Original Article

Ameliorative Potential Of Cyclopyrrolones (Zopiclone) On Gaba$_a$ Receptor In Neuropathic Pain

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DOI: 10.47750/pnr.2023.14.S02.82

Abstract

Cyclopyrrolone (Zopiclone) is a nonbenzodiazepine, regulating the GABAA 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. Animal studies using inflammatory and neuropathic pain models revealed that α1-sparing (non-sedative) agonists have antihyperalgesic activity without diminishing effectiveness over time. 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. In the current investigation, Cyclopyrrolones (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 Cyclopyrrolones (Zopiclone) will be helpful in the management of neuropathic pain, even though further preclinical and clinical research is clearly required.

Key words: Zopiclone, $GABA_A$ receptor, Neuropathic Pain

Introduction

Dysesthesia (abnormal or altered sensations) and allodynia (pain from stimuli that don't typically induce pain) can both be brought on by damaged nerves. Neuropathic pain is the discomfort brought on by a condition that affects the sensory nerves. The pain could be constant or sporadic. It may feel like a burning, stinging, tingling, or prickling pain (1)(2)**.** The available therapy, which can include pharmacologic and nonpharmacologic, pharmacological, and invasive treatments, generally only offer symptomatic treatment. The pharmacological approach has the most comprehensive body of research, and it is currently advised to use antidepressants (tricyclics and serotonin-norepinephrine reuptake inhibitors) and anticonvulsants (gabapentin and pregabalin) as first-line therapy (3,4).

Pregabalin, gabapentin, tricyclic antidepressants, and serotonin-norepinephrine reuptake inhibitors (duloxetine, venlafaxine), which are considered first-line therapies for nerve pain, are calcium-channel-acting modulators (5,6). Ionotropic and metabotropic glutamate (mGlu) receptor are the means through which glutamate mediates its actions. G protein-coupled receptors, or mGlu receptors, are classified into 3 clusters, one through three. 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 GABAA receptor, Cyclopyrrolones (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 α1, α2, α3 or α5—as well as two subunits and a α2 subunit are present in benzodiazepine-sensitive $GABA_A$ receptors in a 2:2:1 stoichiometry (10,11). $GABA_A$ receptors with α1 subunit were discovered to have anxiolytic capabilities (12), but α2 and α3 subunit-containing $GABA_A$ 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). Although these substances are in biomedical studies, they have not yet been recognized for use in humans (17). Clobazam is a 1–5 BZD that is prescribed for seizures and now all types of anxiety. In a variety of pharmacological studies conducted on men, it appears to have fewer psychomotor and cognitive negative effects compared to clonazepam and lorazepam (18,19). Accordingly, Cyclopyrrolones (Zopiclone) is a nonbenzodiazepine may be a suitable drug to investigate the antihyperalgesic effects of $GABA_A$ agonists in preliminary human pain studies.Although an antihyperalgesic action of Cyclopyrrolones (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 Cyclopyrrolones (Zopiclone) is a nonbenzodiazepine in a neuropathic pain model in rats and correlated this to its pharmacodynamic activity effects 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

Cyclopyrrolones (Zopiclone) or Vehicle supplied orally in a total volume of 5ml/Kg, suspended in 0.5% methyl cellulose and 0.9% sodium chloride. 5, 10, and 20 mg/kg doses was examined.

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 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 Cyclopyrrolones (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 Cyclopyrrolones (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. Cyclopyrrolones (Zopiclone) Produces Antiallodynic Effect in Neuropathic Pain Evoked by PINP

The Randall-Selitto test revealed that oral Cyclopyrrolones (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). Cyclopyrrolones (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.

