PHARMACOLOGICAL IMPACT OF CHOLESTEROL-LOWERING DRUG AS ADJUNCTS TO FLUOXETINE AND SILDENAFIL IN ATTENUATION OF NEUROPATHIC PAIN IN RATS

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DECLARATION

I, hereby declared that the presented work in the thesis entitled "**Pharmacological Impact of Cholesterol-Lowering Drug as Adjuncts to Fluoxetine and Sildenafil in Attenuation of Neuropathic Pain in Rats"** in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of **Dr. Bimlesh Kumar**, working as **Professor**, in the **Department of Pharmacology** of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of another investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis "**Pharmacological Impact of Cholesterol-Lowering Drug as Adjuncts to Fluoxetine and Sildenafil in Attenuation of Neuropathic Pain in Rats"** submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the **Department of Pharmacology** , is a research work carried out by Indu Melkani, 41900351, is bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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Abstract

Neuropathic pain (NP), a complex and debilitating condition associated with type 2 diabetes mellitus (T2DM) and nerve injury, requires effective therapeutic strategies. This study evaluates the potential analgesic and neuroprotective effects of sildenafil (SD), fluoxetine (FLX), and lovastatin (LOVA) in two NP models: high-fat diet (HFD) with low-dose streptozotocin (STZ) induced diabetic neuropathy and spinal nerve ligation (SNL). In the HFD + STZ model, Sprague dawley male rats (weighing 250–300 g) were studied over 35 days. HFD was administered from day 1 to day 35, and STZ (35 mg/kg, i.p.) was injected on day 14 to induce T2DM and NP. The study included fourteen experimental groups (n=8), consisting of normal control, diabetic control, Pregabalin-treated (30 mg/kg) group, and treatment groups receiving LOVA (2 mg/kg, 4 mg/kg), FLX (5 mg/kg, 10 mg/kg), and SD (10 mg/kg, 20 mg/kg) alone or in combination. Various assessments were conducted, including lipid profile, body weight (percent change from initial to final day), glucose tolerance assessment (GTA), oxidative stress markers, inflammatory mediators and histopathological analysis. In the SNL model, a 28-day study was performed where L5/L6 spinal nerve ligation was conducted on day 1, inducing chronic NP from day 1 onward. The study involved fifteen experimental groups (n=6), including normal control, SNL control, sham-operated control, Pregabalin-treated (30 mg/kg) group, and treatment groups receiving SD, FLX and LOVA individually or in combination. Evaluations included body weight, biochemical parameter, oxidative stress markers, inflammatory mediators and histopathological examination. The findings from both models revealed significant pain attenuation, reduction in oxidative and inflammatory markers, and neuronal protection in drug-treated groups compared to controls. Histopathological examination confirmed neuroprotection, while metabolic assessments in the diabetic model indicated improved glucose regulation and lipid metabolism. These results suggest that SD, FLX, and LOVA either alone or in combination, may serve as promising therapeutic agents for NP associated with diabetes and nerve injury, warranting further clinical exploration.

Behavioral assessments revealed a significant decrease in withdrawal thresholds in mechanical allodynia (pin-prick test) and thermal hyperalgesia (acetone drop test and tail-flick test) in the HFD-STZ and SNL groups compared to controls ($p<0.05$). In the pin prick test, untreated neuropathic groups displayed heightened sensitivity, with significantly lower paw withdrawal thresholds. Acetone drop test results indicated exaggerated cold hypersensitivity in neuropathic groups, which was markedly alleviated following treatment, particularly in the combination therapy group. Tail-flick latency was significantly reduced in untreated groups, whereas treatment with SD, FLX, and LOVA restored withdrawal latencies toward control levels, with combination therapy showing the most pronounced effect. Rota-rod performance assessment demonstrated significant motor impairment in neuropathic groups, as reflected by decreased latency to fall ($p<0.05$). Treatment with SD, FLX, and LOVA improved motor coordination, with combination therapy displaying the highest improvement.

Biochemical analysis showed elevated oxidative stress markers, including malondialdehyde (MDA) and myeloperoxidase (MPO) activity, in EC group. In contrast, antioxidant markers such as reduced glutathione (GSH) were significantly depleted. Treatment reversed these biochemical imbalances, with a notable reduction in MDA and MPO levels and restoration of

GSH levels ($p<0.05$). Pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), were significantly elevated in untreated neuropathic groups. However, administration of SD, FLX, and LOVA led to a marked reduction in these inflammatory mediators, with combination therapy showing the strongest anti-inflammatory response.

Histopathological examination revealed extensive nerve degeneration, axonal swelling, and demyelination in untreated neuropathic groups. Treatment with SD, FLX, and LOVA preserved nerve morphology, reduced inflammatory infiltration, and maintained myelin integrity. The combination therapy group demonstrated the highest degree of neuroprotection, as evidenced by reduced cellular damage and preserved nerve architecture.

Lipid profile analysis revealed a significant increase in LDL (118 mg/dl vs. 15.33 mg/dl in the normal group), TG (179 mg/dl vs. 23.98 mg/dl), and total cholesterol, while HDL levels dropped significantly (25.55 ± 1 mg/dl vs. 42.56 ± 0.94 mg/dl). Single-drug treatments provided moderate improvements; however, combination therapies, especially the triple combination of (SD+FLX+LOVA- Low dose) yielded the most remarkable reductions (LDL: 55 mg/dl, HDL: 35.65 ± 0.65 mg/dl, TG: 98 mg/dl, and total cholesterol: 87 mg/dl), restoring lipid homeostasis more effectively.

NP assessments demonstrated amplified pain sensitivity in NP induced rats. In the heat hyperalgesia test, hind paw withdrawal latency dropped to 4.13 ± 0.56s in the NP group but improved significantly with triple therapy, reaching 16.99 ± 0.12s by day 35. Similarly, the heat allodynia test showed a latency decline to 11.58 ± 0.023s in the NP group, which improved to 20.95 ± 0.11s with combination therapy. The acetone drop test for cold allodynia indicated a latency increase to 7.49 ± 0.032s in NP rats, which was reduced to 2.03 ± 0.11s following triple therapy. Improvements were evident from day 21, with maximum efficacy by day 35. In mitigating biochemical, oxidative, inflammatory, and histopathological alterations

Biochemical and Oxidative Stress Markers: NP induction resulted in a significant elevation of protein levels (4.96 ± 0.066 gm), malondialdehyde (MDA) (7.5 ± 0.067 gm), and lipid peroxidation (LPO), alongside a marked reduction in key antioxidant enzymes, including glutathione (GSH) (12.76 ± 0.5 gm vs. 49.55 ± 1.22 gm in normal rats), catalase (1.32 ± 0.065 gm vs. normal 4.59 ± 0.039 gm), and superoxide dismutase (SOD) (5.56 ± 0.26 U/mg vs. 16.73 ± 0.23 U/mg in normal rats). Treatment with SD, FLX, and LOVA, particularly in combination, significantly modulated these markers in a dose-dependent manner. Among single-drug treatments, FLX (H) and SD (H) demonstrated the most notable improvements in oxidative stress parameters. However, the triple combination (SD+FLX and LOVA) at low doses) exhibited the greatest efficacy, reducing MDA (2.7 ± 0.056 gm) and restoring GSH (44 ± 0.78 gm), catalase (4.59 ± 0.039 gm), and SOD (15.04 ± 0.056 U/mg) levels to near-normal values.

Inflammatory Markers: NP induction significantly elevated pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) (42.3 ± 0.85 pg/ml vs. 7.4 ± 0.76 pg/ml in normal rats) and interleukin-6 (IL-6) (35.76 ± 0.14 pg/ml vs. lower levels in normal rats). Among single-drug therapies, FLX (H) and SD (H) effectively reduced TNF-α and IL-6 levels. Combination therapies, particularly LOVA (L) + FLX (H) and LOVA (L) + SD (H), demonstrated superior

anti-inflammatory effects. The triple combination therapy resulted in the most significant reductions, lowering TNF-α (12.88 ± 1.32 pg/ml) and IL-6 (9.05 ± 0.092 pg/ml), approaching normal physiological levels, underscoring its potential for inflammation control in NP.

Histopathological Findings: Microscopic examination of the sciatic nerve in NP rats revealed severe pathological alterations, including disrupted nerve fiber alignment, loss of myelin sheath integrity, increased inflammatory cell infiltration, and axonal degeneration. While individual drug treatments showed partial neuroprotection, combination therapies demonstrated superior neurorestorative potential. The triple combination therapy (SD+FLX+LOVA) resulted in the most remarkable histological improvements, including near-normal nerve morphology, reduced inflammation, and preserved myelin sheath integrity, suggesting a synergistic neuroprotective effect in NP management.

This study provides compelling evidence that multi-drug combination therapy, particularly the triple combination of SD, FLX and LOVA exerts superior therapeutic efficacy in mitigating oxidative stress, reducing neuroinflammation, and preserving sciatic nerve integrity in NP. These findings suggest a novel pharmacological approach for diabetic neuropathy management, highlighting the potential clinical relevance of combination therapies in alleviating NP-related complications.

In SNL model the effect of SD, FLX and LOVA with alone and conjunction. Behavioral assessments revealed a significant decrease in withdrawal thresholds in mechanical allodynia (pin-prick test) and thermal hyperalgesia (acetone drop test and tail-flick test) in the HFD-STZ and SNL groups compared to controls ($p<0.05$). In the pin prick test, untreated EC groups displayed heightened sensitivity, with significantly lower paw withdrawal thresholds. Acetone drop test results indicated exaggerated cold hypersensitivity in neuropathic groups, which was markedly alleviated following treatment, particularly in the combination therapy group. Tail-flick latency was significantly reduced in untreated groups, whereas treatment with SD, FLX, and LOVA restored withdrawal latencies toward control levels, with combination therapy showing the most pronounced effect. Rota-rod performance assessment demonstrated significant motor impairment in neuropathic groups, as reflected by decreased latency to fall ($p<0.05$). Treatment with SD, FLX, and LOVA improved motor coordination, with combination therapy displaying the highest improvement. Motor coordination (Rota-Rod test), analgesic response (Tail Immersion test), and oxidative biomarkers (Catalase, Glutathione, SOD, and MDA levels) were assessed over a five-week period. Results demonstrated significant impairments in the neuropathic (NP) group, with progressive motor dysfunction and heightened oxidative stress. While single-drug treatments exhibited partial recovery, combination therapies yielded superior outcomes. By day 28, the triple combination (SD+FLX+LOVA) markedly restored motor coordination (20.89±0.17), tail withdrawal latency (16.41±0.12), and oxidative balance, evidenced by increased catalase (3.98±0.039 U/mg), glutathione (43.42±0.78 µmol/min/mg), and SOD (15.34±0.056 U/mg) activity, alongside reduced MDA levels (2.98±0.065 nM/mg). These findings highlight the synergistic efficacy of the combination therapy, suggesting its potential as a novel approach for managing diabetic neuropathy. Protein estimation revealed a significant increase in protein levels in the SNL group (7.2 ± 0.14 g/dL) compared to the normal control (4.5 ± 0.21 g/dL), with

monotherapies and combination therapies demonstrating dose-dependent reductions. Among individual treatments, FLX exhibited the most pronounced effect, while the triple combination of SD, FLX, and LOVA (low-dose) achieved the lowest protein levels (4.98 ± 0.041 g/dL), indicating synergistic regulation of protein metabolism.

Inflammatory markers, including IL-6 and TNF-α, were significantly elevated in the SNL group (570 ± 3.23 pg/mg and 620 ± 3.56 pg/mg, respectively), reflecting severe neuroinflammation. FLX showed superior efficacy in lowering these markers compared to SD and LOVA, with the greatest reduction observed in the triple combination therapy (IL-6: 95 ± 3.45 pg/mg; TNF-α: 111 ± 3.78 pg/mg), demonstrating the strongest anti-inflammatory synergy. Histopathological analysis revealed extensive axonal degeneration and inflammatory cell infiltration in the SNL group, while combination therapies, particularly low-dose SD + FLX + LOVA, exhibited the most significant structural recovery, reducing nerve edema and preserving nerve integrity.

The therapeutic efficacy of these interventions was further supported by improvements in oxidative biomarkers, including reduced MDA and enhanced SOD and CAT activity. Notably, pain relief was observed earlier in combination therapy groups, with low-dose SD + FLX + LOVA exhibiting improvements from day 7, while monotherapies showed significant effects only after day 28. The neuroprotective effects of these drugs were attributed to their complementary mechanisms, including inhibition of NF-κB and TLR4-mediated inflammation, TRPV and AMPA receptor modulation, and PI3K/Akt pathway activation.

In conclusion, this study highlights the superior efficacy of low-dose combination therapies over monotherapies in mitigating NP, reducing inflammation, and promoting neuroprotection. The findings underscore the potential of multi-targeted pharmacological approaches in early-stage neuropathy management, providing a strong foundation for future clinical applications.

This study demonstrates that NP induced by SNL is associated with significant oxidative stress, protein dysregulation, and inflammatory cytokine overexpression, particularly IL-6 and TNF-α. Among the monotherapies, FLX exhibited the most potent anti-inflammatory and neuroprotective effects, followed by simvastatin SD and LOVA. However, combination therapies provided the most pronounced therapeutic benefits, with the low-dose SD + FLX + LOVA group exhibiting the greatest reduction in protein expression, inflammatory cytokines, and oxidative stress markers.

Histopathological analyses confirmed the neuroprotective benefits of these treatments, with combination therapies showing enhanced nerve fiber integrity and reduced inflammatory infiltration. The mechanistic basis of these effects includes inhibition of NF-κB and TLR4-mediated inflammation, PI3K/Akt pathway activation, antioxidant upregulation, and modulation of serotonergic and ion channel activities.

Overall, the findings highlight the therapeutic potential of multi-targeted combination therapies in NP management, suggesting that lower drug concentrations may provide synergistic neuroprotection and earlier symptom relief.

Keywords: NP, Spinal nerve ligation, High-fat diet, Streptozotocin, OGTT.

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ABBREVIATION	FULL FORM
5HT	5-Hydroxytryptamine Receptors
5-HT	5-Hydroxytriptamine
AAD	Arachidonic Acid
AMPK	5' Monophosphate Activated Protein Kinase
AUC	Area Under The Curve
B (TrkB)	Tyrocin Kinase B
BDNF	Brain Derived Neurotropic Factor
BDNF	Brain Derived Neuropathic Factor
CAM	Calmoduline
CCI	Chronic Constriction Injury
cGMP	Cyclic Guanosine Monophosphate
DMSO	Dimethylsulfoxide
DNA	Deoxyriboneuclic Acid
DRG	Dorsal Root Ganglione
DXH	Duloxetine
eNOS	Endothelial Nitric Oxide
ERK	Extracellular Signal Regulated Kinase
Etc.	Extra
FLX	Fluoxetine
FLXL	Fluoxetine Low Dose
GABA	Gamma Amino Butyric Acid
GSH	Glucocorticoid-Suppressible Hyperaldosteronism.

His	Histamin
HIV	Human Immunodeficiency Virus
i.e.	That is
IC50	Inhibitory Concentarion
MAPK	Mitogen Activated Protein Kinase
MOA	Mechanism Of Action
MPO	Myeloperoxidase
NA	Noradrenaline
NF-κB	Nuclear Factor Kappa
NGF	Nerve Growth Factor
NMDA	N-Methyl, D-Asparted Aspartic Acid
NO	Nitric Oxide
NP	Neuropathic Pain
NRTN	Neurturin
NSAIDS	Non-Steroidal Anti-Inflammatory Drugs
NTs	Neurotransmitters
P38	Protein 38
PDE	Phosphodiesterase Inhibitor
PDEi	Phosphodiaestarase Inhibitors
PKG	Protien Kinase
PKG-L	Protien Kinase- L Receptor
PK-PD	Pharamcokinatic And Pharmadinamic
PSL	Partial Schatic Nerve Ligation
pSNL	Partial Sciatic Nerve Ligation
SD	Sildenafil
SDH	Sildenafil High Dose
SDL	Sildenafil Low Dose
SEM	Standard Error Mean
SERCA	Sarco-Endoplasmic-Reticulum
SERT	Serotonin Reuptake Transporter

SNCV	Sensory Nerve Conduction Velocity
SNI	Spread Nerve Injury
SSRI	Selective Serotonin Reuptake Inhibitor
STZ	Straptozotocin
TNF-α	Tumour Necrosis Factor
TRPV1	Tyrosinase Related Protein 1
CRPS	Complex regional pain syndrome
MS	Multiple Sclerosis
HIV	Human Immuno deficiency Virus

CHAPTER 1
INTRODUCTION

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1.1. Neuropathic pain (NP)

NP remains a major problem, with many patients unable to successfully manage the devastating symptoms, and complete eradication of the disease remains an elusive goal. This has prompted the exploration of alternative treatment options. NP is a debilitating condition affecting the somatosensory nervous system, arising from various causes such as damage to primary afferent nerves, the spinal cord, lumbar and cervical radiculopathy, as well as metabolic disorders like diabetes, HIV, and cancer. The human body responds to various stimuli such as touch, taste, and smell through the sensory system, which processes these sensations as part of its functional mechanisms. It is characterized by symptoms including spontaneous pain, dysesthesia, hyperalgesia, and allodynia, often leading to severe chronic sensory damage. When exposed to external triggers like sharp objects, edges, or forces, pain receptors (nociceptors) are activated, leading to the sensation of pain. Once the stimulus is removed, nociceptors typically return to their inactive state. However, prolonged activation of these receptors can lead to damage to the somatosensory part and nerve cells, resulting in a condition known as NP.

NP is directly linked to somatosensory system dysfunction and can arise from various pathological conditions, including high blood glucose levels (as seen in diabetes), varicella-zoster virus (shingles), toxicity from certain chemotherapy drugs, chronic alcohol abuse, peripheral nerve compression, spinal cord injury, abnormal nerve signaling, improper immune responses, and genetic disorders. Once NP affects the PNS and CNS both, it can lead to prolonged activation of irritating fibers, like- α, β, δ, and C fibers, significantly impacting the quality of life. Pain fibers acts in the body with the several degrees of stimulus. For instance, in normal conditions, touching a smooth or furry surface may feel soothing, but in individuals with peripheral nerve injuries, the same stimulus can cause pain; a condition known as allodynia, “where pain arises from a non-painful stimulus”. Correspondingly, “when pain arise from painful (noxious) stimuli” exceeds the pain threshold, it activates all pain receptors, called hyperalgesia, where the individual experiences an exaggerated pain response. Additionally, when pain manifests as sensations like sharp needle pricks or burning sensations, the phenomenon is referred to as “paraesthesia”. These terminologies and mechanisms are vital in understanding and characterizing the complex nature of NP.

1.2. Prevalence of NP

Nowadays the ratio of NP upgraded progressively, the prevalence of NP has increased day by day, with its global impact ranging between 3% and 17%. Numerous studies conducted by researchers annually highlight this growing concern. The graph below illustrates the year-by-year rise in NP cases, reflecting its escalating presence worldwide. The prevalence of diabetes has increased by 20% to 30% from the previously estimated rates of 7% to 10% (Baskozos et al. 2023). The reported prevalence of differs based on the population studied and the diagnostic approaches employed. For instance, research conducted in the United States estimated NP prevalence at 7.1%, whereas European studies have reported higher rates, exceeding 10.6%. Several articles from 2014 present research data on the prevalence of NP through 2024–2024. NP is present in all populations, "It tends to be more prevalent among women than among men" (Bannister et al. 2020) However, according to Purwata et al., the risk is higher in males (Purwata et al. 2015). Its prevalence increases with age over 50 (Cavalli et al. 2019) which means older people also contribute to the prevalence of NP (Purwata et al. 2015; Watson and Sandroni 2016).

