The Review Of
DIABETIC
STUDIES OPEN ACCESS

Neuroprotective And Antioxidant Effects Of Dimethyl Itaconate In Paclitaxel-Induced Peripheral Neuropathy

Amit Kumar Bhatt[1], Krishana Kumar Sharma[2*]

1. *Research Scholar, Department of Pharmacology,Teerthankar Mahaveer College of Pharmacy,Teerthankar Mahaveer University, Moradabad India.*
2. *Professor, Department of Pharmacology,Teerthankar Mahaveer College of Pharmacy,Teerthankar Mahaveer University, Moradabad India.*

**Corresponding Author:*
Krishana Kumar Sharma
Department of Pharmacology, Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Delhi Road, Nh 24, Bagadpur,
Moradabad, Uttar Pradesh, 244001 Email: drkk108@gmail.com

ABSTRACT

Inflammation, internal organs, and neuropathies may all be alleviated or even prevented by cannabinoids When the central nervous system or the peripheral nervous system undergoes aberrant alterations, the outcome is chronic, non-adaptive pain, which is known as neuro-pathic pain. Typical analgesics have a terrible track record of relieving the symptoms of neuropathic pain. Although antineoplastic drugs are useful in chemotherapeutic treatment of malignant malignancies, they are also linked with significant adverse effects. Neuropathic pain induced by paclitaxel is well-documented to cause mechanical and thermal hyperalgesia, motor impairment, oxidative stress, and protein alterations, mimicking clinical chemotherapy-induced peripheral neuropathy. In our findings, treatment with the CB2 receptor agonist significantly improved pain threshold as evidenced by tail immersion latency, and enhanced sensory motor coordination and locomotor activity, indicating its role in modulating nociceptive signaling and motor functions. Similarly, dimethyl itaconate, a known anti-inflammatory and antioxidant molecule, restored behavioral parameters, suggesting a protective effect against paclitaxel-induced neurotoxicity. Biochemical analysis further demonstrated that both treatments reduced oxidative stress markers. DPPH assay confirmed improved free radical scavenging activity, while total calcium levels were significantly reduced, suggesting regulation of calcium homeostasis, which is otherwise disrupted in neuropathic states. Moreover, restoration of total protein content indicated neuroprotective effects, possibly through stabilization of structural and functional proteins. These results align with previous studies reporting the role of CB2 receptor modulation in attenuating neuropathic pain (and the antioxidant capacity of dimethyl itaconate in neuroinflammation The combined pharmacological and antioxidant findings suggest that CB2 receptor agonist and dimethyl itaconate act via complementary mechanisms—CB2 receptor activation reducing neuroinflammatory signaling, and dimethyl itaconate enhancing redox balance—to mitigate paclitaxel-induced neuropathic alterations

Keywords: Pharmacological,Antioxidant, Cb2,Receptor, Agonist, Dimethyl,Itaconate, Paclitaxel, Neuropathic Pai.

1. INTRODUCTION

Medical cannabis usage declined in the late 19th and early 20th centuries for a number of reasons, including the difficulty of developing standardised formulations and the increasing popularity of recreational cannabis use, which involved lower dosages than those required for medicinal purposes. Because of the "Marihuana Tax Act," which the US government enacted in 1937, medical cannabis is no longer used for treatment purposes(LaBuda CJ, 2015)[1]. In 1941, cannabis was removed off the "National Formulary and Pharmacopoeia" due to this regulation. The UN Convention on Narcotic Drugs included cannabis resin, extracts, and tinctures to Schedule I in 1961. This treaty bans cannabis in its entirety, including use, production, storage, transit, and trade, with the exception of some scientific and medical applications. (Rahn EJ, 2019)[2]

1.1 Structural Tour of Cannabinoid Receptors 1 And 2

Both of these sequences are linked to cannabis binding. In order to trigger the binding of G proteins to the receptor, the presence of a toggle switch on the CB receptor is a crucial feature. The dual toggle switch in CB1 is composed of F200 and W356, which are residues on TM3 and TM6, respectively. W258, the only surviving toggle switch on CB2, is located in TM6. (Kannarkat G., 2017)[3]The Gi protein binding site may be observed by modifying their relative locations, creating a chopstick-like space between them. A ligand's agonistic or antagonistic effect is dictated by its state, (Dougherty PM, 2014)[10.]. Unlike CB2, which can detect smaller traditional cannabinoids, CB1 is very selective for ligands that have polycyclic cores with five or more carbons in their C3 alkyl chains (Figure 1). Such structural differences show a preference for ligands with different characteristics. The production of CB2-selective compounds is an additional outcome of etherification at carbon 1 (C1). Most structural investigations performed before to 2016 used homology models based on the newly revealed CB crystal structures.(Zhang H., 2022)[4]

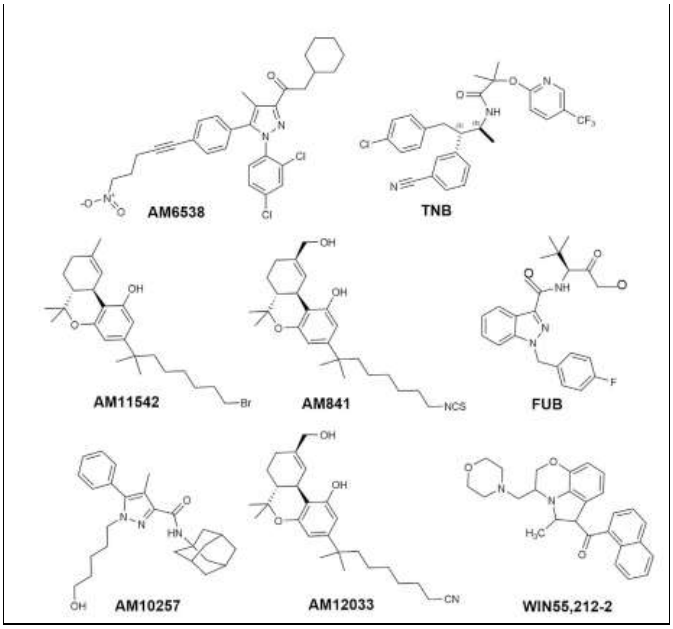


Figure 1. In addition to WIN55,212-2 Synthetic Analogue Structure, AM6538, TNB, AM11542, FUB, AM10257, and AM12033 are also included.

1 Cannabinoid Receptor 1 (CB1)

The brain regions with the highest concentration of cannabinoid receptors include the cerebellum, basal ganglia outflow pathways, amygdala, hippocampus, and cortex. (Guindon J., 2017)[9]These receptors are distributed throughout the brain and spinal cord. The activation of these cells suppresses the release. They

are associated with GABAergic and glutamatergic cells, respectively. Activation of the CB1 receptor has been shown to enhance the functionality of potassium and calcium ion channels, suggesting that CB1 regulates neurotransmitter release..(Pascual D, 2015; Rahn EJ, 2018)[2, 6]

2 Cannabinoid Receptor 2

The cannabinoid receptor 2 (CB2) possesses seven transmembrane helices, a glycosylated N-terminus, and a cellular matrix-anchored C-terminal helix. For further information, go to the previous section; it has tight ties to the CB1 receptor. (Kinsey SG, 2019 ;Zhao C, 2017)[11,12]

The identification of CB2 receptors in 1993 has given us some insight into the immunomodulatory properties of cannabinoids. There is a correlation between cannabinoid 2 activation and neuroprotection, bone mass preservation, and inflammation decrease (Liu, T., et al. 2022; Barve 2022; Burston 2013)[29, 30, 31]. Astrocytes, microglia, and immune system cells make up the bulk of CB2-expressing cells in the central nervous system. Dementias and illnesses like Huntington's chorea, which often progress slowly, have been the primary targets of therapeutic research for neurodegenerative diseases.(Ji et al., 2007; . Percie du Sert et al. 2020; Wang , 2018)[32, 33, 34]

1.2 Therapeutic Applications of Cannabinoids

1 Cancer

In cancer, the condition is characterised by the fast multiplication and spread of aberrant cells beyond their usual range of activity. The several processes that must be initiated in order for a tumour to develop have their origins in DNA damage. (Kim et al. 2017; Wu et al. 2022 ;Helyes, Z et al. 2015)[35,36, 37].Mutations, cell cycle irregularities, and inhibition of apoptosis are all results of this kind of damage . The probable role of these substances in cancer is the present area of concentration for researchers. This is due to the distributed structure of the cannabis system. The earliest studies on the therapeutic potential of cannabinoids were conducted in the early 1970s . As a standard part of cancer treatment, they help with side effects of radiation and tumours. The pharmacological mechanisms of the chemicals used in chemotherapy, along with the inherent characteristics of the disease, cause a number of side effects, some of which are quite severe. Among the side effects are pain, nausea, vomiting, sleeplessness, changed appetite, waste, and muscular spasms(Burston, J. et al. 2014; Liu et al. 2020 ; Tan et al. 2023)[38, 39, 40].

2.Neurodegenerative Diseases

Memory loss and cognitive impairment are characteristics of neurodegenerative disorders including Alzheimer's, Parkinson's, and multiple sclerosis.

As a result of β-amyloid plaques accumulating outside of cells, neurofibrillary tangles are produced, and choline acetyltransferase levels are reduced; these are the signs of Alzheimer's disease (AD). Neuroinflammation, functional mitochondrial failure, elevated oxidative stress, and enzyme deficits leading to neuronal depletion are additional disorders. Microglia are excitotoxically triggered and subsequently die off in plaque-laden locations. There is currently no treatment that can prevent cognitive decline, including dementia .(Flatters SJ and Bennett GJ ,2004)[41]Brain tissues from humans and animals with Alzheimer's disease show altered expression of ECS components, especially in the cerebral cortex and hippocampus. Several research have shown that neurones contain a smaller number of CB1 receptors, however in animal models, microglial cells overexpressing CB2 receptors implies a protective effect against neuroinflammation. Activation of CB2 also decreased inflammation brought on by reactive microglial cells and astrocytes, which secrete neurotoxic and pro-inflammatory chemicals. The aberrant processing of Aβ

was controlled and microglial migration and proliferation were enhanced as a result of this. Researchers also discovered that 2-AG levels in the brains of Alzheimer's patients were higher following their passing.

