Barnett et al., 2022; Škubník et al., 2021). It is the drug of choice for childhood blood cancers, but unfortunately, it can cause dose-dependent neuropathy, giving rise to dose limitation or even a complete discontinuation of therapy and treatment failure (Li et al., 2020). Peripheral neuropathy develops in 78 % of patients and 44% patients reported pain receiving vincristine (VCR) (Lavoie et al., 2015). In another clinical study, 34.9% of patients experienced Vincristine induced peripheral neuropathic pain (VIPNP) (Anghelescu et al., 2011). The clinical diagnostic features of VIPNP are typically variable (Li et al., 2020) and include gait dysfunction, constipation, weakness, urinary retention, tingling, paresthesia and loss of ankle stretch reflexes, some of the features being in a symmetrical, distal, "glove and stocking-like" distribution. The sensory symptoms of VIPNP are predominant

with respect to severity and frequency compared with other chemotherapeutic drugs (Madsen et al., 2019; Said & Tsimberidou, 2014). However, the exact mechanism underlying VIPNP remains unclear. Nonetheless, vincristine targets microtubules and impairs retrograde and anterograde neuronal transport, and this is considered as an underlying cause of altered sensory neuronal function. Other proposed mechanisms include swelling of intracellular axonal mitochondria leading to the release of calcium ions and apoptosis, increased level of substance P in the spinal cord (Chiba et al., 2022), involvement of adenosine signaling in pain transmission (Zhou et al., 2022), increased release of substances including TNF-α (tumor necrosis factor-α), interleukin-1 [IL-1], nitric oxide [NO], interleukin-6 [IL-6]) from glial cells, Langerhans cells and macrophages, increased concentration of nitric oxide synthase (NOS) in the dorsal horn, downregulation of IL-10 in dorsal horns of spinal cord, increased 5-hydroxytryptamine (5-HT2A) receptors on dorsal horn and dorsal root ganglion (DRG) neurons, increased reactive oxygen species (ROS), which affect serine protease activity and decreased endorphins in the spinal cord and DRG, and increased serine proteinase that inactivates endorphins (Addington & Freimer, 2016; Thibault et al., 2008). Moreover, it has been reported that L-ascorbic acid reverses Vincristine induced astrocyte activation (Li et al., 2021). It also potentiates the effects of vitamin E (Sisignano et al., 2014) in neuropathic pain and concentration of vitamin C in plasma is lower in postherpetic neuralgia patients (Sekiguchi & Kawabata, 2013).

The impact of vincristine on the central nervous system has been under active investigation as there are also other central derangements that may contribute to the development and expression of VIPNP. In this regard, serotonin is considered to be a key neurotransmitter that contributes to pain control via descending pathways (Heijmans et al., 2021) and serotonin transporter knockout mice disclose less hyperalgesia but heightened neuropathic pain. In addition, serotonin at the cortical level and in mesolimbic system, has an inhibitory effect on pain, while in the spinal cord and the periphery, it is pronociceptive. What is more, serotonin receptors modulate both sensory and emotional components of pain, and 5-HT3 and 5-HT7 receptors have a bimodal role in controlling pain centrally (de Kort et al.,2022; Neugebauer, 2020).

Central dopaminergic activation in the periaqueductal grey/dorsal raphe system induces anxiety-free analgesia and oxytocin release onto target cells in the

ventrolateral periaqueductal grey inhibits spinal cord sensory neurons, producing antinociception thought to be critical in neuropathic pain models (Iwasaki et al., 2023; Taylor et al., 2019). In addition to this, both dopamine itself and D2 receptors in the striatum and spinal cord are implicated in generating highly effective analgesia (Wang et al., 2021). Furthermore, overexpression of monoamine oxidase B in the dorsal root ganglion and spinal cord has been reported during chemotherapy induced neuropathy, whilst inhibition of this enzyme ameliorates the overall pathology (Ouyang et al., 2023). Additionally glial α7 nAChR activation by agonists contributes towards amelioration of pain and inflammation both centrally and peripherally (Hone & McIntosh, 2023).

In a chemotherapy induced neuropathy model, descending noradrenergic inhibition in the spinal cord is elevated prompting the suggestion that chemotherapy augmented noradrenergic inhibition may stem from an adaptive mechanism to increased peripheral nociceptive input (Costa et al., 2020; Sałat, 2020; Song et al., 2020). In addition to this, during chemotherapy induced peripheral neuropathy, adenosine 1A receptor is also suppressed in the spinal cord (Liu et al., 2023).

Vincristine tends to produce greater neurotoxicity compared to other Vinca alkaloids (Grisold et al., 2012) and this may be ascribed to microtubule destabilization during mitosis (metaphase) and interference with axonal transport (Nie et al., 2017; Stubblefield et al., 2009; Triarico et al., 2021). Currently, no standard treatment is available for VIPNP rather than merely managing it (Lee et al., 2019). Presently, the anti-epileptics (pregabalin and gabapentin), tricyclic antidepressants (amitriptyline), a serotonin-noradrenaline reuptake inhibitor (duloxetine), selective serotonin reuptake inhibitors (fluoxetine, citalopram and paroxetine), an opioid agonist (tramadol), an NMDA antagonist (ketamine), pyridoxine, and pyridostigmine are used in different combinations for the management of complex neuropathic pain (Addington & Freimer, 2016; Köker et al., 2021; Stubblefield et al., 2009; Triarico et al., 2021). Such therapies modify serotonergic, adrenergic, excitatory amino acid or opioidergic pathways to produce anti-allodynic effects with unsatisfactory clinical outcomes associated with serious adverse effects, including dependence (Cho et al., 2021). Considering the complex nature of neuropathic pain and the serious efficacy and tolerability issues of currently available therapies, there is an ongoing search for potential molecules, both natural and synthetic (Chung & Kim, 2022), for the treatment of vincristine induced peripheral neuropathic pain (VIPNP).

In relation to this quest, naturally occurring coumarins consist of fused benzene and pyrone rings (Common and IUPAC names: benzopyran-2-one and chromen-2-one). These compounds exhibit low toxicity, good bioavailability, high potency, and a broad-spectrum activity against various diseases. Natural coumarins have antioxidant, neuroprotective, anti-inflammatory, antidiabetic, antidepressant, and anticonvulsant properties whereas methoxy coumarins possess antioxidant and anti-Alzheimer's disease properties (Chaurasiya et al., 2022; Keri, Budagumpi et al., 2022; Marchant et al., 1985; Mishra et al., 2020; Zhu et al., 2018). 6,7,8-Trimethoxycouamrin (678TMC) (IUPAC name: 6,7,8-trimethoxychromen-2-one; molecular weight: 236.22 g/mol; molecular formula: $C_{12}H_{12}O_5$) is an example of naturally occurring methoxycoumarins having pale yellow color and melting point 104-105^{0}C (Son, et al., 2015), UV Lambda max (CHCl3): 240, 295 and 341 nm (Semple et al., 1999) is found in *Cryptocarya bracteolate, Sapium discolor, Platypodium elegans, Sapium sebiferum, Zanthoxylum lemairie* and *Zanthoxylum rugosum*. It is the inhibitor of human carbonic anhydrase-I (Davis et. al., 2013), has higher effect than the standard drug and has less toxicity (LD_{50} 2.1g/kg) and minimal effects on drug-metabolizing enzymes (Son et al., 2015) and possesses antioxidant potential (Whang et al., 2005). 678TMC has also antiinflamatory activity is ear (Zhang, et. al., 2019). It can cross BBB, and can be absorbed from intestine. It is an inhibitor of p-glycoprotein and it is non-inhibitor of 2C9, 2D6, 2C19 and 3A4. It is non-carcinogen (Cheng et al., 2012). However, the pharmacological potential of 678TMC in VIPNP, its interaction with opioidergic or serotonergic pathways, and associated changes in brain neurotransmitters in addition to vitamin C levels, and plasma level of TNF-α, have not been explored. The purpose of the current study therefore, was to examine the pharmacological potential of 678TMC in a model of VIPNP. The investigation was focused on any possible mechanism of action through its interaction with opioidergic or serotonergic pathways and the associated changes in the concentrations of vitamin C and neurotransmitters in brain areas as well as the level of TNF-α in plasma.

2 Materials and Methods

2.1 Ethical Approval

Experimental procedures were approved by the Research Ethical Committee (REC) of the University (COMSATS University Islamabad, Abbottabad Campus) with approval notification number: PHM.Eth/CS-M01-017-1038 and complied with the UK Animals (Scientific Procedures) Act 1986.

2.2 Materials

CAT-I camera, syringes (1 ml, 3 ml, and 5 ml), Plexiglas boxes, dissection box, Eppendorf tubes, black plastic sheets, water bath, mouse restraint boxes, water bottles, mouse cages with lids, lenses, Von Frey boxes with mesh floor, ice packs, ear buds, weighing balance, white papers, pencils, tags for cages, gloves, masks, sanitizer, funnel, beakers, volumetric flasks, pipettes, filter papers (0.45 μm), filtration assembly, glass vials for brain samples with caps, and analytical balance.