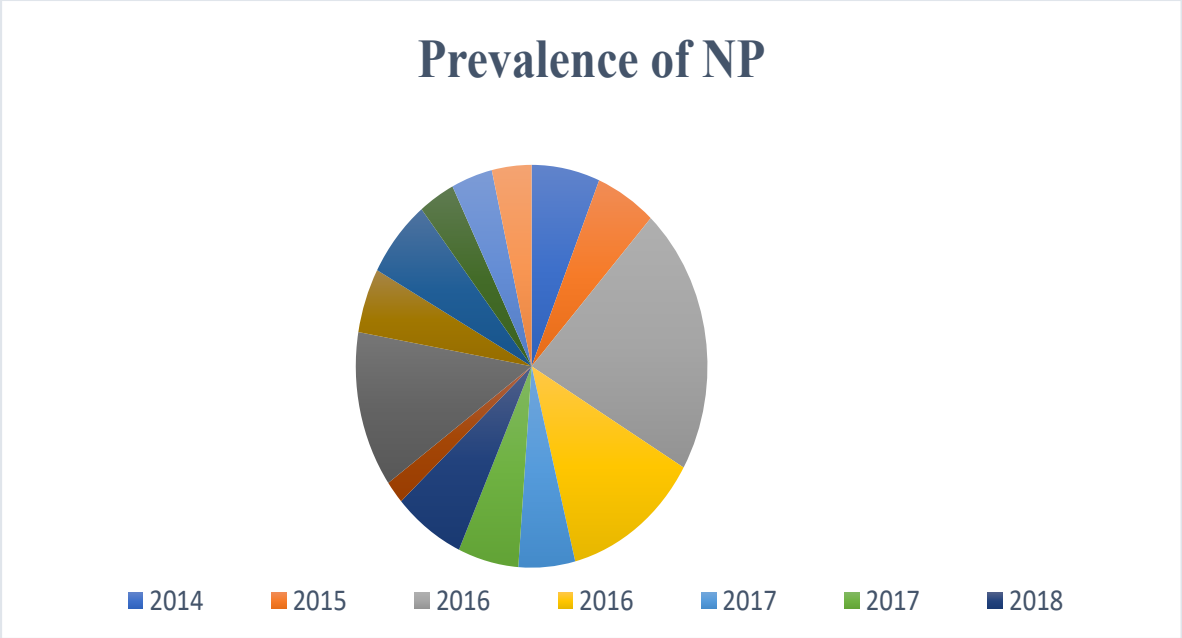


Figure 1. Prevalence of NP: Studies have shown different prevalence rates

In the United States, the prevalence was reported as 7.1%, whereas in Europe, it reached 10.6%, likely influenced by regional and demographic differences. These variations can be

attributed to differences in diagnostic criteria and characteristics. Additionally, the prevalence of NP is higher in certain conditions such as diabetes, where it can range between 20-30% (Lueshen et al. 2014). The prevalence of NP differs in rheumatic diseases (rheumatoid arthritis (RA), osteoarthritis (OA), and fibromyalgia). For instance, in RA, NP is commonly associated with higher disease related issues, poorer functional status, and diminished quality of life (Muthuraman and Singh 2012). Studies cited within the article reported NP prevalence in RA patients to be between 13% to 35% (Garip et al. 2015). Xu, Zhang, and Huang (2016) conducted a study focusing on the prevalence of NP. According to their findings, the prevalence of NP was approximately 11%. The researchers highlighted the complexity of diagnosing NP was part of a broader discussion on the treatment of NP and contributed valuable data to understanding the scope of this condition globally (Xu, Zhang, and Huang 2016). The study "PNP: A Mechanism-Related Organizing Principle Based on Sensory Profiles" by Colloca et al. (2017) examines the prevalence and nature of NP through sensory profiling. Utilizing quantitative sensory testing on 902 patients, researchers identified three distinct sensory profiles: sensory loss, thermal hyperalgesia, and mechanical hyperalgesia. Each profile signifies different underlying pain mechanisms. The study found that these profiles' prevalence was approximately 15%, highlighting the intricacy of NP and emphasizing the individualized treatment approaches (Colloca et al. 2017; Oladele et al. 2025). Another study found a notably lower prevalence of just under 5%. This research highlighted the significant variability in the occurrence of NP across different places and conditions (Pai et al. 2018). A conducted comprehensive study that revealed that approximately 10% of the public suffers from NP. This condition, characterized by a complex, “chronic pain state that usually is accompanied by tissue injury” which including various disease (i.e. diabetes, shingles, and nerve trauma). The findings of Feldman et al. highlight the substantial burden of NP on individuals and emphasize the need for enhanced therapeutic strategies and management protocols to address this prevalent issue. Early diagnosis of NP is important as it resulting in an best treatment selection strategies (Feldman et al. 2019). Subsequent research by Rosenberger et al. (2020) documented an even higher prevalence, estimating that approximately 28% of individuals experience NP. This higher prevalence rate underscores the widespread nature of the condition and its substantial impact on public health. NP can arise from a variety of causes, including diabetes, shingles, and nerve injuries which affecting the “quality of life”. The findings from Rosenberger et al. highlight the unavoidable needs for improved diagnostic measures and more effective treatment options to address this significant health issue (Mian et al. 2025). Early intervention

and comprehensive pain disease control measures are essential to alleviate the suffering from NP and to reduce its overall burden on healthcare systems (Rosenberger et al. 2020). NP remains a significant and challenging condition affecting many individuals worldwide. Clavo et al. (2021) reported that approximately 9% of the patient experiences NP, highlighting the widespread nature of this chronic pain condition (Clavo et al. 2021). Additionally, a study by Mitsikostas et al. (2022) observed a higher prevalence, estimating that around 15% of individuals suffer from NP (Mitsikostas et al. 2022). Also, Doneddu et al. (2023) found the prevalence to be close to 12% (Leoni et al. 2025; Scholz et al. 2019).

NP prevalence varies significantly across regions, influenced by methods used, demographic factors, and socioeconomic conditions. In the United Kingdom, the S-LANSS questionnaire identified an 8% prevalence of NP, while a postal questionnaire revealed 48% of participants experienced chronic pain, with 8% being neuropathic in origin. Specific conditions like trigeminal neuralgia (26–27 cases per 100,000 people/year) and phantom limb pain (1–2 cases per 100,000 people/year) were highlighted. In France, the DN4 questionnaire reported a prevalence of 7% in 2008, decreasing slightly to 6.9% in subsequent studies, with higher rates in middle-aged individuals, manual workers, and rural residents. Germany, using telephone interviews, recorded a 6.5% prevalence, with patients reporting significant pain intensity. In the Netherlands, the Integrated Basic Attention Information database found an overall NP prevalence of 8.2 cases per 1,000 patients/year, with specific conditions like mononeuropathy (4.3 cases), carpal tunnel syndrome (2.3 cases), diabetic peripheral neuropathy (0.72 cases), and postherpetic neuralgia (0.42 cases). NP predominantly affected females, peaking in individuals aged 70–79. Canada reported a higher prevalence of 17.9% through telephone surveys, with females and economically disadvantaged individuals more affected. In Austria, an unspecified method identified a 3.3% prevalence, highest among those aged 41–50 (26%) and 51–60 (25%). Overall, NP prevalence varies widely across regions and is Conditioned by factors which include age, gender, socioeconomic status, and occupational exposure, with some regions reporting increasing rates over time while others show a decline (Posso, Palmeira, and Vieira 2016; Mian et al. 2025). These searching highlight the widespread impact of NP and the necessity for effective management strategies.

1.3. Causes and types of NP

NP can result from various causes, including diabetes, herpes zoster infections, and nerve injuries, leading to chronic and severe pain that significantly impairs quality of life. The data

from these studies underscore the importance of early diagnosis, improved treatment protocols, and comprehensive management approaches to address this pervasive health issue. Effective intervention and tailored therapeutic strategies are essential to improve patient outcomes and reduce the burden on healthcare systems (Doneddu et al. 2023). In summary, the reported prevalence of NP varies significantly across different studies, with the highest recorded at 30% and the lowest just under 5%. NP is not a single disease it comprehends a variety of diseases including drug-induced NP, disease-induced NP, and injury-induced NP. These different types of NP are further classified in Table 1. These diseases often do not exhibit symptoms in early stages, however, once they become chronic, they can affect the somatosensory system in the brain, considerably influencing the "quality of life" of the patient. NP leads from many diseases mentioned in Figure 1. It depicts the types (Feldman et al. 2019; Muthuraman, Singh, and Jaggi 2011; Roos 2021; Mitsikostas et al. 2022; Xu, Zhang, and Huang 2016; Van Hecke et al. 2014; Sommer, Leinders, and Üçeyler 2018; Selvy et al. 2021; Abers, Shandera, and Kass 2014; Shin et al. 2019; Stevens et al. 2019; Ölmestig et al. 2017).

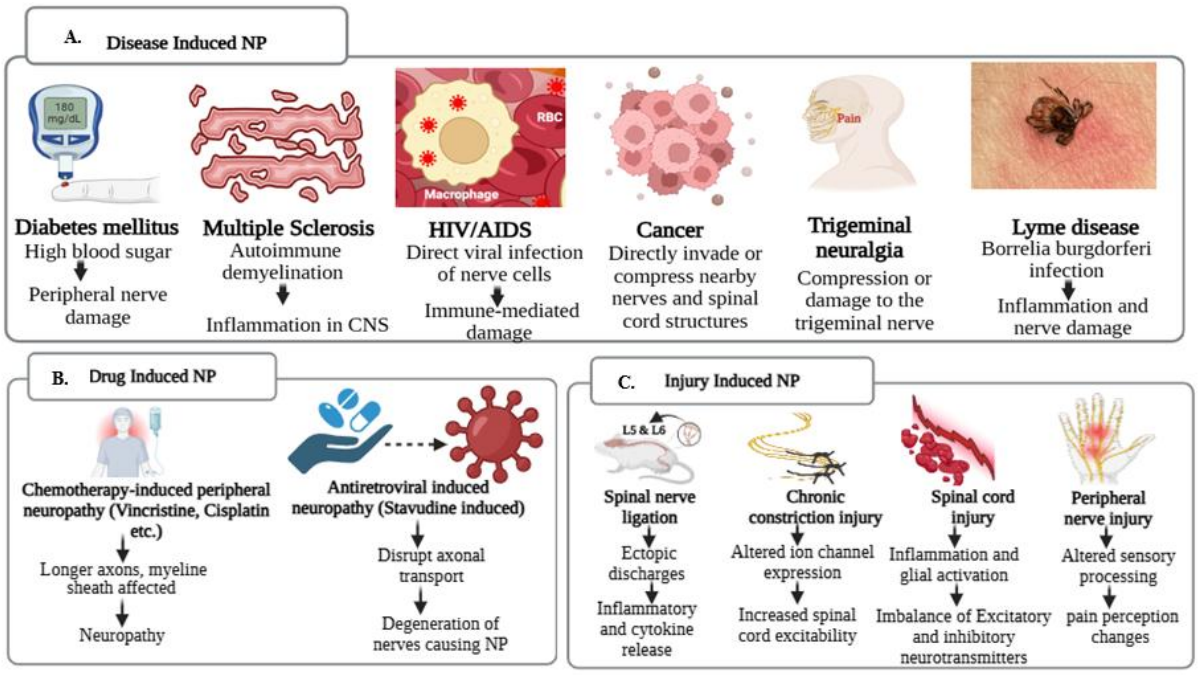


Figure 2. Types of NP: A. Disease Induced NP **B.** Drug Induced NP **C.** Injury Induced NP

NP into three primary categories: disease-induced NP, drug-induced NP, and injury-induced NP. Diseases such as diabetes mellitus, multiple sclerosis, HIV/AIDS, cancer, and trigeminal neuralgia can induce NP. NP induced by drugs like chemotherapy-induced Chemotherapeutic agents like vincristine and cisplatin induce peripheral neuropathy by affecting the longer

axons and myelin sheaths and Antiretroviral drugs that induce neuropathy, such as Stavudine, disrupt axonal transport and cause nerve degeneration, ultimately leading to NP. NP Chronic constriction injury is caused by injuries like spinal nerve ligation, which cause inflammatory cytokines to be released and ectopic discharges. This changes ion channel expression, making the spinal cord more excitable (Figure 2).

Inflammation, glial activation, and an imbalance of excitatory and inhibitory neurotransmitters are the consequences of spinal cord injury. Peripheral Nerve Injury alters sensory processing to modify pain perception. Numerous conditions, medications, and physical injuries can cause neurological pain, each with its unique mechanisms and manifestations. This detailed classification shows NP complexity. Certain patient, particularly with having insulin resistance, cancerous development, infections, have elevated occurrence of NP (Anil et al. 2016).

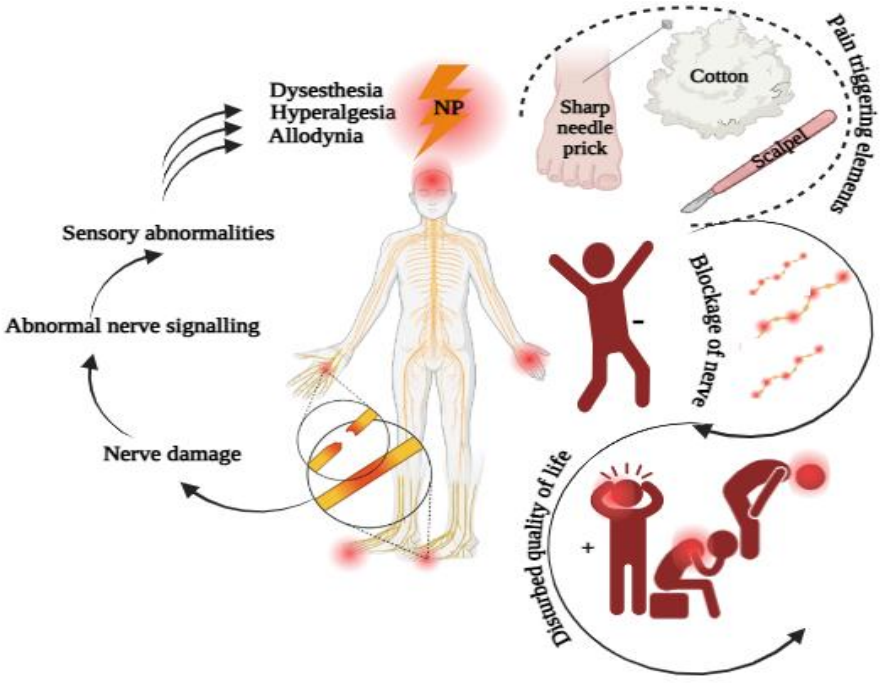


Figure 3. NP: Complex mechanisms and consequences of NP

In an early stage of neuropathy, there will be a transitory activation of pain fibres (particularly AC and Aδ) known as nociceptive pain. Aδ fibers are thin myelinated fibers, almost with a diameter of 1-5µm and a conduction velocity of 3-30m/s. They are associated with cutaneous and mechanoreceptor functions for nociception and cold sensation. The C fibers, have a diameter of around 0.2-1.5µm and a conduction velocity of 0.5-2.0m/s, responsible for transmitting pain and warmth sensations. NP is a complicated chronic condition which

characterized by “pain that transpires from the damage and dysfunction” (abnormal processing of sensory signals) of the somatosensory nervous system. It is emphasized through various aetiologies such as nerve compression, trauma, diabetes, HIV, cancer, MS, CRPS, postherpetic and trigeminal neuralgia (Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al. 2019).

The depicted the origination of NP from nerve damage, which can result from various causes such as injury, surgical procedures, or diseases like diabetes (Figure 3). This damage leads to abnormal nerve signaling, characterized by heightened sensitivity and misfiring of pain signals that are not typically associated with pain. The abnormal signaling induces sensory abnormalities, including dysesthesia (unpleasant abnormal sensations), hyperalgesia (increased sensitivity by noxious stimuli), and allodynia (Severe pain in non-noxious stimuli). Triggering elements for NP include sharp needle pricks, cotton touch, and minor injuries, which can elicit severe pain responses. Furthermore, the blockage or dysfunction of nerve pathways exacerbates these sensory abnormalities and pain sensations. Consequently, individuals with NP experience a significantly disrupted quality of life, marked by both emotional distress and physical discomfort. This underscores the cyclical nature of neuropathic pain, where nerve damage initiates a cascade of abnormal signaling and sensory disturbances, perpetuating the pain cycle and severely impacting the individual's overall well-being. NP involves a complex interaction of physiological changes along with significant emotional and mental challenges (Figure 3). Although several treatments, including antiepileptic and antidepressant medications, are available, the intricate mechanisms and pathophysiology of NP often make monotherapy insufficient for long-term symptom control. The phenomenon called "wind-up" explains how repeated stimulation of nociceptors gradually amplifies the central nervous system's pain response, resulting in abnormal sensations like tingling and numbness, as well as heightened sensitivity to stimuli (hyperalgesia and allodynia). Sensitization occurs through both peripheral and central pathways. Following nerve damage, increased excitability and spontaneous nerve discharges feeling like burning, shooting, or electric shock-like pain. Chemical mediators also play a crucial role by stimulating neurotransmitters (like glutamate, substance P, CGRP, and prostaglandins). They directly influence neuroplasticity, neurotrophins, and sensitization of pain perception. Furthermore, the presence of pro-inflammatory factors elevates oxidative stress, activating key elements like TNF-α and IL-6, which intensify pain symptoms.

Dysregulation of sodium and calcium channels further increases neuronal excitability, promoting the development of NP.

1.4. Treatment of NP

Accurate diagnosis and effective treatment require an in-depth understanding of NP's mechanisms and clinical manifestations. Further research is necessary to develop targeted interventions that can relieve symptoms and enhance the "quality of life" for individuals (Neerati and Gudimandula 2024).

Table 1. Treatment of NP

Class	Drug	Mechanism of Action	Dose	Challenges	Reference
Anticonvulsants (First-Line treatment)	Pregabalin	Act on α2δ subunit	150 mg/day	Limited efficacy, dizziness and somnolence	(Zarei et al. 2016; Kaur et al. 2025)
	Gabapentin	Acts in VGCC via binding in α2δ subunit	1 00–300 mg/day	High interpatient variability in response	(In et al. 2024)
SNRIs (Antidepressants) (First-Line treatment)	Duloxetine	Inhibits reuptake of serotonin and norepinephrine	60–120 mg/day.	Nausea and unsuitable in severe liver or renal impairment	(Alfaro-Rodríguez et al. 2024; P. R. Wilson et al. 2008)
SSRI (Antidepressants) (First-Line treatment)	Fluoxetine	Inhibits reuptake of serotonin			
TCAs (Antidepressants) (First-Line treatment)	Amitriptyline	Inhibits reuptake of serotonin and norepinephrine; has antihistaminic and anticholinergic effects	25–150 mg/day.	Sedation, dry mouth, weight gain	(Alhowiti and Mirghani 2024; P. R. Wilson et al. 2008)
Topical Analgesics (Second-Line treatment)	Capsaicin 8% Patch	Activates TRPV1 receptors, leading to desensitization of pain fibers	30–60 minutes	Severe burning sensation	(Moreno-Alonso et al. 2024; Kopalli et al. 2025)
	Lidocaine Patch 5%	Blocks sodium channels, stabilizing neuronal membranes, and inhibiting pain signal conduction	Applied once daily	Ineffective for widespread or systemic pain	(Mao et al. 2024)
Opioids (Second-Line treatment)	Tramadol	Binds to μ-opioid receptors and inhibits reuptake of serotonin and norepinephrine	75 mg/kg	Dependency and tolerance.	(Edinoff et al. 2021)
Botulinum Toxins (Third-Line treatment)	Botulinum Toxin A	Inhibits release glutamate and substance P, reducing peripheral sensitization	Dosage varies; administered by healthcare professionals.	Safety is uncertain.	(Targe and Bhayani 2022)

As knowledge and therapeutic options advance, care for those with NP will continue to improve. At last, NP is a challenging condition caused by “damage or dysfunction” in the nervous system, and its management often requires more than a single medication. This is because the mechanisms underlying NP are complex and vary between individuals (Shi et al. 2024). As a result, combination therapy is frequently employed to provide better pain relief by targeting multiple pathways simultaneously. The use of drug combinations is driven by the multifaceted nature of NP. It arises from various processes like damage in the nerve, further inflammation, and then enhance central sensitization which leading the difficulty to manage from a single medication. For example, anticonvulsants like gabapentin or pregabalin help reduce overactive nerve signals, while antidepressants such as amitriptyline or duloxetine modify how the brain perceives pain. Combining these medications allows for a more comprehensive approach to managing pain. Moreover, combination therapy enhances efficacy by synergizing the effects of different drugs, often enabling the use of lower doses. This reduces the risk of side effects compared to relying on higher doses of a single medication. For instance, the combination of an antidepressant with an anticonvulsant not only tackles nerve hypersensitivity but also improves mood, which can indirectly help with pain management. Common combinations include antidepressants with anticonvulsants, opioids with other classes of drugs, or systemic medications paired with topical treatments like lidocaine patches or capsaicin creams. These combinations are tailored to the patient’s specific condition, ensuring that the treatment is as effective as possible. In essence, the need for combination therapy arises from the complex and diverse nature of NP. By addressing multiple mechanisms, optimizing efficacy, and minimizing side effects, these combinations offer a more personalized and effective approach to pain management. While traditional treatments often involve combining drugs like anticonvulsants, antidepressants, and opioids, new and innovative combinations are continually being explored to improve outcomes.

Managing chronic NP is particularly difficult due to its resistance to conventional analgesics. While several promising drugs are available, unconventional options like tricyclic antidepressants have been utilized as first-line therapies but often fail to provide sufficient relief. Consequently, a multimodal or combination therapy approach is being considered to enhance the duration of analgesic effects and minimize adverse reactions. Sildenafil (SD), a selective phosphodiesterase type 5 (PDE5) inhibitor, is widely recognized for treating conditions like erectile dysfunction and pulmonary hypertension, as well as for its neuroprotective properties, including its role in neurogenesis. The antinociceptive effects of

SD are believed to involve cyclic guanosine monophosphate (cGMP) and interactions with GABA receptors. It has also demonstrated therapeutic efficacy in previous models of NP. Fluoxetine (FLX), a well-known selective serotonin reuptake inhibitor (SSRI), has been considered due to its potential benefits in pain management, despite conflicting evidence. It is commonly used for mood, behavioral, and depressive disorders. Our previous report already established the development of chronic constriction injury model in rats with effective treatment using SD and FLX (Melkani, Kumar, Panchal, Gulati, Gill, Jyoti, Pandey, and Kumar 2019). CCI induction led to the development of characteristic NP symptoms in rats, closely resembling those observed in humans. An enhanced therapeutic effect for NP was observed following the administration of the SD-FLX combination in CCI-induced rats. Notably, SD appears to enhance the effectiveness of FLX. This improvement may stem from the synergistic interaction between the two drugs, each acting through distinct yet complementary mechanisms. Therefore, this combination may serve as a promising and effective therapeutic approach for managing NP (Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, and Kumar 2019).

The present investigation finding out the result after administering the all-encompassing a combination of drugs like; FLX (a selective serotonin reuptake inhibitor), (a phosphodiesterase inhibitor), and Lovastatin (LOVA) (an HMG-CoA inhibitor) in different models of NP in rats. Rats fed a high-fat diet (HFD) are often observed to develop insulin resistance, though not necessarily progressing to hyperglycemia or diabetes. HFD is considered an effective approach to initiating insulin resistance. Streptozotocin (STZ), on the other hand, is commonly used to induce diabetes by causing β-cell death through DNA alkylation. While high doses of STZ lead to severe insulin secretion impairment resembling type 1 diabetes, low doses result in mild impairment of insulin secretion, mimicking the later stages of type 2 diabetes. Combining HFD with low-dose STZ has emerged developing type II diabetic neuropathy (a rat model that replicates the progression from insulin resistance to β-cell dysfunction). Diabetes is a chronic progressive metabolic disorder that affects brain cells and leading the secondary complications which includes neuropathy, retinopathy, nephropathy, and cardiomyopathy (Sood et al. 2020) confirming via changes in the biochemical pathways after performing the tests in the preclinical as well as clinical studies. The ailment of these complications cannot treat easily it becomes a major challenge to treat worldwide. Finally, it defined as, “NP is a complicated disorder that is characterized by aberrant pain perception”. It provokes a series of actions brought on by nerve damage or injury that cause the release of different chemicals, channels and other substances that

contribute to peripheral sensitization. Glial cell activation in the dorsal root ganglia amplifies pain signals even more by raising ATP, H+, and glutamate levels, leading to central sensitization and dysregulation of ion channels leading the excitation.

Alternatively, the SNL is a well-established experimental approach for studying peripheral neuropathic pain (Dupuis et al. 2017). Its induction typically requires surgical exposure involving the separation of paraspinal musculature and removal of transverse processes, which may lead to localized tissue damage. This additional trauma can potentially alter the pathophysiological mechanisms underlying neuropathic pain by causing targeted physiological modifications (Chung et al. 2015).