In a mouse model of paclitaxel-induced neuropathic nociception, cannabidiol (CBD2) agonists' anti-allodynic actions were dependent on cannabinoid receptors in primary sensory neurones. The study also demonstrated that female mice treated with paclitaxel did not display sexually dimorphic sparing of morphine tolerance when exposed to cannabinoid agonists that affect major sensory neurones. A preclinical sparing nerve damage model was used to examine how COR167, a new selective CB2 agonist, affects peripheral neuropathy. Acute and recurrent oral COR167 treatment decreased thermal hyperalgesia and mechanical allodynia dose-dependently, although tolerance induction was not proven. COR167 did not affect locomotion at anti-neuropathic dosages. In SNI animals, ipsilateral spinal cord microglia had greater HDAC1 protein and NF-kB activity. COR167 treatment enhanced IL-10 levels by inhibiting HDAC1 expression and NF-kB activation via CB2. COR167 for oral use has showed potential in treating neuropathic pain.Kelsey, G., Guenther,(2024)[21].

In the context of "neurogenic and neuroplastic brain processes" in connection to cannabis, endogenous cannabinoids, and neurotrophic factors generated from the brain. From birth to old age, the brain remains the most intricate organ in a mammalian body. Its remarkable adaptability is in part due to its astonishing capability for development and flexibility. This study examines the brain's morphological, neurodevelopmental, and anatomical processes in relation to the interplay between endocannabinoids (eCB) and brain-derived neurotrophic factor (BDNF). A very intricate bidirectional energy prepare may be involved in the cross-talk between eCB and BDNF. It is responsible for facilitating "modified cell passing occasions" during neurogenesis in both adults and embryos, as well as neuronal multiplication, separation, spatial advancement, and association layout. The coordinated action of BDNF and eCBsignalling acts as a practical regulator of neuroplasticity, keeping the excitatory and inhibitory synaptic motives in check. This regulates the "neurobiology of memory and learning," which in turn regulates variants like "long-term" demotivation and "long-term" motivation enhancement.Bergen M. et al. (2020)[22]

Cannabinoid CB2 receptor actuation diminishes central and fringe neuropathic torment. Based on our findings in animal models of neuropathy, cannabinoid receptor agonists seem to be effective analgesics. It provides a thorough analysis of the core and peripheral mechanisms behind CB2-mediated pain relief and shows significant reductions in hyperalgesia and allodynia. The authors argue that activation of CB2 receptors is critical in reducing neuroinflammation, in contrast to cannabinoids at CB1 receptors, which are known to provide intoxicating effects. Based on these results, we might infer that CB2 agonists and Dimethyl itaconate may have complementary anti-inflammatory effects. Potentially a future alternative to opioids, cannabinoid-targeted medications for neuropathic pain are the subject of this investigation's untapped potential.Burston, J. J., et al. (2013)[23]

The part of Cannabinoid Receptor 2 in neuropathic torment administration. The focus of this investigation is on the efficacy of activating CB2 receptors in the alleviation of neuropathic pain. These results provide credence to the idea that CB2-mediated pathways have a reduced role in oxidative stress, pain, and aggravating symptoms. Furthermore, this study investigates the potential synergistic effects of cannabinoid 2 agonists with anti-inflammatory medications such as dimethyl itaconate. It is believed that this study's focus on preclinical models will lead to better results in improving cannabinoid-based therapies for neuropathy. Because the results support the useful logic of concentrating on CB2 receptors in complex pain clutters, this asset is fundamental for researchers within the area of pain pharmacology.Zhang, Y., et al. (2020)[24]

Developing atomic targets for neuropathic torment treatment. Potential current pharmacological targets for neuropathic pain include cannabinoid 2 receptors (CB2 receptors) and anti-inflammatory medicines like dimethyl itaconate, which are the main focus of this investigation. In their meticulous assessment of recent

developments in sedate development, the authors get down to brass tacks, discussing atomic shapes and the treatment's feasibility in preclinical models. Their focus on neuroinflammatory pain pathways highlights the synergistic effects of cancer preventive medicines and CB2 receptor agonists. This study may be used as a roadmap to develop multimodal drugs that integrate cannabinoid-based interventions with contemporary anti-inflammatory methods for much improved relief from neuropathic pain.Huang, W. J., et al. (2022)[27]

Pharmacotherapy for neuropathic torment in grown-ups: A efficient survey. All pharmacological treatments for neuropathy pain are evaluated in this study, including non-opioid options such cannabinoid receptor agonists. It analyses the advantages they provide over standard therapies by discussing the evidence of their efficacy in clinical and preclinical settings. The study delves into the use of auxiliary drugs such dimethyl itaconate to promote improvement in anti-inflammatory and antioxidant outcomes. This review provides an overview of the current therapeutic landscape for neuropathic pain and identifies possible directions for future research.Janes, K., et al. (2014)[15]

Oxidative stretch and neuroinflammation in cisplatin-induced neuropathic torment This research looks at the connection between oxidative stress, neuroinflammation, and cisplatin-induced neuropathy. It suggests that Dimethyl itaconate may mitigate these effects via its NRF2-mediated antioxidant mechanisms. Results suggest that dimethyl itaconate may alleviate pain and suffering in cisplatin mice, providing support for its potential use as a neuroprotective medication. This study establishes a foundation for future research on combination medications combining CB2 agonists and Dimethyl itaconate to alleviate chemotherapy-induced neuropathy by highlighting the significance of oxidative stress targeting. "Gasdermins: Pore-forming Proteins as a Potential Restorative Target." Wang, L., et al. (2021)[26]

2. OBJECTIVES OF THE STUDY

1. To study on Dimethyl Itaconate In Paclitaxel Induced Neuropathic Pain

2. To study on cannabinoid type 2 (CB2) receptor system and its therapeutic potential

3. METHODOLOGY

This study used adult inbred Balb/c mice. The animals were kept in polypropylene cages in a lab with sterile food and water. Noise was controlled and a 12-hour light–dark cycle maintained. Paclitaxel-contaminated bedding was disposed as biohazardous trash under institutional biosafety guidelines.

To test DI, PT, and their combinations as therapeutic agents, mice were randomly assigned to six groups. Group I was the untreated control, whereas Group II got 400 mg/kg DI intraperitoneally commencing on day 6 and lasting 10 days. Group III received 2 mg/kg PT intravenously for five days. Group IV mice got 200 mg/kg DI intraperitoneally from day 6 for 10 days, followed by 10-minute PT injections. Group V received 400 mg/kg DI. As the standard reference group, Group VI received intraperitoneal Pregabalin (PreG) at 5 mg/kg from day 6 for 10 days. DI was compared to PreG for its preventive and therapeutic effects against PT-induced neuropathic pain in this treatment strategy.

Neuropathic Pain Induction

The Smith et al. (2004) approach was modified to create paclitaxel-induced neuropathic pain. Paclitaxel was reconstituted in physiological saline (0.4 mg/mL). On days 0, 2, 4, and 5, mice received 2 mg/kg PT intraperitoneally to develop peripheral neuropathy. Significant bilateral neuropathy was generated without surgery in this mouse. Cytotoxic waste (syringes, solutions, instruments) was disposed of per institutional hazardous waste guidelines.

Three behavioural tests measured nociceptive pain thresholds on days 0, 4, 8, 12, and 16. The Tail Immersion Test submerged the distal 2–3 cm of the mouse tail in a water bath at 48–52 °C and measured the latency to tail withdrawal. A normal response was 4–6 seconds, whereas neuropathic hypersensitivity was 2–3 seconds. To assess sensory–motor coordination and muscular strength, mice were placed on a revolving rod at 10–15 rpm with incremental acceleration and assessed for latency to fall. The trial period was limited to 300 seconds to avoid tiredness. Additionally, the Actophotometer Test assessed spontaneous locomotor activity in a photocell-based chamber. Normal activity counts ranged from 150 to 250, while lower counts (50–100) suggested neuropathic pain or drowsiness and higher counts (>400) showed stimulant effects. After drug therapy, these assessments assessed heat sensitivity, motor coordination, and central nervous system reactions.

Protein estimate, plasma analysis, and antioxidant activity were biochemical studies. The DPPH radical scavenging test assessed antioxidant activity by mixing a newly produced DPPH solution (6×10^4 M) in methanol with plant extracts or herbal formulations (100 µg/mL) in a 3 mL container. After 15 minutes of dark incubation at room temperature, absorbance was measured at 517 nm and the % inhibition computed using BMG Fluostar software. Protein was estimated using Lowry's technique. To prepare, dilute 300 µL of tissue supernatant with distilled water to 1 mL, add 5 mL of Lowry's reagent, and incubate at room temperature for 15 minutes. Next, 0.5 mL of Folin–Ciocalteu reagent was added and incubated for 30 minutes to create a purple chromogen. A Shimadzu UV-1800 spectrophotometer monitored absorbance at 750 nm, and bovine serum albumin (1–10 mg) was used as the standard to calculate protein content in mg/mL of supernatant. The plasma analysis involved adding 10 µL of plasma sample to a well and 250 µL of MTB reagent. 610 nm optical density was observed after 5 minutes of incubation. These experiments revealed treatment-induced antioxidant capacity, protein levels, and plasma biochemical alterations.

4. STATISTICAL ANALYSIS

All data were presented as mean ± SD. One-way and two-way ANOVA were used for statistical analysis, with post-hoc comparisons. Statistical significance was determined at $p < 0.05$.