2.3 Chemicals

678TMC was purchased from Biosynth, United Kingdom. HPLC-grade water was obtained from commercial suppliers locally available in Pakistan. Acetonitrile, Acetone, Vitamin C, Methanol, Sodium dihydrogen phosphate, perchloric acid, Dopamine, Serotonin, Adenosine, and their metabolites were purchased from reputed commercial sources. TNF-α ELISA kit was purchased from the Cayman Chemical Company, Ann Arbor, MI, USA.

2.4 Experimental Animals

Male BALB/c mice, randomly selected, weighing 21-25 g, were kept at 23-25 ^{0}C on a 12h-12h light-dark cycle, and they were provided with ad libitum access to food and water.

The animals were divided into the following groups (n = 6 per group):

a. Saline vehicle subacutely 10 ml/kg intraperitoneally [i.p.]

b. Vincristine subacutely 0.1 mg/kg i.p.

c. Vincristine subacutely 0.1 mg/kg i.p. +Gabapentin 75 mg/kg i.p.

d. Vincristine subacutely 0.1 mg/kg i.p. +678TMC 10 mg/kg i.p.

e. Vincristine subacutely 0.1 mg/kg i.p. +678TMC 15 mg/kg i.p.

f. Vincristine subacutely 0.1 mg/kg i.p. +678TMC 20 mg/kg i.p.

2.5 Induction of Vincristine-induced Peripheral neuropathic pain (VIPNP)

Vincristine (VCR) was administered once daily i.p. at 0.1 mg/kg dose subacutely for fourteen consecutive days. Behavioral tests (tail immersion, cold allodynia, Von Frey filament static pressure and dynamic allodynia) were performed on days 0, 4, 7, 10, and 14 of VCR treatment protocol (Zhu et al., 2021).

2.6 Behavioral tests

2.6.1 Thermal hyperalgesia

The tail immersion test was used for evaluation of thermal hyperalgesia. Mice were positioned in light restraining cages in such a way that the tail protruded. Animals

were habituated to their surroundings 30 min before experimentation. The terminal portion of the tail (3cm) was dipped carefully in water maintained at 54 ± 0.5 °C. Tail withdrawal latency (TWL) was quantified, using a stop watch, in seconds. Readings in triplicate were taken, and a cut-off duration of 15 seconds was applied to prevent any tissue injury (Khan et. al., 2019; Nawaz et. al., 2018; Sewell and Spencer 1976) .

2.6.2 Static mechanical allodynia

The von Frey filaments were used for gauging the static mechanical allodynia. Mice were placed in Plexiglas cages having dimensions of 30 cm×24 cm (*H*×*D*) with a fine metal mesh (1.3cm×1.3 cm apertures) to allow direct access to mice paws from below (Bardin et al., 2009). Prior to the experiment, mice were habituated with the experimental condition. Von Frey filaments with different nominal weight ranges (0.008 – 8 g) were applied to the left hind paw for 2 s. If mice responded to a selected filament, then a lower force was used, but if mice did not respond to the selected filament, then a filament of an upper force was applied. This process was repeated further four times after the response to first filament. A cut-off time of 6 seconds was imposed to measure the pain threshold (Chaplan et al., 1994; Shahid et al., 2017) .

2.6.3 Dynamic mechanical allodynia

Following a 30 min habituation period, dynamic allodynia was evaluated using a cotton bud which was lightly brushed on the mid plantar area of the left hind paw. Abrupt paw withdrawal, flinching or licking was evaluated as a paw reaction. Mice that responded to brushing within 8 seconds were included in the test. Readings in triplicate were taken and a 15 second cut-off period was imposed (Khan et al., 2019; Shahid et al., 2017).

2.6.4 Acetone drop-induced cold allodynia

Mice were kept on wire netting bottomed cages for a habituation period of 10 min. using a syringe connected to a blunt needle, 50 µl acetone was applied carefully to hind paw without touching it. The paw withdrawal response to the cooling effect of each acetone drop was noted within period of 0.5 seconds to 15 seconds (Khan et al., 2019; Shahid et al., 2017). The response was classed into four categories (0 = no withdrawal response; 1 = brisk or flick of the paw; 2 = repeated flicking of the paw; 3 = repeated flicking and licking of the paw). The test was performed in triplicate to each hind paw and a mean value was calculated (Bardin et al., 2009; Deuis et al., 2017).

2.7 Mechanism of 678TMC analgesic or antiallodynic effects

The possible mechanism of the analgesic and antiallodynic effects of 678TMC in VIPNP was explored by examining the role of opioidergic or serotonergic pathways using specific receptor-specific antagonists (naloxone or ondansetron) (Ali et al., 2022; Tokhi et al., 2023).

2.8 Quantification of TNF-α in plasma

Blood was collected from mice after decapitation by direct cardiac puncture. It was centrifuged and the plasma was kept at the specified temperature stipulated by the manufacturer of the ELISA kit and the TNF-α concentration was quantified according to the manufacturer's instructions (Zhou et al., 2018).

2.9 Acetic acid induced writhing test

The food and water was removed from the animals were 120 minutes prior to this experiment. Vehicle at dose 10 ml/kg, Diclofenac sodium at 50 mg/kg dose and 678TMC at the three mentioned doses were injected IP to the animals 30 minutes before administration of 1 percent acetic acid IP. The dose of vehicle and Acetic acid was 10 ml/kg and after 5 minutes of administration of acetic acid, the number of constrictions were recorded for each animal for continuous 20 minutes (Collier, et al., 1968).

2.10 Neurochemical Analysis:

2.10.1 Sample preparation

Following behavioral assessment, post-mortem (after decapitation) whole brains were removed and the tissues of the three brain areas (frontal cortex, hippocampus and striatum) were dissected on ice chilled plates, precisely weighed, and kept at -80°C. Homogenization of the tissues was carried out in chilled perchloric acid (0.2%) following cold centrifugation (4°C; 12,000 rpm) and the supernatant was then separated. Once the samples were prepared, they were passed through a 0.45mm filter and then analyzed using HPLC (Arif et al., 2022; Rehman et al., 2020).

2.10.2 Chromatographic Conditions

A Waters Alliance separation model 2690 with a UV-detector system was used for the chromatographic analysis (USA) coupled to a C18 stainless steel column with a 5µm particle size (250× 4.6 mm). For vitamin C, serotonin, dopamine and noradrenaline analysis, methanol, HPLC grade water (5:95, v/v) and 20 mM monobasic sodium phosphate were used with isocratic elution at 280 nm with column maintained at 35 °C and a flow rate set at 0.5 mL/min. For the analysis of adenosine, inosine and

hypoxanthine, solvent of HPLC grade water and acetonitrile in 95:5 v/v ratio for preparation of 0.01M monobasic sodium phosphate was used with isocratic elution at 260 nm, with the column at 25 ℃ and a elution rate set at of 1.0 ml/min (Arif et al., 2022; Rehman et al., 2020).

2.10.3 Standard Preparation and Determination of Sample Concentration

Standard stock solutions were made by dissolving 1.0 mg of each of the following compounds in 10 mL of HPLC-grade water: vitamin C, dopamine, 5-HT, noradrenaline, adenosine, inosine, and hypoxanthine. The standard stock solution was then diluted to provide various concentrations ranging from 100 to 500 ng/mL from which, calibration curves were constructed. The samples were loaded into the HPLC system, and a 20 µL sample volume was extracted for injection (EmpowerTM). Then, a calibration curve was constructed against the peak area (A) of each sample (y) and the concentration (x) using linear regression (Arif et al., 2022; Tokhi et al., 2023; Rehman et al., 2020).

2.11 Statistical analysis

Data was presented as mean value ± SEM (n = 6 animals) and analyzed statistically with the assistance of Graph Pad Prism 8. One-way ANOVA with Tukey's test for multiple comparisons in post hoc analysis was used to determine the significance of results keeping level of significance at 0.05. The results were assumed to be significant was at $p<0.05$. For analyzing the development of VIPNP, t-test was used.

3 Results:

3.1 Development of thermal hyperalgesia

Treatment with vincristine 0.1 mg/kg i.p. daily for 14 days resulted in the development of thermal hyperalgesia indicated by a notable decrease in mouse tail immersion latency on days 7 – 14 in comparison with the saline vehicle treated animal group (Fig. 1A).

