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EFFECTS OF SELECTIVE GABA-A AGONISTS MODULATOR IN RATS WITH NEUROPATHIC PAIN

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Abstract:

Neuropathic pain is the discomfort brought on by a condition that affects the sensory nerves. Dysesthesia and allodynia can both be brought on by damaged nerves. Zopiclone is a nonbenzodiazepine, regulating the GABA receptor, thus relaxes the nerves and brain. The role of glycinergic and γ-aminobutyric acid (GABA)ergic neurons in this process has been widely described. Benzodiazepine-sensitive $GABA_A$ receptors contain at least one of the following α subunits α1, α2, α3 or α5. In animals, α1-sparing (non-sedative) agonists showed an antihyperalgesic activity in inflammatory and neuropathic pain models without losing efficacy after repeated treatment. In the current investigation, zopiclone decreased allodynia and hyperalgesia brought on by models for Disease-Induced Neuropathy Pain (DINP), Paclitaxel-Induced Neuropathic Pain (PINP), and Chronic Constriction Injury (CCI). Its effect was dose-dependent, and one of its mechanisms of action was probably the sequential activation of spinal neurons at the supraspinal site of action. The current study further supports the likelihood that zopiclone will be helpful in the management of neuropathic pain, even if additional preclinical and clinical investigations are unquestionably necessary.

Introduction

Neuropathic pain is the discomfort brought on by a condition that affects the sensory nerves. Dysesthesia (abnormal or altered sensations) and allodynia (pain from stimuli that don't typically induce pain) can both be brought on by damaged nerves. The pain could be constant or sporadic. It may feel like a burning, stinging, tingling, or prickling pain (1)(2). Available treatments essentially provide only symptomatic relief and may include nonpharmacological, pharmacological, and interventional therapies. Most extensive evidence is available for pharmacological treatment, and currently recommended first-line treatments include antidepressants (tricyclic agents and serotonin-norepinephrine reuptake inhibitors) and anticonvulsants (gabapentin and pregabalin) (3,4).

Current treatments are usually dispensed without precision, and calcium-channel-acting modulators (pregabalin, gabapentin), tricyclic antidepressants, and serotonin-noradrenalin reuptake inhibitors (duloxetine, venlafaxine) represent first-line treatment options for neuropathic pain (5,6). Glutamate mediates its effects via ionotropic and metabotropic glutamate (mGlu) receptors. mGlu receptors are G protein-coupled receptors that are classified into three clusters,

group I-III. It is well established that glutamate is a critical neurotransmitter in peripheral and central pain signaling pathways. mGlu5 receptors are expressed throughout these pathways from the skin, spinal cord, dorsal horn neurons and the spino-thalamic tract and are, therefore, strategically located to modulate pain signaling at distinct levels of the nervous system (7).

As a nonbenzodiazepine that controls the GABA receptor, zopiclone calms the nerves and the brain. Glycinergic and GABAergic neurons have been extensively documented as playing a part in this process (8,9). At least one of the following subunits—1, 2, 3, or 5—as well as two subunits and a 2 subunit are present in benzodiazepine-sensitive GABAA receptors in a 2:2:1 stoichiometry (10,11). GABAA receptors with 1 subunit were discovered to have anxiolytic capabilities (12), but 2- and 3 subunit-containing GABAA receptors were found to be substantially in charge of the spinal antihyperalgesic effects (13,14). (15).

In experimental model, α1-sparing (non-sedative) in inflammatory and neuropathic pain models, BDZ agonists demonstrated antihyperalgesic effects without diminishing effectiveness following repeated administration (15,16) . Such compounds are under clinical development but are not yet available for use in human beings (17). Clobazam is a 1–5 BZD prescribed in all forms of anxiety and in epilepsy. It seems to exert less cognitive and psychomotor side effects compared with clonazepam and lorazepam in a wide range of pharmacodynamic tests in man (18,19). Therefore, Zopiclone is a nonbenzodiazepine may be a suitable compound to test the antihyperalgesic effect of $GABA_A$ agonists in exploratory pain studies in human beings. Although an antihyperalgesic action of Zopiclone is a nonbenzodiazepine in rats is likely, it has not been proven so far. In a set of experiments, we therefore investigated the antihyperalgesic and sedative effects of Zopiclone is a nonbenzodiazepine in a neuropathic pain model in rats and correlated this to its pharmacodynamic activity properties with Pregabalin.