One such promising combination includes SD, FLX, and LOVA, which may offer a groundbreaking approach to managing NP by targeting multiple mechanisms involved in its pathology. Choosing Combination is a Game-Changer for NP. The combination of SD, FLX, and LOVA works synergistically to address various pathways associated with NP Like as follows below. SD (Phosphodiesterase-5 Inhibitor) is primarily known for its role in improving blood flow by enhancing nitric oxide-mediated vasodilation. In the context of NP, it has been shown to improve microvascular blood flow and reduce oxidative stress, which are important to nerve damage. By restoring vascular health and reducing inflammation, SD helps alleviate pain and promotes nerve regeneration. FLX (Selective Serotonin Reuptake Inhibitor) enhances serotonin levels in the CNS, which modulating pain perception. It not only helps in reducing the hyperactivity of pain pathways but also improves the emotional and psychological well-being of patients, which is often impaired in chronic pain conditions. Additionally, FLX exhibits anti-inflammatory properties, further contributing to its effectiveness in managing NP. LOVA (Statin), commonly used for lowering cholesterol, has shown potential benefits in NP due to its anti-inflammatory and neuroprotective effects. It reduces pro-inflammatory (cytokines, oxidative stress etc.), which are major contributors to nerve damage and pain sensitization. LOVA also enhances nerve repair mechanisms, making it a valuable component in this combination.

1.5. Combination approach

A combination of SD, FLX and LOVA has shown promising results as a more effective approach compared to pregabalin for the management of neuropathic pain. SD, a PDE5 inhibitor, enhances blood flow and has neuroprotective effects that complement the pain-relieving properties of FLX, a SSRI known to modulate pain perception through serotonin

pathways. Additionally, LOVA, a lipid-lowering agent, exhibits anti-inflammatory and neuroprotective benefits by reducing oxidative stress and improving vascular function. This combined approach offers a multimodal mechanism that targets various pain pathways, providing superior relief with potentially fewer side effects compared to the use of PB alone, which primarily targets calcium channels but may cause dizziness, sedation, and tolerance issues over time (P. Wilson et al. 2008).

The combination of SD, FLX, and LOVA represents a promising and novel approach to NP treatment. By targeting key factors such as vascular health, inflammation, oxidative stress, and pain perception, it offers a well-rounded solution to address the complex nature of this condition. Additionally, using lower doses of each drug in combination reduces the likelihood of side effects, enhancing the safety and tolerability of the therapy for patients. This groundbreaking combination has the potential to transform the management of NP, bringing hope to those who have not experienced relief from conventional treatments. With further clinical research and validation, it could set a new benchmark for treating this challenging and debilitating condition.

CHAPTER 2
LITERATURE REVIEW

2.1. Perception of pain and NP

It is an emotional and unpleasant sensory from actual or potential tissue damage. Its safest mechanism and prompting necessary responses to avoid further harm. Pain is detected by nociceptors, which are sensory receptors responsive to the stimuli (mechanical, thermal, and chemical). Than nociceptors transmit signals to the brain, where they are interpreted as pain (Otari & Upasani, 2015; Pasero, 2004).

NP is a subtype of chronic pain, occurs because of damage or dysfunction within the somatosensory system itself. Unlike pain which leads direct injury to tissues, NP stems from pathological changes in nerve function. This type of pain can arise from various conditions such as diabetes, shingles, spinal cord injuries, or chemotherapy. These nerves have special fibers (i.e. Aδ and C fibers) that regulate pain pathways (Edwards et al., 2008). The stress activates the fibers from the social, emotional, and physical stress where the body feels uncomfortable leading to unpleasant sensations and the condition can be acute or chronic (Table 2). However, nociceptive pain occurs when the tissue becomes injured and then activates the pain pathways via Aδ and C fibers in the body once the stress is removed it will become a normal stage, moreover, in NP where the pain fibers are fully damaged and unable to send the message to the brain and brain to the body that condition is the worst condition or cannot be treated by the one medicine properly because in this condition the direct attacking the somatosensory nervous system which activates Aδ and C fibers (Ueda, 2008).

Table 2. Types of pain and their distinguished characters (Ibuki et al., 1996; Shim et al., 2005)

S. No.	Aspects	Pain	Nociceptive pain	NP
1.	Description	Causing pain due to unpleasant sensory and emotional experience	Tissue injury can activate the nociceptors	Pain because of the damage in the somatosensory nervous system (Solinas et al., 2022)
2.	Mechanism	Activation of nociceptors	Activation of nociceptors	Aberrant signalling of nociceptors
3.	Fiber	Aδ, and C	Aδ, and C	Aβ, Aδ, and C
4.	Condition	It can be acute and chronic	Resolve with treatment and healing	Chronic and lethal
5.	Example	Cut and burn	Broken bone	Diabetic neuropathy and postherpetic neuralgia

2.2. Pain fibers and development of NP

Pain fibers can be categorized into two major types: Aδ and C fibers which have arranged as thinly myelinated and unmyelinated respectively. Aδ fibers transmit fast, sharp pain, while C fibers transmit slow, dull, and aching pain. The Aδ fibers are triggered via mechanical and thermal stimuli, while the C fibers are activated through mechanical, thermal, and chemical stimuli (Gebhart, 2013). When these fibers are activated, they bind with the receptors or may act directly to activate the pain signals, which initiate the sensation in the body. The abnormal sensation leads to damage in the nerve cells, altering the excitability and inhibitory actions somatosensory systems. This can result in abnormal spontaneous firing, causing shooting, burning, or electrical shock-like pain (Scholz & Woolf, 2007; Shim et al., 2005).

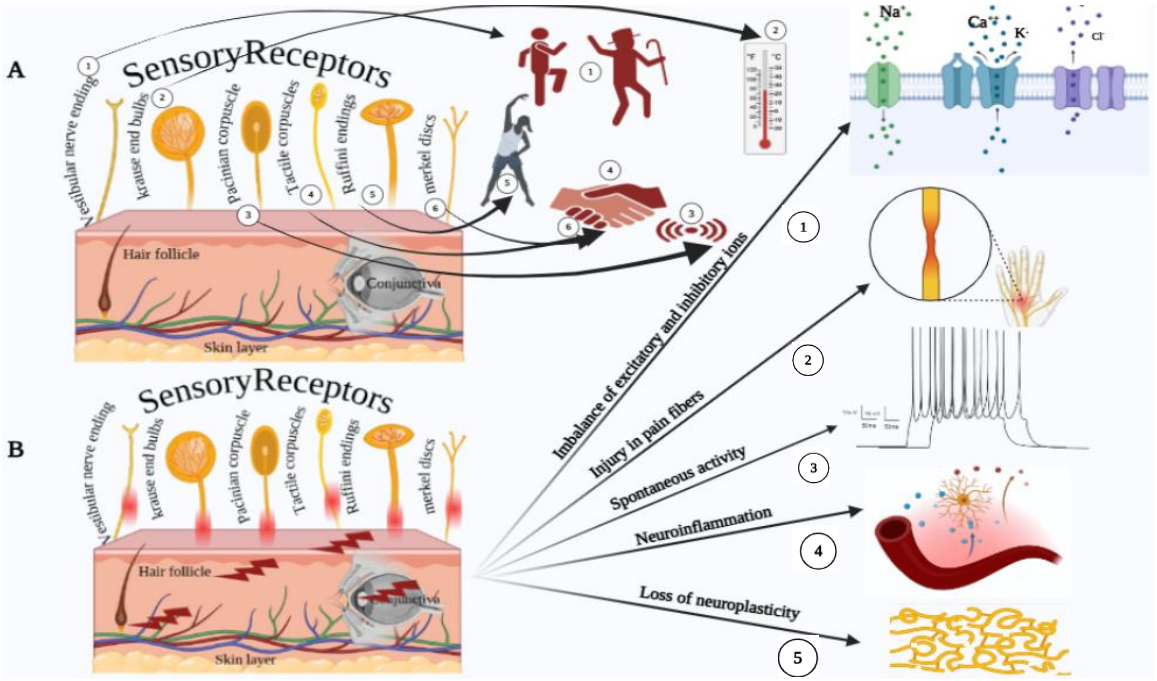


Figure 4. Sensory receptors: A. Location of normal sensory receptors with proper functioning 1. Vestibular nerve ending; Associated with balance and spatial orientation, 2. Krause end bulbs; Detect cold temperatures, 3. Pacinian corpuscles; Sensitive to vibration and pressure, 4. Tactile corpuscles (Meissner's corpuscles); Detect light touch, 5. Ruffini endings; Respond to skin stretch, 6. Merkel discs; Detect light touch and texture. **B. Pathological alteration after injury in the sensory receptors**: 1. Injury and Neuroinflammation, 2. Ion Imbalance, 3. Spontaneous Activity and Pain, 4. Neuroinflammation (Esplugues, 2002; Jannini et al., 2009; Schumacher, 2011)

The pain is characterized by hyperalgesia, allodynia, and paraesthesia. These characteristics are to understanding the mechanisms of NP. The nerve fibers compromised, malfunctioning, or harmed. These impaired nerve fibers transmit inaccurate signals to other pain centers. Several sensory receptors are available for the particular respective sensation, if the singnals

receive any improper message then it converts into injury and leads to diseases. Like the dermal papillae have tactile corpuscles (Meissner's Corpuscles) for the light touch sensitivity in the skin. After injury, tactile corpuscles can lead to hyperalgesia and allodynia. Pacinian corpuscles (lamellar corpuscles) are located in the deep skin like the dermis and subcutaneous tissues, and they are mechanoreceptors sensitive to vibration and pressure. Its disbalance leads to improper signaling sensation (García-Mesa et al., 2021) .

Loss of Ruffini Endings leads to abnormal sensations. If the spatial orientation is disturbed through the vestibular nerve ending causing the NP. The merkel discs are located in the epidermis and hair follicle for the light touch (García-Mesa et al., 2021). Cold sensation of eye conjunctiva and vagina sensation specialised through Krause End Bulbs, if damaged its lead the dysesthesias (more pain sensation in the cold). The illustration (Figure 4) compares normal sensory receptor function with the pathological changes that lead to chronic pain, such as NP. It depicts how various skin receptors, like Pacinian corpuscles and Meissner's corpuscles, typically detect stimuli and transmit signals through Aδ and C nerve fibers to the brain, guided by balanced ion channels. However, when injury or inflammation occurs, this balance is disrupted, leading to abnormal, spontaneous nerve activity that triggers persistent pain sensations. The image also emphasizes how neuroinflammation exacerbates this pain by further sensitizing the affected nerves, offering insight into potential therapeutic targets for managing chronic pain.

2.3. Specialized receptors and nerve fibers

The somatosensory system plays a crucial role in detecting changes both within the body and in the surrounding environment through specialized receptors and nerve fibers. These receptors are categorized based on the specific types of stimuli they respond to. Mechanoreceptors; are sensitive to physical forces such as pressure, stretching, and vibration, commonly found in the skin, joints, and auditory structures (Gracely et al., 1992). Chemoreceptors; monitor chemical changes; internal ones detect variations in blood glucose, osmotic pressure, and gas concentrations, while external types are involved in sensing taste and smell (Park, 2023). Photoreceptors; located in the retina, enable vision by converting light stimuli into neural signals. Thermoreceptors; detect temperature changes, with separate receptors for warmth and cold. Nociceptors; are responsible for detecting harmful stimuli, including physical injury, extreme temperatures, or chemical threats. Sensory information is carried by three major groups of nerve fibers such as group A, B, and C fibers which differ in their speed of

conduction, diameter, and myelination. Various fibers involved in NP are given below (Jelicic Kadic et al., 2014; Park, 2023):

1. **Group A fibers;** are myelinated and transmit signals rapidly. They include:
   i. **Alpha (α) fibers:** Large fibers (12–20 μm) with high conduction speeds (70–120 m/s) for proprioception and voluntary movement control.
   ii. **Beta (β) fibers:** Medium-sized fibers (5–12 μm) conducting signals at 30–70 m/s, involved in sensing touch and pressure.
   iii. **Gamma (γ) fibers:** Smaller fibers (3–6 μm) with conduction speeds of 15–30 m/s, responsible for regulating muscle tone.
   iv. **Delta (δ) fibers:** Smaller still (2–5 μm), transmitting sharp pain and cold sensations at 12–30 m/s.
2. **Group B fibers;** are lightly myelinated, with diameters below 3 μm and conduction speeds of 3–15 m/s, transmitting autonomic preganglionic signals.
3. **Group C fibers;** are unmyelinated and conduct signals slowly, with speeds of 0.5-2.3 m/s and diameters between 0.4–1.3 μm. These includes dorsal root C fibers, which relay pain and temperature signals, and sympathetic C fibers, which control involuntary functions like heart rate and gland secretion. The physiological characteristics of nerve fibers, particularly myelination and diameter, significantly affect their signal transmission properties. Myelinated fibers enable faster communication due to saltatory conduction, making them suitable for immediate sensory responses and motor control. Unmyelinated fibers, although slower, are essential for carrying persistent pain signals and autonomic functions, reflecting the nervous system's adaptation to diverse sensory demands.

2.4. Types, causes, pathophysiological processes, and treatment options for NP

NP can result from damage to the both (peripheral or central) nervous system, leading to persistent or abnormal pain sensations. Each category highlights the causes, pathophysiological processes, and treatment options that are crucial for understanding and managing this complex condition effectively (Lisney & Devor, 1987; Pasero, 2004). The classification provides a comprehensive view to support clinical decision-making and research on personalized pain therapies (Table 3).

Table 3. Various types of NP, highlighting their distinctive features, underlying causes, and treatment strategies

S.No.	Type of NP	Description	Causes/Mechanisms	Initial Treatment	Alternative Treatments	References
1	**Postherpetic Neuralgia (PHN)**	Unilateral pain in dermatomes caused by herpes zoster virus.	Nerve damage from herpes zoster infection.	Monotherapy (e.g., anticonvulsants, antidepressants).	Combination therapy if monotherapy fails.	(Niemeyer et al., 2024; Watson et al., 1991)
2	**Complex Regional Pain Syndrome (CRPS)**	Chronic pain affecting limbs, often diagnosed by a neuropathic specialist.	Abnormal pain response following injury or surgery.	Antispastic drugs, bisphosphonates, physical therapy, psychological therapy.	Invasive procedures if necessary.	(Frare et al., 2024; Muthuraman et al., 2010)
3	**Diabetic Polyneuropathy (DPN)**	Pain and sensory abnormalities in feet, potentially extending to thighs, lower legs, and hands.	Long-term diabetes mellitus causing nerve damage.	First-line medications (e.g., antidiabetic drugs).	Alternate therapies (second-line drugs, natural/synthetic drugs, drug combinations) if first-line drugs fail.	(Javed et al., 2015; Zhu et al., 2024)
4	**Trigeminal Neuralgia (TN)**	Chronic pain in the trigeminal nerve distribution.	Compression or irritation of the trigeminal nerve.	Carbamazepine, clonazepam, antiepileptics, baclofen.	Surgery if medications fail; botulinum toxin as a pre-surgical option.	(Ashina et al., 2024; Niemeyer et al., 2024)
5	**Post-Amputation Pain (Phantom Pain)**	Pain in the area surrounding the amputation site, or felt in the missing limb.	Neuroplastic changes and peripheral nerve damage.	Strong opioids, gabapentin, tramadol, lidocaine infusions, amitriptyline, calcitonin, pregabalin, physiotherapy, psychotherapy.	Neurodestruction, peripheral nerve stimulation, spinal cord lesions.	(Kovalenko et al., 2024; Melzack et al., 2001)
6	**Post-Traumatic Pain (PTP) and Post-Operative Pain (PPP)**	Chronic pain resulting from trauma or surgery.	Incomplete tissue healing or damage during injury/surgery.	Prophylactic measures (e.g., epidural anesthesia, local anesthetics), gabapentin/pregabalin, lidocaine infusions.	Causative management, lidocaine (5%), systemic drugs, and interventional methods.	(Bharde, 2024)
7	**Neuropathic Cancer Pain**	Pain related to cancer, its treatment, or related conditions.	Cancer metastasis, anticancer therapies, cancer-related or unrelated diseases.	Tramadol for moderate pain; opioids for severe pain.	Drug combinations (e.g., opioids, pregabalin, gabapentin), duloxetine and venlafaxine post-chemotherapy.	(Mulvey et al., 2024)
8	**NP due to HIV Infection**	Pain resulting from HIV infection or its antiretroviral therapy.	Direct viral effects or side effects of antiretroviral drugs.	Capsaicin patches (8%), gabapentin, lamotrigine.	Lidocaine patches (5%), amitriptyline, pregabalin (less effective).	(Dworkin et al., 2010; Surnar et al., 2021)

9	**Central NP (CNP)**	Pain resulting from central nervous system (CNS) injuries.	CNS injury (e.g., stroke, SCI).	Pregabalin, tricyclic antidepressants (TCAs) for insomnia and depression.	Continued pharmacotherapy and supportive therapies.	(Mora-Escobedo et al., 2024; Naderi et al., 2014; Sun et al., 2021)
10	**Peripheral Nerve Injury and NP**	Pain related to damage of peripheral nerves, influenced by age.	Nerve damage leading to hypersensitivity.	Minocycline (inhibitor of pro-inflammatory polarized microglia).	Various supportive and pharmacological treatments.	(Guo et al., 2021; Mora-Escobedo et al., 2024; X. H. Zhao et al., 2015)
11	**Osteoarthritis (OA) NP**	Joint pain associated with osteoarthritis, involving central and peripheral pain pathways.	Joint degeneration, inflammation, and sensitization of pain pathways.	Centrally acting drugs, analgesics, anti-inflammatory drugs.	Hydrotherapy, physiotherapy, heat/cold packs, electrical stimulation, bisphosphonates (under investigation).	(Bannister et al., 2017; Demir et al., 2021)

NP manifests in various forms, each with distinct characteristics, underlying causes, and treatment approaches. Conditions such as diabetic polyneuropathy, postherpetic neuralgia and complex regional pain syndrome originate from nerve damage caused by infections, injuries, or chronic illnesses like diabetes. Initial treatments typically include medications like anticonvulsants, antidepressants, or antispastic drugs, with alternative options involving combination therapies, invasive procedures, or surgery when first-line treatments fail. Specific conditions, such as trigeminal neuralgia and phantom pain, may require specialized treatments like strong opioids, gabapentin, or botulinum toxin, while NP related to cancer or HIV infection is managed with tailored pharmacological regimens. Central NP, resulting from CNS damage, and pain due to peripheral nerve injuries or osteoarthritis, also demand a combination of pharmacotherapy and supportive therapies to alleviate symptoms and improve patient outcomes.

Table 4. Hallmarks of NP (Pottabathini et al., 2016)

S.No.	Aspects	Allodynia	Hyperalgesia	Paraesthesia
1.	**Description**	Pain arising with non-painful stimulus	Pain arising with painful stimulus	Abnormal sensation via painful sensation
2.	**Mechanism**	Sensitization via central and peripheral pathway	Activation of nociceptors and pain mediators	Dysfunction nerve signalling
3.	**Example**	Feather, Cotton, etc.	Pin-prick etc.	Damaged nerve

Painful and non-painful stimuli have distinct characteristics. The differentiation is clear: a noxious stimulus that activates the pain fibers is called hyperalgesia, while a non-noxious stimulus that activates the pain fiber is called allodynia. Additionally, pain arising from injured tissues can lead to abnormal sensations such as tingling and numbness, which is called paraesthesia. Various chemicals act as pain mediators which activate the pain fibers and leading the NP.

2.5 Fundamental of NP development: Mechanistic point of view

The pathophysiology involves a multifaceted interaction among numerous nociceptive mediators, including glutamate, substance P, calcitonin gene-related peptide, prostaglandins, and various lipid-derived compounds (Table 5). Glutamate, a principal excitatory neurotransmitter, significantly contributes to enhanced neuronal excitability and the development of central sensitization. This hyperexcitability amplifies nociceptive signal processing. Substance P further modulates nociceptive pathways by facilitating synaptic transmission and initiating neurogenic inflammation, thereby intensifying pain perception (Ueda, 2008). Calcitonin Gene-Related Peptide (CGRP) is a key contributor to heightened nociceptive sensitivity. It facilitates peripheral sensitization, a state in which nociceptors exhibit increased responsiveness to painful stimuli. Moreover, CGRP is actively involved in neurogenic inflammation by promoting the release of pro-inflammatory mediators. Prostaglandins, a class of bioactive lipid compounds, further exacerbate NP by enhancing peripheral sensitization and pain signalling, thereby intensifying nociceptive transmission and the subjective pain experience (Jia et al., 2018). NGF is instrumental in the aberrant sprouting and structural reorganization of sensory nerve fibers, leading to amplified nociceptor sensitivity and connectivity. NGF also drives neuroinflammatory cascades, perpetuating chronic pain states. Neurotrophins, a family of neuronal growth regulators, modulate neuronal survival, synaptic plasticity, and pain signaling within both PNS and CNS (Ahmad et al., 2022). Disruptions in neurotrophin signaling are implicated in the initiation and maintenance of NP. Pro-inflammatory cytokines, notably TNF-α and IL-6, mediate neuroimmune responses and augment peripheral sensitization (Naseem et al., 2023; H. Zhao et al., 2017). ROS, as potent oxidative agents, further aggravate pain processing by inducing oxidative stress, damaging neuronal tissues, and activating intracellular nociceptive pathways. These molecules collectively contribute to persistent pain by reinforcing a chronic pro-nociceptive state.

At the molecular level, injured peripheral nerves trigger the upregulation of voltage-gated sodium (Nav1.3, Nav1.7, Nav1.8, Nav1.9) and calcium channels (Cav2.2, Cav3.2), leading to aberrant neuronal excitability and pathological signaling characteristic of neuropathy. Additionally, factors such as NGF released near spared nerve fibers can upregulate ion channels and receptor expression in adjacent uninjured fibers, contributing to expanded sensitivity and ectopic pain (Fornasari, 2017; St. John Smith, 2018). Understanding the integrative roles of these biochemical mediators offers insight into novel therapeutic strategies aimed at interrupting maladaptive pain circuits, potentially improving outcomes in patients suffering from neuropathic pain. (Boyce-Rustay et al., 2009). Spontaneous activity in C-nociceptors triggers secondary alterations in central sensory processing, leading to heightened excitability within the spinal cord. This hyperexcitability is further intensified by the downregulation of potassium channels, including Kv1.2, Kv1.4, and Kv7.2/Kv7.3, following nerve injury. Additionally, the dorsal horn of the spinal cord contains various receptors, such as TRPV1 and TRPA1, which play a crucial role in modulating the excitability of sensory neurons. The graphical representation (Figure 4) illustrates how, under normal conditions, these receptors bind to specific molecules, initiating a balanced response. However, in cases of injury or dysfunction, the binding of certain chemicals disrupts this equilibrium, altering the balance between inhibitory and excitatory signals.