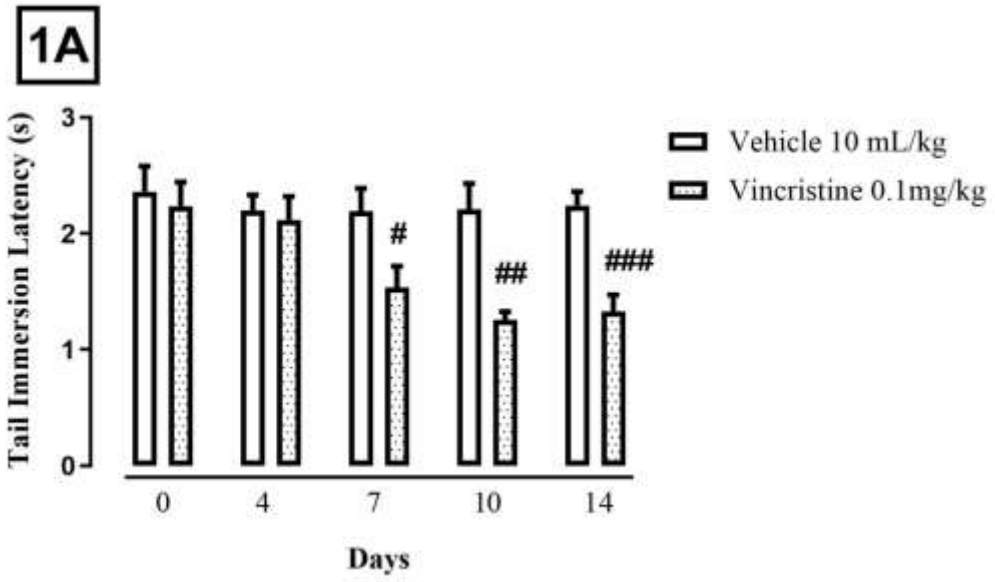

Figure 1A - Development of vincristine induced thermal hyperalgesia in the mouse tail immersion test. Significance of reduced response latencies (mean ± SEM) compared with saline vehicle treated control: #p<0.05, ##p<0.01 and ###p<0.001.

3.2 Effect of 678TMC and gabapentin on thermal hyperalgesia

678TMC reversed vincristine induced thermal hyperalgesia at 15 mg/kg 60 min after administration. The 20 mg/kg dose of 678TMC reversed the vincristine hyperalgesia from 30 to 90 min after administration. Gabapentin (positive control) at a higher dose (75 mg/kg) produced a notable improvement in response latency throughout the testing phase as compared to the vincristine treated hyperalgesic group (Fig. 1B).

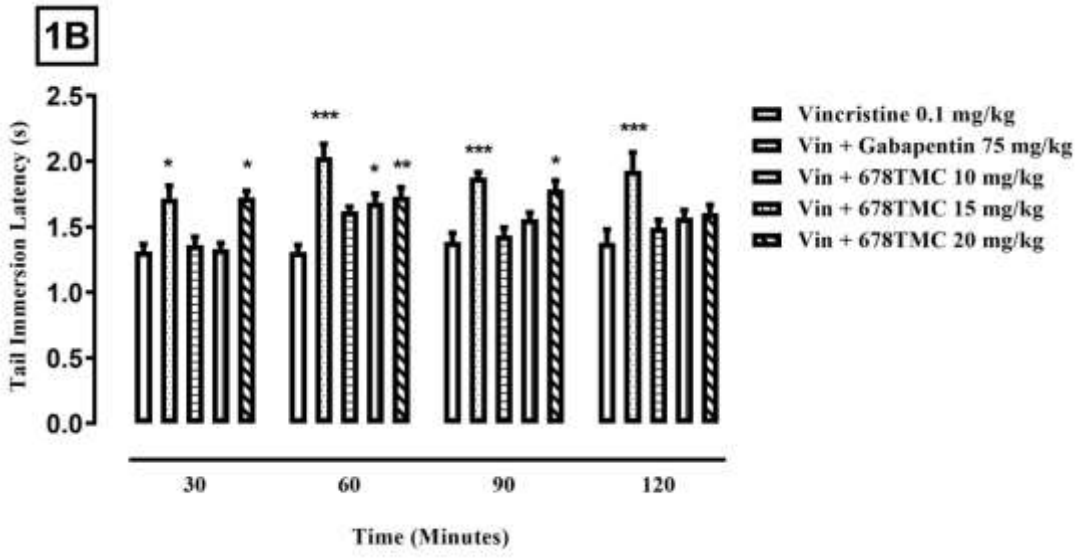

Figure 1B - Effect of 678TMC and gabapentin on vincristine induced thermal hyperalgesia in the mouse tail immersion test. Significance of differences in response

latencies (mean ± SEM) compared with the vincristine treated group: *p<0.05, **p<0.01; ***p<0.001

3.3 Development of static mechanical allodynia

Vincristine daily treatment in the 14-day protocol led to the development of static allodynia as evidenced by decreased paw withdrawal thresholds from treatment day 7 to 14 in comparison with controls (Fig. 2A).

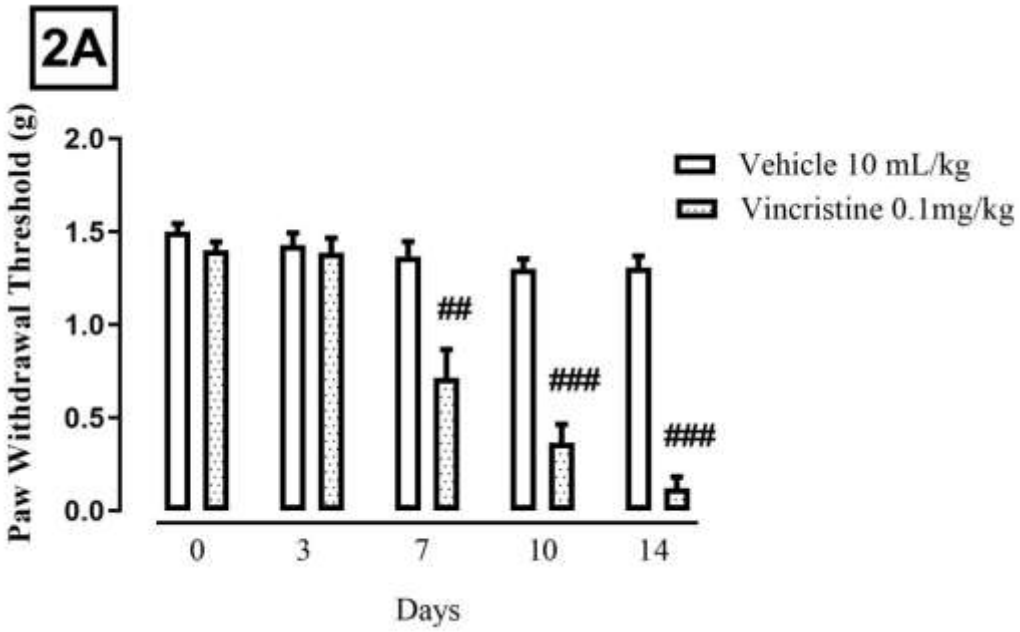

Figure 2A - Development of vincristine-induced mechanical allodynia (static) in the von Frey filament mouse paw withdrawal threshold test. Significance of reduced paw withdrawal threshold compared with saline vehicle treated control: ##p<0.01 and ###p<0.001.

3.4 Effect of 678TMC and gabapentin on static mechanical allodynia

There was a substantial improvement (i.e., elevation of paw withdrawal threshold) in vincristine-induced mechanical allodynia produced by 678TMC at the two higher doses at 30 to 90 min and at 10 mg/kg dose at 60 and 90 min after its administration. Similarly, gabapentin reversed the vincristine provoked suppression of paw withdrawal threshold up to 2h after dosing (Fig 2B).

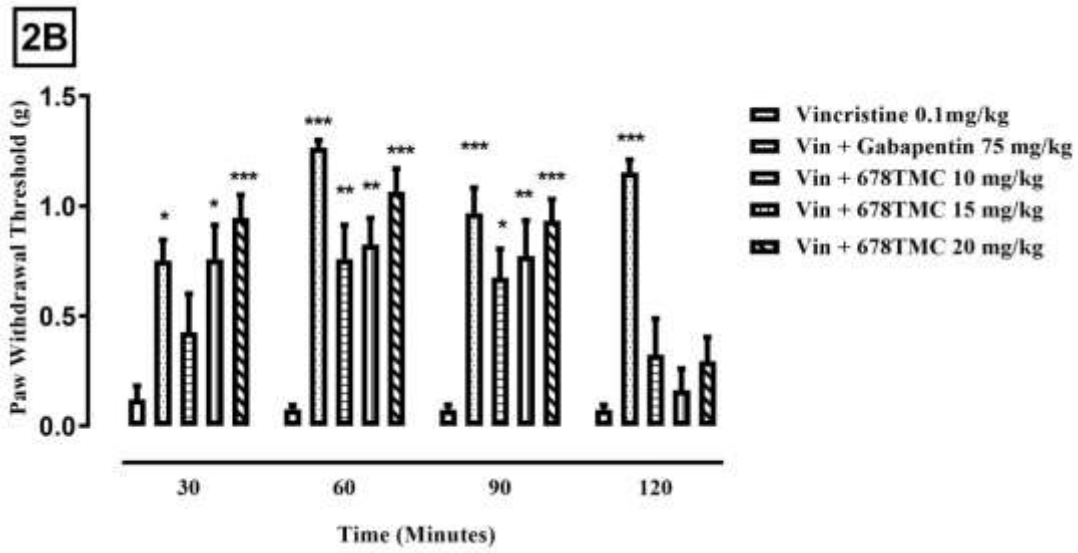

Figure 2B - Effect of 678TMC and gabapentin on vincristine induced static mechanical allodynia in the mouse paw withdrawal threshold test. Significance of differences in paw pressure thresholds (mean ± SEM) compared with the vincristine treated group: *p<0.05, **p<0.01 and ***p<0.001

3.5 Development of vincristine induced dynamic mechanical allodynia

Fourteen-day treatment with vincristine gave rise to the development of dynamic allodynia revealed by a decrease in response time following light brushing of mouse paw. The onset of effect occurred on days 7, 10 and 14 compared to controls. (Fig 3A).