2. Materials and Methods

The trials were carried out on 25 Wistar rats (150–250 g). The tests were conducted according to the guidelines established by the institution's animal welfare committees. The animal housing procedure was carried out at 23 2 °C, 50 1% relative humidity, and 23 2 °C, with a 12-12 h light-dark cycle. At the School of Pharmacy, Bharat Institutional of Technology Meerut, studies involving animals were approved by the animal care committee and met CPCSEA requirements. Rats underwent a 3-day adaption period before to the test. All investigators were kept secret from the randomization and treatments. Three males and three females out of a total of six animals were distributed evenly among the two groups.

2.1. Drugs

Zopiclone or Vehicle suspended in 0.5% methyl cellulose and 0.9% NaCl and administered orally in a total volume of 5ml/Kg. Doses of 5,10 and 20 mg/kg were tested.

2.2. Neuropathic pain

2.2.1. Chronic Constriction Injury (CCI) model

The CCI surgery was carried out as reported earlier in order to produce NP models. All operations were performed in a clean setting. Sodium pentobarbital (50 mg/kg) was used to anaesthetize the

rats before the left sciatic nerve was loosely ligated in four places with 4-0 chromic gut sutures. Sham surgery comprised a similar technique without closure of the sciatic nerve.
Rats aged 7 to 8 weeks were subjected to the chronic constriction injury (CCI) model. A unilateral CCI to the left sciatic nerve close to the trifurcation was carried out. By bluntly cutting through the biceps femoris, the sciatic nerve was exposed at the mid-thigh level close to the sciatic trifurcation. Three chromic gut ligatures were loosely tied around the nerve with roughly 1 mm of spacing after 5 to 7 mm of the nerve had been cleared of adherent tissue. The ligatures were tightened until the hindlimb twitched momentarily. Layers were used to seal the incision. These rats' postoperative behaviour suggested that they experienced hyperalgesia, allodynia, and potentially even spontaneous pain (or dysesthesia) (20).

2.2.2. Paclitaxel-induced neuropathic pain (PINP)

Low doses of paclitaxel (1 or 2 mg/kg i.p.) have been shown to evoke pain syndrome in an experimental model of neuropathy without causing systemic toxicity or motor impairment in mice. A peripheral neuropathy characterised by long-lasting tactile (mechanical) allodynia, endoneural edoema of the sciatic nerve, and cold allodynia has been observed following paclitaxel administration on four alternate days (days 0, 2, 4, and 6; with a cumulative dose of 4 or 8 mg/kg). On the fifth day of paclitaxel treatment, changes in pain thresholds have been seen, and they endure for about 3 weeks after the last dose (21–23). Rats treated with vincristine and paclitaxel exhibit strong mechanical and cold hypersensitivity but little to no heat hyperalgesia.

2.2.3. Disease-induced neuropathy models

In rat model of subcutaneous STZ-induced diabetes, hyperalgesia and hyper-responsivity of C-fibers develop during a period of approximately 2–3 weeksv (24,25). significant degree of hyperalgesia develops in mice after 4 weeks of single dose STZ (200 mg/kg) administration.

2.2.4. Mechanical sensitization

We measured mechanical sensitization before and seven days after surgery. The mechanical sensitivity of von-Frey filaments was examined. Paw withdrawal thresholds (PWTs) were averaged over five or four assessments for each time point. Depending on the amount of pressure used, the system may measure, store, and display the test readings in grammes. PWT measurements were taken alternately on the injured paw and the uninjured paw.
Mechanical sensitization was tested for 4 hours following oral administration of zopiclone (5, 10, or 20 mg/kg) or a vehicle (n = 6 rats/dose). Rats were secrifized immediately following the behavioural testing to evaluate the brain concentration of zopiclone. After being dissected, brains were frozen at -20°C for later processing.