Patients with NP commonly report a range of symptoms, like, constant burning pain, characterized by a deep, aching sensation that is present continuously. Another frequently reported symptom is stabbing or shooting pain, which is described as sharp and electric-like, occurring suddenly or lasting for a longer duration. Allodynia (Sorkin & Yaksh, 2009), is a condition in which stimuli that ordinarily cause pain, such as mild touch or contact with clothing, hyperalgesia (Boyce-Rustay et al., 2009; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019), an increase in sensitivity to painful stimuli, Paraesthesia (Lisney & Devor, 1987), which include tingling, prickling, or numb feelings, can be localized to one area of the body or generalized. Also typical are numbness in the hands and feet and weakness, especially in the legs. Either as a direct result of the pain itself or as a side effect of medicine, NP frequently results in despair and anxiety.

Table 5. Various factors involved in the mechanism of NP

S.N.	Mechanism	Result	Reference
1.	Glutamate	Acts as an excitatory neurotransmitter, contributing to neuronal hyperexcitability and central sensitization	(Barygin et al., 2017)
2.	Substance P	Mediate's pain transmission and inflammation	(Atas et al., 2021)
3.	Calcitonin Gene-Related	Enhances pain sensitivity by promoting peripheral	(Fuchs et al., 2010)
4.	Peptide (CGRP)	Sensitization and facilitating neurogenic inflammation	(Fuchs et al., 2010)
5.	Prostaglandins	Contribute to peripheral sensitization and inflammation, amplifying pain signals	(Janjic et al., 2018; Millan, 1999)
6.	Bradykinin	Damaged tissue can activate nociceptors and cause pain	(Fuchs et al., 2010)
7.	Nerve Growth Factor (NGF)	Facilitates sprouting of nerve fibers, enhances nociceptor sensitivity, and promotes neuroinflammation	(Ashraf et al., 2016; Kinnman & Levine, 1995)
8.	Neurotrophins	Alter neuronal survival, plasticity, and pain processing in CNS and PNS.	(Kulkarni & Dhir, 2007; Millan, 1999)
9.	Cytokines	Proinflammatory cytokines, such as TNF-α and IL-6, contribute to neuroinflammation and peripheral sensitization.	(Muthuraman et al., 2010)
10.	ROS	Resultant nerve damage and activation of pain pathways.	(Tarquini et al., 2017)
11.	voltage-gated sodium channels (VGSCs	involved in the generation of action potentials, which are the electrical signals that transmit pain signals	(Lai et al., 2003)
12.	Gene expression	Nerve damage can change the expression of genes which can lead to changes in the way that pain signals are processed and perceived	(Navarro et al., 2007; Ueda, 2008)
13.	TRPV1 (Transient Receptor Potential Vanilloid 1)	After nerve injury, TRPV1 is activated which allows the influx of ca^{2+} ion, resultant generation and propagation of pain signals	(Schumacher, 2011)
14.	Transient Receptor Potential Ankyrin 1 (TRPA1)	Increased sensitivity and expression from nerve injury leads to pain response (Chemical induced)	(Nilius et al., 2012)
15.	Sodium Voltage-Gated Channel α Subunit 1.3 (Nav1.3)	Hyperexcitability due to re-expression in the injured neuron as well as non-neuronal cells	(Wood, 2006)
16.	Sodium Voltage-Gated Channel α Subunit 1.7 (Nav1.7)	Increased excitability and signals of pain in sensory neurons after nerve injury	(Cummins et al., 2004)
17.	Sodium Voltage-Gated Channel α Subunit 1.8 (Nav1.8)	Altered pain signalling and sensitivity due to upregulation and activation of threshold level	(Thakor et al., 2009)

18.	Sodium Voltage-Gated Channel α Subunit 1.9 (Nav1.9)	Hypersensitivity and amplification in pain signals due to increased activation and expression of the sensory neurons due to damage in the neuron	(Lolignier et al., 2015)
19.	Calcium Voltage-Gated Channel α -2/δ Subunit 2.2 (Cav2.2)	Calcium influx leads release of level of neurotransmitters increases	(Khanna et al., 2019)
20.	Calcium Voltage-Gated Channel α -1H (Cav3.2)	Hyperexcitability due to overexpression and enhanced activity	(Tomita et al., 2020)
21.	Potassium Voltage-Gated Channel (Kv1.2)	Downregulation of potassium can reduce neuronal activity leads neuronal inhibition	(Zhang et al., 2021)
22.	Potassium Voltage--Gated Channel (Kv1.4)	Increase the neuronal excitability which changes the expression and reduced potassium current	(Cao et al., 2010)
23.	Potassium Voltage--Gated channel (Kv7.2/Kv7.3)	Reduced potassium current leads to hyperexcitability	(Mannelli et al., 2017)

The responsiveness of neurons and their heightened sensitivity are influenced by various molecular processes that affect the experience of pain. Certain neuropeptides, such as Substance P and CGRP, contribute to the transmission of pain signals and inflammatory responses within the nervous system. Meanwhile, glutamate serves as a stimulating neurotransmitter, playing a role in the amplification of pain processing. moreover, compounds like prostaglandins and bradykinin intensify pain by promoting inflammation and increasing sensitivity in affected areas. Proinflammatory cytokines and reactive oxygen species (ROS) exacerbate nerve damage and pain pathways, while nerve growth factor (NGF) and neurotrophins modify neuronal plasticity and promote neuroinflammation. TRPV1 and TRPA1 receptors, as well as voltage-gated sodium channels (VGSCs) such as Nav1.3, Nav1.7, Nav1.8, and Nav1.9, are essential for the generation and propagation of pain signals. Neurotransmitter release and neuronal hyperexcitability are also influenced by calcium channels, including Cav2.2 and Cav3.2. Neuronal excitability is regulated by potassium channels, which include Kv1.2, Kv1.4, and Kv7.2/Kv7.3. Their dysfunction leads pain sensitivity. The processing is further influenced by changes in gene expression that occur as a result of nerve injury, rendering these mechanisms critical targets for therapeutic intervention (Boyce-Rustay et al., 2009; Finnerup et al., 2021).

Initially, the nerve fibers (axons) or their protective sheaths (myelin) are damaged due to various factors like diabetes, infections, certain medications, or traumatic injuries. When the protective covering of nerves is impaired, their capacity to transmit electrical impulses efficiently is hindered, leading to communication breakdown between the brain, spinal cord, and other parts of the body (Sisein, 2014). This disruption can affect movement, sensation, and involuntary functions, giving rise to various symptoms. The advancement of nerve impairment is often linked to cellular damage caused by inflammation and an imbalance of reactive molecules. (Dalakas, 2001). Factors like excessive glucose levels in diabetics can cause metabolic disturbances, leading to the production of harmful substances that damage nerves. Adding, the buildup of sorbitol, due to inadequate glucose metabolism, can cause excessive water intake by the nerve cells, resulting in swelling and further nerve damage. The combined effect of these mechanisms impairs the patient sensory and motor functions, leading to the characteristic symptoms of peripheral neuropathy. Peripheral neuropathy involves a series of complex mechanisms that lead to the pain and sensory disturbances characteristic of this condition. Initially, nociceptors, which are sensory receptors responding to potentially harmful stimuli, can become sensitized upon exposure to inflammatory mediators i.e. prostaglandins,

bradykinin and serotonin. This sensitization, often following nerve injury, results in heightened sensitivity (allodynia) and increased pain response (hyperalgesia). Abnormal excitability in the sensory neurons, particularly C and Aδ fibers, contributes to sensations like burning and stinging pain. This irregular excitability is driven by sodium channels, including Nav1.7, Nav1.8, and Nav1.9, and other voltage-gated ion channels. In conditions like phantom limb pain, the brain undergoes significant cortical reorganization, with the extent correlating to pain intensity. This adaptation shows the brain's plasticity in chronic pain conditions. In the spinal dorsal horn, glutamate plays a key role in pain signaling via NMDA receptors, with substance P and CGRP further amplifying the process. Reduced inhibitory control in the spinal cord can cause sensory distortions like the thermal grill illusion, which results from disrupted temp and pain pathways, highlighting the balance between excitatory and inhibitory signals. Peripheral sensitization boosts nociceptor activity, leading to allodynia and hyperalgesia, while central sensitization lowers the pain threshold, making the nervous system more reactive. Abnormal nerve activity and spontaneous discharges further drive chronic pain. Peripheral nerve injury triggers molecular changes, sensitizing dorsal horn neurons and prolonging C-fiber activation. This leads to the release of excitatory neurotransmitters and neuropeptides, altering neuronal membrane properties and weakening inhibition (Figure 5). The resulting hyperactivity of dorsal horn neurons is a key feature of NP (Barygin et al., 2017; Costigan et al., 2009; Stroberg et al., 2003).

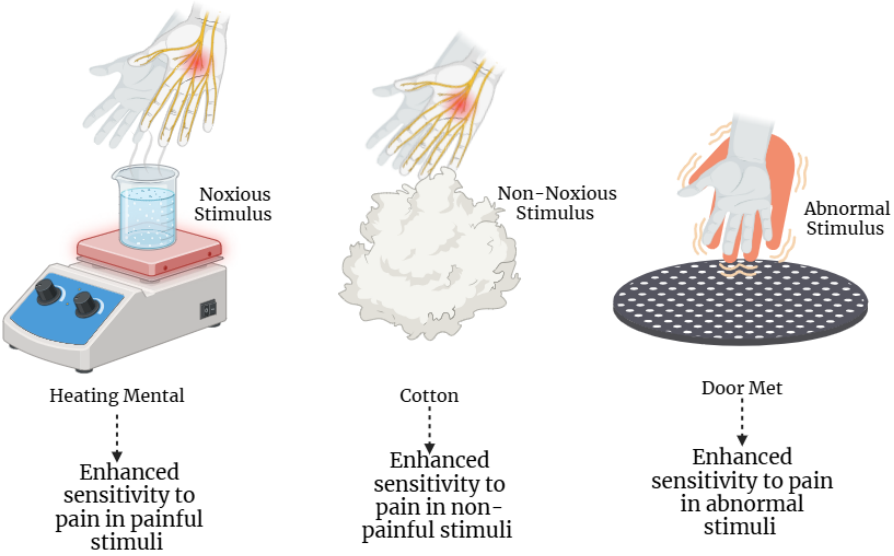


Figure 5. Pain Perception: Sensitization Responses

2.6. Mechanism of sympathetically maintained pain:

Sympathetically maintained pain involves both direct and indirect mechanisms. NP can result from the coupling of efferent sympathetic signals with nociceptive input. For example, the presence of noradrenaline in subcutaneous tissue following sustained sympathetic blockade can induce pain and hyperalgesia. Stimulation of the sympathetic trunk can similarly lead to increased pain and sensitivity because of sympathetic and nociceptive pathways. This interaction is notably observed in conditions like complex regional pain syndrome (CRPS), where sympathetic activation exacerbates pain and dysfunction. The pathophysiology of NP is multifaceted, involving nociceptor sensitization, abnormal excitability of afferent neurons, cortical reorganization, spinal dorsal horn facilitation, inhibitory disinhibition, and sympathetically maintained mechanisms (Colloca et al., 2017; Stroberg et al., 2003).

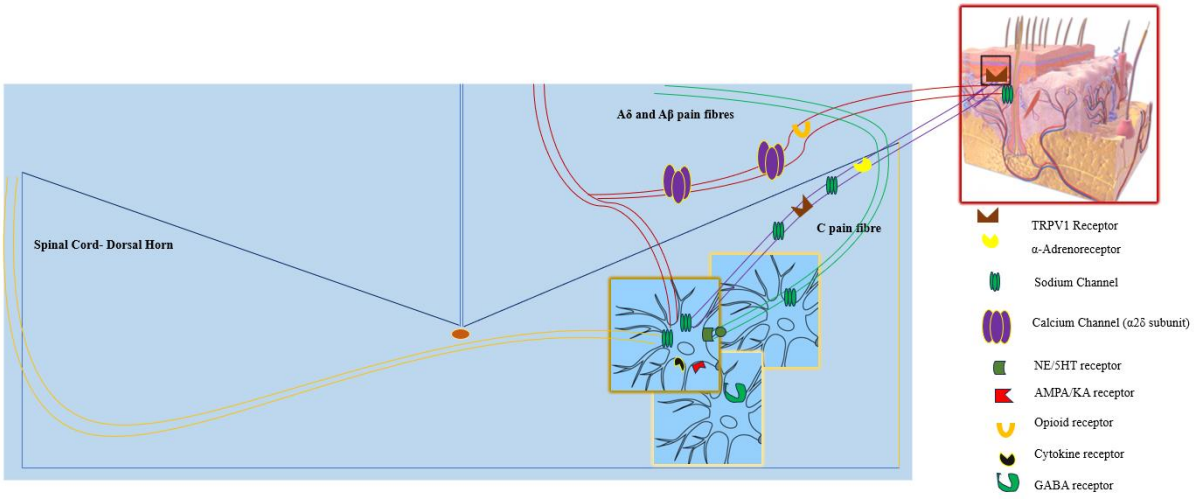


Figure 6: Dorsal horn of the spinal cord with the arrangement of receptors and channels

Due to their role in the emergence and duration of chronic pain, central and peripheral sensitizations are significant in NP. After an injury or other nerve damage, the CNS and the PNS, respectively, become more receptive to pain signals. This occurrence, which can result in a long-lasting increase in pain, is known as central and peripheral sensitization. Normally the CNS organizing and manages all the organs, and transmits nerve fibers In the CNS and PNS, respectively, a damaged nerve might emit substances that can stimulate other nearby nerves. Even if there is no apparent injury, these stimulated nerves can still transmit pain signals to the nervous system. The CNS and PNS may begin to process pain differently in the future as a result of this over time (Figure 6). Individual differences affect how well people will respond to central and peripheral sensitization. Over time, some individual's pain may gradually get

better, whilst other people can still have chronic pain. An individual's response to treatment cannot be predicted. A recent study that was published in the journal Pain Medicine discovered that alterations linked to peripheral sensitization. The research revealed that treatment can undo these modifications (Nickel et al., 2012). Histamine, CGRP, proteases, substance P, cytokines, bradykinin, NO, 5HT, and chemokines are just a few of the chemicals that are released at the site of an injury during the earliest stages of an injury. The growth of peripheral sensitization is facilitated by these drugs. Glial cells in the dorsal root ganglia are triggered by impulses from visceral afferent neurons. The central sensitization that results from this activation is caused by a persistent rise in ATP, H+, and glutamate levels.

2.7. Preclinical models of NP

NP categorized into surgical, diabetic, post-herpetic, drug-induced, disease-induced, and other models. Each model has a distinct methodology, with a primary focus on inducing nerve injury or dysfunction to simulate pain conditions. Surgical models, like CCI, SNL etc., are widely used to study pain mechanisms through direct nerve damage and typically result in hypersensitivities to thermal and mechanical stimuli. Diabetic neuropathy models, such as the Streptozotocin-induced rat model, reflect the nerve damage caused by chronic hyperglycemia, leading to various types of allodynia and hyperalgesia. Post-herpetic neuralgia models, like the Varicella Zoster virus model, replicate the pain caused by viral infections, focusing on mechanical hypersensitivity. Drug-induced neuropathy models simulate nerve damage caused by chemotherapeutic agents, alcohol consumption, and anti-HIV drugs, providing insight into the mechanisms behind these treatments' neuropathic side effects. Disease-induced models, such as those for diabetes and cancer-induced bone pain, investigate the pathophysiological changes associated with these conditions, including altered nerve function and hypersensitivity. Additionally, models like inherited neuropathies and optogenetic approaches offer unique insights into genetic and molecular mechanisms of pain. While these models help advance our understanding of NP, each has limitations related to variability, ethical concerns, and the degree to which they replicate human conditions (Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, & Kumar, 2019).

Table 6. Models of NP in animals for clinical studies.

Sr. No.	Animal Model	Principle	Characterization/Advantages	Animal used	Limitations	Symptoms (emphasized)	Reference
Surgical Models							
1	Chronic constriction injury (CCI)	Four loose ligatures (Unilateral/bilateral) around sciatic nerve	a. The neuroanatomical (behavioural as well as anatomical) changes over the time in short period. b. Mimics the painful neuropathy as human. c. It is reliable and easily reproducible model d. This is suitable for assessing cold allodynia.	Rats, mice	a. Changes to heat and mechanical stimuli seen with unilateral CCI (CCI) are transient, lasting four weeks or less. b. Variability of degree of damage from animal to animal	Thermal and mechanical hypersensitivities	(Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, & Kumar, 2019)
2	Spare nerve injury (SNI)	Ligation & transaction of 'tibial and peroneal nerves' leaving the sural nerve intact	a. Distinct and separate anatomical distribution of injured nerve b. Hypersensitivities are maintained for atleast 6 months	Rats, mice	a. Produces difficult in performing behavioral tests as it causes degeneration of axons b. Hypersensitive area in the limb is difficult to test as it is lateral part of the paw.	Cold and mechanical hyperalgesia/allodynia	(Thakur & Srivastava, 2016)
3	Spinal nerve ligation (SNL)	Tight ligation of spinal nerves L5, L6 and L7	a. Experimental variability is less	Rats (L5, L6) Macaca fascicularis (L7)	a. Extensive muscle injury surgery can differ outcomes and make the mechanism more complex.	Mechanical allodynia	(Decosterd & Woolf, 2000)
4	Partial sciatic nerve ligation (Seltzer model)	Ligation at high thigh (ipsilateral sciatic nerve)	Surgical procedure which consumes less time.	Rats, mice	a. Changes in the Dorsal Root Ganglion (DRG) are difficult to study	Mechanical and cold allodynia	(Seltzer et al., 1990)
5	Brachial plexus avulsion (BPA) model	Lesion made in brachial plexus	Valid model long lasting mechanical and cold allodynia	Rats	Lesion made in brachial plexus may lead plasticity of CNS.		(Challa, 2015)

6	Sciatic Transection Nerve model	Transection of sciatic nerve at mid-thigh level Completely	Suitable for simulating phantom limb pain	Rats	Ethical considerations issues		(Hammarberg et al., 2000)
7	Spinal Nerve Transection (SNT) model	Incision made along spinal nerve	May stimulate limb pain	Rats	Lacks the inflammatory component		(Hsu et al., 2017)
8	Sciatic cryoneurolysis	Freezing of the sciatic nerve	Useful for postoperative and chronic pain and only for mononeuropathy	Rats, mice	Reduces weight loss and not validated by anti-neuropathic drug.	Thermal hyperalgesia and mechanical allodynia	(DeLeo et al., 1994)
Diabetic neuropathy models							
9	Streptozotocin induced rat model	Persistent hyperglycemia-induced injury to the nerves	a. Earlier development of diabetes followed by neuropathy (4–8 weeks) b. Reduce sensory and motor nerve conduction velocity.	Rats	a. Non obese diabetes mice develop autoimmune T-cell mediated insulin dependent diabetes due to inheritable polygenic immunodeficiency. b. Not validated by antineuropathic drug.		(Pham et al., 2019)
10	Chinese hamster neuropathic model	Persistent hyperglycemia-induced injury to the nerves	a. No reduction on nerve fibres diameter b. Conduction velocity of both motor and sensory components of hind lamb nerves (16%–22%) decreased	Chinese hamster	a. PDN was less severe than human DNP b. Need further studies for validation.	Thermal, cold and mechanical allodynia/hyperalgesia	(Kennedy et al., 1982)
11	HFD-fed female C57BL6/J mice model	Persistent hyperglycemia-induced injury to the nerves	a. Induced obesity or prediabetic-related neuropathy.	Mice	a. Loss of intradermal nerve fiber b. Absent atrophy c. Cannot induced chronic diabetic neuropathy. d. Not validated by antineuropathic drugs		(Obrosova et al., 2007)