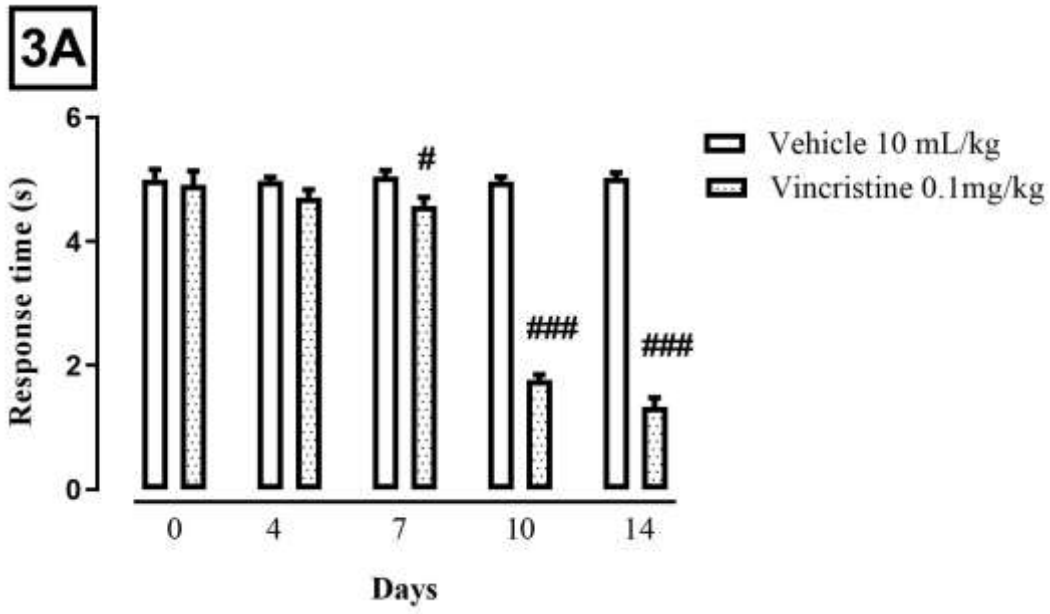

Figure 3A - Development of vincristine induced dynamic allodynia revealed by a decreased response time in mice following light brushing of the plantar surface of their hind paws. Significance of reduced response latencies (mean ± SEM) compared with the saline vehicle treated control: #p<0.05 and ###p<0.001

3.6 Effect of 678TMC and gabapentin on vincristine induced dynamic mechanical allodynia

678TMC ameliorated vincristine induced dynamic mechanical allodynia 60 - 90min after administration of the 15 – 20 mg/kg doses and 10 mg at 90 min after its administration which caused increased response times to light plantar paw brushing. Likewise, gabapentin evoked an increase in response times throughout the whole 120 min testing period after injection of this positive control versus the vincristine treated group (Fig 3B).

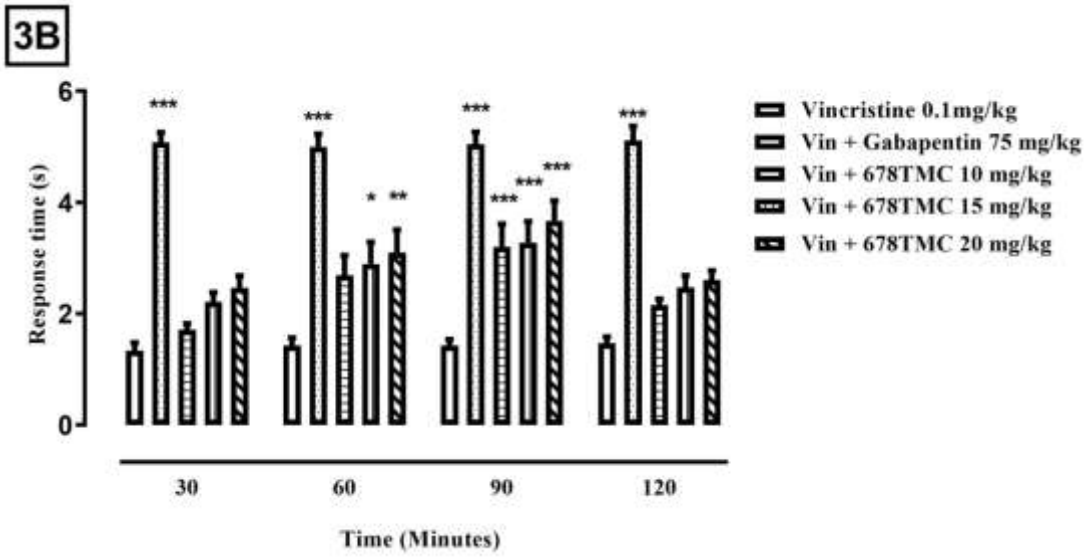

Figure 3B - Effect of 678TMC and gabapentin on vincristine induced dynamic mechanical allodynia in response to light brushing of mouse paw plantar surface. Significance of differences in response times (mean ± SEM) compared with the vincristine treated group: *p<0.05, **p<0.01 and ***p<0.001

3.7 Development of vincristine induced cold allodynia

The 14-day vincristine treatment protocol generated cold allodynia shown by a steady increase in the intensity of response score in response to acetone application from days 7 to 14 of the protocol (Fig 4A).

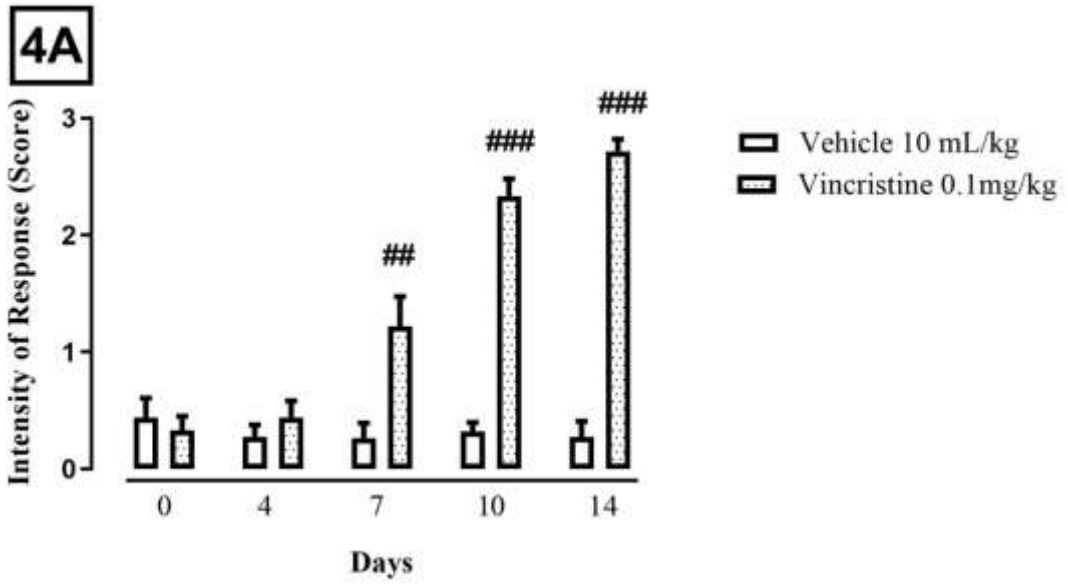

Figure 4A - Development of vincristine cold allodynia, signified by an increased response score to application of acetone to mouse paw plantar surface. Significance of increased response score compared with saline vehicle treated control: ##p<0.01 and ###p<0.001

3.8 Effect of 678TMC and gabapentin on vincristine induced cold allodynia.

678TMC significantly reversed vincristine induced cold allodynia in response to mouse paw acetone application. The 678TMC reversal was substantiated by a reduced intensity response score. After 120 min, the reversal was evident with the 10 mg/kg dose and both the 15 and 20 mg kg doses maintained the vincristine cold allodynia reversal throughout the test period. Gabapentin also reversed the cold allodynia to vincristine throughout the test period (Fig 4B).