2.2.5. von Frey Test

It is usual practise to measure mechanical allodynia in rodents using the von Frey test (26). Each animal in this study underwent the von Frey test seven days after CCI. Rats were put in a plastic box with a mesh bottom and given 30 minutes to get used to it. The middle plantar surface of the paw was then subjected to a series of tactile stimuli consisting of von Frey filaments (0.4, 0.6, 1,

2, 4, 6, 8, and 15 g), beginning with the 2-g filament. The "up-down method" was used, and the withdrawal threshold was computed as 50% withdrawal threshold (g)=(10^{Xf+kd})/10,000, where Xf is the value of the von Frey stimulus last applied (in log units), k is a tabular value for the response pattern, and d is the distance between consecutive filaments applied (in log units) (26). For the following studies, only animals meeting the requirements for mechanical allodynia (50% withdrawal threshold <4g) were chosen. Animals were divided into groups so that the average body weight on Day 7 and the 50% withdrawal threshold were the same for each group. The von Frey test was performed both before and two hours after the medication was administered.

3. Results & Discussion

3.1. Zopiclone Produces Antiallodynic Effect in Neuropathic Pain Evoked by PINP

The Randall-Selitto test revealed that oral Zopiclone had a stronger antiallodynic effect than pregabalin in PINP-induced mechanical allodynia as evidenced by a decline in rat paw pressure thresholds (PPTs). Neuropathic pain frequently exhibits the sign of mechanical allodynia (16). Zopiclone in oral doses of 25, 50, and 100 mg/kg restored the developed mechanical allodynia in the tested time points namely 60, 120, and 180 min after treatment (Figure 1). Only at higher tested doses (50 and 100 mg/kg) did pregabalin, used as a positive control, have a consistent antiallodynic effect that persisted for 180 minutes (*Figure 1and 2*). However, rats given the vehicle showed mechanical allodynia, as evidenced by a substantial reduction in the PPT of operated paws compared to unoperated ones (Figure 1and 2). The dosages of these drug used in this study had no significant effects on the withdrawal threshold and did not elicit abnormal behavior in rats.

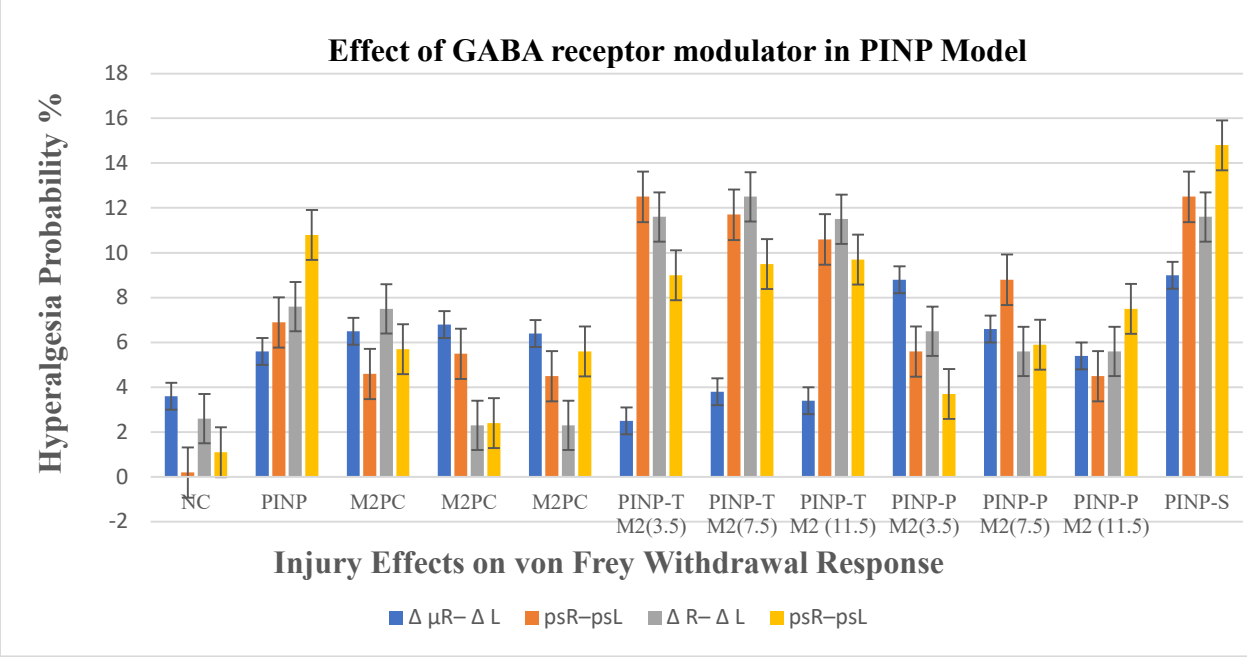

Fig:1 Effects of Zopiclone on paw withdrawal latency (PWL) and paw withdrawal threshold (PWT) Mechanical allodynia evoked by Paclitaxel-Induced Neuropathic Pain (PINP) in rats.