Post-herpetic neuralgia models							
12	Varicella Zoster virus model	Injection of viral infected cells in the footpad	a. Anxiety like pattern of ambulation (reduced entry into the central area of the open arena) that is positively correlated with mechanical hypersensitivity.	Rats, mice	a. Infection with all viral strains was associated with a dose-dependent mechanical hypersensitivity but not a thermal or cool hypersensitivity	Mechanical hypersensitivity	(Hasnie et al., 2007)
13	Non-viral model	Afferents with resiniferotoxin	a. Ultrastructural damage of myelinated fibers in RTX-treated rats	Rats	a. Damage to myelinated afferent fibers	Thermal and mechanical sensitivity	(Pan et al., 2003)
Drug induced NP models							
14	Chemotherapeutic agents (Paclitaxel, oxaliplatin, vincristine, cisplatin)	Axon damage (axonopathy) leading to injury to nerves later spreads out centrally	Causes disruption of microtubule structure that interfere with axoplasmic transport to produce nerve damage.	Rats, mice, guinea pigs, rabbits	Chances of development of orthostatic hypotension, paralytic ileus and impotency	Numbness, distal dysparathesia,	(Miltenburg & Boogerd, 2014)
15	Chronic alcohol consumption	Alcohol administration over a long time (approximately 70 days)	a. Impairment of axonal transport as well as cytoskeletal properties. b. Increased NFκβ activity, caspase 3 activity as well as PKC activity.	Rats	Augments the level of hormones like epinephrine, stress hormone (cortisol and adrenaline) as well as corticosterone	Allodynia, spontaneous burning pain, hyperalgesia	(Chopra & Tiwari, 2012)
16	Anti-HIV drugs induced NP (Nucleoside reverse transcriptase inhibitors like zalcitabine)	Distal axonal degeneration causing inflammation and hence damage the nerves.	a. Significantly increased expression of phospho-p38 in microglia. b. Significant change in thigmotactic behavior	Mice, Rats, Rabbits	Reveal mechanisms of neuropathy but do not mimic HIV disease complexities.	Mechanical hypersensitivity	(Huang et al., 2017)
17	Formalin induced NP	ROS induced oxidative stress leading to tissue injury followed	a. Biphasic nociceptive behavior is produced i.e., Phase 1 and Phase 2 b. Produce long lasting thermal and mechanical hyperalgesia.	Mice, Rats, Dogs	Time perception (critical ability of animals and humans in their daily	Thermal and mechanical hyperalgesia	(Abdeen et al., 2021)

		by nociceptive response	c. A comparable pattern of activity in dorsal horn neurons is produced. d. NO is involved in the central mechanism of peripheral neuropathy.		activities) is distorted due to altered emotional states on formalin injection.		
18	Pyridoxine induced NP	Declined distal limb proprioception and sensory ataxia occurs depicting neuropathy	Pure degeneration of peripheral sensory neurons.	Rats, Mice	Associated with permanent sensory abnormalities	Hyperalgesia	(Albin & Albers, 1990)
Disease induced NP models							
19	Diabetes induced NP	Persistent hyperglycaemia induced oxidative stress causing nerve damage	a. Modifies axon–glia interactions at PNS nerves. b. Affects axonal energy metabolism c. Affects ion conductance particularly. d. Large myelinated motor nerve fiber dysfunction	Rats, Mice	Only mild neurophysiologic deficits are produced.	Mechanical allodynia and hyperalgesia	(Nitta et al., 2002)
20	Cancer induced NP (Bone pain)	Multiple mechanisms like ectopic sprouting and sensitization of sensory nerve fibres due to cancer	a. Induction of neuroplastic changes in the spinal cord and supraspinally b. Cytokines and chemokines released causes nociceptive signalling and sensitization in bone	Rats, Mice	Tumor burden within spinal cord leads to hind leg paralysis that does not allow assessment of neuroceptive behaviors,	Hyperalgesia and allodynia	(Bennett, 2010)
21	Inflammatory induced NP	Inflammatory mediators (TNF-α, Interleukins, nerve growth factor) induced spontaneous discharge in pain fibers results in	Abnormal spontaneous activity within 1–2 days after nerve injury	Rats, Mice	Not readily amenable sometimes	Allodynia (High regular and clock like discharge) Hyperalgesia	(Xie et al., 2006)

		greater sensitivity to peripheral stimulation					
22	HIV induced neuropathy	Distal degeneration of axons leads to peripheral nerve damage	a. Multifocal inflammation due to hyperactive macrophage response. b. Monocytes as well as pro-inflammatory cytokines influx in DRGs and peripheral nerves causing neuronal injury.	Rats, mice, rabbits	Lack of evaluation for morphological abnormalities	Hyperalgesia, allodynia	(Jimenez-Andrade et al., 2008)
Other models							
23	Uremic peripheral neuropathy (Surgical method)	Compression of median nerves (especially in carpel tunnel) due to surgical induction of uraemia	a. Decrease in nerve conduction velocity, b. Increase in amyloid disposition. c. Acute and chronic renal failure models can be used to evaluate uremic neuropathy.	Rats, mice	Variable results are produced due to extent of compensatory response produced by remaining normal tissue.	Hyperthyroidism	(Krishnan & Kiernan, 2007)
24	Inherited induced neuropathy (Charcot-Marie-Tooth neuropathy and Tomasulo's neuropathy)	Mutations in genes i.e., PMP22, P0 and connexin 32	a. Muscle weakness of the lower limbs. b. Severe demyelation, decreased nerve conduction velocity, lower grip strength.	Transgenic mouse and rats	Variability as well as reproducibility issues sometimes.		(Kumar et al., 2018)
25	Optogenic approach	Visualization of signaling events and manipulation of cellular activities by light and molecular genetics	a. Powerful approach in assessment of LTMR (low threshold mechano receptors) derived pain. b. Ensures reproducibility of results. c. This model helps in selective stimulation or inhibition or silencing of subpopulations to elucidate complete neuronal circuitry. d. Targeting specificity of neurons	Rats, mice, rabbits	a. Difference in neuronal activity due to optogenic stimulation and stimulus to skin in animals. b. Level, consistency, time course as well as specificity of opsin expression among animals.	Mechanical allodynia	(Tsuda, 2019)

2.8 High-Fat Diet (HFD) + Streptozotocin (STZ) and Spinal Nerve Ligation (SNL) model

In HFD + STZ model of diabetic neuropathy mirrors the effects of type 2 diabetes. It affects the normal functions of peripheral nerves. This model combines a HFD, which leads to obesity and insulin resistance, with STZ, which induces pancreatic β-cell destruction, resulting in persistent hyperglycemia (Ahlawat & Sharma, 2018). Over time, this chronic hyperglycemic state causes nerve damage, including altered nerve conduction, sensory deficits, and the development of NP. The HFD + STZ model provides insights into the mechanisms of diabetic peripheral neuropathy (DPN), particularly in terms of oxidative stress, inflammation, and nerve dysfunction. By using both models, we can explore the distinct and combined contributions of traumatic and metabolic factors in the development of neuropathy. This dual-model approach allows for a comprehensive understanding of NP and provides a robust platform for evaluating potential treatments that target both nerve injury and the underlying metabolic conditions that aggravate it.

The SNL model is a highly reliable and effective method for inducing neuropathy by ligating specific spinal nerves, which leads to a consistent and well-defined nerve injury (LaBuda & Little, 2005; Pottabathini et al., 2016). This model closely mimics the pathophysiology of human NP, particularly focusing on mechanical allodynia, a hallmark symptom observed in clinical conditions. It allows for a high degree of experimental control, making it ideal for studying the mechanisms behind nerve injury and pain. Additionally, the model enables the testing of potential therapies and produces chronic pain behaviors that last for weeks or even months, making it valuable for long-term therapeutic studies and exploring the molecular pathways involved in neuropathy. SNL model is widely regarded as one of the most effective and reliable methods for inducing NP in animal studies. This model involves the ligation of the L5 and L6 spinal nerves in rats, which leads to localized nerve injury and initiates a “complex cascade of physiological changes”. The pressure exerted by ligation leads to nerve fiber injury, inflammation, and nociceptor activation, prompting the release of pro-inflammatory cytokines and increasing neuronal sensitivity. Disruptions in ion channel function, particularly in Na^{+}, Ca^{2+}, and K^{+} channels, heighten neuronal excitability, playing a key role in spontaneous pain and intensified pain perception. To evaluate pain responses in this model, behavioral assessments are commonly used, such as mechanical allodynia testing with von Frey filaments and thermal hyperalgesia measurements using hot or cold plate tests. Observation of spontaneous pain behaviors such as excessive grooming or limb guarding. Additionally, motor function changes are evaluated through gait analysis or limb use scoring. The SNL model also

involves Wallerian degeneration distal to the injury site and induces neuroinflammation, characterized by the "activation of immune cells" like macrophages and microglia. These immune cells release cytokines (pro-inflammatory), which exacerbate nerve damage and promote sensitization. At the molecular level, the SNL model is marked by altered ion

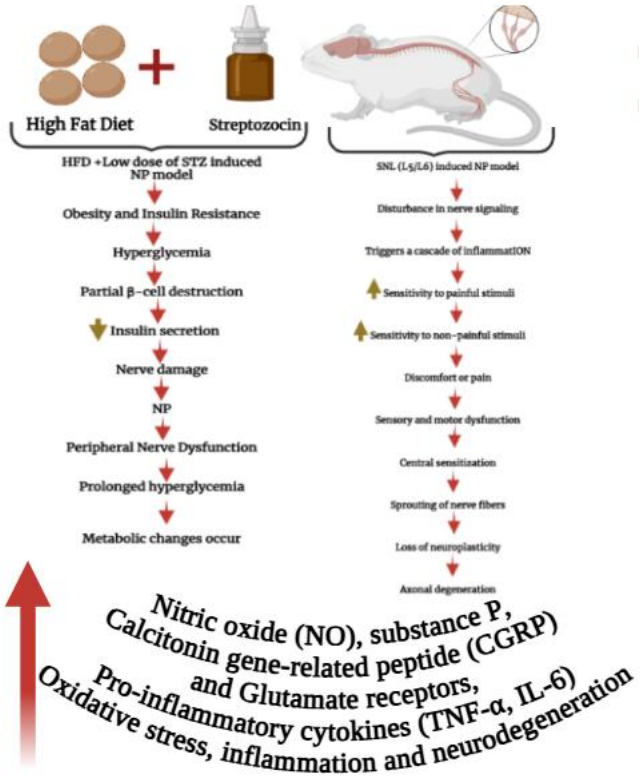


Figure 7. Mechanisms and Consequences of NP Induced by HFD + STZ and SNL

Increased expression of sodium channels (Nav1.7, Nav1.8), calcium channels (Cav2.2), and transient receptor potential (TRP) channels (TRPV1) contributes to neuronal hyperexcitability. Similarly, activation of glial cells in the spinal cord, particularly astrocytes and microglia, promotes chronic inflammation and central sensitization. These processes lead to neuroplastic adaptations and prolonged pain perception, even after the initial injury has healed (Balzani et al., 2021; Cummins et al., 2007; Stevens et al., 2019). From a pain transmission perspective, peripheral sensitization occurs due to the release of cytokines at the site of injury, which heightens the sensitivity of nociceptors and leads to increased neuronal firing. The pain signal is transmitted to the spinal cord, where the "release of neurotransmitters" like glutamate and substance P activates second-order neurons, intensifying the pain response. Over time, repeated stimulation results in central sensitization, where the central nervous system becomes abnormally responsive to stimuli, contributing in persistence of chronic pain (Chung et al., 2009). In SNL model also highlights the dysfunction of descending pain pathways. Normally, these pathways from the brainstem help inhibit pain signal transmission at the spinal level, but

in NP, this inhibitory control is impaired, which exacerbates the pain perception. Overall, the SNL offers the pathophysiology of NP, as it replicates many of the basic features observed in human patients. It provides a clear understanding of the neural and inflammatory processes that underlie chronic pain conditions, making it an ideal choice for evaluating potential therapies aimed at treating neuropathy. However, the model does come with some challenges, including the need for expertise in performing the procedure to avoid unintended nerve damage and ensuring the success of the ligation (Figure 7). The surgical process can be time-consuming, and the model may not be suitable when a more selective manipulation of peripheral nerve innervation is required (Seltzer et al., 1990). Despite these limitations, the SNL model remains a gold standard for studying NP due to its reliability, reproducibility, and relevance to human pain conditions (Lindenlaub & Sommer, 2000).

The "International Association for the Study of Pain (IASP) NP Special Interest Group (NeuPSIG)" has established evidence-based guidelines for the pharmacological management of NP. These guidelines categorize medications into three tiers based on the strength of evidence from randomized controlled trials (RCTs) and expert consensus.

First-line treatments include SNRIs and TCAs, calcium channel α2-δ ligands (gabapentin and pregabalin), and lidocaine (5% patch, topically). These medications have shown consistent efficacy across multiple RCTs and are recommended as primary treatment options. Second-line treatments consist of opioids and tramadol, which are generally reserved for cases where first-line therapies are inadequate. However, in specific clinical situations, their use as first-line options may be considered. Third-line treatments include medications with limited or inconsistent supporting evidence but may still be appropriate for select patients. The guidelines highlight that combining multiple effective medications can enhance pain relief compared to monotherapy, though it may also increase side effects, drug interactions, and overall treatment costs. Since many patients achieve only partial symptom relief in clinical trials, a stepwise treatment approach incorporating combination therapy is often necessary. These recommendations exclude paediatric patients and individuals with trigeminal neuralgia, which require separate treatment protocols (Dworkin et al., 2010). Additionally, conditions such as fibromyalgia and irritable bowel syndrome, which do not involve a distinct lesion in the somatosensory nervous system, are not classified as NP (Dworkin et al., 2010).

This complex condition often managed with various pharmacological treatments. Below is a comprehensive summarizing several medication classes used in NP management, including their mechanisms of action, common brand names, starting dosages, titration approaches, maximum dosages, duration, side effects and precautions (Table 7).

Various treatments are available for managing NP, ranging from pharmacological interventions to non-pharmacological therapies. Traditional treatments include analgesics, anticonvulsants, antidepressants, and topical agents. Opioids are sometimes prescribed for severe cases, though their use is limited due dependency. Anticonvulsants like gabapentinoids and certain antidepressants, particularly SNRIs, are commonly used for their efficacy in modulating pain pathways. Topical treatments, such as capsaicin creams and lidocaine patches, provide localized relief for some patients. Additionally, non-pharmacological approaches such as physical therapy, acupuncture, and cognitive behavioral therapy have shown benefits in pain management. However, for the specific needs of our study, we have chosen to focus on three treatments: SD, FLX, and LOVA. These compounds were selected based on their potential to address the underlying mechanisms of NP effectively. Preliminary research has suggested that they have promising therapeutic effects, particularly in modulating neuroinflammatory responses, neural excitability, and other pathophysiological changes associated with neuropathy. The targeted nature of these treatments makes them an ideal choice for investigating potential therapies within our experimental models.

2.9. Importance of Combination Therapy

Chronic discomfort that is often refractory to single-drug therapy. Combination therapy has emerged as a superior approach by targeting multiple pathways, enhancing efficacy, and reducing adverse effects associated with high-dose monotherapy. It involves various mechanisms, including central sensitization, peripheral nerve damage, inflammatory processes, and altered neurotransmitter activity. A single drug often addresses only one aspect, leaving other contributing factors untreated. Hence, combining agents with different mechanisms of action enhances therapeutic effectiveness and mitigates side effects.

Table 7. Comparative analysis of combination vs. Single drug therapy

S.No.	Points	Single Drug Therapy	Combination Therapy
1	Mechanism of Action	Targets a single pathway	Acts on multiple pathways
2	Efficacy	Limited efficacy	Improved pain relief
3	Side effect	Higher at therapeutic dose	Reduce due to lower doses of age drug
4	Tolerance	More likely over time.	Less risk due to synergistic actions.
5	Adaptability.	Not flexible	Flexible
6	Example.	Gabapentin	Gabapentin with nortriptyline
		FLX	FLX with SD

2.10. Multimodal Approach to NP Management

NP arises from complex mechanisms, necessitating a combination therapy approach to achieve optimal relief. Peripheral sensitization, triggered by nerve injury, leads to hyperexcitability, which can be mitigated by drugs such as gabapentin that reduce calcium influx. TCAs complement this effect by blocking sodium channels, providing a dual mechanism for pain inhibition. Central sensitization, characterized by excessive pain signaling within the spinal cord, often requires NMDA receptor antagonists like ketamine, which, when combined with opioids or anticonvulsants, enhance analgesic efficacy. The inflammatory component of NP is addressed by COX-2 inhibitors, which reduce inflammation, but their effectiveness is further amplified when used alongside pregabalin, a drug that modulates neurotransmitter release. Additionally, deficiencies in descending serotonergic inhibition contribute to persistent pain. Serotonin-norepinephrine reuptake inhibitors (SNRIs) work to restore this balance, and their combination with anticonvulsants results in more comprehensive symptom relief.

Clinical and preclinical findings highlight the superiority of combination therapy over monotherapy in NP management. Presenting the case like, 52-year-old diabetic with peripheral neuropathy, gabapentin alone provided inadequate relief. However, the introduction of duloxetine significantly improved symptoms while allowing for lower doses of both medications, thereby reducing adverse effects. Similarly, a 55-year-old patient suffering from postherpetic neuralgia experienced limited benefits from amitriptyline alone. The addition of pregabalin led to enhanced pain control, improved sleep quality, and better overall well-being. Preclinical studies further support these observations, demonstrating that a combination of gabapentin and amitriptyline resulted in superior pain relief compared to monotherapy. Animal models showed notable improvements allodynia (mechanical) and hyperalgesia (thermal), reinforcing the benefits, targeting multiple pain pathways simultaneously. By addressing different pain mechanisms while minimizing side effects, combination therapy proves to be a more effective strategy in NP management, offering superior patient outcomes compared to single-drug treatments.

2.10.1. SD

SD, chemically known as “1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidine-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine citrate”, is a widely utilized pharmacological agent initially developed to address erectile dysfunction (Jk et al., 2012). Since its approval in 1998, SD has also been used to manage pulmonary hypertension under

the brand name Revatio® and has shown effectiveness in treating Raynaud's phenomenon (Roustit and Cracowski, 2013). Initially researched by Pfizer under the code name UK-92,480, SD was originally intended for the treatment of hypertension and angina pectoris. However, it did not yield significant benefits for hypertension and had a minimal effect on angina (Carson, 2003). As illustrated in Figure 8, SD citrate has a molecular weight of 666.7g/mol and is water-

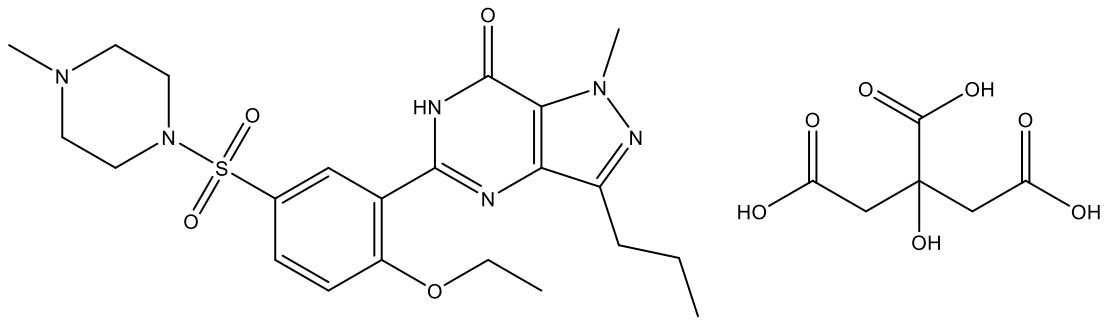


Figure 8. Chemical structure of SD citrate

soluble, aromatic compound (Sawatdee and Srichana, 2013). Beyond its well-known effects in sexual health, SD also exhibits promising therapeutic benefits for central nervous system (CNS) disorders, with studies demonstrating its ability to reduce memory impairment (Puzzo and Sapienza, 2008), mitigate neurological deficits, enhance neurogenesis, and support recovery following stroke in animal models (Charriaut-Marlangue et al., n.d.; Joseph et al., 2004). Clinically, SD has been shown to improve symptoms of multiple sclerosis (Uthayathas et al., 2007). The primary pharmacological action of SD involves selective inhibition of phosphodiesterase-5 (PDE5), an important enzyme responsible for the hydrolysis of cyclic guanosine monophosphate (cGMP) (Carson and Lue, 2005; Corbin and Francis, 1999). By inhibiting PDE5, SD raises cGMP levels, thereby enhancing its biological effects. This action is attributed to SD's structural similarity to cGMP, allowing it to bind to allosteric sites and modify the enzyme's catalytic activity (Francis et al., 2010; Xiao et al., 2007). The interaction between SD and the cGMP pathway contributes to various beneficial outcomes, such as smooth muscle relaxation, which is crucial for its efficacy in erectile dysfunction and other clinical indications.

2.10.1.1. Mechanisms of Action

SD exerts significant therapeutic effects in NP by targeting various neurophysiological pathways. One of its primary mechanisms involves inhibiting PDE5, which results in the accumulation of cGMP, a key mediator of smooth muscle relaxation and vasodilation. This enhanced signaling improves blood flow, including in peripheral and central nervous tissues,

which supports neuronal survival and function by alleviating nerve ischemia. SD also enhances the release of nitric oxide (NO) from endothelial cells, which further activates guanylyl cyclase, increasing intracellular cGMP levels. This pathway promotes neuroprotection by mitigating oxidative stress and reducing excitotoxicity. Notably, NO plays a role in modulating neurotransmitter signaling, thereby influencing pain transmission pathways. Additionally, SD's modulation of NMDA receptor activity through increased NO synthesis promotes calcium influx into neurons. This helps restore normal synaptic activity and reduce hyperexcitability, a hallmark of NP. Enhanced calcium signaling through this mechanism also supports synaptic plasticity, crucial for adaptive changes in the nervous system during injury recovery. Moreover, elevated cGMP levels stimulate GABA release, contributing to a balanced excitatory and inhibitory neurotransmission environment.

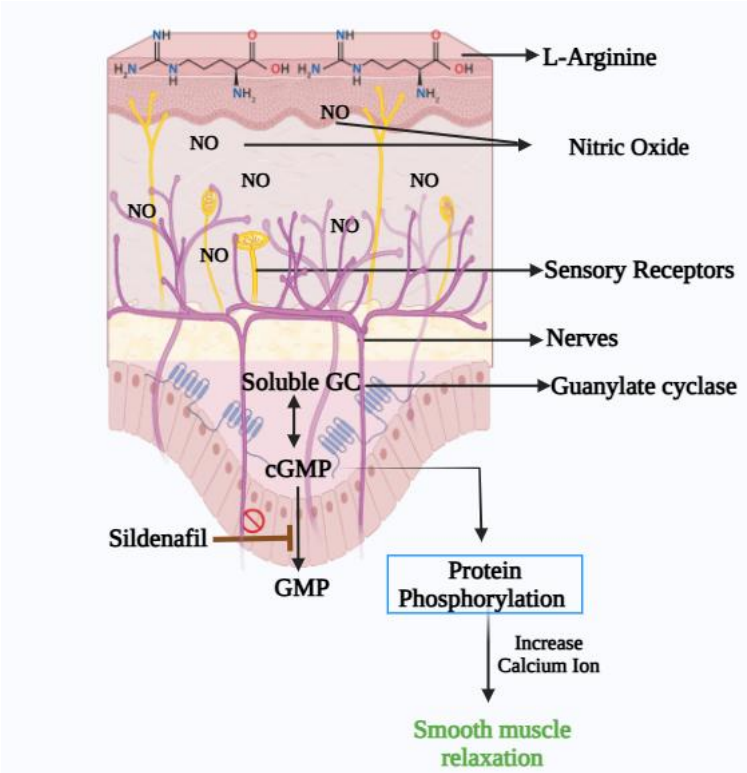


Figure 9. Mechanism of SD

This balance is critical in preventing the hyperactive neuronal states associated with chronic pain syndromes. The drug's ability to promote GABAergic signaling further underscores its potential as a modulator of pain pathways. SD also appears to impact immune pathways by reducing inflammation within neural tissues as showing in the Figure 9. By maintaining endothelial function, it limits the infiltration of inflammatory cytokines, contributing to a

neuroprotective microenvironment (Araiza-Saldaña et al., 2010; Singh et al., 2001; Knott et al., 2017) (Boswell-Smith et al., 2006).