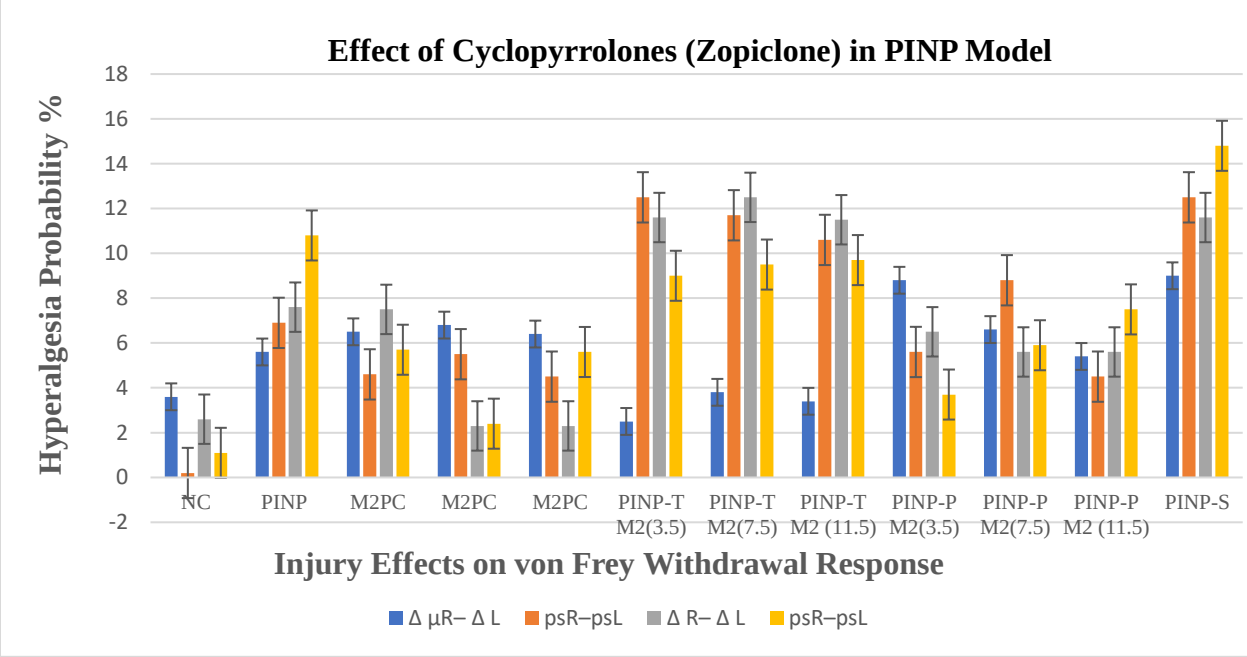

Fig:1 Effects of Cyclopyrrolones (Zopiclone) on paw withdrawal latency (PWL) and paw withdrawal threshold (PWT) Mechanical allodynia evoked by Paclitaxel-Induced Neuropathic Pain (PINP) in rats.

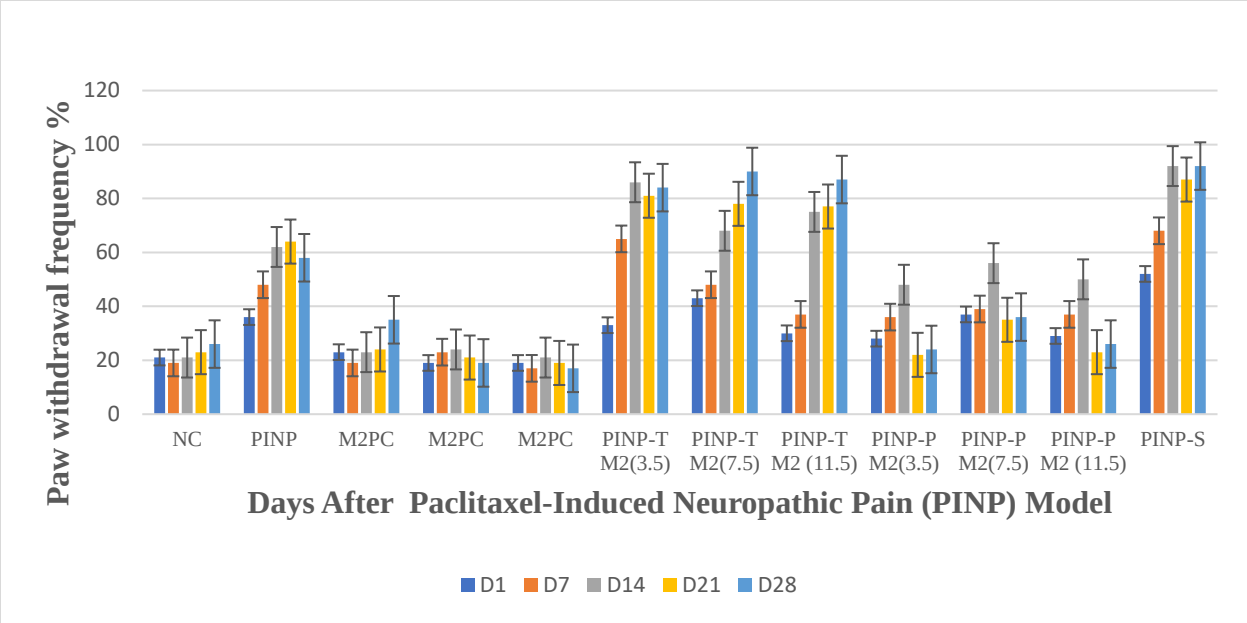

Fig:2 Effects of Cyclopyrrolones (Zopiclone) on PWL and PWT Von Frey evoked by Paclitaxel-Induced Neuropathic Pain (PINP) in rats.