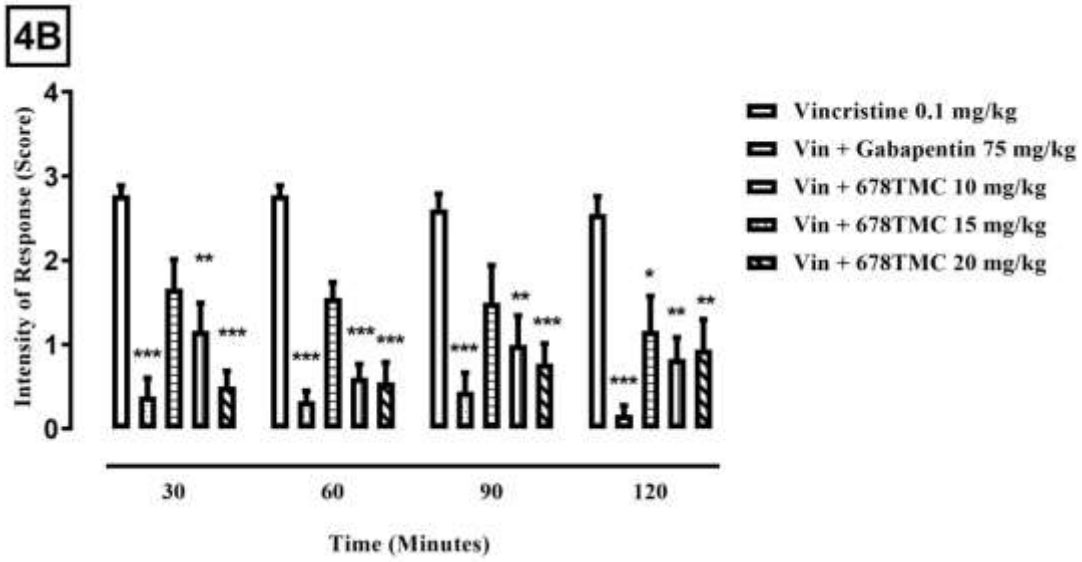

Figure 4B - Effect of 678TMC and gabapentin on vincristine induced cold allodynia in response to mouse paw plantar surface application of acetone. Significance of differences in response scores (mean ± SEM) compared with the vincristine treated group: *p<0.05, **p<0.01 and ***p<0.001

3.9 Involvement of either a serotonergic or opioidergic mechanism in the thermal anti-hyperalgesic activity of 678TMC

678TMC (15 and 20 mg/kg) reversed vincristine induced thermal hyperalgesia by increasing the tail-immersion latency, and this reversal was inhibited by 10 min pretreatment with both the naloxone (1.0 mg/kg) and ondansetron (1.0 mg/kg) reducing the tail immersion latency to a comparable level with the vincristine protocol group, but not inhibited by naloxone against 678TMC 15 mg/kg dose, (Fig 5A).

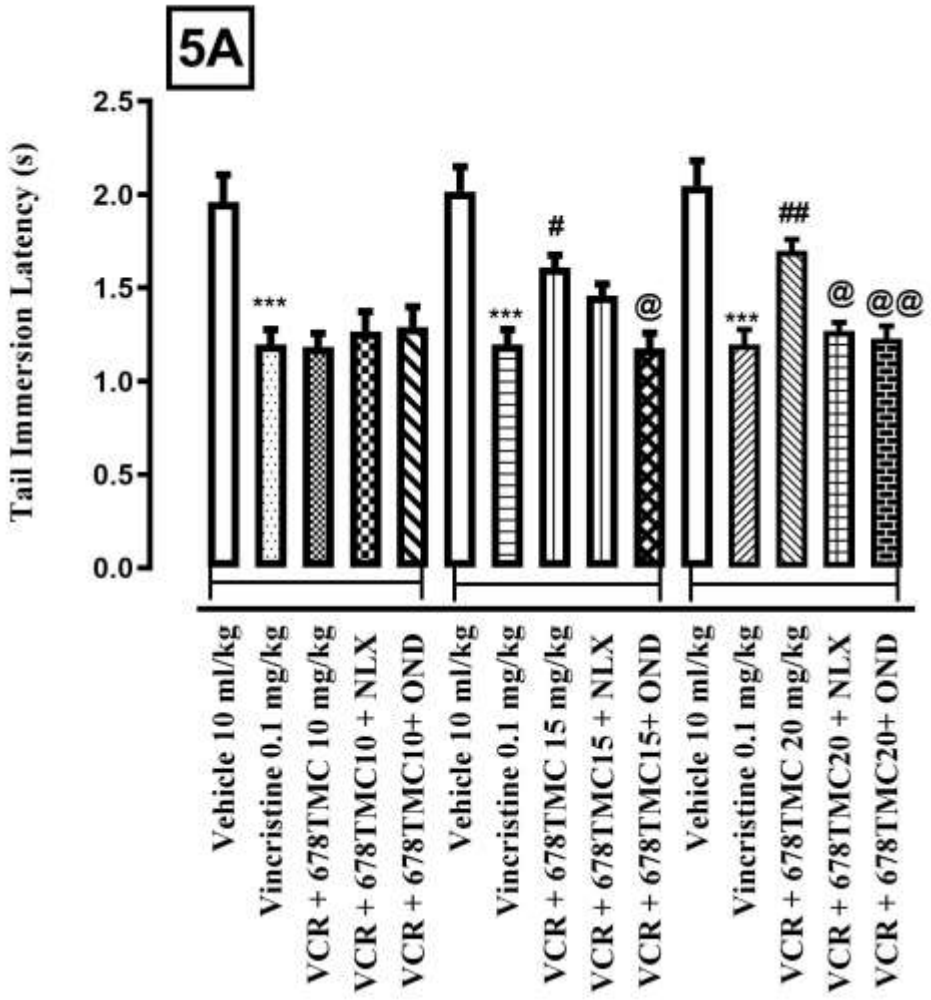

Figure 5A - Effect of naloxone (Nlx) or ondansetron (Ond) 10 min pretreatment on 678TMC activity against vincristine induced hyperalgesia in the mouse tail immersion test. Significance of difference from the vehicle, vincristine (VCR) and 6,7,8-

Trimethoxycoumarin (678TMC) treated groups are ***P<0.001, #P<0.5, ##P<0.01, ###P<0.001 and @P<0.5, @@P<0.01, @@@P<0.001 respectively.

3.10 Involvement of either a serotonergic or opioidergic mechanism in the mechanical static anti-allodynic activity of 678TMC

678TMC at all the three doses reversed vincristine-induced mechanical static allodynia by increasing the threshold pressure for paw withdrawal. This anti-allodynic effect of 678TMC was antagonized by both ondansetron (1.0 mg/kg) and Naloxone (1.0 mg/kg). Naloxone in contrast, did not modify the anti-allodynic activity of 678TMC 10 mg/kg dose (Fig 5B).

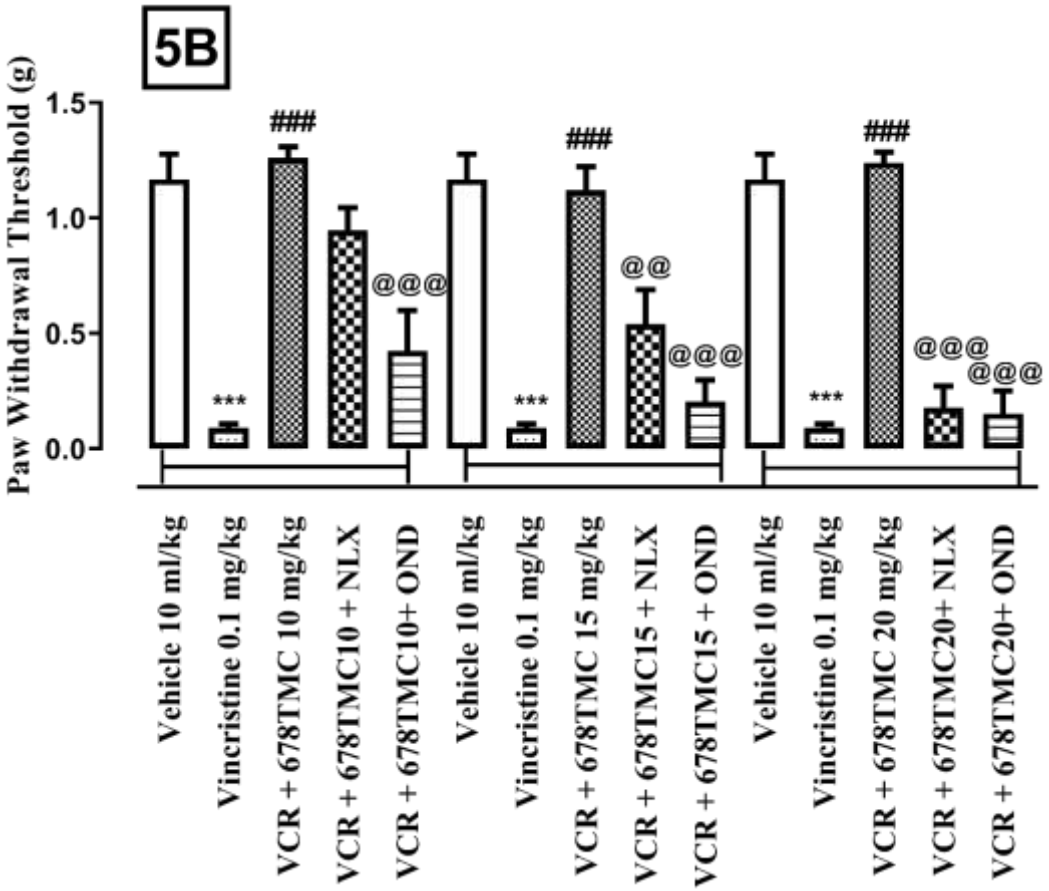

Figure 5B - Effect of ondansetron (Ond) or naloxone (Nlx) 10 min pretreatment on 678TMC activity against vincristine induced allodynia in the mouse paw pressure test. Significance of difference from the vehicle, vincristine (VCR) and 6,7,8-Trimethoxycoumarin (678TMC) treated groups are ***P<0.001, #P<0.5, ##P<0.01, ###P<0.001 and @P<0.5, @@P<0.01, @@@P<0.001 respectively.