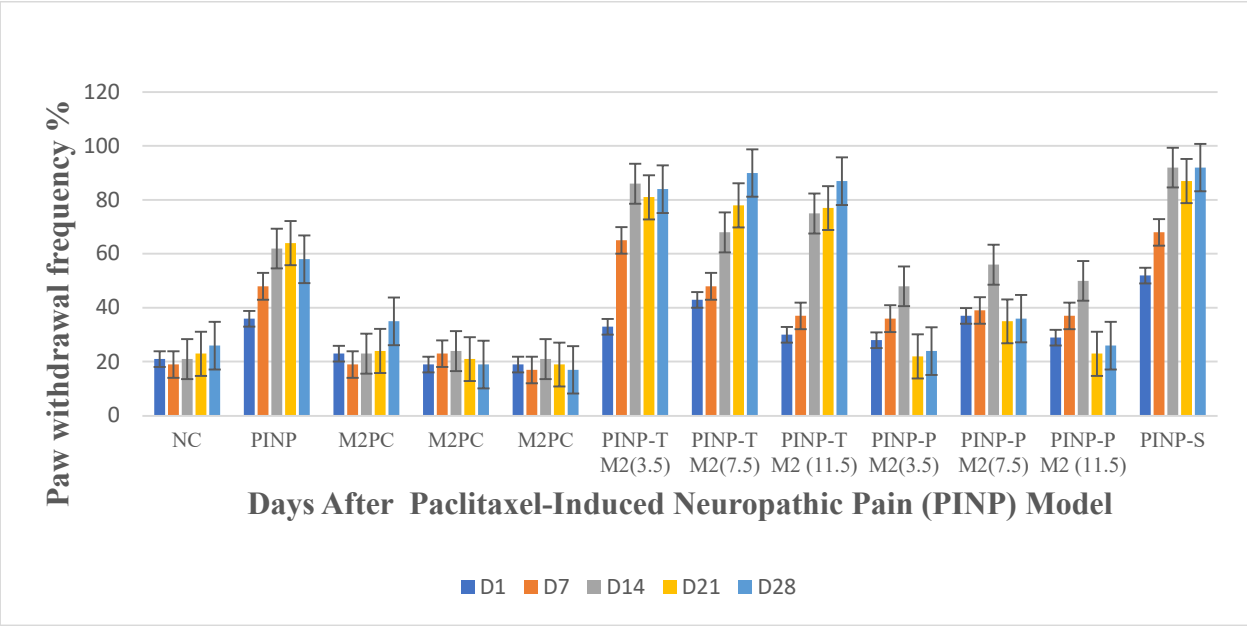

Fig:2 Effects of Zopiclone on paw withdrawal latency (PWL) and paw withdrawal threshold (PWT) Von Frey evoked by Paclitaxel-Induced Neuropathic Pain (PINP) in rats.

3.2. Zopiclone Produces Antiallodynic Effect in Neuropathic Pain Evoked by CCI

Although the difference between 7.5 and 11.5 was not statistically significant, zopiclone increased withdrawal threshold and withdrawal latency at doses between 3.5, 7.5, and 11.5 and the impact was dose-dependent in *Figures 3 and 4*. The maximum effect of zopiclone on withdrawal threshold and withdrawal latency was only partially effective in bringing the threshold back to the levels seen in the sham operation. The antiallodynic effect of p.o. administration of zopiclone at 3.5, 7.5, and 11.5 was attenuated by $GABA_A$ receptor modulator Figures 3 and 4. Both the withdrawal threshold and aberrant behaviour in rats were unaffected by the dosages of these modulator utilised in this investigation.