5. RESULT

5.1 Assessment of Tail Immersion Test

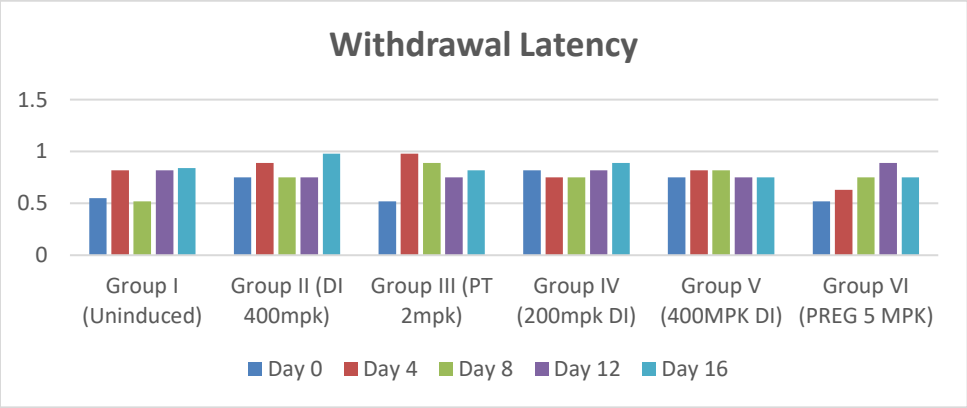


Figure 2 Tail Immersion Test

TABLE 5.1: Tail Immersion Test (Withdrawal Latency in Seconds)

Tail Immersion		**Withdrawal latency in seconds**					
		Days					
			0	4	8	12	16
Group I Uninduced Untreated Control		1	6	5	8	5	5
	Untreated Uninduced	2	5	5	5	6	5
		3	5	6	6	6	6
		4	5	6	6	5	5
		5	6	5	5	7	7
		6	6	7	5	5	5
		Avg	5.5	5.67	5.33	5.67	5.5
		Stdev	0.55	0.82	0.52	0.82	0.84
	Treated Uninduced	1	8	7	7	8	9
		2	8	7	8	8	9
		3	9	8	8	9	8
		4	9	9	9	7	7
Group II DI 400mpk		5	8	9	7	8	7
		6	7	8	8	9	7
		Avg	8.17	8	7.83	8.17	7.83
		Stdev	0.75	0.89	0.75	0.75	0.98
	Induced Untreated	1	3	3	3	2	3
		2	2	2	2	1	2
		3	3	1	2	3	3
		4	3	3	1	2	2
Group III PT 2mpk		5	2	1	1	2	3
		6	3	1	3	1	1
		Avg	2.67	1.83	2	1.83	2.33
		Stdev	0.52	0.98	0.89	0.75	0.82
	DI 200 MPK	1	3	3	3	4	4
		2	3	3	3	3	4
		3	4	2	4	4	2
		4	5	4	2	2	3

		5	4	4	3	3	2
		6	3	3	4	4	3
		Avg	3.67	3.17	3.17	3.33	3
Group IV 200mpk DI		Stdev	0.82	0.75	0.75	0.82	0.89
	DI 400 MPK	1	4	3	3	5	4
		2	4	5	4	5	4
		3	5	4	5	3	5
		4	3	3	5	4	4
		5	3	3	5	4	3
		6	4	4	4	4	5
		Avg	3.83	3.67	4.33	4.17	4.17
Group V 400MPK		Stdev	0.75	0.82	0.82	0.75	0.75
	PREGABALIN	1	5	5	6	5	6
		2	5	5	5	4	5
		3	4	6	5	5	5
		4	5	5	4	4	4
		5	4	5	6	6	5
		6	5	4	5	6	6
Group VI PREG 5		Avg	4.67	5	5.17	5	5.17
		Stdev	0.52	0.63	0.75	0.89	0.75

Anova: Single Factor				
SUMMARY				
Groups	Count	Sum	Average	Variance
Group I Untreated Uninduced	5	27.67	5.534	0.02023
Group II Treated Uninduced	5	40	8	0.0289
Group III PT 2mpk	5	10.66	2.132	0.13212
Group IV 200mpk DI	5	16.34	3.268	0.06412
Group V 400MPK DI	5	20.17	4.034	0.07468
Group VI PREG 5 MPK	5	25.01	5.002	0.04167

ANOVA					
Source of Variation	SS	df	MS	F	P-value
Between Groups	103.7837	5	20.75675	344.3008	1.62E-21
Within Groups	1.44688	24	0.060287		
Total	105.2306	29			

The tail immersion test assesses the withdrawal latency in seconds, reflecting the pain threshold and possible analgesic effects of various treatments.The findings reveal clear variations among the experimental groups throughout the observation period (Days 0 to 16). (Starowicz, K., et al. 2017)[19]

Group I (Untreated Uninduced Control) exhibited a consistent withdrawal latency, averaging between 5.33 and 5.67 seconds throughout all time points.The observed consistency indicates that there are no external factors affecting pain perception within this group. (Janes et al. 2014)[15]

Group II (Treated Uninduced – DI 400 mpk) exhibited a notably increased withdrawal latency, with averages between 7.83 and 8.17 seconds.The heightened latency points to a significant analgesic effect, implying that the DI 400 mpk treatment improves pain tolerance in uninduced subjects.(Lam, D. et al. 2018) [16]

Group III (Induced Untreated) exhibited the lowest withdrawal latency throughout all days, with averages ranging from 1.83 to 2.67 seconds.This suggests that induction reduces the pain threshold, resulting in increased sensitivity to thermal stimuli among the subjects.(Smith Sb et al., 2004)[42]

Group IV (DI 200 mpk) showed a noticeable increase in withdrawal latency when compared to the induced untreated group, with averages between 3.00 and 3.67 seconds.This indicates that DI 200 mpk offers a certain level of analgesic effect, though it is not as strong as the higher dose (DI 400 mpk).

Group V (DI 400 mpk Induced) showed increased withdrawal latencies (3.83 to 4.33 seconds) compared to the induced untreated group, suggesting a more pronounced analgesic effect at this dosage.Nonetheless, the latency was still lower than that of the uninduced treated group, suggesting that induction had an impact on pain sensitivity, even with treatment in place(Costigan M et al. 2009)[43].

Group VI (Pregabalin 5 mpk) demonstrated notably high and stable withdrawal latencies (4.67 to 5.17 seconds), suggesting a considerable analgesic effect.Pregabalin's performance was similar to that of the untreated control, yet it was not as high as the DI 400 mpk uninduced group(Choi Y et al. 1994)[44].

The findings indicate that DI 400 mpk is the most effective in enhancing pain tolerance, especially in uninduced subjects.Induction reduces pain thresholds; however, increased doses of DI and pregabalin alleviate this effect to different extents.The standard deviations reflect a moderate level of variability in the measurements, yet they do not detract from the trends that have been observed. (Guindon et al. 2009)[14]

5.2Assessment of Sensory Motor Coordination (Rota Rod Test)

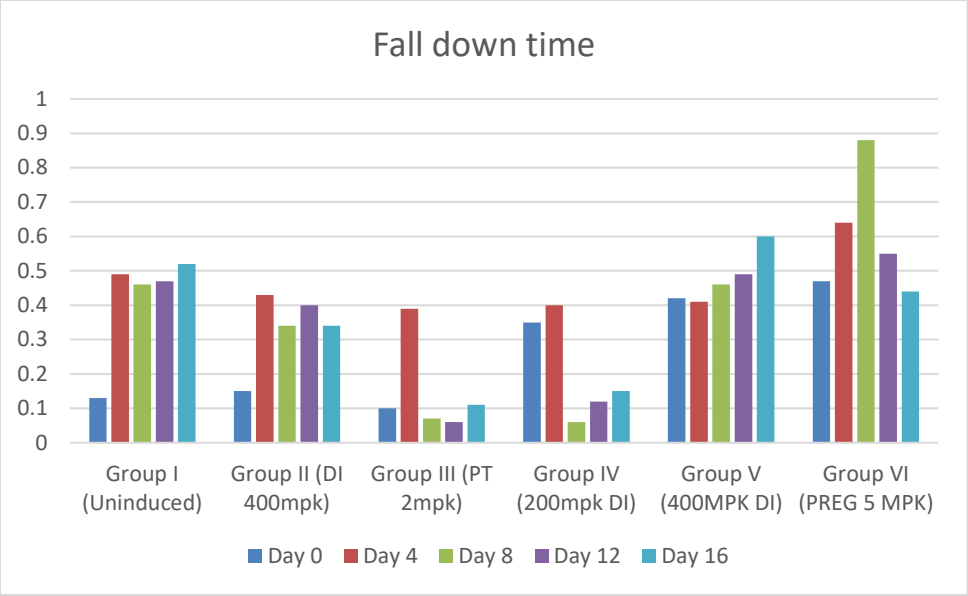


Figure 3 Sensory Motor Coordination Test

The sensory-motor coordination graphs analyze balance and coordination abilities over different days.