3.11 Effects of 678TMC and Diclofenac sodium on acetic acid induced writhing

678TMC at all the three doses and diclofenac sodium used as standard drug at 50 mg/kg dose notably repressed the abdominal constrictions in comparison to the control group (Fig 6).

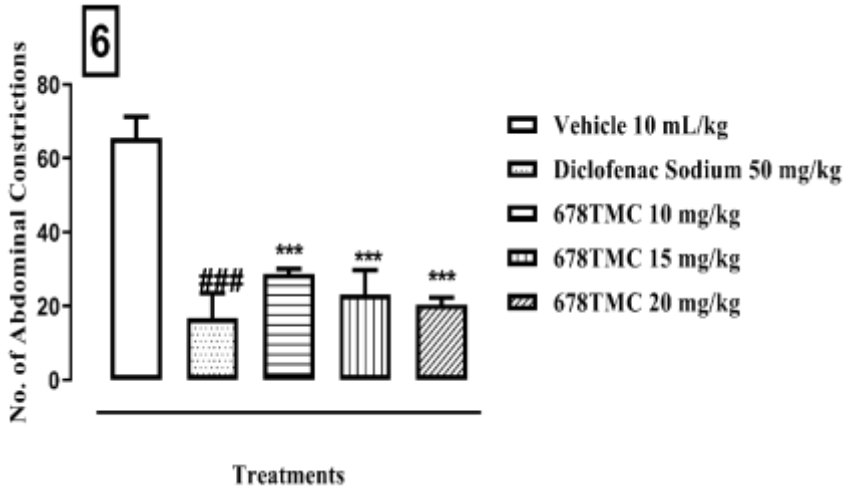

Figure 6: Effects of 678TMC on abdominal constrictions. The number of abdominal constrictions is expressed in mean ± SEM. Significance of differences from vehicle and Diclofenac sodium are ###p < 0.001 and ***p < 0.001 respectively.

3.12 Effect of 678TMC and Gabapentin on the level of TNF-α in plasma:

As shown in Figure 7, the plasma level of TNF-α was increased (30.1 ± 4.04 up to 73.77 ± 6.74 pg/ml) after the development of vincristine induced peripheral neuropathic pain and this effect was significantly reversed by the standard drug gabapentin 75 mg/kg and 678TMC 20 mg/kg (73.77 ± 6.74 down to 25.77 ± 4.06 and 29.1 ± 8.08 respectively).

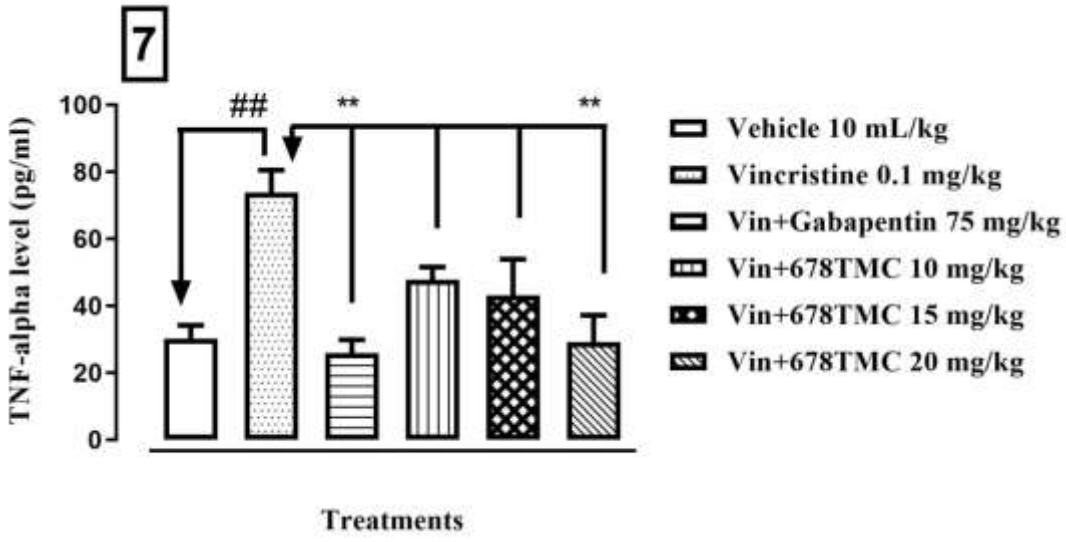

Figure 7. Effect of 678TMC on the level of TNF-α in plasma. The mean ± SEM is expressed in pg/ml. VCR indicates vincristine at 0.1 mg/kg, GBP for gabapentin 75 mg/kg and 678TMC is for 678TMC at 10, 15 and 20 mg/kg doses. Significance of differences from vehicle and VIN are ##p < 0.01 and **p < 0.01 respectively.

3.13 Effect of 678TMC on the Level of Vitamin C and Neurotransmitters in Frontal Cortex:

Vitamin C levels after the development of neuropathic pain repressed that were significantly improved by 678TMC at 20 mg/kg with stronger effect than gabapentin. An upsurge in serotonin levels was observed after the development of neuropathic pain. 678TMC notably repressed the serotonin upsurge at all the three doses and Gabapentin. Noradrenaline was increased non-significantly and 678TMC treatment restored the noradrenaline levels at 20 mg/kg. Upsurges in dopamine levels were observed in the neuropathic brain that was repressed at all three doses of 678TMC but not by Gabapentin. Repression in adenosine was also observed in after the development of neuropathic pain and was restored by 20 mg/kg of 678TMC but not by Gabapentin. However, inosine levels were not altered by Vincristine and hence all the doses of 678TMC and Gabapentin. Upsurge in Hypoxanthine levels was seen in neuropathic pain that was significantly lowered by all doses of 678TMC but not by Gabapentin (Table 1).

Table 1. Frontal cortical levels of vitamin C (VC), serotonin (5-HT), noradrenaline (NA), dopamine (DA), adenosine (AD), inosine (IN), and hypoxanthine (HX) after the vincristine treatment protocol (VCR) and subsequent modification by 678TMC and gabapentin. Significance of differences from the VCR group: *p<0.05, **p<0.01 and ***p<0.001

	Vehicle 10 ml/kg	VCR 0.1 mg/kg	VCR+GBP 75 mg/kg	VCR+678TMC 10 mg/kg	VCR+678TMC 15 mg/kg	VCR+678TMC 20 mg/kg
VC	11.92 ± 1.62	5.03 ± 0.37##	6.9± 0.33*	4.03± 0.39	7.6± 0.54	12.6 ± 0.89**
5HT	10.88 ± 1.57	26.4 ± 4.96##	1.18 ± 0.28***	14.65 ± 2.19*	9.14 ± 1.19**	8.03 ± 0.99**
NA	28.17 ± 2.15	36.46 ± 1.2	29.55 ± 4.69	41.63 ± 5.27	30.4 ± 3.94	13.27 ± 1.86**
DA	10.25 ± 1.56	73.18 ± 4.82###	106.8 ± 4.71**	34.93 ± 3.03*	20.16 ± 2.07***	21.23 ± 2.56**
AD	15.18 ± 1.43	5.25 ± 0.36#	9.03 ± 1.35	10.0 ± 2.88	14.43 ± 1.07	17.13 ± 3.47*
IN	33.93 ± 2.09	38.61 ± 4.11	13.73 ± 1.07	53.13 ± 4.74*	76.0 ± 5.96 **	84.4 ± 4.31***
HX	22.93 ±	85.83 ±	110.5 ± 5.73	29.15 ± 2.58***	35.6 ± 3.95**	29.9 ± 2.88***

	1.13	4.55##				

3.14 Effect of 678TMC on the Level of Vitamin C and Neurotransmitters in the Hippocampus:

Upsurge in the concentrations of serotonin, dopamine, Vitamin C and norepinephrine in the hippocampus after the development of neuropathic pain was notably lowered by 678TMC at all three doses and gabapentin. Repression in hippocampal adenosine after the development of neuropathic pain was restored by 678TMC at two higher doses and gabapentin. Upsurge in hippocampal inosine was significantly repressed by 678TMC at all doses and gabapentin. Repression in in hypoxanthine was significantly restored by 678TMC at the highest dose and gabapentin (Table 2).