Table 8. Mechanisms of SD in NP

Mechanism	Effect
PDE5 Inhibition	Increases cGMP levels, promoting vasodilation and improving tissue perfusion
NO Release	Activates guanylyl cyclase, further elevating cGMP and reducing excitotoxicity
NMDA Modulation	Enhances calcium signaling, restores normal neuronal signaling
GABA Stimulation	Balances excitatory and inhibitory neurotransmission
Anti-inflammatory Effects	Reduces inflammatory cytokines and limits neural inflammation
Oxidative Stress Reduction	Mitigates cellular damage and supports neuronal survival

SD's effects on neurotransmission, vascular health, and inflammation point to its broad therapeutic potential in addressing NP. These mechanisms suggest its usefulness in treating conditions involving nerve damage and disrupted pain processing, supporting the need for further investigation and clinical application. In NP, SD's capacity to boost nitric oxide (NO) production in the endothelium is essential for reducing nerve ischemia and promoting the survival of neurons. Enhanced blood flow not only sustains nerve function but also prevents hypoxic damage, a key contributor to chronic pain. Additionally, SD's role in stabilizing NO levels helps alleviate oxidative stress, a common result of nerve injury. Apart from its vascular effects, SD may also possess indirect anti-inflammatory properties. By supporting endothelial health and preventing vascular dysfunction, it helps limit the influx of inflammatory cells that can worsen NP. Furthermore, SD's influence on endothelial nitric oxide synthase (eNOS) helps maintain its proper function, ensuring continuous NO production while preventing the generation of damaging free radicals.

In conclusion, SD's regulation of the NO-cGMP pathway, along with its protective effects against oxidative stress and inflammation, positions it as a promising treatment for NP. This comprehensive mechanism underscores its potential application beyond erectile dysfunction, offering both neurovascular protection and pain relief.

Table 9. Pharmacological evaluation of SD in the treatment of NP

S. No	Drug (Alone/Combination)	Dose & Route	Animal Model Used	Mechanism of Treatment	Results	Reference
1	SD + Bicuculline + Saclofen	3 ml/kg, i.v.	Randall-Selitto Hyperalgesia Model	cGMP plays a role in antinociception.	GABAB and GABAA receptors contribute to SD action. Reversal by saclofen and bicuculline.	Huang et al., 2010
2	SD + FPL 64176 + KT 5823	10 µL, i.t.	PKG-L-type Calcium Channel Model	cGMP reduces L-type calcium channel activity via PKG activation.	Increased cGMP in the spinal cord reduces nociception.	Kim et al., 2010
3	SD + Streptozotocin	5 mg/kg, 10 mg/kg, p.o.	Diabetic Neuropathy Model	Enhances smooth muscle relaxation via NO-PDE5-cGMP pathway.	Significant reduction in serum glucose and NP.	Sarifakioglu et al., 2004
4	SD + Curcumin	10 g/kg, p.o.	Alcohol-Induced NP	cGMP involvement with GABAA and GABAB receptors.	Improved nerve function, histopathology, and biochemical markers.	Kandhare et al., 2012; Kaur et al., 2017
5	SD + Ellagic Acid	50 µL, i.p.	Formalin-Induced Pain Model	NO-cGMP and opioidergic pathway.	EA shows a significant antinociceptive effect.	Mansouri et al., 2014
6	SD + Streptozotocin + L-NAME + Methylene Blue + Acetic Acid	200 mg/kg (mice), 75 mg/kg (rats), i.p.	Diabetic NP Model	NO-cGMP pathway with L-NAME inhibition.	Increased pain threshold in diabetic animals.	Benkelfat and Boivin, 2006; Patil et al., 2004
7	SD + Cilostazol	0.01, 0.1, 1 mM (SD); 0.1, 1, 10 mM (Cilostazol), i.p.	Migraine with Human Headache Model	PDE5 and PDE3 inhibition increases cGMP.	Affects cGMP hydrolysis in the trigeminal ganglia.	Nordgaard et al., 2013
8	SD + Pregabalin	2.2 mg/kg (SD), 10 mg/kg (Pregabalin), i.v.	Allodynia Model	Synergistic effect via cGMP and ion channel modulation.	54.4% decrease in EC50, synergistic response.	Bender et al., 2010; Vogel, 2007
9	SD + Prazosin + Yohimbine + Atropine + Mecamylamine	3, 10, 30 µg (SD), i.t.	Formalin-Induced Flinching Model	Modulates adrenergic, muscarinic, and nicotinic receptors via cGMP.	PDE inhibition expedites acute and chronic pain relief.	Park et al., 2011
10	SD + CTOP + Naltrindole + GNTI	30 µg, i.t.	Formalin Model	NO–cGMP–K+ channel pathway activation in opioid receptors.	SD facilitates pain modulation in the spinal cord.	Yoon et al., 2008a; Kim et al., 2008;

						Yoon et al., 2008b
11	SD + Adenosine A2A, A2B, A3 Antagonists	30 µg, i.t.	Formalin-Induced Nociception Rat Model	NO-cGMP and adenosine pathway modulate PKG.	cGMP-PKG-K+ channel activation leads to antinociception.	Lee, 2010
12	SD + Glyceryl Trinitrate	1-5.6 mg/kg, i.p.	Streptozotocin-Induced Neuropathy Model	PDE5 inhibition with NO donation enhances cGMP and hyperpolarization.	PDE5 inhibitor + NO donor restores pain sensitivity.	Araiza-Saldaña et al., 2010; Paulus, 2017
13	SD + Cuprizone	25 mg/kg (SD), 0.2% (Cuprizone) in drinking water	Cuprizone-Demyelination Model	Reduces TNF-α and IL-1β while increasing IL-10 via eNOS activation.	Prevents NFkB activation, offering anti-inflammatory effects.	Nunes et al., 2015
14	SD + BCNR	20 mg/kg, i.p.	Erectile Dysfunction Neurodegenerative Model	Modulates cGMP and ERK signaling, enhancing neuroplasticity.	Upregulates neurotrophic factors, improving neuropathies.	Hlaing et al., 2011
15	SD Alone	3 mg/kg, i.p.	Neurotrauma Model	Enhances ERK/CRAB and NO-cGMP pathways in the spinal cord.	Improves allodynia, hyperalgesia, and neuroprotection.	Knott et al., 2017; Bernard, 2000

In summery SD enhancing NO signaling and promoting improved vascular function and blood flow. In addition to this well-known effect, sildenafil activates cGMP-dependent protein kinase (PKG), which in turn opens calcium-activated potassium (KCa) channels. This action leads to the hyperpolarization (vascular smooth muscle cells) and a reduction in intracellular calcium, further facilitating vasodilation (Sisein, 2014). Moreover, SD stimulates the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, resulting in the "upregulation of key antioxidant enzymes" such as GSH and SOD. The enhanced antioxidant defense contributes to a decrease in oxidative stress markers like MDA and ROS. Additionally, by inhibiting toll-like receptor 4 (TLR4) signaling, SD suppresses the production of pro-inflammatory cytokines (a cytokines), thereby reducing inflammation. Finally, its capacity to enhance GABA transmission further mitigates neuronal hyperactivity. Collectively, these mechanisms not only underpin SD vascular benefits but also suggest broader therapeutic implications in managing oxidative stress, inflammation, and neuronal excitability.

2.10.2. FLX

FLX is selective serotonin reuptake inhibitor (SSRI), is primarily used for the mitigation of depression but has also gained recognition for its efficacy in managing NP. The IUPAC name for FLX is "(R, S)-N-methyl-3-phenyl-3-(4-(trifluoromethyl) phenoxy) propan-1-amine" (Figure 10).

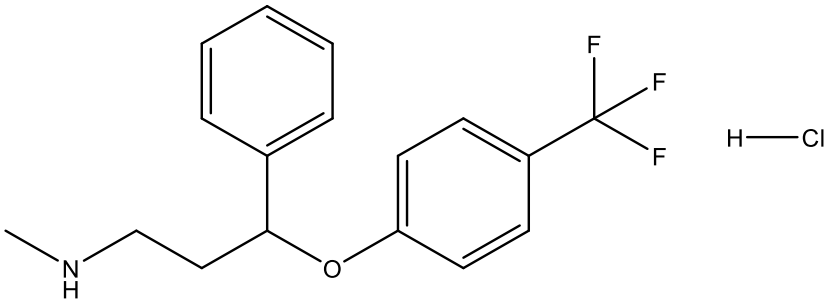


Figure 10. Chemical Structure of FLX

FLX, marketed under the brand name Prozac (Eli Lilly) (Choi et al., 1999; Wong et al., 2005). It enhancing serotonin levels in the synaptic cleft, which is crucial for modulating both mood and pain perception (Wenthur, 2014; Wenthur, 2016). Its role in NP management is has ability modulate central and peripheral mechanisms of pain processing, providing a non-opioid analgesic.

2.10.2.1 Mechanisms of action: It inhibits the reuptake of serotonin and norepinephrine, increasing their availability in the synaptic cleft. This regulation of the serotonergic and adrenergic systems enhances pain relief by activating various receptors, particularly 5-HT1 and 5-HT2, which are involved in pain modulation. Studies using animal models, such as streptozotocin-induced diabetic neuropathy, have demonstrated that FLX elevates synaptic serotonin levels and delays the onset of antinociceptive effects, ultimately reducing pain associated with chronic neuropathic conditions. Additionally, FLX influences multiple signaling pathways that contribute to pain modulation, including the regulation of pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α), which plays a key role in the inflammatory response in neuropathic pain (Catalisano et al., 2024). By enhancing serotonin signaling, FLX helps regulate inflammatory responses by reducing TNF-α production in both the peripheral and central nervous systems. This modulation plays a crucial role in controlling inflammation and alleviating pain hypersensitivity (Catalisano et al., 2024). The anti-inflammatory effects of FLX are essential, as pro-inflammatory cytokines play a role in the onset and continuation of pain in neuropathic conditions (Pușcașu et al., 2023).

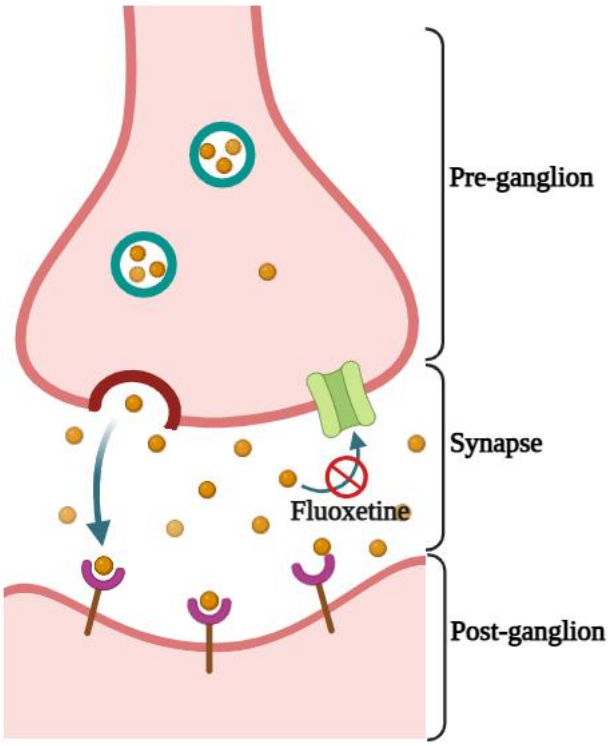


Figure 11. Mechanism of FLX

After releasing of norepinephrine in the dorsal root ganglia has been shown to contribute to the suppression of pain signals by inhibiting central sensitization a crucial mechanism in chronic pain. By modulating adrenergic receptors, increases norepinephrine release, which enhances

descending inhibitory pathways in the nervous system, ultimately increasing to pain reduction. In models of morphine-induced hyperalgesia, FLX has been observed to reduce markers inflammation, like c-Fos and protein kinase-C. Further suggesting that FLX may help prevent the development of chronic pain through these mechanisms (Rashid & Ueda, 2005). Another critical aspect of role in NP management is its potential effect on the blood-brain barrier. Following nerve injury, the disruption of the BBB allows peripheral immune cells to infiltrate the central nervous system, exacerbating neuroinflammation and pain. Evidence suggests that FLX may contribute to the stabilization of the blood-brain barrier, preventing the excessive migration of immune cells into the CNS. This action could further reduce neuroinflammation, which is often a contributor to chronic pain states, including NP. It stabilizing the blood-brain barrier and reducing the inflammatory response, FLX offers a comprehensive approach to managing both acute and chronic NP (Figure 11). Its dual action on both serotonergic and adrenergic systems, along with its influence on inflammatory pathways and the stabilization of the blood-brain barrier, underscores its effective management for NP. Its ability to modulate pain-related signaling pathways, reduce neuroinflammation, and enhance the release of norepinephrine positions. Finally, it enhances serotonin signaling by blocking its reuptake and modulating 5-HT1A receptors, reducing neuronal excitotoxicity. It limits calcium influx by downregulating NMDA receptor activity, preventing damage associated with calcium overload. Moreover, FLX boosts BDNF levels, which are crucial for neuronal repair and synaptic plasticity. Its influence on G-protein coupled inward rectifying potassium (GIRK) channels also helps in reducing neuronal excitability, contributing to overall neural stability.

Table 10. Pharmacological Activity of FLX in treatment of NP

S.No.	FLX Used in NP	Dose	NP Model	Mechanism of Action	Findings	References
1	FLX + ondansetron, pindolol and ritanserin	5–20 mg/kg (FLX), 1–2 mg/kg (others)	STZ	Modulating (serotonin pathways) by "5-HT1 and 5-HT2 receptors except 5-HT3"	Intraperitoneal FLX increases synaptic 5-HT levels, delaying antinociceptive response	(Anjaneyulu & Chopra, 2004)
2	FLX+ morphine sulfate + naloxone + naltrexone	5–20 mg/kg i.p.	writhing model (Acetic acid-induced)	Inhibits monoamine reuptake and affects calcium channels (voltage-sensitive)	Dose-dependent analgesic effect (5–20 mg/kg); 20 mg/kg FLX enhances tail-flick response; co-administration with pindolol augments antinociception	(Mora-Escobedo et al., 2024; Singh et al., 2001; Wang et al., 2023)
3	FLX+clonidine	30 nmol	Pre-injury morphine model	Reduces neuronal hyperactivity markers (c-Fos, PKC) in the spinal dorsal horn	Systemic morphine and FLX activate descending monoaminergic pathways, preventing sensation centrally	(Rashid & Ueda, 2005)
4	FLX + reboxetine, bupropion + venlafaxine	3–30 mg/kg	CCI and SNL models	Combined 5-HT and NA reuptake inhibition modulates pain response	FLX induces flinching in the formalin test and affects allodynia and hyperalgesia; NA reuptake inhibition mediates thermal pain relief	(Pedersen et al., 2005)
5	FLX+ atropine + naloxone + yohimbine	10–20 mg/kg	Diabetic neuropathy	Increases 5-HT levels; modulates cholinergic, opioidergic, and serotonergic (5-HT2A) systems	20 mg/kg FLX significantly reduces pain in tail-immersion and hotplate tests; yohimbine does not affect analgesic response	(Anjaneyulu & Chopra, 2006)
6	FLX + desipramine, paroxetine + ketorolac, reboxetine + duloxetine + gabapentin	10, 30, 56 respectively (mg/kg s.c.)	Rodent pain model	Selective inhibition of NE and 5-HT reuptake; opioid receptor involvement	NRI activity shows higher efficacy in neuropathic and visceral pain than SSRIs alone; NRIs and SRIs demonstrate synergistic effects in visceral pain	(Leventhal et al., 2007)
7	FLX + nortriptyline + amitriptyline	5–10 mg/kg (FLX), 0.5–5 mg/kg (others)	Sciatic nerve cuffing model	Blocks noradrenaline and serotonin uptake; interacts with opioid receptors	Chronic TCA treatment alleviates allodynia; FLX alone is ineffective; delta- and kappa-opioid receptors contribute to TCA effects	(Benbouzid et al., 2008)
8	FLX + bis-selenide + amitriptyline + bupropion	1–30 mg/kg p.o.	CCI	Enhances descending inhibitory pain pathways, increasing 5-HT, DA, and NA levels	FLX and bupropion alleviate CCI-induced depression-like behavior and pain hypersensitivity	(Jesse et al., 2010)

						(Garcia et al., 2010)
9	FLX+ amitriptyline, doxepin + imipramine + nortriptyline + desipramine + bupivacaine + fluvoxamine	0.25–1 mg/kg	CCI model	Modulates G-protein-activated inwardly rectifying K+ and voltage-gated Na+ channels	FLX shows minimal analgesic effect at lower doses compared to TCAs	
10	FLX	5–25 mg/kg i.p.	CCI model	Involvement of CART in FLX-induced anti-hyperalgesia	FLX increases CART fiber population in pain-related brain regions; enhances paw withdrawal latency in neuropathic rats	(Upadhya et al., 2011)
11	FLX	20 mg/kg i.p.	Oxaliplatin induced	Blocks 5-HT2C receptors; modulates PAG and amygdala activity	Stimulates descending inhibitory pathways; reduces 5-HT2C receptor levels in PAG while increasing them in the amygdala	(Baptista-De-Souza et al., 2014)

2.10.3. LOVA

Recent studies have suggested that LOVA, a drug commonly used to manage cholesterol levels, may offer promising therapeutic effects for NP due to its anti-inflammatory and immunomodulatory properties. While LOVA is primarily recognized for its role in inhibiting HMG-CoA reductase, which is involved in cholesterol biosynthesis, it also exerts pleiotropic effects that extend beyond lipid-lowering, particularly in the modulation of immune responses and inflammation. The chemical name of LOVA is "(1S,3R,7S,8S,8aR)-8-[2-(4-hydroxy-6-oxooxan-2-yl)ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2-methylbutanoate" (Figure 12) (Henwood & Heel, 1988; Tomaszewski et al., 2021).

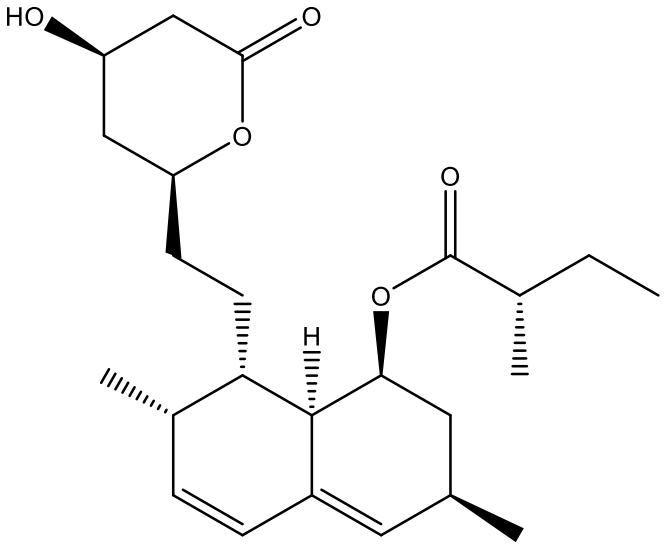


Figure 12. Chemical structure of LOVA

2.10.3.1 Mechanism of LOVA: LOVA can modulate inflammatory pathways, including the inhibition of Toll-like receptor 4 (TLR4) signaling, which is implicated in the innate immune response and neuroinflammation associated with NP (X. H. Zhao et al., 2015). Additionally, LOVA found to reduce the production of pro-inflammatory cytokines (TNF-α, and nitric oxide), which has significant in the maintenance of inflammation followed by pain. These actions suggest that LOVA may act as an effective modulator of the inflammatory processes that contribute to NP, potentially reducing pain perception and promoting recovery. Several animal studies have explored the effects of LOVA on NP models, providing valuable insight into its potential therapeutic mechanisms. In one study, male Swiss mice and male Wistar rats were treated with LOVA at varying doses (0.5, 1, 2, 5, and 10 mg/kg) for 24 hours. The results revealed a remarkable reduction in levels of TNF-α, IL6 and nitric oxide, demonstrating the drug's anti-inflammatory effects and its potential to alleviate pain associated with

neuroinflammation (Goncalves et al., 2011; Neerati & Gudimandula, 2024). Another study using male Sprague-Dawley rats showed that LOVA inhibited TLR4 signalling and blocked lipopolysaccharide (LPS)-induced immune activation, suggesting that the drug can suppress immune responses that contribute to neuroinflammation in NP (Peng et al., 2019). Further research involving adult male Wistar rats with sciatic nerve crush injury revealed that LOVA administration restored anti-inflammatory, antioxidative, and immunomodulatory properties, suggesting its potential to improve recovery and reduce NP in peripheral nerve traumatic models (Ghayour et al., 2017). Additionally, a study involving formalin-induced

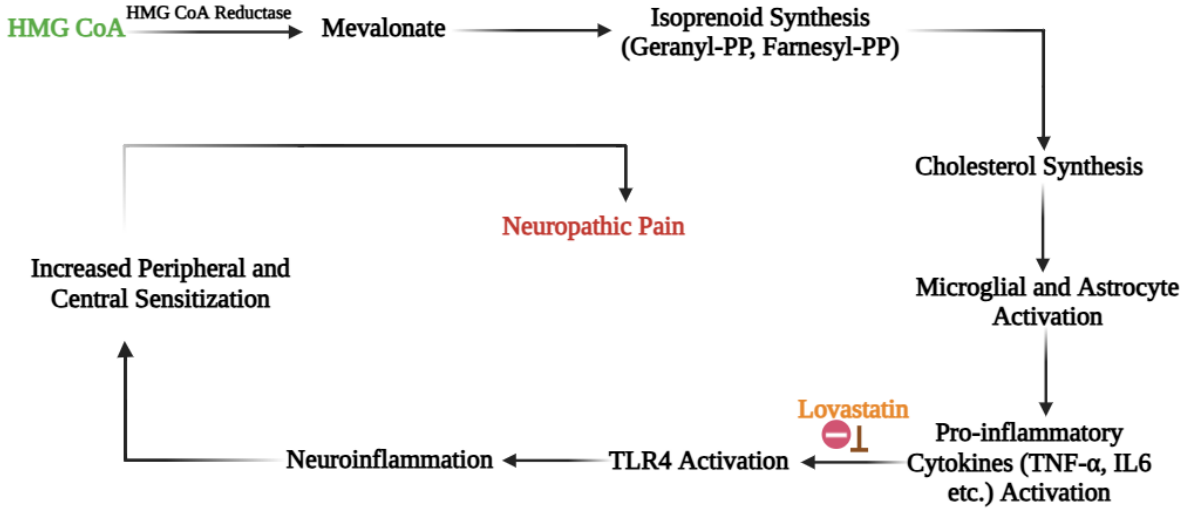


Figure 13. Mechanism of LOVA

pain in adult male mice demonstrated that LOVA downregulated pain and inflammation in both inflammatory and neurogenic areas, further supporting its role in modulating pain responses (Mirhadi, 2011). Lastly, the accumulating evidence from animal studies highlights the potential of LOVA as a novel therapeutic approach for managing NP. Its ability to modulate inflammatory pathways, reduce oxidative stress, and influence immune signalling mechanisms positions it as a promising adjunct therapy, particularly for patients whose pain is resistant to conventional treatments. (Figure 13). LOVA has neuroprotective activity as follows.