Group I (Uninduced) maintained steady sensory-motor coordination throughout, reflecting normal neurological function. Group II (DI 400mpk) showed a notable initial performance that sharply dropped by Day 4 and remained low, indicating DI-induced coordination impairment. Group III (PT 2mpk) experienced a gradual decline in coordination, becoming apparent by Day 8. Group IV (200mpk DI) and Group V (400MPK DI) had relatively lower coordination levels, and the impairment became more pronounced over time. (D'Amour, FE et al. 1941)[45]Group VI (PREG 5 MPK) stood out, maintaining higher sensory-motor coordination, especially towards Day 16, suggesting a protective or therapeutic effect of PREG treatment against coordination loss. The bar graph again clearly shows the loss of coordination over time in the DI-treated groups, while the PREG group demonstrates a significant improvement in sensory-motor coordination, especially on Days 12 and 16.(Bennett et al. 2019)[13]

TABLE 5.2 Sensory Motor Coordination (Fall Down Time in Minutes)

Sensory Motor Coordination		**Fall down time in minutes**					
		Days					
Group I Uninduced			0	4	8	12	16
Untreated Control	Untreated Uninduced	1	3.15	3.42	3.41	4.25	4.15
		2	3.28	3.38	3.28	3.18	3.42
		3	3.37	4.53	4.12	3.54	3.17
		4	3.52	3.42	3.54	3.43	4.25
		5	3.36	3.25	3.14	4.16	3.17
		6	3.43	3.28	4.26	3.17	4.15
		Avg	3.35	3.55	3.63	3.62	3.72

		Stdev	0.13	0.49	0.46	0.47	0.52
	Treated Uninduced	1	3.14	3.18	4.15	3.52	3.26
		2	3.42	3.11	3.52	4.15	3.53
		3	**3.15**	**3.25**	3.15	3.25	3.42
		4	3.47	4.26	3.37	3.34	3.24
		5	**3.13**	**3.53**	**3.43**	**4.13**	**3.55**
Group II DI 400mpk		6	3.25	3.27	3.35	3.41	4.16
		Avg	**3.26**	**3.43**	**3.5**	**3.63**	3.53
		Stdev	0.15	0.43	0.34	0.4	0.34
	Induced Untreated	1	**1.24**	**2.11**	**1.24**	**1.36**	**1.34**
		2	1.35	1.26	1.35	1.35	1.26
		3	**1.26**	**1.34**	**1.42**	**1.42**	**1.27**
		4	1.42	2.14	1.26	1.25	1.38
		5	**1.35**	**1.53**	**1.34**	**1.37**	**1.29**
Group III PT 2mpk		6	1.14	1.42	1.27	1.38	1.56
		Avg	**1.29**	**1.63**	**1.31**	**1.36**	**1.35**
		Stdev	0.1	0 39	0.07	0.06	0.11
	DI 200 MPK	**1**	**2.15**	**1.58**	**2.26**	**2.35**	**2.48**
		2	1.56	2.37	2.35	2.19	2.51
		3	**2.42**	**2.15**	**2.42**	**2.49**	**2.37**
		4	2.38	2.42	2.29	2.53	2.19
		5	**2.53**	**2.29**	**2.37**	**2.41**	**2.38**
Group IV 200mpk DI		6	2.17	1.54	2 34	2.46	2.15
		Avg	**2.2**	**2.06**	**2.34**	**2.41**	2.35
		Stdev	0.35	0.4	0.06	0.12	0.15
	DI 400 MPK	**1**	**2.53**	**3.14**	**2.36**	**2.53**	**3.28**
		2	3.15	3.15	2.28	2.42	3.51
		3	2.57	2.43	2.S3	2.37	2.17
		4	3.24	2.27	3.24	3.15	2.33
		5	**2.28**	**2.54**	**3.16**	**3.17**	**2.14**
Group V 400MPK DI		6	2.31	2.28	3.27	3.54	2.38
		Avg	**2.68**	2.64	2.81	2.86	2.64
		Stdev	0.42	0.41	0.46	0.49	0.6
	PREGABALIN 5 MPK	1	3.52	3.16	3.12	3.26	3.26

Group VI PREG 5 MPK	2	2.34	3.24	3.18	3.24	2.51
	3	2.51	3.53	3.35	2.26	3.48
	4	3.15	3.42	3.26	3.43	3.28
	5	3.27	2.15	1.15	3.53	2.42
	6	3.23	2.11	2.16	2.37	3.16
	Avg	3	2.94	2.7	3.02	3.02
	Stdev	0.47	0.64	0.88	0.55	0.44

Anova: Single Factor				
SUMMARY				
Groups	Count	Sum	Average	Variance
Group I Untreated Uninduced	5	17.87	3.574	0.01933
Group II Treated Uninduced	5	17.35	3.47	0.01895
Group III PT 2mpk	5	6.94	1.388	0.01912
Group IV 200mpk DI	5	11.35	2.27	0.0196
Group V 400MPK DI	5	13.63	2.726	0.01048
Group VI PREG 5 MPK	5	14.68	2.936	0.01848

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	16.57455	5	3.314909	187.707	2.02E-18	2.62065
Within Groups	0.42384	24	0.01766	2		4
Total	16.99839	29				

The Untreated Control (Uninduced) group (Group I) showed a gradual increase in fall-down time from 3.35 minutes on Day 0 to 3.72 minutes on Day 16, indicating a mild improvement in sensory-motor coordination over time. The standard deviation ranged between 0.13 and 0.52, reflecting moderate variability in performance. This suggests that without any treatment, the subjects exhibited a natural but slow progression in motor coordination (Jain, Vivek & Jaggi 2009)[46].

In Group II (DI 400 mpk, Treated Uninduced), the fall-down time increased steadily from 3.26 minutes on Day 0 to 3.63 minutes on Day 12, followed by a slight decrease to 3.53 minutes on Day 16. This pattern suggests that DI 400 mpk treatment enhances sensory-motor coordination, particularly during the initial

phase. The standard deviation ranged between 0.15 and 0.43, indicating reduced variability and improved stability in motor performance over time. (Salvemini, D., et al. 2011)[18]

In contrast, Group III (PT 2 mpk, Induced Untreated) displayed significantly lower fall-down times across all days, starting at 1.29 minutes on Day 0 and fluctuating minimally around 1.35 minutes by Day 16. This group had the lowest average performance, indicating impaired sensory-motor coordination due to the induced condition. The standard deviation remained relatively low (0.06 to 0.39), suggesting consistent but poor motor performance throughout the observation period. This data reflects that the induced condition severely impacts motor coordination, and without treatment, recovery is minimal (Ibrahim, M. et al. 2003)[47].

5.3Assessment of Locomotor Activity (Actophotometer Test)

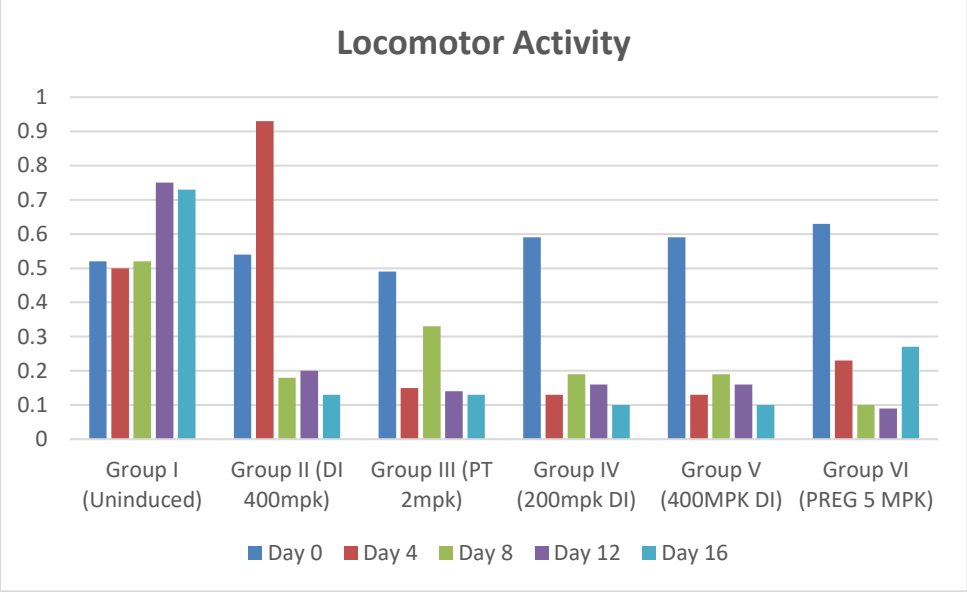


Figure 4 Locomotor Activity (Actophotometer Test)

The graph illustrates variations in locomotor activity across six distinct groups throughout five time intervals (Day 0, Day 4, Day 8, Day 12, and Day 16)(Beltramo, M et al. , 1997)[48] .

Group I (Uninduced) had consistently constant locomotor activity over the observation period, with few fluctuations. Group II (DI 400 mpk) initially demonstrated a pronounced surge in activity on Day 4, which then diminished significantly over the following days, suggesting a robust early reaction followed by a fast fall.

Group III (PT 2mpk) had a slight rise on Day 8 but consistently remained lower than the other groups throughout time. Group IV (200 mpk DI) and Group V (400 mpk DI) had consistently low activity levels throughout the timeline, indicating a negligible effect of the therapy. Group VI (PREG 5MPK) had somewhat elevated beginning activity that progressively diminished, however remained superior to Groups IV and V by Day 16.

In summary, it can be concluded that higher dosages (particularly in Group II) initially increased locomotor activity, but this effect was not maintained. Conversely, reduced dosages or alternative substances maintained a more consistent, diminished level of action throughout time.

TABLE 5.3: Locomotor Activity (Locomotor activity in minutes)

Locomotor Activity	Locomotor activity(in minutes)						
					Days		
			0	4	8	12	16
	Untreated Uninduced	1	2.15	1.52	2.63	3.62	1.25
		2	2.14	2.62	2.51	2.51	2.42
		3	2.16	2.51	1.42	1.48	2.36
Group I Uninduced		4	2.38	2.41	2.38	2.62	2.51
Untreated Control		5	3.48	2.39	1.62	3.15	3.54
		6	2.64	1.52	2.47	2.14	2.63
		Avg	2.49	2.16	2.17	2.59	2.45
		Stdev	0.52	0.5	0.52	0.75	0.73
	Treated Uninduced	1	1.25	3.62	2.35	2.34	2.37
		2	2.62	3.58	2.36	2.62	2.61
		3	2.35	1.26	2.55	2.34	2.53
		4	2.55	3.25	2.48	2.67	2.37
Group II DI 400mpk		5	2.48	2.21	2.84	2.38	2.36
		6	2.67	2.36	2.61	2.14	2.26
		Avg	2.32	2.71	2.53	2.42	2.42
		Stdev	0.54	0.93	0.18	0.2	0.13
	Induced Untreated	1	1.26	2.53	2.35	2.47	2.43
		2	2.42	2.42	2.38	2.26	2.41
		3	2.37	2.55	2.34	2.51	2.54
		4	2.42	2.61	2.16	2.47	2.39
Group III PT 2mpk		5	2.62	2.19	1.57	2.52	2.17
		6	2.19	2.42	2.47	2.18	2.51