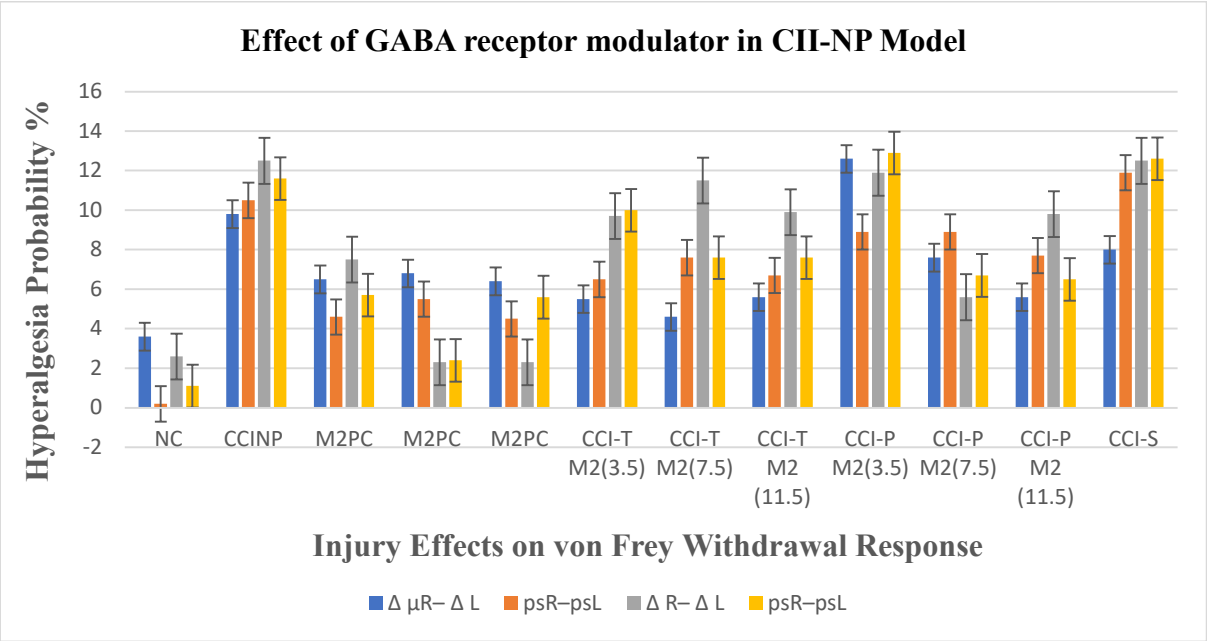

Fig. 3: Effects of Zopiclone on paw withdrawal latency (PWL) and paw withdrawal threshold (PWT) Von Frey evoked by chronic constriction injury (CCI) in rats.