2.10.3.2. Immunomodulatory and Neuroprotective Effects of Statins

Recent research underscores that statins, in addition to their cholesterol-lowering properties, play crucial immunomodulatory and neuroprotective roles. Their therapeutic potential extends to managing immune-mediated disorders and offering protection against neuronal damage (Althanoon et al., 2020).

2.10.3.2.2. Immunomodulatory Actions

Statins have shown promise in preventing and reversing experimental autoimmune encephalomyelitis, a widely used model for MS (Solinas et al., 2022). These drugs modulate immune activity in a manner similar to interferon-based therapies, promoting a shift from a pro-inflammatory to an anti-inflammatory state. Clinical studies with statins like simvastatin and atorvastatin have demonstrated a significant reduction in the size and frequency of new lesions detected by MRI in MS patients. Statins also regulate the activity of T-cells by suppressing their proliferation, downregulating activation markers, and stimulating IL-4 production. Although some pro-inflammatory effects, such as increased secretion of interferon and interleukin-12, were observed, combining statins with interferon-1b enhanced their anti-inflammatory efficacy (Song et al., 2025).

2.11.2. Neuroprotective Role of LOVA

LOVA, a lipophilic statin, has emerged as a promising agent for neuroprotection, particularly in the context of ischemic stroke. One of its primary mechanisms involves enhancing the origination of endothelial NO, a critical molecule for vascular function and neuronal protection. By increasing NO bioavailability, LOVA supports “improved cerebral blood flow, reduces oxidative stress, and mitigates neuronal injury”. Furthermore, LOVA modulates endothelial nitric oxide synthase (eNOS) activity, preventing eNOS uncoupling and sustaining NO synthesis. This modulation plays a central role in reducing ischemic brain injury and facilitating recovery. In addition to these vascular benefits, LOVA demonstrates anti-inflammatory effects by reducing neuroinflammation, which can exacerbate neuronal damage in ischemic and degenerative conditions (Ghayour et al., 2017; Mirzaie et al., 2022).

2.10.3.3 Role of TLRs in Neuroprotection and NP

TLRs are crucial components of the innate immune system, playing key roles in recognizing pathogens and modulating inflammatory responses. Recent evidence suggests that TLRs are particilated in the origination and maintenance of NP. Overactivation of TLRs, particularly TLR4, can lead to the release of pro-inflammatory chemokines and cytokines, contributing neuronal pain signalling followed by hypersensitivity. Statins, including LOVA, have been shown to downregulate TLR expression and signaling pathways. By inhibiting TLR4 activation, statins can reduce pro-inflammatory cytokines, thereby alleviating neuroinflammation and NP. This effect may represent an additional mechanism by which statins exert neuroprotective and analgesic benefits. LOVA is multifaceted neuroprotective

effects, including NO modulation, anti-inflammatory actions, and TLR inhibition, make it a promising therapeutic candidate for managing both ischemic conditions and NP.

LOVA exhibits multiple neuroprotective and analgesic mechanisms that contribute to its therapeutic potential in neurological disorders. One key mechanism is the enhancement of endothelial nitric oxide (NO) production, which improves cerebral blood flow and minimizes neuronal injury during ischemic events. By promoting NO availability, LOVA supports vascular function and reduces oxidative damage. Additionally, LOVA plays a role in modulating endothelial nitric oxide synthase (eNOS) activity, ensuring continuous NO synthesis while preventing eNOS uncoupling—a condition that could otherwise result in harmful oxidative stress. This regulatory effect on eNOS highlights its significance in maintaining vascular and neuronal health. The drug also reduces oxidative stress, offering protection to neurons from ischemic damage. By minimizing free radical formation and oxidative injury, LOVA helps maintain cellular integrity in the brain. Another crucial aspect of LOVA's neuroprotective profile is its anti-inflammatory action. It mitigates neuroinflammation, a key factor contributing to neuronal damage and disease progression, thereby promoting recovery in brain tissues. Moreover, LOVA has shown the ability to inhibit Toll-like receptor 4 (TLR4) signaling, which plays a role in immune activation and NP. By reducing pro-inflammatory cytokine production through TLR4 inhibition, LOVA helps alleviate pain and inflammation, making it a valuable candidate for managing both neuroinflammation and NP (Chaves et al., 2017; X. H. Zhao et al., 2015).

Table 11. Pharmacological Activity of LOVA in treatment of NP

S.No.	Dose	NP Model	Mechanism of Action	Findings	References
1	Variable dosage	Sciatic nerve injury model	Acts as a TLR4 antagonist and selectively inhibits LPS-induced TLR4, NF-κB	Inhibited production of nitric oxide, ROS, and pro-inflammatory markers (TNF-α, IL-6, COX-2); reduced phagocytic activity in BV-2 microglial cells	(Peng et al., 2019)
2	10 or 25 mg/kg	Autoimmune Neuritis (EAN) model	Inhibits leukocyte proliferation and migration; independent of cholesterol reduction	Prevented peripheral nerve conduction deficits and morphological nerve injury; reduced cellular infiltrates in sciatic nerves; diminished myelin-stimulated splenocyte proliferation; shortened disease course without hepatotoxic or myotoxic effects	(Sarkey et al., 2007)
3	10 mM	Apoptotic signal transduction (staurosporine-induced apoptosis model)	Potentiates caspase-3 activity and increases the expression of the pro-apoptotic GTPase RhoB	Enhanced neuronal apoptosis when combined with apoptotic stimuli; raised concerns for careful use in patients with neurological diseases	(Föcking et al., 2004)
4	20-40 mg/kg	peripheral neuropathy induced by statin therapy (Clinical cases)	Long-term administration of statins may carry a risk of peripheral neuropathic complications, possibly due to underlying neurotoxicity.	Published reports and clinical observations point to a slight but plausible association between statin use and peripheral nerve impairment. Statin-induced neuropathy should be suspected when other differential diagnoses are excluded.	(Chong et al., 2004)
5	2 mg/kg and 5 mg/kg	Sciatic nerve crush injury in male Wistar rats	Anti-inflammatory, immunomodulatory, and antioxidant properties	LOVA at 5 mg/kg significantly "accelerated nerve regeneration and functional recovery" Morphometric parameters, including axonal number and myelin thickness, were notably higher compared to controls.	(Ghayour et al., 2017)
6	2, 5, 10 mg/kg (rats); 0.5, 1, 5 mg/kg (mice)	Carrageenan-induced paw edema and peritonitis models of acute inflammation	Anti-inflammatory, anti-nociceptive, modulation of leukocyte migration, inhibition of TNF-α and iNOS activity	LOVA attenuated paw edema formation, reduced leukocyte migration, and demonstrated dose-dependent anti-nociceptive effects. It inhibited myeloperoxidase release from neutrophils and decreased TNF-α and iNOS expression.	(Goncalves et al., 2011)
7	1, 5, 10, 20, 40, 80, 100 mg/kg (ip, 4 days)	Formalin-induced pain and inflammation model in mice	Analgesic and anti-inflammatory effects; dose-dependent inhibition of biphasic pain responses	LOVA significantly reduced pain responses in both early and late phases of the formalin test. It demonstrated a dose-dependent analgesic and anti-inflammatory effect.	(Mirhadi, 2011)
8	20 mg daily for >6 months	Human clinical study on peripheral neuropathy	Evaluation of sensory and motor nerve conduction features through electrodiagnostic study	Significant differences in the "amplitudes of the peroneal motor nerve (p=0.048) and sural sensory nerve (p=0.036)"; no significant occurrence of peripheral neuropathy but evidence of peripheral 9nerve involvement	(Emad et al., 2018)

9	2 mg/kg and 5 mg/kg (ip, daily for 7 days post-injury)	SCI model in female adult Wistar rats	Exhibits anti-inflammatory, antioxidant, and anti-apoptotic properties; reduces apoptosis and gliosis; modulates cytokine expression by upregulating IL-10 and downregulating TNF-α and IL-1β; improves stereological parameters	Both LOVA-treated groups showed significant improvements in biochemical markers (except MDA), neuronal and glial cell density, and neurological function (assessed via BBB and NBT tests), with the 5 mg/kg dose demonstrating a more pronounced neuroprotective effect	(Mirzaie et al., 2022)
10	20 mg daily (oral)	Case study: 48-year-old doctor with hypercholesterolaemia treated with LOVA who developed exercise-induced myopathy	Inhibition of HMG-CoA reductase leads to reduced mevalonate production, which decreases the synthesis of cholesterol and ubiquinone (Coenzyme Q). Reduced CoQ levels in mitochondria can impair electron transfer and energy metabolism, potentially contributing to muscular lactic acidosis and myopathy.	The patient experienced quadriceps soreness, fatigue, and severe cramps during exercise. CPK levels were significantly elevated (initially 1400 U/l, and remaining at 800 U/l for 6 months after discontinuing treatment). Despite improved lipid profiles (LDL decreased from 4.65 to 3.1 mmol/l and HDL increased from 1.55 to 1.66 mmol/l), muscle symptoms persisted, suggesting LOVA-induced myopathy.	(Pj et al., n.d.)

The combination of SD, FLX, and LOVA offers a promising therapeutic approach for managing NP by targeting distinct yet interconnected pathways. SD enhances nitric oxide (NO) bioavailability through phosphodiesterase-5 inhibition, improving neural blood flow and reducing hyperalgesia. FLX modulates neurotransmission by inhibiting excessive glutamate activity, preventing excitotoxicity, and enhancing glycinergic inhibition, thereby suppressing pain signals. LOVA exhibits anti-inflammatory and neuroprotective effects by downregulating TLR4 expression, mitigating oxidative stress, and stabilizing neuronal functions. The synergistic effects of this combination lie in LOVA's potentiation of SD and FLX actions. By preserving NO signaling and reducing inflammatory disruptions, LOVA amplifies SD's vascular and neuroprotective benefits. Concurrently, LOVA supports FLX by enhancing inhibitory pathways, countering excitatory neurotransmission, and creating a neuroprotective environment. This comprehensive interaction addresses multiple facets of NP pathophysiology, offering a novel and multifactorial treatment strategy to reduce pain and protect neural integrity. In this combination SD, FLX and LOVA for managing NP it appears to potentiate the effect of SD and FLX. SD, FLX and LOVA exhibit strong neuroprotective properties by targeting multiple receptors, ion channels, and intracellular signaling pathways, making them highly valuable for addressing NP.

CHAPTER 3
HYPOTHESIS

3.1. Scope of the study

The high-fat diet combined with streptozotocin (HFD/STZ) rat model is a widely used experimental approach to induce diabetes in animals. In this model, animals are first fed a diet rich in fat (and occasionally sugar), which leads to the development of insulin resistance, hyperinsulinemia, and impaired glucose tolerance. Following this dietary phase, low dose STZ, a compound toxic to pancreatic β-cells, is administered to significantly decrease the number of functioning β-cells. Together, these interventions simulate the pathological features of type 2 diabetes, but within a much shorter timeframe than that observed in human disease progression (Ahlawat & Sharma, 2018; Kumar et al., 2018; Olugbuyi et al., 2022). More than 50% of all diabetic individuals experience diabetic neuropathy. This condition is commonly associated with reduced sensation in the feet and weakened foot muscles, which can lead to difficulty in walking. A major contributing factor is oxidative stress, which activates inflammatory and apoptotic pathways. These processes are linked to diminished levels of neurotrophic factors, disruptions in neurovascular function, neuronal demyelination, and heightened autoimmune responses (Ahmad et al., 2022; Filppula et al., 2021). Therefore, one approach to prevent the progression of diabetic neuropathy is to reduce oxidative stress and inflammation. The main strategy to prevent or delay the onset of diabetes and its complications, including neuropathy, is to keep strict glycemic control. The primary therapies for diabetic neuropathy include TCAs (e.g., amitriptyline), SNRIs (such as duloxetine and venlafaxine), and gabapentin, a calcium channel α2-δ ligand. Despite their use, only around one-third of patients experience partial symptom relief (De Vry et al., 2004; Kulkarni & Dhir, 2007; Nishikawa & Nomoto, 2017). Cholesterol may play a role in the cause of depression. Statins lower cholesterol levels and reduce the risk of cardiovascular issues. There is clinical evidence that statins also have neuroprotective benefits that aren’t fully explained by their effect on blood cholesterol. The effectiveness of traditional antidepressants may depend on serotonergic neurotransmission, which can be influenced by changes in cholesterol levels. Statins are widely used in managing dyslipidemia and are also recommended for individuals with CAD, diabetes, stroke, hypertension, or CKD, regardless of lipid levels. Their advantages extend beyond cholesterol reduction (Althanoon et al., 2020; Grover et al., 2014). They have several other positive effects, known as "pleiotropic effects," which include combating inflammation, acting as antioxidants, inhibiting cell proliferation, promoting

apoptosis, regulating the cell cycle, and modulating the immune response. Statins, originally derived from fungal sources, function by blocking the enzyme HMG-CoA reductase, a crucial component in the cholesterol synthesis process. They are categorized as either lipophilic or hydrophilic, both types exhibiting "pleiotropic effects." Lipophilic statins, such as LOVA, simvastatin, fluvastatin, atorvastatin, and pitavastatin, readily cross cell membranes and tend to have lower liver selectivity (Filppula et al., 2021; Klotz, 2003). Hydrophilic statins (rosuvastatin and pravastatin) cannot easily cross cell membranes and therefore require special transport. They are more selective for liver tissues. In many studies, statins were used as additional therapy. Adjuvants enhance the effectiveness of other medications. Evidence suggests statins boost immune reactions and germinal center development. LOVA acts as an effective adjuvant to strengthen the immune response to the H1N1 influenza vaccine. When combined with the vaccine, LOVA increases the production of H1N1-specific antibodies and stimulates T-cell activity and cytokine release. This indicates a strong mixed Th1/Th2 immune response. Furthermore, this evidence strongly suggests it helps enhance the overall immune system by developing the germinal center. While this drug is commonly known for lowering cholesterol, it may improve immune responses when used with vaccines and may also assist in treating certain mental health disorders. A 6-week randomized placebo-controlled clinical trial examined the effectiveness and safety of LOVA as an additional treatment alongside FLX for major depressive disorder (MDD). This study reported that LOVA, as an added treatment, may help patients with MDD when used with FLX (Ghanizadeh & Hedayati, 2013; Vital et al., 2025). Statins, including LOVA, serve as the main treatment for lowering LDL cholesterol and reducing cardiovascular risk. LOVA can also be combined with other medications such as niacin, fibrates, or intestinal cholesterol absorption inhibitors to improve lipid control, highlighting its role in managing dyslipidemia.

Statins, including LOVA, have shown potential benefits in treating neuropathic pain in preclinical models through cholesterol-independent mechanisms such as reducing inflammation, acting as antioxidants, and modulating nerve activity. These findings suggest that LOVA may help ease neuropathic symptoms when combined with standard treatments. However, clinical data present a different view, with some studies linking statin use to the development of neuropathy, which raises questions about their role. In research conducted LOVA at a dose of 20 mg/day was administered as an adjunct treatment for schizophrenia over eight weeks. The results

showed no significant differences in PANSS scores between the LOVA and placebo groups (Ghanizadeh et al., 2014; Ghanizadeh & Hedayati, 2013). Moreover, both groups tolerated the treatment well, with no major adverse effects observed.Numerous studies have reported that statins can cause neuropathy, indicating a paradox in their treatment of neuropathic pain. Statin use has been associated with a low risk of developing peripheral neuropathy, estimated at around 12 cases per 100,000 people annually, or approximately 6 in 10,000 patients—roughly 1 in every 10,000 treated for a year. Individuals on long-term statin therapy (typically over a year) may experience symptoms such as numbness, tingling, pain, tremors in the hands or feet, and difficulty maintaining balance while walking. Reports suggest that polyneuropathy occurs more often with atorvastatin than with fluvastatin. Importantly, the exact mechanism by which statins might cause neuropathy remains unclear. One theory, supported by various studies, suggests that because statins inhibit cholesterol synthesis, they may disrupt nerve membrane function.

Peripheral nerve injury caused by trauma, disease, or exposure to certain toxins can result in chronic neuropathic pain that often does not respond to conventional analgesics. Rat models simulating post-traumatic peripheral neuropathic pain have been instrumental in uncovering the underlying mechanisms of neuropathic pain and aiding the development of novel therapeutic options. Although these models are most directly related to post-traumatic neuropathies such as causalgia, they are also considered relevant for neuropathic conditions arising from diabetes or surgical procedures like SNL (Bennett et al., 2003). One major advantage of the SNL model is that the fourth lumbar dorsal root allows access to the intact afferent axons of the sciatic nerve.

SD, a PDE5 inhibitor, alleviates diabetes-induced pain by enhancing neurovascular function and markedly boosting the formation of functional blood vessels within the sciatic nerve (Pușcașu et al., 2023). It exhibits neuroprotective properties by stimulating neurotrophic factors that aid in neuron survival and regeneration. However, the involvement of the NO/cGMP signaling pathway in regulating cytokine activity and oxidative stress following nerve injury in autonomic neurons remains poorly understood (Garcia et al., 2014; Wang et al., 2015). The cGMP is synthesized by soluble guanylate cyclases within the cytoplasm and is specifically broken down by the enzyme PDE5. cGMP plays a key role in regulating vascular tone, axonal navigation, and synaptic adaptability. Notably, individuals with diabetic peripheral

neuropathy who receive SD for erectile dysfunction have reported improvements in neuropathic symptoms (Wang et al., 2015).

Patients with cardiovascular disease often take statins because of their cholesterol-independent benefits, including increased nitric oxide (NO) activity in blood vessels. Many of these patients also experience erectile dysfunction, which can be treated with SD, a selective phosphodiesterase type 5 inhibitor. Both SD and statins can activate the NO-cGMP pathway.

A recent study showed that FLX hydrochloride improves insulin resistance and glucose tolerance. Research has found that oral FLX can lower fasting blood glucose levels in individuals without diabetes. Additionally, a meta-analysis of five large clinical trials indicated that T2DM patients treated with FLX hydrochloride have significantly lower fasting blood glucose and triglyceride levels compared to those on a placebo. FLX hydrochloride may also increase insulin sensitivity. While the mechanisms behind FLX's effects on lowering blood glucose, promoting weight loss, and enhancing insulin resistance are not yet fully understood, these findings suggest that FLX might improve insulin resistance and treatment outcomes for non-depressed individuals with type 2 diabetes. Statins could potentially enhance the effects of antidepressants because literature reports provided evidence that LOVA can improve the antidepressant-like effects of a low dose of FLX in rats and may increase FLX's therapeutic impact on neuropathic pain. Due to side effects, limited effectiveness, and insufficient focus on nerve repair and regeneration, current treatment options for neuropathic pain are often inadequate. Only a few therapeutic approaches are available, and their effectiveness in managing neuropathic pain is generally low. Developing effective treatment options is complicated by the complex interactions between numerous pathophysiological systems involved in the onset and progression of neuropathic pain.

3.2. Rationale of the study

No research currently exists on LOVA as an adjunct to FLX and SD in reducing neuropathic pain in rats. The way these treatments work together to address neuropathic pain in rats induced by a high-fat diet, small doses of streptozotocin, and L5 spinal nerve ligation is unknown. This gap highlights the need to explore their potential to treat neuropathic pain in rats. Therefore, it is essential to create more effective treatment plans with fewer side effects. Drug combinations can also help avoid some negative effects that arise from high doses of a single medication.

CHAPTER 4
AIM AND OBJECTIVES

4.1. Aim of the work

Pharmacological impact of cholesterol-lowering drug as adjuncts to fluoxetine and sildenafil in attenuation of neuropathic pain in rats

4.2. Objectives

1. Induction of NP in experimental animals using

   a. High fat diet (HFD) and low dose of Streptozotocin induced NP

   b. Spinal nerve ligation (SNL) induced NP

2. Pharmacological investigation of co-administration of cholesterol-lowering drug as adjuncts to fluoxetine and Sildenafil in neuropathic rats

CHAPTER 5
EXPERIMENTAL WORK

5.1 Materials:

5.1.1. Chemicals

Table 12 provides a detailed list of the various chemicals utilized in the study.