3.2. The antiallodynic effect of cyclopyrrolones (Zopiclone) in neuropathic pain caused by CCI

Although the difference between 7.5 and 11.5 was not statistically significant, Cyclopyrrolones (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 Cyclopyrrolones (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 Cyclopyrrolones (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.

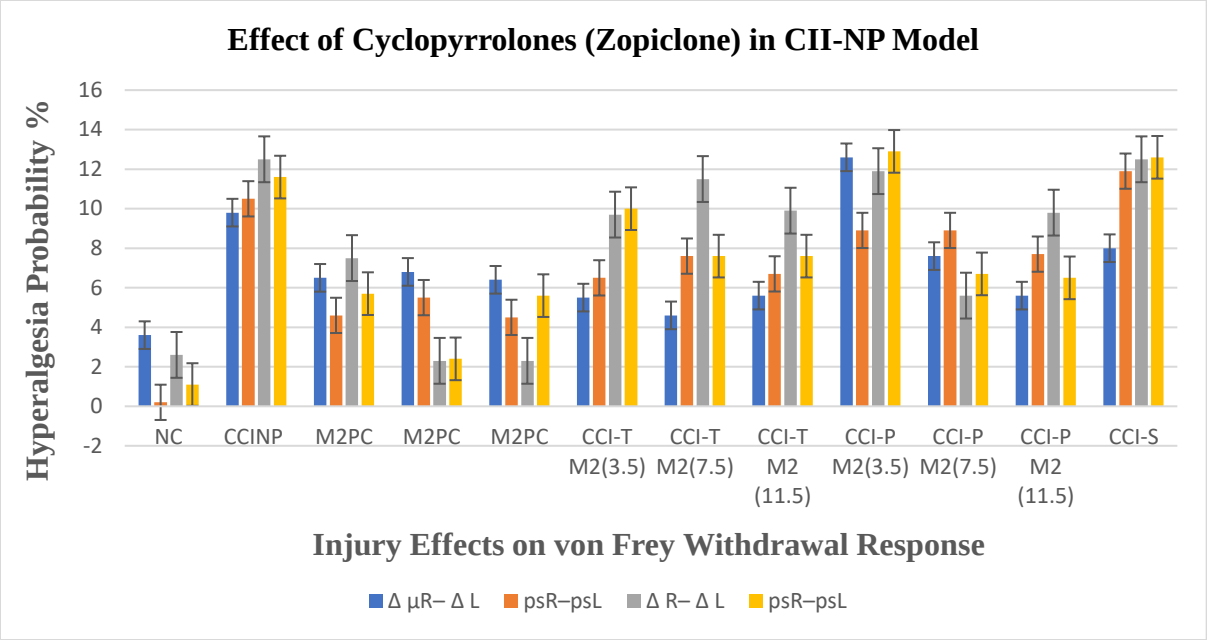

Fig. 3: Effects of Cyclopyrrolones (Zopiclone) on PWL and PWT Von Frey evoked by (CCI) in rats.