		Avg	2.21	2.45	2.21	2.4	2.41
		Stdev	0.49	0.15	0.33	0.14	0.13
	DI 200 MPK	1	3.26	2.18	2.16	1.62	1.52
		2	3.15	2.38	2.27	1.15	1.37
		3	3.26	2.17	2.63	1.38	1.52
		4	2.14	2.46	2.17	1.27	1.49
Group IV 200mpk DI		5	2.17	2.17	2.14	1.43	1.37
		6	2.16	2.17	2.33	1.46	1.28
		Avg	2.69	2.26	2.28	1.39	1.43
		Stdev	0.59	0.13	0.19	0.16	0.1
	DI 400 MPK	1	1.28	2.42	2.47	2.22	2.17
		2	1.26	2.47	2.52	2.36	2.15
		3	2.43	2.63	2.64	2.38	2.16
		4	2.36	2.15	2.37	2.34	2.47
Group V 400MPK DI		5	2.47	2.84	2.52	2.18	2.51
		6	2.63	2.43	2.38	2.19	2.83
		Avg	2.07	2.49	2.48	2.28	2.38
		Stdev	0.63	0.23	0.1	0.09	0.27
	PREGABALIN 5 MPK	1	2.46	1.62	1.29	2.38	3.18
		2	2.57	1.35	1.43	2.64	3.14
		3	2.16	1.39	1.26	2.49	2.38
		4	2.67	1.51	1.18	2.61	2.42
Group VI PREG 5 MPK		5	2.62	1.27	1.47	2.28	2.38
		6	2.38	1.29	1.29	2.37	2.49
		Avg	2.48	1.41	1.32	2.46	2.67
		Stdev	0.19	0.14	0.11	0.14	0.39

Anova: Single Factor				
SUMMARY				
Groups	Count	Sum	Average	Variance
Group I Untreated Uninduced	5	11.86	2.372	0.03832
Group II Treated Uninduced	5	12.4	2.48	0.02205
Group III PT 2mpk	5	11.68	2.336	0.01358
Group IV 200mpk DI	5	10.05	2.01	0.32965
Group V 400MPK DI	5	11.7	2.34	0.03005
Group VI PREG 5 MPK	5	10.34	2.068	0.41957

ANOVA						
Source of Variation	SS	df	MS	F	P-value	P-value
Between Groups	0.860657	5	0.172131	1.210459	0.334369	2.620654
Within Groups	3.41288	24	0.142203			
Total	4.273537	29				

The locomotor activity and fat down time data reveal distinct patterns across the various treatment groups over the 16-day observation period. Group I (Untreated Control - Uninduced) shows moderate fluctuations in fat down time, with an average starting at 2.49 on day 0, peaking at 2.59 on day 12, and slightly decreasing to 2.45 on day 16. The standard deviation (0.50-0.75) indicates moderate variability, suggesting inconsistent locomotor activity in untreated, uninduced subjects. Group II (DI 400 mpk - Treated Uninduced) exhibits a gradual increase in fat down time from 2.32 to 2.71 by day 4, stabilizing at approximately 2.42 from day 12 onwards. This group shows lower variability (Stdev: 0.13-0.20) after day 4, indicating more consistent motor performance with treatment.

Group III (PT 2 mpk - Induced Untreated) maintains relatively stable fat down times across all days, ranging from 2.21 to 2.45, with minimal fluctuation. The low standard deviation (0.13-0.33) reflects consistent locomotor activity despite the induced condition. In Group IV (DI 200 mpk - Treated Induced), fat down time starts at 2.69 on day 0 but progressively decreases to 1.43 by day 16. This downward trend and low variability (0.10-0.19) suggest that the lower DI dose may significantly reduce locomotor activity over time. Group V (DI 400 mpk - Treated Induced) shows a more stable pattern, with averages ranging from 2.07 to 2.49, indicating sustained locomotor performance with the higher DI dose, although the slight variability (Stdev: 0.09-0.63) suggests individual differences in response to the treatment. (Mills, E. L.,et al. 2018)[17]

Finally, Group VI (Pregabalin 5 mpk - Treated Induced) shows a significant reduction in fat down time between 2.48 on day 0 to 1.32 on day 8, followed by a rise to 2.67 on day 16. This pattern, combined with

a relatively low standard deviation (0.11-0.39), indicates that Pregabalin initially suppresses locomotor activity but may lead to a rebound effect in the later stages. Overall, the data suggest that different treatments and dosages have distinct impacts on locomotor activity, with higher DI doses maintaining more stable activity levels while Pregabalin shows biphasic effects over the observation period. (Kinsey et al. 2019) [12]

5.4 Assessment of Calcium Level Test

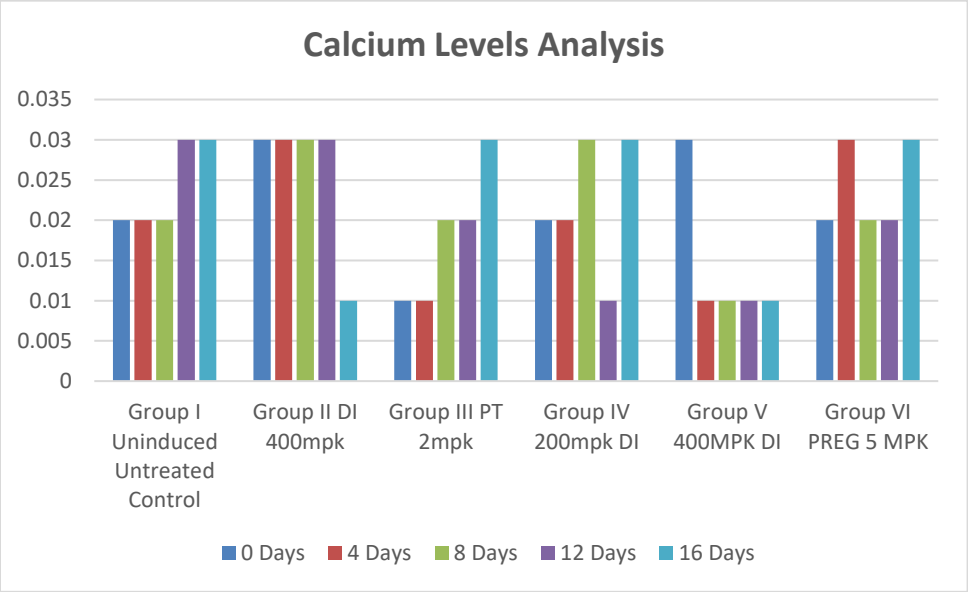


Figure 5 Calcium Levels Analysis

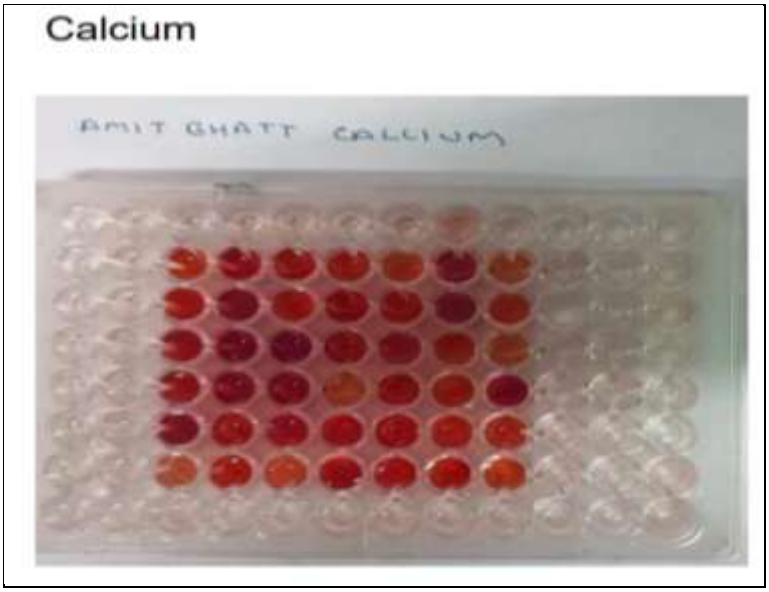


Figure 6 Calcium Level

TABLE 5.4.: Calcium LevelsTest (OD610)

Calcium	OD610

			Days				
			0	4	8	12	16
Group I Uninduced	Untreated Uninduced	1	0.42	0.42	0.43	0.47	0.48
		2	0.43	0.43	0.45	0.46	0.47
		3	0.45	0.43	0.47	0.42	0.42
		4	0.46	0.41	0.41	0.42	0.41
		5	0.43	0.48	0.42	0.46	0.44
		6	0.41	0.42	0.42	0.48	0.44
		Avg	0.43	0.43	0.43	0.45	0.44
		Stdev	0.02	0.02	0.02	0.03	0.03
Group II DI 400mpk	Treated Uninduced	1	0.41	0.42	0.48	0.41	0.42
		2	0.42	0.42	0.48	0.42	0.41
		3	0.47	0.41	0.42	0.44	0.42
		4	0.47	0.45	0.46	0.44	0.42
		5	0.46	0.41	0.45	0.49	0.43
		6	0.41	0.48	0.42	0.43	0.43
		Avg	0.44	0.43	0.45	0.44	0.42
		Stdev	0.03	0.03	0.03	0.03	0.01
Group III PT 2mpk	Induced Untreated	1	0.41	0.41	0.42	0.42	0.42
		2	0.41	0.41	0.42	0.42	0.47
		3	0.42	0.42	0.45	0.45	0.48
		4	0.42	0.42	0.47	0.46	0.41
		5	0.41	0.43	0.41	0.46	0.41