Table 2. Hippocampal levels of vitamin C (VC), serotonin (5-HT), noradrenaline (NA), dopamine (DA), adenosine (AD), inosine (IN) and hypoxanthine (HX) after the vincristine treatment protocol (VCR) and subsequent modification by 678TMC or gabapentin. Significance of differences from the VCR group: *p<0.05, **p<0.01 and ***p<0.001

	Vehicle 10 ml/kg	**VCR 0.1 mg/kg**	**VCR+GBP 75 mg/kg**	**VCR+678TMC 10 mg/kg**	**VCR+678TMC 15 mg/kg**	**VCR+678TMC 20 mg/kg**
VC	1.87 ± 0.19	5.08 ± 0.27###	2.23 ± 0.17***	2.08 ± 0.25***	1.9 ± 0.29***	2.48 ± 0.33***
5HT	4.2 ± 0.22	55.32 ± 4.86###	21.03 ± 2.23**	14.08 ± 2.24***	4.52 ± 0.47***	2.18 ± 0.31***
NA	2.77 ± 0.24	16.13 ± 1.77###	5.13 ± 0.75***	2.95 ± 0.14***	3.82 ± 0.36***	5.13 ± 0.81***
DA	5.78 ± 0.57	73.04 ± 4.33###	33.05 ± 2.74***	12.35 ± 1.12***	9.76 ± 0.94***	6.45 ± 0.62***
AD	14.43 ± 1.08	0.34 ± 0.05###	9.25 ± 0.84***	4.70 ± 0.55	5.7 ± 0.81**	7.48 ± 1.26***
IN	51.97 ± 3.35	131.1 ± 5.88###	0.4 ± 0.06***	55.63 ± 4.14***	54.45 ± 2.86***	42.13 ± 2.6***
HX	28.37 ± 3.04	14.96 ±	0.28 ± 0.05**	22.08 ± 1.71	24.43 ± 0.68	26.5 ± 2.2*

		1.82#				

3.15 Effect of 678TMC on the Level of Vitamin C and Neurotransmitters in Striatum:

Striatal serotonin augmented after the development of neuropathic pain i.e., after administration of Vincristine. 678TMC has significantly repressed the serotonin levels at all three doses and gabapentin (Table 3).

Table 3. Striatal levels of vitamin C (VC), serotonin (5-HT), noradrenaline (NA), dopamine (DA), adenosine (AD), inosine (IN), and hypoxanthine (HX) after the vincristine treatment protocol (VCR) and subsequent modification by 678TMC or gabapentin. Significance of differences from the VCR group: *p<0.05, **p<0.01 and ***p<0.001

	Vehicle 10 ml/kg	**VCR 0.1 mg/kg**	**VCR+GBP 75 mg/kg**	**VCR+678TMC 10 mg/kg**	**VCR+678TMC 15 mg/kg**	**VCR+678TMC 20 mg/kg**
VC	2.45 ± 0.27	1.24 ± 0.18	2.13 ± 0.24	1.45 ± 0.17	0.26 ± 0.04	0.8 ± 0.12
5HT	3.48 ± 0.54	14.33 ± 1.35###	2.15 ± 0.46***	3.62 ± 0.48***	2.9 ± 0.36***	2.8 ± 0.3***
NA	0.8 ± 0.07	0.36 ± 0.04	1.73 ± 0.23	1.33 ± 0.19	1.34 ± 0.21	0.6 ± 0.07
DA	27.1 ± 2.12	25.67 ± 2.66	28.38 ± 3.2	7.58 ± 1.48*	5.6 ± 0.58**	5.46 ± 0.84**
AD	3.78 ± 0.34	1.93 ± 0.31	72.03 ± 4.39***	4.48 ± 0.68	1.86 ± 0.27	2.3 ± 0.44
IN	15.53± 2.02	4.24 ± 0.78#	14.83 ± 1.61*	15.33 ± 2.64*	21.65 ± 2.78**	22.26 ± 3.12**
HX	9.38 ± 1.55	8.58 ± 1.12	6.57 ± 1.16	12.9 ± 0.85	9.62 ± 1.43	9.82 ± 1.61

4 Discussion:

Vincristine-induced peripheral neuropathic pain (VIPNP) has been extensively studied, and diverse cellular and molecular targets have been explored for their potential to ameliorate VIPNP. Testing of various natural and synthetic compounds, primarily those with antioxidant and anti-inflammatory potential in the attenuation of VIPNP, has been performed using various mechanisms (X.-J. Chen, Wang, & Song, 2020; Fouda, et al., 2023; A. Khan et al., 2021; Khan et al., 2019; Khodaei et al.,

2022; Lee et al., 2020; Starobova et al., 2019; Zhou et al., 2018; Zhou et al., 2020; Zhu et al., 2021).

In the present study, vincristine administration caused a significant decrease in tail withdrawal latency when assessed on days 7 – 14 supported by the other studies (Amirkhanloo et al., 2020; Khan et al., 2019; Kim et al., 2021). The suggested mechanisms of VIPNP include swelling of mitochondria in the axons intracellularly, leading to the release of calcium ions and apoptosis; raised levels of substance P in the spinal cord (Chiba et al., 2022); involvement of altered adenosine signaling in pain transmission (Zhou et al., 2022); increased release of substances (TNF-α, that is, Tumor Necrosis Factor-α, Interleukin-1 [IL-1], nitric oxide [NO] and Interleukin-6 [IL-6]) in macrophages, glial cells and Langerhans cells; down-regulation of IL-10 in spinal dorsal horns; increased nitric oxide synthase (NOS) in the dorsal horn of the spinal cord, increased 5-Hydroxytryptamine (5-HT) 2A subtype receptors in the dorsal horn and dorsal root ganglion (DRG) neurons; increased reactive oxygen species (ROS), which are responsible for affecting the activity of serine protease and decrease endorphins in the Spinal Cord and DRG; and increased serine proteinase that inactivates endorphins (Addington & Freimer, 2016; Thibault et al., 2008). The administration of 678TMC reversed the decrease in tail withdrawal latency time in seconds caused by vincristine, as also reported in other studies of vincristine, where other test drugs were applied (Akbar et al., 2020; Khan et al., 2019b; Kim et al., 2021). The positive control, gabapentin, showed robust effects against thermal hyperalgesia, which is supported by several studies in rats and mice at different doses (Khan et al., 2021; Khan et al., 2022; Singh et al., 2019). The reported mechanisms of action of gabapentin of inhibiting VIPNP include calcium channels inhibition by binding to α2δ- 1 subunit, inhibition the release of neurotransmitters including substance-P, Glutamate and Calcitonin-Gene-Related-Peptide (CGRP), reducing the inflammation (by decreasing the astrocytes number and inhibiting activation of microglia), Inhibition of NMDA receptors, decreasing Protein Kinase-C's over expression, Inhibiting the anterograde trafficking, decrease in the trafficking of complexes of β4abound Cav2.1 in plasma membrane, activation of pain inhibitory pathway (descending) and decrease in the TRPV1 channels expression (Kukkar et al., 2013; Patel & Dickenson, 2016).

Vincristine administration resulted a significant decrease in the readings of paw withdrawal threshold in comparison to that control group on days 7 – 14, as also

reported in other studies in mice (Khan et al., 2021; Nawaz et al., 2018; Sahranavard et al., 2022) and rats (Kim et al., 2020; Singh et al., 2019), and VIPNP is associated with an increased expression of Cyclooxygenase-2 (Cox-2), TNF-α,Tumor Necrosis Factor-α, Interlukin-1 β and NF-κB (nuclear factor-kappa B) (Ali et al., 2022) involving the Aβ fibers and C-fibers (Ameyaw et al., 2014; Nagi et al., 2011). Administration of 678TMC reversed the decrease in paw withdrawal threshold caused by vincristine, as observed in other studies on Cisplatin and Vincristine, where other test drugs were applied (Akbar et al., 2020; Lee et al., 2020; Kim et al., 2020). The positive control, gabapentin, caused robust effects against paw withdrawal threshold, which is supported by several studies in rats and mice at different doses (Akbar et al., 2020; Ali et al., 2022; Basit et al., 2022; Jain et al., 2022; Khan et al., 2021). Vincristine-induced mechanical allodynia, dynamic (VIMAD) was successfully developed by decreasing time of withdrawal latency on days 7 – 14 of VCR administration compared to the control group when the plantar surface of the hind paw of the animal was stroked lightly, which is supported by other studies (Field et al., 1999; J. Khan et al., 2019b). Administration of 678TMC reversed the decrease in the withdrawal latency induced by vincristine, as observed in other studies where other test drugs were applied (Akbar et al., 2020; Vashistha et al., 2017). The positive control, gabapentin, showed robust effects against VIMAD, which is supported by a previous study (Khan et al., 2019b). Vincristine-induced cold allodynia (VICA) was successfully developed by increasing the intensity of the response of animals to cold stimulus compared to the control group, which is augmented by other studies (Ali et al., 2022; Fouda et al., 2023). Administration of 678TMC reversed the increase in the intensity of the response of animals caused by vincristine, which was also observed in another study where coenzyme Q10 was used as the test drug (Elshamy et al., 2022). The positive control, gabapentin, caused robust effects against VICA, which is supported by a previous study (Forouzanfar et al., 2023).

It has been reported that several compounds with carbonic anhydrase inhibitory potential have attenuated neuropathic pain (Akgül et al., 2022; Carta et al., 2015). An injury of the peripheral nerves reduce potassium chloride co-transporter (Neuron specific) which affect the spinal GABA-ergic networks negatively, resulting in allodynia. It is known that carbonic anhydrase inhibitors decrease depolarization of GABA receptors, so they produce analgesia (Supuran, 2018). It is evident from previous study that 678TMC is a strong inhibitor of carbonic anhydrase (K_i 0.0097

micromole) (Davis et al., 2013) thereby suggesting its possible mechanism responsible for amelioration of VIPNP. The acetic acid initiates a response in animals characterized by continuous multiple constriction in the abdomen, the whole-body movement and repressed motor response collectively known as writhing. The effect of 678TMC on the writhing's induced by acetic acid was evaluated using diclofenac sodium as standard drug for assessing the tonic nociception. 678TMC repressed the writhing significantly which implies that 678TMC has got peripheral antinociceptive potential (Nawaz et al., 2018).