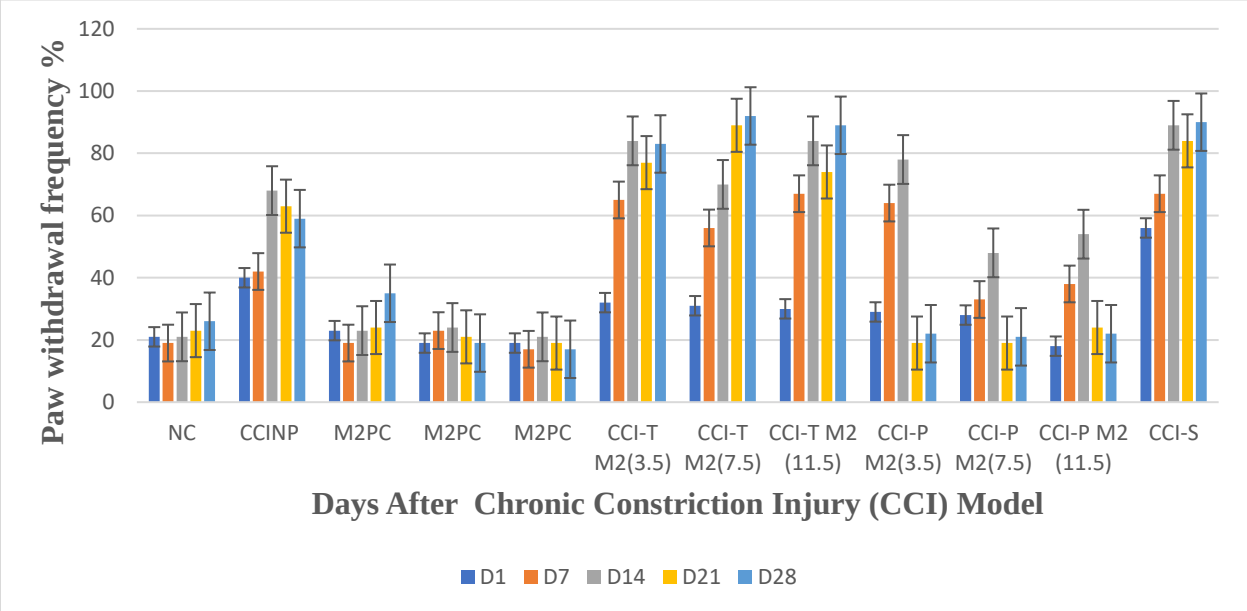

Fig. 4: Effects of Cyclopyrrolones (Zopiclone) on PWL and PWT Mechanical allodynia evoked by (CCI) Pain in rats.

3.3. The antiallodynic effect of cyclopyrrolones (Zopiclone) on neuropathic pain caused by DINP

Antiallodynic effect of $GABA_A$ agonists, the time course of the increase in threshold produced by oral drug administration illustrated in Fig. 5 and 6. The mechanical stimulus (in the range of 1.0-3.0 g) required to cause a quick withdrawal reaction in the wounded hind paw in reaction to von Frey hair activation was reduced for all rats involved in this investigation. Depending upon the dose, the threshold was maximally raised and then gradually decreased to control over a duration of 2–5 hrs. As indicated in Figs. 5 and 6, a somewhat shorter anti-allodynic duration were noticed following the efficacious doses of 3.5, 7.5, and 11.5.

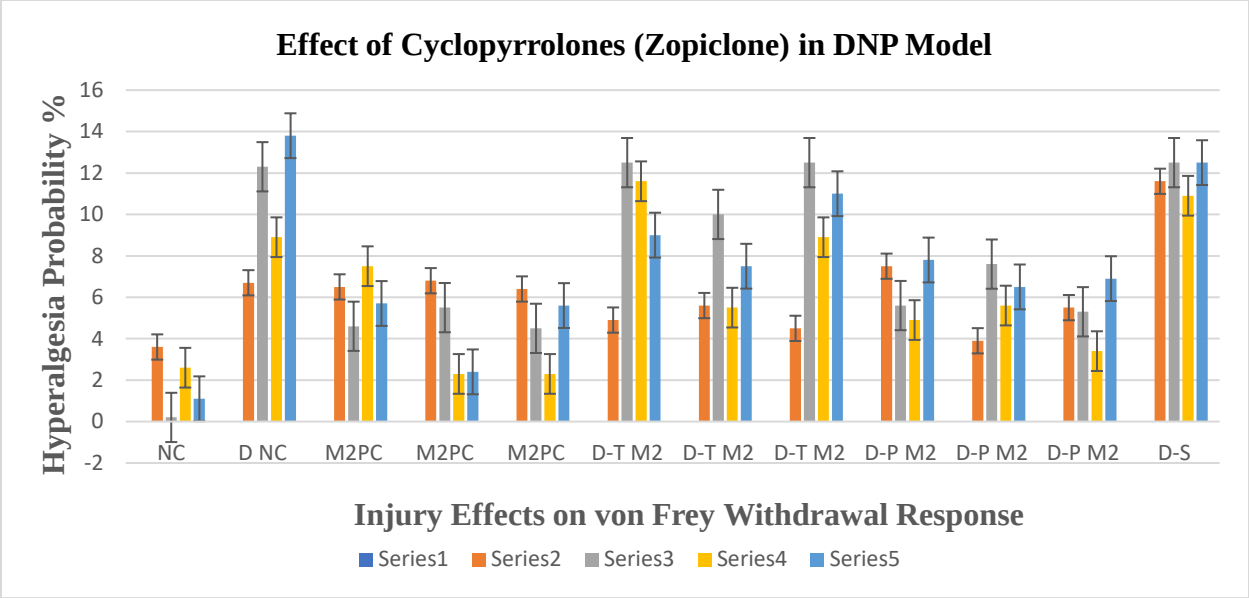

Fig. 5: Effects of Cyclopyrrolones (Zopiclone) on PWL and PWT Von Frey evoked by Disease-Induced Neuropathic Pain in rats.