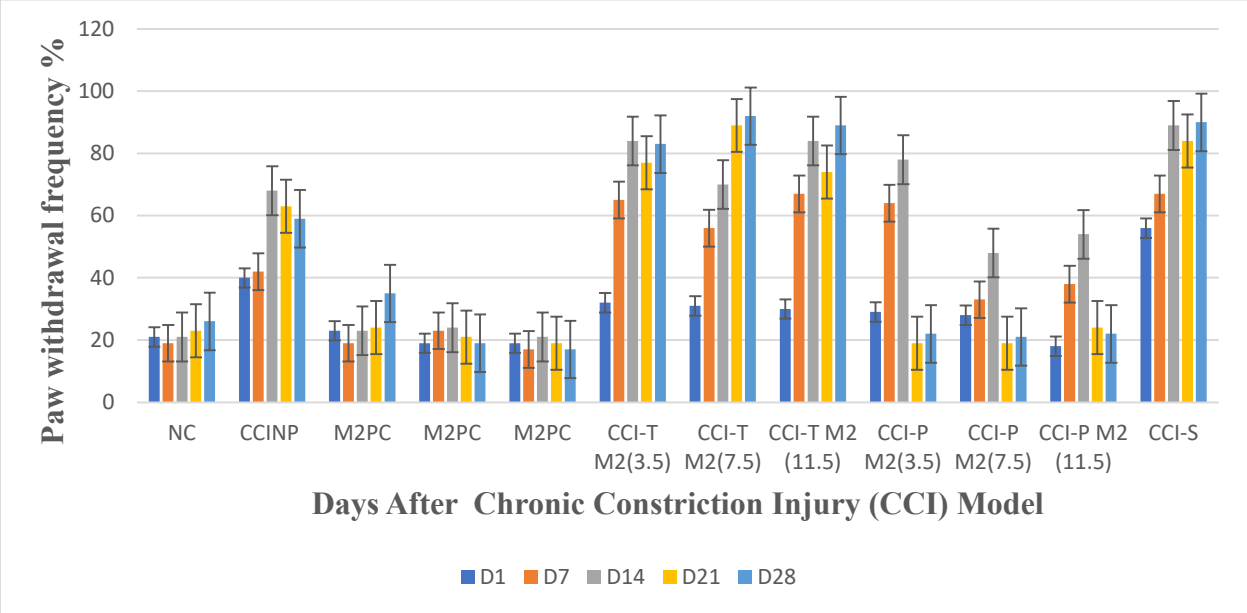

Fig. 4: Effects of Zopiclone on paw withdrawal latency (PWL) and paw withdrawal threshold (PWT) Mechanical allodynia evoked by chronic constriction injury (CCI) Pain in rats.

3.3. Zopiclone Produces Antiallodynic Effect in Neuropathic Pain Evoked by DINP

All rats entered into this study displayed a significant decrease in the magnitude of the mechanical stimulus (in the range of 1.0–3.0 g) necessary to evoke a brisk withdrawal response in the injured hind paw in response to von Frey hair stimulation. Antiallodynic effect of GABA agonists, The time course of the increase in threshold produced by oral drug administration illustrated in *Fig. 5 and 6*. Threshold was maximally increased and then gradually decreased to control over a 2–5-h period, depending upon the dose. A somewhat shorter anti-allodynic time course was observed after the effective doses 3.5, 7.5, and 11.5, as shown in Fig. 5and 6.

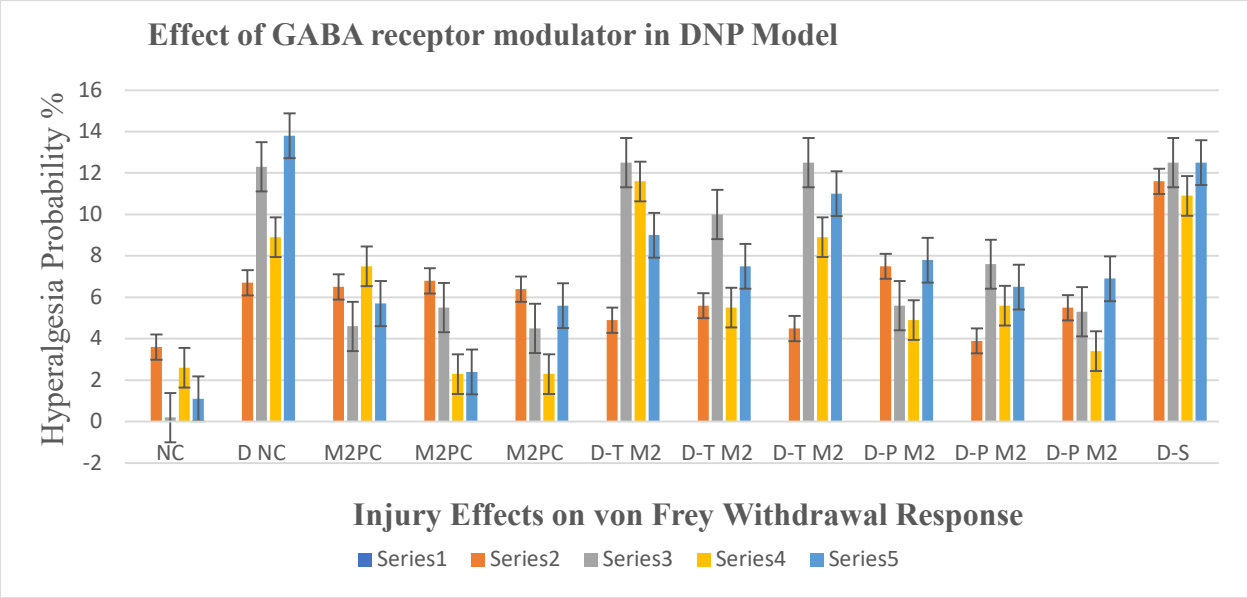

Fig. 5: Effects of Zopiclone on paw withdrawal latency (PWL) and paw withdrawal threshold (PWT) Von Frey evoked by Disease-Induced Neuropathic Pain in rats.