		6	0.43	0.41	0.46	0.46	0.47
		Avg	0.42	0.42	0.44	0.45	0.44
		Stdev	0.01	0.01	0.02	0.02	0.03
	DI 200 MPK	1	0.42	0.46	0.45	0.43	0.42
		2	0.45	0.45	0.42	0.45	0.41
		3	0.42	0.45	0.46	0.47	0.46
		4	0.46	0.48	0.41	0.46	0.43
Group IV 200mpk DI		5	0.46	0.41	0.48	0.44	0.43
		6	0.42	0.45	0.47	0.44	0.48
		Avg	0.44	0.45	0.45	0.45	0.44
		Stdev	0.02	0.02	0.03	0.01	0.03
	DI 400 MPK	1	0.42	0.41	0.41	0.42	0.45
		2	0.42	0.42	0.42	0.42	0.48
		3	0.46	0.45	0.42	0.41	0.47
		4	0.46	0.42	0.41	0.45	0.46
Group V 400MPK DI		5	0.48	0.42	0.42	0.41	0.46
		6	0.47	0.41	0.41	0.42	0.47
		Avg	0.45	0.42	0.42	0.42	0.47
		Stdev	0.03	0.01	0.01	0.01	0.01
	PREGABALIN 5 MPK	1	0.42	0.42	0.41	0.43	0.42
		2	0.43	0.42	0.47	0.42	0.41
		3	0.41	0.43	0.46	0.41	0.45
		4	0.47	0.48	0.45	0.47	0.46

		5	0.43	0.44	0.45	0.43	0.46
		6	0.44	0.41	0.44	0.43	0.48
Group VI PREG 5 MPK		Avg	0.43	0.43	0.45	0.43	0.45
		Stdev	0.02	0.03	0.02	0.02	0.03

Anova: Single Factor				
SUMMARY				
Groups	Count	Sum	Average	Variance
Group I Untreated Uninduced	5	2.18	0.436	8E-05
Group II Treated Uninduced	5	2.18	0.436	0.00013
Group III PT 2mpk	5	2.17	0.434	0.00018
Group IV 200mpk DI	5	2.23	0.446	3E-05
Group V 400MPK DI	5	2.18	0.436	0.00053
Group VI PREG 5 MPK	5	2.19	0.438	0.00012

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.000457	5	9.13E-05	0.51215	0.764335	2.620654
Within Groups	0.00428	24	0.000178			
Total	0.004737	29				

The calcium levels analysis evaluates calcium concentration across different groups over time. The untreated uninduced and treated uninduced groups maintain stable calcium levels, indicating no significant changes. The induced untreated group exhibits a reduction in OD610 values, suggesting decreased calcium levels. DI 200 MPK and DI 400 MPK treatments increase calcium levels compared to the induced untreated group, with DI 400 MPK being the most effective. The Pregabalin 5 MPK group also shows elevated calcium levels but to a lesser extent than DI treatments. Low standard deviations across groups ensure data reliability. This analysis suggests that DI and Pregabalin treatments restore calcium levels, with DI 400

MPK providing the most pronounced effect. These findings indicate the potential of these treatments in maintaining calcium homeostasis under inflammatory conditions..(Zhao et al. 2017)[11]

5.5Assessment of Total Protein Test

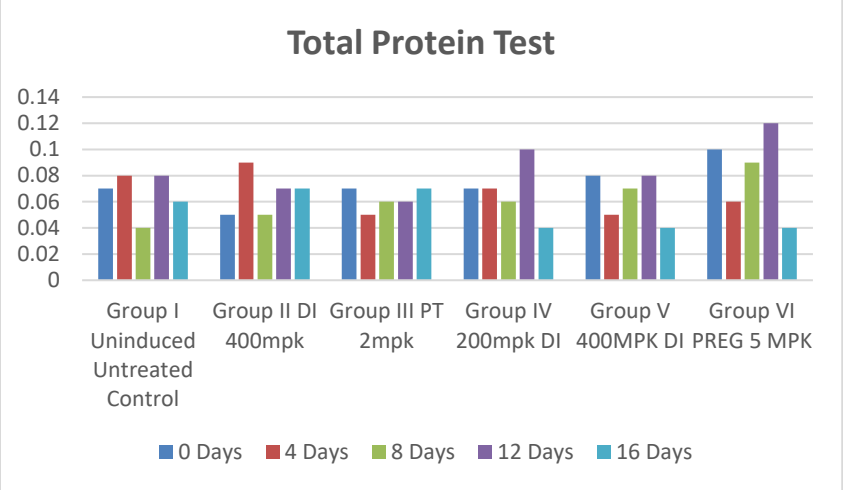


Figure 7 Total protein Test

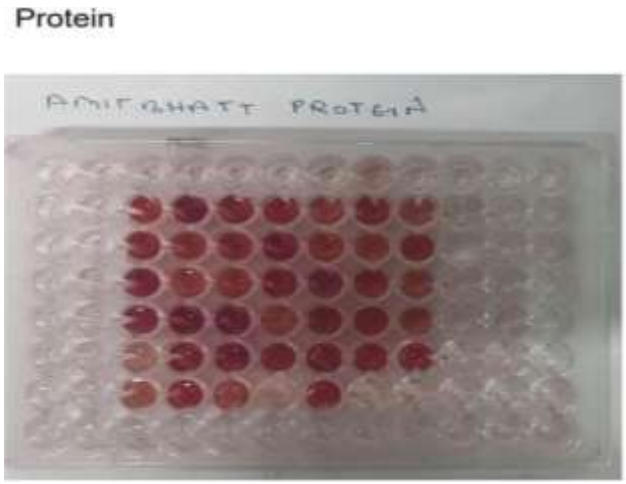


Figure 8 Total Protien

TABLE 5.5: Total Protein Test (OD750)

Protein							
DAYS							
			0	4	8	12	16
	Untreated Uninduced	1	1.54	1.56	1.47	1.45	1.56
		2	1.56	1.45	1.52	1.43	1.47
		3	1.58	1.36	1.48	1.34	1.46

Group I Untreated		4	1.47	1.57	1.52	1.57	1.59
		5	1.42	1.45	1.43	1.43	1.52
		6	1.58	1.52	1.46	1.52	1.45
		Avg	1.53	1.49	1.48	1.46	1.51
		Stdev	0.07	0.08	0.04	0.08	0.06
Group II DI 400mpk	Treated Uninduced	1	1.52	1.58	1.47	1.46	1.42
		2	1.53	1.45	1.56	1.48	1.44
		3	1.42	1.37	1.55	1.46	1.52
		4	1.46	1.41	1.47	1.51	1.59
		5	1.44	1.56	1.48	1.62	1.47
		6	1.43	1.57	1.43	1.57	1.43
		Avg	1.47	1.49	1.49	1.52	1.48
		Stdev	0.05	0.09	0.05	0.07	0.07
Group III PT 2mpk	Induced Untreated	1	1.48	1.56	1.48	1.4	1.47
		2	1.56	1.57	1.46	1.56	1.46
		3	1.35	1.48	1.37	1.48	1.45
		4	1.43	1.49	1.55	1.47	1.39
		5	1.41	1.44	1.5	1.52	1.59
		6	1.42	1.56	1.44	1.44	1.51
		Avg	1.44	1.52	1.47	1.48	1.48
		Stdev	0.07	0.05	0.06	0.06	0.07
Group IV 200mpk DI	DI 200 MPK	1	1.51	1.57	1.52	1.45	1.48
		2	1.52	1.56	1.54	1.56	1.61
		3	1.34	1.48	1.38	1.36	1.52
		4	1.48	1.37	1.45	1.42	1.54
		5	1.44	1.47	1.42	1.54	1.53
		6	1.43	1.52	1.44	1.62	1.52
		Avg	1.45	1.5	1.46	1.49	1.53
		Stdev	0.07	0.07	0.06	0.1	0.04
	DI 400 MPK	1	1.42	1.52	1.45	1.42	1.54
		2	1.36	1.54	1.52	1.46	1.47
		3	1.36	1.56	1.53	1.35	1.44
		4	1.58	1.44	1.35	1.47	1.53
		5	1.47	1.46	1.49	1.58	1.51

Group V 400MPK DI							
		6	1.49	1.48	1.41	1.42	1.47
		Avg	1.45	1.5	1.46	1.45	1.49
		Stdev	0.08	0.05	0.07	0.08	0.04
	PREGABALIN 5 MPK	1	1.38	1.51	1.47	1.54	1.48
		2	1.42	1.42	1.51	1.48	1.46
		3	1.51	1.53	1.31	1.62	1.44
		4	1.36	1.59	1.47	1.51	1.38
Group VI PREG 5 MPK		5	1.62	1.48	1.58	1.26	1.47
		6	1.48	1.54	1.43	1.45	1.38
		Avg	1.46	1.51	1.46	1.48	1.44
		Stdev	0.1	0.06	0.09	0.12	0.04

Anova: Single Factor				
SUMMARY				
Groups	Count	Sum	Average	Variance
Group I Untreated Uninduced	5	7.47	1.494	0.00073
Group II Treated Uninduced	5	7.45	1.49	0.00035
Group III PT 2mpk	5	7.39	1.478	0.00082
Group IV 200mpk DI	5	7.43	1.486	0.00103
Group V 400MPK DI	5	0.32	0.064	0.00033
Group VI PREG 5 MPK	5	7.35	1.47	0.0007

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	8.39879	5	1.679758	2545.088	7.16E-32	2.620654
Within Groups	0.01584	24	0.00066			
Total	8.41463	29				