In the frontal cortex, vincristine significantly increased dopamine concentrations in the current study. It has been demonstrated that dopamine levels in the frontal cortex significantly decrease after neuropathic pain develops due to CCI (chronic constriction injury) (Kang et al., 2023) without changing the levels of 5HT while restraint stress increases dopamine in frontal cortex. The concentration of dopamine in the frontal cortex was decreased by 678TMC after vincristine treatment, whereas the positive control, gabapentin, caused a further increase in its concentration. Moreover, increased activation of the serotonergic system in the CCI model of neuropathic pain has been previously demonstrated (Palazzo et al., 2006), which is consistent with the current study in which the positive control and 678TMC significantly reversed the increase in the level of 5HT after development of VIPNP. In another study, 5HT-2A knockout mice did not develop neuropathic pain with vincristine (Thibault et al., 2008), and the frontal cortex has been reported to play a role in pain processing (Metz et al., 2009). To the best of our knowledge, no study has quantified the 5HT levels in the frontal cortex after the development of vincristine-induced neuropathic pain. Administration of an inhibitor of serotonin synthesis (para chlorophenyl methyl ester HCl) also increased analgesic action, which is consistent with our findings (Khanna and Bhatia, 2003). The effect of VIPNP on the concentration of Vitamin C in the frontal cortex in VIPNP is not yet been studied. VIPNP was diminished by the intrathecal administration of l-ascorbic acid(Li et al., 2021). Our results showed that the concentration of vitamin C was significantly repressed by vincristine in the frontal cortex compared to the control group, and 678TMC significantly reversed the decrease caused by vincristine, whereas the positive control did not. The plasma concentration of vitamin c has been demonstrated to be lower in patients with postherpetic neuralgia (Chen et al., 2009; Wang et al., 2020), which supports the results of this study. Lower vitamin C in the frontal cortex may lead to increased

reactive oxygen species and, hence, increased neuropathic pain. However, the effect of norepinephrine concentration in the frontal cortex on VIPNP has not yet been studied. However, SSRIs and SNRIs have shown significant effects on attenuating VIPNP (Katsuyama et al., 2014). The concentration of norepinephrine slightly increased (non-significantly) in the frontal cortex after vincristine which was reversed by 678TMC non-significantly. The concentration of Adenosine in frontal cortex was repressed significantly after development of neuropathic pain i.e., after administration of Vincristine 0.1 mg/kg IP and was recovered 678TMC at 20 mg/kg but not by standard drug suggesting that the mechanism of 678TMC in attenuating VNIP is different from that of gabapentin. It has already been shown that inhibition of adenosine kinase and the resultant increase in the level of adenosine significantly relieves neuropathic pain (Little et al., 2015) which strongly supports our findings. Moreover, activation of A1 receptors has shown significant effects against VIPNP (Kim et al., 2020). Similarly, activation of A1, A2A, and A3 receptors inhibits neuropathic pain (Zhou et al., 2022). This study is the first to report a decrease in adenosine levels in the frontal cortex after VIPNP development. No study has been found till date regarding the effects of Vincristine on the metabolites of adenosine in frontal cortex. The level of the metabolite of adenosine, hypoxanthine, in the frontal cortex was increased after the development of neuropathic pain, that is, after administration of Vincristine 0.1 mg/kg IP and was decreased to 678TMC but not by gabapentin; once again suggesting the difference of mechanism of action. The increase in its level by vincristine may be due to increased catabolism of adenosine in the frontal cortex, which was inhibited by 678TMC. No significant changes were observed in inosine levels in the current study.

In the current study, upsurge the levels of vitamin C, dopamine, serotonin and norepinephrine in the hippocampus was observed after development of VIPNP and was repressed by 678TMC with effects comparable to that of gabapentin. It has been demonstrated that the Hippocampus is involved in pain processing and abnormalities in it are associated with chronic pain (Mokhtari et al., 2019). However, the effect of VIPNP on the concentration of vitamin C in the hippocampus in VIPNP is not yet been studied. Moreover, the inhibition of dopamine receptors (D1 and D2) in the hippocampus inhibits analgesia (Rezaee et al., 2020), supporting the current findings. Decreased levels of 5HT in the hippocampus were observed in the CCI model (Jiang et al., 2018), which is not consistent with the current study and may be due to

differences in its mechanism. In another study, VCR failed to develop neuropathic pain in the 5HT-2A knockout (Thibault et al., 2008).

5-hydroxytryptamine type 3 receptors have been shown to have role in the potent analgesia caused by tramadol. Additionally ondansetron a 5-hydroxytryptamine type 3 receptor antagonists modulate Opioid induced hyperalgesia (Liang et al., 2011), and inhibits tramadol induced analgesia (Arcioni et al., 2002). In our study, the administration of ondansetron reversed the effects of 678TMC on VIPNP, implying the role of the 5-hydroxytryptamine type 3 receptors in the antinociceptive effect of 678TMC. Multiple anticancer drugs modify 5-hydroxytryptamine type 3 receptors; however, vincristine has no such effect (Nakamura et al., 2018). 5-HT3 receptors also mediate formalin-induced hyperalgesia and allodynia (Bravo et al., 2012). Opioid receptors have been shown to be involved in the analgesic effect of many drugs (Li et al., 2023) including tramadol and morphine in attenuating neuropathic pain (Omara et al., 2023). Almost all the analgesic drugs are having low efficacy in managing neuropathic pain while the opioids essentially provide strong relief against Neuropathic pain in in moderate and severe cases although its relief is for short time (Nafziger & Barkin, 2018; Smith, 2012). Moreover, naloxone, an opioid receptors antagonist results in strong analgesia (Sawynok et al., 1979). In our study, the administration of naloxone reversed the effects of 678TMC on VIPNP, implying the role of opioid receptors in the antinociceptive effect of 678TMC.

To the best of our knowledge, no study has quantified 5HT and norepinephrine levels in the hippocampus after the development of VIPNP. The concentration of Adenosine in Hippocampus was significantly decreased by vincristine, significantly increased by standard drugs and 678TMC at higher doses. It has already been shown that inhibition of adenosine kinase and the resultant increase in the level of adenosine significantly relieves neuropathic pain (Little et al., 2015), which supports our findings. This study is the first to report a decrease in adenosine levels in the hippocampus after the development of VIPNP. The level of the metabolite of adenosine, Inosine, in Hippocampus was increased after development of VIPNP and was repressed by gabapentin and 678TMC which supports our findings because the levels of adenosine are opposite to it. Repression in hypoxanthine level was observed after development of VIPNP which was recovered by 678TMC at the highest dose while the standard drug has lowered it further suggesting that 678TMC is better as compared to standard drug with respect to its effects on the neurotransmitters and its metabolite. No study

has been found till date regarding the effects of Vincristine on the two metabolites of adenosine (Inosine and hypoxanthine) in Hippocampus.

In Striatum, no significant alteration in the level of vitamin C, dopamine and nor epinephrine was observed after the development of VIPNP in the current study while the level of 5HT was increased which was repressed by 678TMC with comparable effects to that of the gabapentin. Moreover, ondansetron reversed VIPNP in the mechanistic experiment in our current study, which supports our findings, and 678TMC acts by targeting the serotonergic system. To the best of our knowledge, no study has been conducted on the quantification of 5HT levels in the striatum after development of VIPNP. It has been shown in another study that there is no significant effect of Vincristine on the level of norepinephrine in brainstem and telencephalon (Cheney et al., 1973). No significant alterations were observed on the level of adenosine and its metabolites Inosine and hypoxanthine in Striatum after the development of VIPNP.

The current study revealed that the upsurge in the level of TNF-α in plasma caused by VCR was significantly repressed by 678TMC at the highest dose and gabapentin. An increase in the level of TNF-α after the development of VIPNP has been reported in another study (Zhou et al., 2018), supporting our results.

Conclusion: 678TMC attenuates VIPNP and is a potential candidate for further studies. 678TMC acts by targeting the 5-HT3 receptors and opioidergic system to attenuate VIPNP. 678TMC at the highest dose significantly lowered TNF-α levels in blood. Our results are the first to report changes in the levels of neurotransmitters in the frontal cortex, striatum, and hippocampus after VIPNP development. Interestingly, in reversing the changes in the levels of some neurotransmitters in the brain caused by Vincristine, 678TMC showed stronger effects than the positive control.

Limitations of the Study: More specific receptor studies, especially with 5-HT3 or opioid knock-out animals, in addition to an evaluation of possible activity of 678TMC in modifying other inflammatory mediators at higher 678TMC doses would have been beneficial. Furthermore, 678TMC could be screened at other targets or receptors for treating vincristine induced peripheral neuropathic pain. Addressing these limitations is warranted in further investigative studies for the future.

Conflict of interest:

The authors declare no competing interest.

Funding:

Any agency did not fund the current research work.

Submission declaration:

The work described above has not been published formerly and it is not under consideration for publication somewhere else. All authors approved the publication and if accepted in this journal, will not be published elsewhere.

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