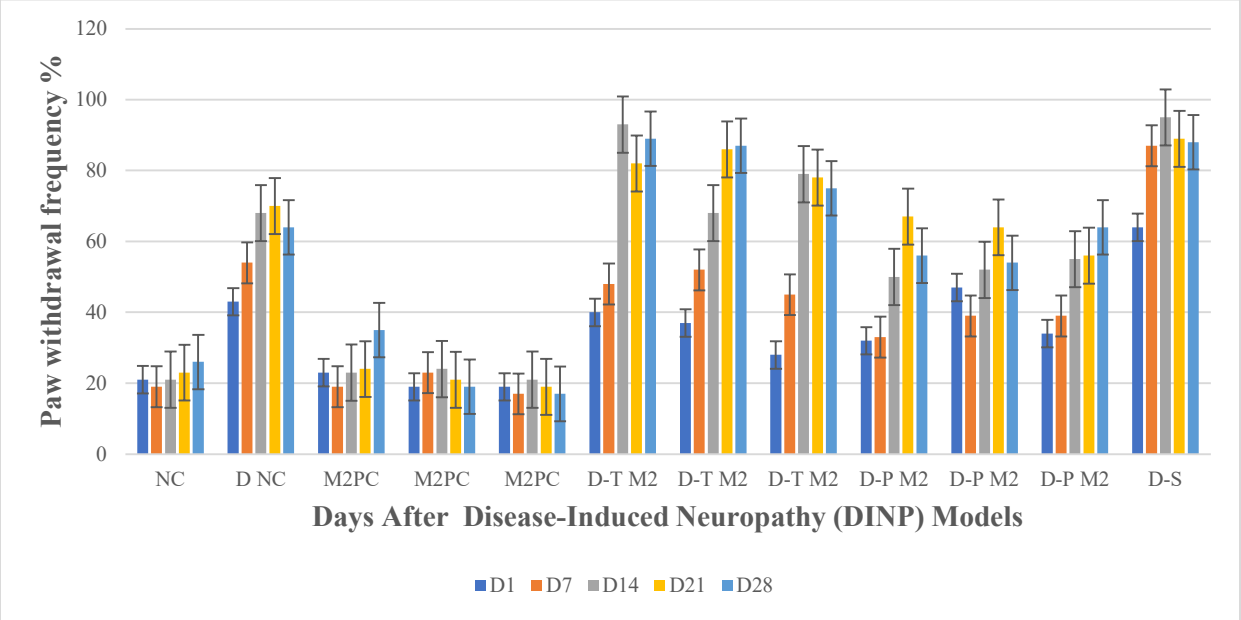

Fig. 6: Effects of Zopiclone on paw withdrawal latency (PWL) and paw withdrawal threshold (PWT) Mechanical allodynia evoked by Disease-Induced Neuropathic Pain in rats.

In this study, we demonstrated the antiallodynic efficacy of pregabalin in combination with other teat drug, namely Zopiclone regulating the GABA receptor, in the rat CCI, PINP& Disease induced neuropathic pain model. The antiallodynic effect was most prominent of zopiclone, when pregabalin was slandered drug analysis of this effects. Consistent with the findings reported in the literature regarding the use of pregabalin (anticonvulsant), Zopiclone (regulating the GABA receptor), for neuropathic pain, the results from our single-drug experiment indicated antiallodynic effects of Zopiclone of these drugs. The von Frey test showed reduced mechanical allodynia in most subgroups.

4. Conclusions

This study showed that, when compared to pregabalin, the effects of zopiclone may be a viable neuropathic pain treatment. With this combination method, it is possible to get the same therapeutic benefit as high-dose monotherapy while utilising lower dosages of each component medicine and perhaps with fewer side effects. In the present study, Zopiclone inhibited both allodynia and hyperalgesia induced by Chronic Constriction Injury (CCI) model, Paclitaxel-induced neuropathic pain (PINP) and Disease-Induced Neuropathy Models. Its effect was dose-dependent and probably involved the supraspinal site of action and the sequential activation of spinal neurons as one of its mechanisms of action. Although further preclinical and clinical studies are clearly required, the present study further reinforces the probable usefulness of zopiclone in the treatment of neuropathic pain.

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