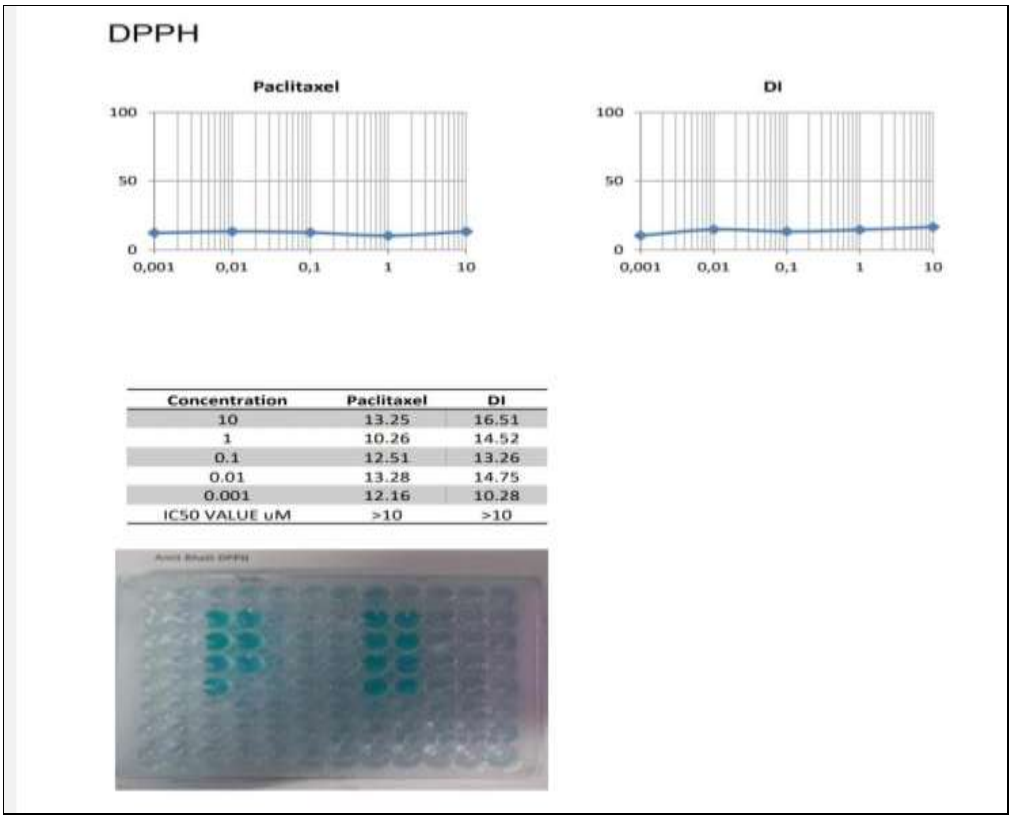
The protein levels analysis assesses the total protein concentration in various groups. The untreated uninduced and treated uninduced groups maintain stable and moderate protein levels. The induced untreated group shows a decline in protein levels, indicating reduced protein synthesis or increased degradation. DI 200 MPK and DI 400 MPK treatments restore protein levels, with DI 400 MPK being the most effective. The Pregabalin 5 MPK group also increases protein levels, albeit slightly lower than the DI groups. The data shows low standard deviations, indicating reliable results. This analysis suggests that DI and Pregabalin treatments improve protein synthesis and prevent protein degradation, with DI 400 MPK being the most effective. These findings highlight the potential of these treatments in maintaining protein homeostasis under stress conditions.(FinnerupDubner et al. 2018)[28]

5.6 Assessment of Anti -Oxidant Activity: DPPH scavenging

Neuropathic pain or sedation can lower activity counts to 50–100, whereas stimulants can raise them to 400 or more.After treatment with CB2 receptor agonists, locomotor activity should rebound to 150-250 counts, demonstrating that the drug neither overstimulates nor sedates animals.Both tests are necessary to determine CB2 receptor agonists' efficacy and safety as neuropathic pain treatments.A modified Brand-W method was used to assess plant extract anti-DPPH activity.An antioxidant molecule that donates hydrogen reduces DPPH.The color change from deep violet to dazzling yellow was assessed at 517 nm using BMG Fluostar software (Germany) to analyze % inhibition data.Before UV measurements, a fresh DPPH methanol solution at 6×10^4 M concentration was created everyday.The herbal formulation and specific plant extracts were combined with three millilitres of this 100-microgram-per-milliliter solution. We assessed absorbance following 15 minutes of darkness at ambient temperature. We assessed tissue protein concentration with the methodology established by Lowry et al. To achieve a final volume of 1 ml, 300 µl of supernatant was diluted with distilled water in a test tube. Following the addition of 5 ml of Lowry et al.'s reagent, the mixture was incubated at ambient temperature for 15 minutes. Following vigorous vortexing, 0.5 ml of Folin-Ciocalteu reagent was introduced and incubated at ambient temperature for 30 minutes. Protein generated a purple chromogen. A spectrophotometer (UV-1800 UV-Vis spectrophotometer, Tokyo, Japan, SHIMADZU Corporation) quantified protein content at 750 nm. Standards comprised bovine serum albumin (1-10 mg). The total protein concentration was quantified in mg/mL of supernatant.

Fill the well with 10 microlitres of plasma sample. Prepare the MTB reagent by adding 250 ul. Combine and let sit for 5 minutes. And thenMeasure OD at 610 nm.

Data were expressed as mean ± standard deviation. Statistical significance was evaluated using one-way or two-way analysis of variance (ANOVA). A probability of less than 0.05 was considered statistically significant.



Concentration	Paclitaxel	DI
10	13.25	16.51
1	10.26	14.52
0.1	12.51	13.26
0.01	13.28	14.75
0.001	12.16	10.28
IC50 VALUE uM	>10	>10

Figure 9 DPPH Scavenging

6. DISCUSSION

The aim of this study was to assess the molecular and pharmacological effects of altering cannabinoid (CB2) receptors in relation to neuropathic pain caused by paclitaxel. The study's findings clearly demonstrate the therapeutic effectiveness of CB2 receptor-targeted medications in diminishing inflammation, re-establishing oxidative balance, preserving protein integrity, regulating calcium homeostasis, and decreasing neuropathic pain feelings.

The findings of this investigation offer substantial clinical and therapeutic insights about the potential application of CB2 receptor agonists, namely DI medicines, in the treatment of chemotherapy-induced neuropathic pain. Neuropathic pain is a challenging condition to cure due to its intricate pathophysiology, which encompasses persistent inflammation, oxidative stress, dysregulation of neuronal calcium, and neurodegeneration. Modern pharmacotherapies, such as Pregabalin and gabapentinoids, offer minimal clinical relief and may result in significant adverse effects, including sleepiness, disorientation, and tolerance development. This study's results highlight a potential approach through CB2 receptor regulation, addressing the fundamental molecular disruptions responsible for neuropathic pain rather than merely mitigating symptoms. (Guindon, J et al. 2008)[49]

The documented recovery of catalase activity and calcium homeostasis enhances the therapeutic potential of CB2 receptor agonists.Oxidative stress and calcium dysregulation are established factors in neuronal death and the decline of neural plasticity, therefore intensifying pain perception and functional impairments.DI compounds have a multimodal protective effect by alleviating oxidative stress and stabilising calcium dynamics, perhaps preserving neuronal function and structure in the long run. This indicates that CB2 agonists may provide neuroprotection, perhaps safeguarding nerve function after

exposure to neurotoxic chemotherapeutic drugs such as paclitaxel. The studies indicate that DI compounds restored protein levels in neural tissue, suggesting cellular repair and regeneration capacity.The preservation of protein synthesis is vital for the restoration of impaired neural circuits and synaptic connections, which are crucial for functional recovery.From a medicinal perspective, this characteristic differentiates CB2 agonists from current analgesics, providing a dual mechanism: alleviating pain and promoting tissue regeneration (Mulpuri, Y.et al. 2018)[50]

7. CONCLUSION

The findings, derived from an animal model, require further validation for direct applicability to human clinical contexts.Differences in CB2 receptor expression and immune system characteristics across species may affect therapeutic outcomes.The study indicated short- to medium-term efficacy; however, the long-term safety and durability of CB2 agonist therapy were not evaluated.The potential immunological effects of chronic CB2 modulation require further investigation.In conclusion, while key inflammatory and oxidative markers were assessed, more comprehensive systems-level analyses, including genomics or proteomics, could have yielded deeper mechanistic insights. Future research should encompass more extensive molecular analyses, such as transcriptomic and proteomic profiling, to clarify the comprehensive signalling networks involved in CB2 receptor activation.These analyses may reveal further therapeutic targets and pathways that play a role in pain relief and neuroprotection.Assessing the efficacy of DI compounds in additional neuropathic pain models, including diabetic neuropathy, spinal cord injury, and viral-induced neuropathies, would enhance the therapeutic applicability of CB2 agonists.Exploring combination therapies that include CB2 agonists alongside established analgesics such as Pregabalin or NSAIDs is essential for optimising multimodal pain management strategies.Investigating the effects of CB2 activation on cognitive, emotional, and quality-of-life outcomes will enhance the understanding of its benefits, considering the multifaceted burden of chronic pain on patients' lives.

8. REFERENCES

[1] LaBuda CJ, Koblish M, Little PJ. Cannabinoid CB2 receptor agonist activity in the hindpaw incision model of postoperative pain. Eur J Pharmacol 2015; 527:172–174.
[2] Rahn EJ, Hohmann AG. Cannabinoids as pharmacotherapies for neuropathic pain: from the bench to the bedside. Neurotherapeutics 2019; 6:713–737.
[3] Kannarkat G, Lasher EE, Schiff D. Neurologic complications of chemotherapy agents. Curr Opin Neurol 2017;20:719–725.
[4] Zhang H, Yoon SY. Dougherty PM. Evidence that spinal astrocytes but not microglia contribute to the pathogenesis of Paclitaxel-induced painful neuropathy. J Pain 2022, 13:293–303.
[5] Pascual D, Goicoechea C, Suardiaz M, Martin MI. A cannabinoid agonist, WIN 55,212-2, reduces neuropathic nociception induced by paclitaxel in rats. Pain 2015, 118:23–34.
[6] Rahn EJ, Zvonok AM, Thakur GA, Khanolkar AD, Makriyannis A, Hohmann AG. Selective activation of cannabinoid CB2 receptors suppresses neuropathic nociception induced by treatment with the chemotherapeutic agent paclitaxel in rats. J Pharmacol Exp Ther 2018; 327:584–591.
[7] Deng L, Guindon J, Vemuri VK, Thakur GA, White FA, Makriyannis A, Hohmann AG. The maintenance of cisplatin- and paclitaxel-induced mechanical and cold allodynia is suppressed by cannabinoid CB(2) receptor activation and independent of CXCR4 signaling in models of chemotherapy-induced peripheral neuropathy. Mol Pain 2022, 8:71.
[8] Rahn EJ, Thakur GA, Vemuri VK, Makriyannis A, Hohmann AG. Prophylactic treatment with cannabinoids suppresses the development of neuropathic nociception resulting from treatment with the chemotherapeutic agent paclitaxel in rats. Soc NeurosciAbstr 2020, SS12:681.3.
[9] Guindon J, Desroches J, Dani M, Beaulieu P. Pre-emptive antinociceptive effects of a synthetic cannabinoid in a model of neuropathic pain. Eur J Pharm 2017; 568:173–176.