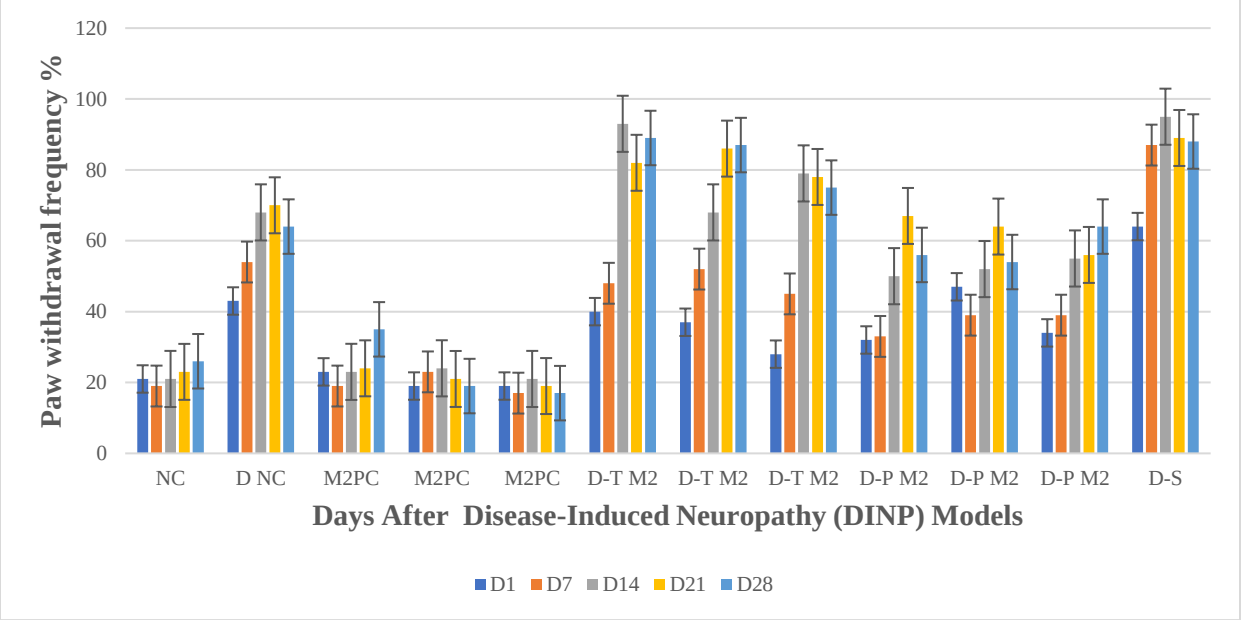

Fig. 6: Effects of Cyclopyrrolones (Zopiclone) on PWL and PWT Mechanical allodynia evoked by Disease-Induced Neuropathic Pain in rats.

In this study, we demonstrated the antiallodynic efficacy of pregabalin in combination with other teat drug, namely Cyclopyrrolones (Zopiclone) regulating the $GABA_A$ receptor, in the rat CCI, PINP & Disease induced neuropathic pain model. The antiallodynic effect was most prominent of Cyclopyrrolones (Zopiclone), when pregabalin was slandered drug analysis of this effects. Consistent with the findings reported in the literature regarding the use of

pregabalin (anticonvulsant), Cyclopyrrolones (Zopiclone) (regulating the $GABA_A$ receptor), for neuropathic pain, the results from our single-drug experiment indicated antiallodynic effects of Cyclopyrrolones (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 Cyclopyrrolones (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, Cyclopyrrolones (Zopiclone) inhibited both allodynia and hyperalgesia induced by (CCI) model, Paclitaxel-induced neuropathic pain (PINP) and Disease-Induced Neuropathy Models. Its effect was dose-dependent, and one of its action mechanisms was likely the sequential activation of spinal neurons at the supraspinal site of action. The current study further supports the likelihood that cyclopyrrolones (Zopiclone) are useful in the treatment of nerve pain, even if more clinical and preclinical research is needed.

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