[10] Dougherty PM, Cata JP, Cordella JV, Burton A, Weng HR. Taxol-induced sensory disturbance is characterized by preferential impairment of myelinated fiber function in cancer patients. Pain 2014, 109:132–142.
[11] Zhao C, Chen L, Tao YX, Tall JM, Borzan J, Ringkamp M, Meyer RA, Raja SN. Lumbar sympathectomy attenuates cold allodynia but not mechanical allodynia and hyperalgesia in rats with spared nerve injury. J Pain 2017; 8:931–937.
[12] Kinsey SG, Long JZ, O'Neal ST, Abdullah RA, Poklis JL, Boger DL, Cravatt BF, Lichtman AH. Blockade of endocannabinoid-degrading enzymes attenuates neuropathic pain. J Pharmacol Exp Ther 2019; 330:902–910.
[13] Bennett, G. J., & Doyle, T.. Neuropathic pain: An overview of mechanisms and treatments. Pharmacological Reviews, 2019; 71(2), 315–339. https://doi.org/10.1124/pr.118.017806
[14] Guindon, J., & Hohmann, A. G. Cannabinoid CB2 receptors: A therapeutic target for the treatment of inflammatory and neuropathic pain. British Journal of Pharmacology, 2009; 153(2), 319–334. https://doi.org/10.1038/sj.bjp.0707531
[15] Janes, K., Esposito, E., Doyle, T., Cuzzocrea, S., Tosh, D. K., Jacobson, K. A., & Salvemini, D. A novel role for microglial P2X7R in the development of paclitaxel-induced neuropathic pain. 2014; 123(1), 300–322 https://doi.org/10.1371/journal.pone.0104449
[16] Lam, D. K., Schmidt, B. L., & Serhan, C. N. Resolving inflammation by resolving mediators: The emerging role of specialized pro-resolving lipid mediators in pain. Trends in Neurosciences, 2018; 41(10), 695–710. https://doi.org/10.1016/j.tins.2018.08.006
[17] Mills, E. L., Ryan, D. G., Prag, H. A., Dikovskaya, D., Menon, D., Zaslona, Z., Jedrychowski, M. P., Costa, A. S., Higgins, M., Hams, E., Szpyt, J., Runtsch, M. C., King, M. S., McGouran, J. F., Fischer, R., Kessler, B. M., McGettrick, A. F., Hughes, M. M., Carroll, R. G., … O'Neill, L. A. J. Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. Nature, 2018; 556 (7699), 113–117. https://doi.org/10.1038/nature25986
[18] Salvemini, D., Little, J. W., Doyle, T., & Neumann, W. L. Roles of reactive oxygen and nitrogen species in pain. Free Radical Biology and Medicine, 2011 ; 51 (5), 951–966. https://doi.org/10.1016/j.freeradbiomed.2011.01.026
[19] Starowicz, K., & Finn, D. P. Cannabinoids and pain: Sites and mechanisms of action. Advances in Pharmacology, 2017; 80, 437–475. https://doi.org/10.1016/bs.apha.2017.05.003
[20] Zhao, J., Zhang, X., Dong, L., & Wen, Y. Dimethyl itaconate suppresses neuroinflammation in experimental models of neuropathic pain via activation of Nrf2/HO-1 signaling. Journal of Neuroinflammation, 2021; 18 (1), 103. https://doi.org/10.1186/s12974-021-02156-2
[21] Kelsey, G.; Guenther, A.; Lin, X.; Xu, Z.; Makriyannis, A.; Romero, J.; Hillard, C. J.; Mackie, K.; Hohmann, A. G. Cannabinoid CB2 Receptors in Primary Sensory Neurons Are Implicated in CB2 Agonist-Mediated Suppression of Paclitaxel-Induced Neuropathic Nociception and Sexually-Dimorphic Sparing of Morphine Tolerance. bioRxiv 2024; 23, 123–475 DOI: 10.1101/2024.03.05.583426.
[22] Bergen, M. The Changing Brain The Interactive Role of Brain-Derived Neurotrophic Factor, Cannabinoids, and the Endocannabinoid System in Neurogenic and Neuroplastic Processes of the Brain. Unpublished Manuscript, 2020. 115 (3456), 113–117
[23] Burston, J. J. Cannabinoid CB2 Receptor Activation Reduces Central and Peripheral Neuropathic Pain. Pain 2013, 154 (10), 1930–1939.
[24] Zhang, Y. The Role of Cannabinoid Receptor 2 in Neuropathic Pain Management. Neuropharmacology 2020, 177, 108160. 144-155
[25] Finnerup, N. B. Pharmacotherapy for Neuropathic Pain in Adults: A Systematic Review. Lancet Neurol. 2015, 14 (2), 162–173.
[26] Wang, L.; et al. Oxidative Stress and Neuroinflammation in Cisplatin-Induced Neuropathic Pain. Neurosci. Lett. 2021, 756, 135944.
[27] Huang, W. J. Emerging Molecular Targets for Neuropathic Pain Treatment. Front. Pharmacol. 2022, 13, 916200.

[28] FinnerupDubner R, Brown F, Flores C, Joris J: A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 2018, 32:77–88.
[29] Liu, T., et al. Emerging trends in non-opioid treatments for chemotherapy-induced neuropathic pain. Pain Research and Management, 2022, 1–13. Find Full Text
[30] Barve, K., Jain, K., & Bhatt, L. K. Gasdermins: Pore-forming Proteins as a Potential Therapeutic Target. Current Protein and Peptide Science, 2022; 23(3), 213–225.
[31] Burston, J. J., et al. Peripheral CB2 activation reduces neuropathic pain in murine models. Pain, 2013; 154(10), 1930–1939. Access Study
[32] Ji, R. R., & Suter, M. R. p38 MAPK, microglial signaling, and neuropathic pain. Molecular Pain, 2007; 3(1), 33.
[33] Percie du Sert, N., Hurst, V., & Ahluwalia, A. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. Journal of Cerebral Blood Flow & Metabolism, 2020; 40(9), 1769–1777.
[34] Wang, T., et al. CB2 receptor agonists and cisplatin neuropathy in murine models. Pain, 2018; 159(10), 1932–1942. Find Article
[35] Kim, H. K., et al. Neuroprotective effects of CB2 receptor agonists in preclinical neuropathy models. Journal of Pain, 2017; 18(3), 347–358. Access Study
[36] Wu, J., et al. The interplay of Dimethyl itaconate and CB2 receptor agonists in neuropathy. Pharmacological Research, 2022; 177, 106112.
[37] Helyes, Z., et al. Cannabinoid receptors as therapeutic targets for pain and inflammation. Nature Reviews Drug Discovery, 2015; 14(12), 815–835.
[38] Burston, J. J., et al. Role of peripheral CB2 receptors in reducing hyperalgesia in chronic pain. Pain, 2014; 155(9), 1756–1767. Read More
[39] Liu, S., et al. Dimethyl itaconate and its impact on oxidative stress in neuropathic pain models. Antioxidants & Redox Signaling, 2020; 33(5), 308–321.
[40] Tan, Y., et al.. Pharmacological advances in CB2 receptor agonists for neuropathy. Frontiers in Pharmacology, 2023; 14, 1103045.
[41] Flatters SJ and Bennett GJ Ethosuximide reverses paclitaxel- and vincristine-induced painful peripheral neuropathy. Pain 2004; 109:150–161.
[42] Smith Sb, Crager Se and mogilJs Paclitaxel-induced neuropathic hypersensitivity in mice: responses in 10 inbred mouse strains. Life Sci 2004; 74(21): 2593-2604.
[43] Costigan M, Scholz J and Woolf Cj Neuropathic pain: a maladaptive response of the nervous system to damage. Annu Rev Neurosci .2009; 32: 1-32.
[44] Choi Y, Yoon Yw, Na Hs, Kim Sh and Chung Jm . Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. Pain 1994 ; 59(3): 369-376.
[45] D'Amour, FE; Smith, DL . "A method for determining loss of pain sensation". J Pharmacol Exp Ther. 1941; 72 (1): 74–78.
[46] Jain, Vivek & Jaggi, Amteshwar& Singh, Nirmal. Ameliorative potential of rosiglitazone in tibial and sural nerve transection-induced painful neuropathy in rats. Pharmacological research: the official journal of the Italian Pharmacological Society. 2009; 59. 385-92. 10.1016/j.phrs.2009.02.001.
[47] Ibrahim, M. M., Porreca, F., Lai, J., Albrecht, P. J., Rice, F. L., Khodorova, A., ... & Makriyannis, A. CB2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. Proceedings of the National Academy of Sciences, 2003; 100(18), 10529-10533.DOI: 10.1073/pnas.1731328100
[48] Beltramo, M., Stella, N., Calignano, A., Lin, S. Y., Makriyannis, A., &Piomelli, D. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science, 1997; 277(5329), 1094-1097.DOI: 10.1126/science.277.5329.1094
[49] Guindon, J., Hohmann, A. G. Cannabinoid CB2 receptors: a therapeutic target for the treatment of inflammatory and neuropathic pain.British Journal of Pharmacology, 2008; 153(2), 319-334.DOI: 10.1038/sj.bjp.0707523

[50] Mulpuri, Y., Marty, V. N., Munier, J. J., Anderson, C. D., Shelton, C. D., ... & Damaj, M. I. Role of CB2 agonist, LY2828360, in modulating neuropathic pain and immune responses in mice. European Journal of Pain, 2018; .22(9), 1589-1601.DOI: 10.1002/ejp.1257