TABLE 12. Index of chemicals

Chemicals	Manufacturer
Acetone	BB chemie Pvt. Ltd., India
Acetonitrile	Deejey corporation, Amrtisar, India
Bovine Serum Albumin (BSA)	Loba Life, Loba Chemie, Mumbai, Maharastra India
Copper Sulphate	Loba Life, Loba Chemie, Mumbai, Maharastra India
Disodium Hydrogen Sulphate	Loba Life, Loba Chemie, Mumbai, Maharastra India
Dimethyl Sulphoxide (DMSO)	Finar Pvt. Ltd., India
Fluoxetine (FLX)	Yarrow biochem. Pvt. Ltd., India
Glacial Acetic Acid	Deejey corporation, Amrtisar, India
Molondialdehyde (MDA)	Sigma Aldrich Pvt. Ltd., India
Lovastatin (LOVA)	Yarrow biochem. Pvt. Ltd., India
Pyridine	BB chemie Pvt. Ltd., India
Sildenafil (SD)	Yarrow biochem. Pvt. Ltd., India
Sodium Potasium tartrate	Loba Life, Loba Chemie, Mumbai, Maharastra India
Sodium Carbonate	Loba Life, Loba Chemie, Mumbai, Maharastra India
Sodium Hydroxide	Loba Life, Loba Chemie, Mumbai, Maharastra India
Sodium Citrate	Loba Life, Loba Chemie, Mumbai, Maharastra India
Standard GSH reagent	CDH Pvt. Ltd., New Delhi, India
Streptozotocin (STZ)	Anjan Chemical, Amritsar, Punjab, India
Sulfosalicylic Acid	CDH Pvt. Ltd., New Delhi, India
Tetramethoxypropane (TMP)	Sigma Aldrich Pvt. Ltd., India
Thiobarbituric Acid (TBA)	Finar Pvt. Ltd., India
Thiopental Sodium	Loba Life, Loba Chemie, Mumbai, India
Tetramethyl Benzamide	Lobachemie Pvt. Ltd., Mumbai, India
Trichloro Acetic Acid	CDH Pvt. Ltd., New Delhi, India
1,1,3,3 Tetramethoxy Propane	CDH Pvt. Ltd., New Delhi, India

5.1.2 Equipment and Apparatus

Table 13 provides a detailed list of the various equipment's and apparatus utilized in the study.

Table 13. Index of equipment and apparatus

Equipment	Model/Manufacturer
Cooling Centrifuge	M-1214, Remi, Vasai, Mumbai, India
Digital Weighing Balance	Wensar Weighing Scales Limited, Chennai, India
Eddy's hot plat	Inco Pvt. Ltd. India
ELISA	Perlong Medical Equipment, Chaina
FTIR-ATR Spectrophototometer	Infra 3000, Analytical Technologies Limited, India
High Performance Liquid Chromatography	LC-20AD Shimadzu Co. Ltd., Japan
Homogeniser	Remi Moter Division., Vasai
Hot air oven	Cadmach Drying Oven, Cadmach Machinery Ltd., (India)
Magnetic Stirrer	Remi 5mlh, Vasai, Mumbai, India
Mechanical Shaker	Neolab, Mumbai, India
Membrane filter	Pall Corporation, Mumbai, India
Micro centrifuge tubes	Sigma-Aldrich Ltd., Powai, Mumbai, India
Micropipette	Perfit, India
pH meter	Phan, Labindia, Thane West, Maharashtra, India
Refrigerator	Kelvinator International, (India)
Rota rad apparatus	Inco Pvt. Ltd. (India)
Tissue homogeniser	Remi Rq 127 A, Vasai, Mumbai, India
Ultra sonication bath	Loba Life, Loba Chemie, Mumbai, India
UV Spectrophotometer	UV-1800, Shimadzu Co. Ltd., Japan
Vacuum Filtration Pump	Millipore, India
Vortex Mixer	REMI Instrument Division, India

5. 2. Methodology

5.2.1. Characterstics of the active pharmaceutical ingredients (API)

5.2.1.1 Physical description of the drugs

The characterization of pharmaceutical compounds checked with a visual examination, where the sample is observed under normal and white light to assess its color, texture, and crystalline nature (Jendrzejewska et al., 2023). This is then compared with a reference standard for consistency. The odor test involves taking a small amount of the powdered drug and gently wafting it toward the nose without direct inhalation to determine its odor profile, which may be odorless, amine-like, or musty. To find the melting point, place 2-3 mg of the powdered sample in a capillary tube, insert it into a Melting Point Apparatus, and gradually increase the temperature. Record the temperature at which the sample completely melts and

compare it with standard values. For the solubility test, small samples (approximately 10 mg) of each drug are added to various solvents, including water, ethanol, methanol, and DMSO (Dimethyl Sulfoxide). The mixture is stirred well, and the solubility or precipitation behaviour is observed and documented to establish the solubility profile of each compound.

5.2.1.2. FTIR analysis

FTIR, performed to identify the functional groups and molecular vibrations characteristic of SD, FLX, and LOVA. Each compound in its solid form was finely ground using an agate mortar to achieve uniform particle size and consistency. For sample preparation, approximately 1–2% of each drug (by weight) was mixed with spectroscopic-grade potassium bromide (KBr) and pressed into transparent pellets using a hydraulic press. The FTIR spectra were then collected using an FTIR spectrometer, scanning in the range of 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1} and 16 scans per sample to ensure accurate and high-quality spectral data (Kuthati et al., 2019). Before sample measurement, a background scan was conducted using a blank KBr pellet to eliminate environmental interferences such as atmospheric moisture and CO_2. The obtained spectra were subjected to background subtraction and baseline correction for clearer visualization of absorption peaks. The functional group regions and fingerprint regions were analyzed for characteristic bands associated with SD, FLX, and LOVA, including peaks indicative of amine, aromatic, ester, and hydroxyl functional groups. All experimental conditions and spectral observations were documented for further interpretation and validation.

5.3. Pharmacological Evaluation of NP

5.3.1. Experimental animals

For the study, Sprague Dawley (male/250-300g) rats, were selected. They were procured from National Institute of Pharmaceutical Education and Research (NIPER), Mohali, India and housed in Central Animal House, Lovely Professional University, Phagwara, Punjab, India. Maintain light and dark monotonous (12–12 h), fed over commercially Feed and water were provided freely without restriction. The animals were kept in cages at a temperature 25 ± 2°C and relative humidity 55–65% This study received approval and authorization from the Institutional Animal Ethics Committee (**LPU/IAEC/2021/79**) Lovely Institute of Technology (Pharmacy), Punjab.

5.4. Effect of drug treatment on diabetes-induced NP using a HFD and low dose STZ in rats

This study utilized a model combining a HFD and STZ to assess the effects of various drugs on diabetic neuropathy. On the first day of the experiment, all groups, except the normal control, were provided with HFD, while the control group received a standard diet. A 14-day period on HFD induced metabolic changes, including insulin resistance and prediabetic conditions (Figure 14).

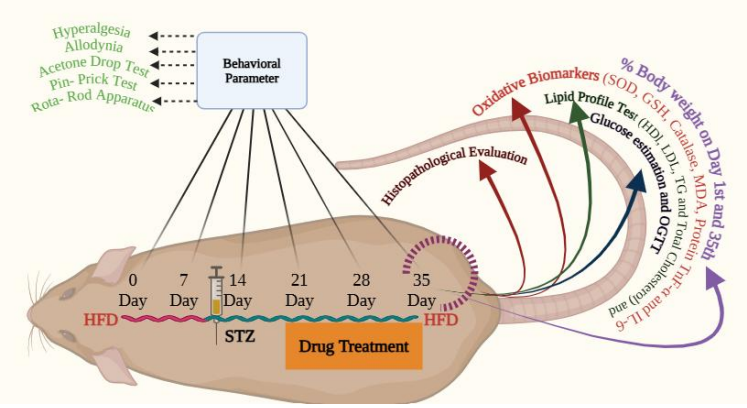


Figure 14. Design for HFD+STZ Induced NP Model

On the 14th day, diabetes was induced by administering STZ (35 mg/kg, i.p.) freshly prepared in citrate buffer (pH 4.4). Progressive hyperglycemia and NP were monitored through behavioral tests, assessing symptoms such as Thermosensitiveand mechanical hyperalgesia. Throughout the study, animals continued to receive pellet feed and plain water. The detailed mixture of the HFD is presented in Table 14.

Table 14: Dietary composition of HFD (Ahlawat & Sharma, 2018)

Constituents	Diet (g/kg)
Powdered NPD	365
Lard	310
Casein	250
Cholesterol	10
Vitamin and mineral mix	60
DL-Methionine	03
Yeast powder	01
Sodium chloride	01

On the 21st day, drug treatment commenced and persisted until the 35th day. The study comprised 14 groups (n=8 per group), each subjected to distinct pharmacological interventions. The normal control group (Group I) received no treatment, while the diabetic

control group (Group II) was subjected to HFD and STZ without any drug intervention (Table 15).

Table 15. Experimental protocol: Diabetes-induced NP using a HFD and low dose STZ in rats (Ahlawat & Sharma, 2018; Mohan et al., 2017)

Group (n=8)	Treatment	Dose
I	Normal Control	Without treatment
II	Experimental Control (NP)	HFD+STZ (35 mg/kg)
III	Pregabalin (PB) + NP	30 mg//Kg
IV	LOVA (LOV^{L}) + NP	2 mg/Kg
V	LOVA (LOV^{H}) + NP	4 mg/Kg
V I	FLX (FLX^{L}) + NP	5mg/Kg
VII	FLX (FLX^{H}) + NP	10 mg/Kg
VIII	SD (SD^{L}) + NP	10 mg/Kg
IX	SD (SD^{H}) + NP	20 mg/Kg
X	LOVA (LOV^{L}) + FLX (FLX^{L}) + NP	2 mg/Kg + 5mg/Kg
XI	LOVA (LOV^{L}) + FLX (FLX^{H}) + NP	2 mg/Kg + 10mg/Kg
XII	LOVA (LOV^{L})+ SD (SD^{L}) + NP	2 mg/Kg+10 mg/Kg
XIII	LOVA (LOV^{L})+ SD (SD^{H}) + NP	2 mg/Kg+20mg/Kg
XIV	LOVA (LOV^{L}) + FLX (FLX^{L})+ SD (SD^{H}) + NP	2 mg/Kg+ 5mg/Kg+ 10 mg/Kg

Pregabalin (30 mg/kg) was administered to Group III as a reference treatment for NP. SD was assessed in Groups VIII and IX at doses of 10 mg/kg and 20 mg/kg, respectively. FLX was administered at 5 mg/kg and 10 mg/kg in Groups VI and VII, respectively. LOVA was evaluated in Groups IV and V at doses of 2 mg/kg and 4 mg/kg, respectively. Combination therapy was examined in Groups X through XIV. Specifically, Groups X and XI received SD (10 mg/kg) with FLX (5 mg/kg or 10 mg/kg), while Groups XII and XIII were given SD (10 mg/kg or 20 mg/kg) with LOVA (2 mg/kg). Group XIV received a combination of SD (10 mg/kg), FLX (5 mg/kg), and LOVA (2 mg/kg). Behavioural evaluations were performed at the end of the treatment phase to assess neuropathic pain parameters. These pharmaceutical drugs' neuroprotective and metabolic effects were examined by biochemical and histological investigations. In order to mitigate the metabolic and neurological consequences linked to diabetes, this study intends to shed light on the therapeutic potential of these medications in diabetic neuropathy.

5.4.1. Determination of physiological weight/Body weight

The percentage change in the initial Physiological weight of the animal was measured on day 0 and on the 35th day according to the specified formula (Ahlawat & Sharma, 2018).

$$\frac{\text{Physiological weight (day 35-day 1)}}{\text{Physiological weight 1st day}} \times 100$$

5.4.2. Biochemical tests

5.4.2.1. Measurement of Blood Glucose Level

Blood glucose levels were measured using a "Dr. Morepen Gluco One" glucometer to monitor changes before and after STZ induction. The device uses enzymatic electrochemical technology, where glucose-specific enzymes react with the blood sample to produce an electric current proportional to glucose concentration. This current is converted into a digital reading, providing a quick and accurate method for blood glucose estimation.

5.4.2.2. Oral Glucose Tolerance Test (OGTT)

This test was assessed the tolerance for oral glucose levels. The rats fasted for 12-14 hours prior to the test. Initially, a baseline measurement was recorded. Following that, a dose of 3g/kg of glucose was administered to the rats (6 rats per group) via oral gavage. Blood glucose levels were then collected and measured using a glucometer at intervals of 30, 60, 90, and 120 minutes post-administration (Rubino et al., 2006).

5.4.2.3. Lipid Profile Tests

In the HFD or STZ model, lipid concentration levels need to be checked. On the 35th day, blood samples are collected from the retro-orbital sinus using capillary tubes under light ether anesthesia. The blood is collected in Eppendorf tubes and performed centrifugaton at 3000g for 20 minutes. The serum is separated and used to determine the levels of LDL, HDL, triglycerides (TG), and total cholesterol (Ahlawat & Sharma, 2018; Rifai & Warnick, 2006).

5.4.3. Assessment of drug treatment on behavioural parameters

5.4.3.1 Thermosensitive test: heat hyperalgesia and heat allodynia test

To induce hyperalgesia, rats were placed on Eddy's hot plate, and the response time was recorded in seconds by noting when they licked their hind paw. The plate temperature was kept at 52.5 ± 0.5°C, with a maximum exposure time of 20 seconds. This assessment was carried out on days 0, 7, 21, 28, and 35. For evaluating allodynia, the temperature was adjusted to 45 ± 0.5°C, with a cut-off time of 30 seconds. The sensitivity to non-painful stimuli was measured by observing the withdrawal reflex of the left hind paw on the same days (Ahlawat & Sharma, 2018; Melkani, 2019).

5.4.3.2 Cold test: Tail cold hyperalgesia test

To assess cold sensation using cold hyperalgesia, approximately 1cm of the tail was emersed in cold water (0-4°C). The withdrawal threshold was then measured, with a cut-off time of 20 seconds on the 0, 7, 21, 28 and 35th day (M. Kaur et al., 2017). The tail cold hyperalgesia test is a vital tool for evaluating NP, offering a reliable measure of cold-induced pain sensitivity. It helps in understanding pain mechanisms and assessing the efficacy of therapeutic agents. This test is instrumental in preclinical research, aiding in the development of effective treatments for NP.

5.4.3.3 Paw cold sensitivity (allodynia): Acetone drop test

Evaluating the licking withdrawal response after the cold sensitivity test is crucial for understanding the onset and progression of cold-induced pain in experimental models. The test involves applying 100 µL of acetone to the plantar surface of the rat's hind paw on days 0, 7, 21, 28, and 35, with a 20-second cut-off to avoid tissue harm (Ahlawat & Sharma, 2018).

5.4.3.4 Mechanical hyperalgesia test: Pin-prick test

The mechanism of hyperalgesia was assessed by noting the withdrawal response of rats when a bent gauge needle, positioned at a 90° angle to the syringe was applied without penetrating the skin on the 0, 7, 21, 28 and 35th day. The cut-off time for this evaluation was 20 seconds.

5.4.3.5 Grip muscles test using rota rod apparatus

Muscle strength was checked by measuring the fall off time in a rota-rod apparatus. The apparatus was set to rotate at 25 rpm for a duration of one minute on the 0, 7, 21, 28 and 35th day (Ahlawat & Sharma, 2018; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019).

5.5.4. Tissue preparation: Dissection and homogenization

On the 35th day, this test was conducted after euthanized and subsequently homogenized the sciatic nerve and adjacent portion. The supernatant was obtained from the homogenized mixture by preparing a 10% w/v tissue homogenate in 0.1 M phosphate buffer (pH 7.4). The homogenization was carried out using a Remi Motors homogenizer (India), followed by centrifugation (Sigma, UK), at 10,000g for 15 minutes at 4°C.

5.4.4.1. Estimation of biochemical and oxidative biomarker

5.4.4.1.1. Estimation of total protein content

Protein concentrations were determined using the Lowry method (1951), with bovine serum albumin (BSA) serving as the standard. Absorbance readings were taken at 750 nm using a

spectrophotometer (H. Kaur et al., 2014; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019).

5.4.4.1.2. Estimation of myeloperoxidase (MPO) activity

The estimation was conducted following the method described by Grisham (1994). The absorbance was recorded at 460 nm using a spectrophotometer, and the results were expressed as units of myeloperoxidase activity per milligram of protein per minute (H. Kaur et al., 2014; Melkani et al., 2019; Muthuraman et al., 2011). Assessing MPO activity is crucial in NP studies as it provides insights into the extent of oxidative stress and inflammation at the site of nerve injury. Elevated MPO levels indicate enhanced immune cell activity and the production of reactive oxygen species, which contribute to nerve sensitization and pain progression. Monitoring MPO activity helps evaluate the severity of neuropathic conditions and assess the effectiveness of therapeutic interventions targeting inflammation and oxidative damage.

5.4.4.1.3. Estimation of reduced glutathione (GSH)

A 0.01 mL portion of the supernatant was mixed with 2 mL of phosphate buffer (pH 8.4). Then, 0.5 mL of 5,5′-dithiobis(2-nitrobenzoic acid) and 0.4 mL of double-distilled water were added. The solution was vortexed and analyzed under UV light at 412 nm, with the reaction reaching completion within 15 minutes. The reduced GSH concentration was determined and expressed as μg/mg of protein (Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019; Muthuraman et al., 2011)

5.4.4.1.4. Estimation of Catalase (CAT) activity

The degradation of hydrogen peroxide (H_2O_2) was measured in micromoles every 30 seconds for a duration of 2 minutes. This was done by mixing 3ml of H_2O_2 with phosphate buffer and 0.05ml of tissue supernatant. The mixture was then measured using a spectrophotometer at 240 nm (Choinski & Patterson, 1993; Dworkin et al., 2010; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019; Muthuraman et al., 2011).

5.4.4.1.5. Superoxide dismutase (SOD)

The assay system included 0.1 mM EDTA, 50 mM sodium carbonate, and 96 mM nitro blue tetrazolium (Kumar et al., 2025; Tiwari et al., 2010). In a cuvette, 2 mL of this prepared solution was combined with 0.05 mL of hydroxylamine and 0.05 mL of the supernatant. The auto-oxidation of hydroxylamine was monitored over a duration of 2 minutes at 30-second intervals by recording absorbance at 560 nm using a spectrophotometer (Himedia

Laboratories, Mumbai, India) (Ahlawat & Sharma, 2018; Kumar et al., 2025; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019).

5.4.4.1.6. Estimation of TNF-α, IL-6 and BDNF activity

Tissue homogenates were centrifuged at 10,000g for 10 minutes at 14°C. The supernatant obtained was vortexed, subjected to a second round of centrifugation, and the final supernatant was aliquoted. TNF-α levels were measured using a Rat TNF-α ELISA kit.(E-EL-R2856). Similarly, IL-6 and caspase concentrations were assessed following the protocols outlined by the manufacturer, utilizing the Rat IL-6 ELISA Kit (E-EL-R0015) and BDNF ELISA Kit (E-EL-R1235) from ESSENCE LIFE SCIENCES, Chandigarh (Ahlawat & Sharma, 2018; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019; Muthuraman et al., 2011)

5.4.5. Histopathological studies

At the conclusion of the experiment, sciatic nerve tissues were collected and preserved in 10% formalin. Sections were stained using hematoxylin and eosin and examined under a light microscope at 450× magnification to assess axonal degeneration (Ahlawat & Sharma, 2018; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019).

5.5. Effect of drug treatment on spinal nerve ligation (SNL) induced NP in rats

A surgery-induced animal model can be developed by ligating the L5/L6 spinal nerves under chloral hydrate anesthesia (35 mg/kg, intraperitoneally).

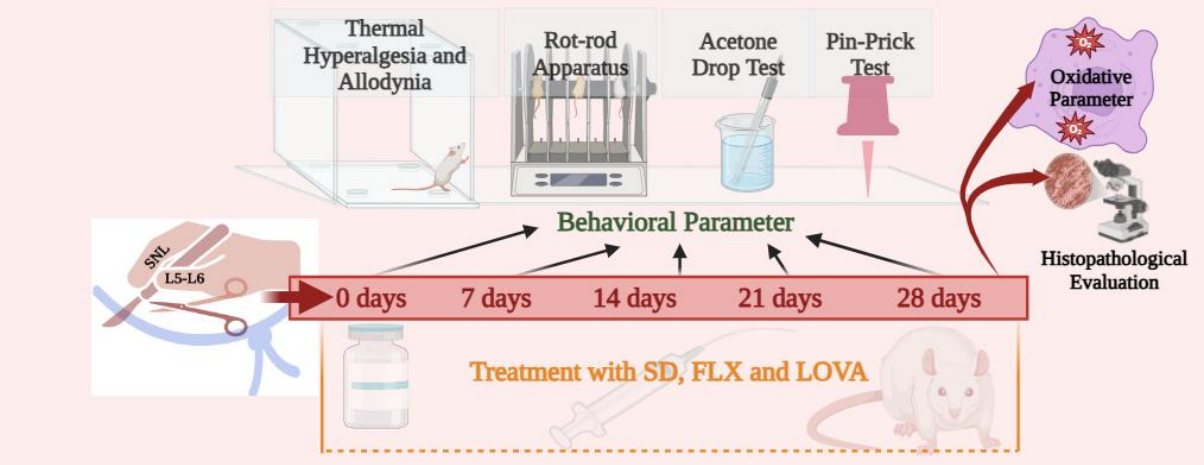


Figure 15. Design for SNL Induced NP Model

The nerve was carefully exposed through surgical procedures and firmly ligated using a 6-0 silk suture, placed between the dorsal root ganglion and the proximal region of the sciatic nerve (Figure 15). The study included treatment groups, each consisting of six animals, with drug administration performed as outlined in Table 16.

Table 16. Experimental Protocol: spinal nerve ligation (SNL) induced NP in rats

Group (n=6)	Treatment	Dose
I	Normal Control	Without treatment
II	Experimental Control (SNL)	L5/L6 spinal nerves were ligated
III	Sham Control	Surgery performed; vehicle administered
IV	Pregabalin (PB) + SNL	30mg/Kg
V	LOVA (LOV^L) + SNL	2mg/Kg
VI	LOVA (LOV^H) + SNL	4 mg/Kg
VII	FLX (FLX^L) + SNL	5mg/Kg
VIII	FLX (FLX^H) + SNL	10mg/Kg
IX	SD (SD^L) + SNL	10mg/Kg
X	SD (SD^H) + SNL	20mg/Kg
XI	LOVA (LOV^L) + FLX (FLX^L) + SNL	2mg/Kg + 5mg/Kg
XII	LOVA (LOV^L) + FLX (FLX^H) + SNL	2mg/Kg + 10mg/Kg
XIII	LOVA (LOV^L)+ SD (SD^L) + SNL	2 mg/Kg+10 mg/Kg
XIV	LOVA (LOV^L)+ SD (SD^H) + SNL	2 mg/Kg20mg/Kg
XV	LOVA (LOV^L) + FLX (FLX^L)+ SD (SD^L) + SNL	2 mg/Kg+ 5mg/Kg+ 10 mg/Kg

In this method, drugs were administered "daily at 10:00AM for 28 consecutive days", beginning on day 0 after SNL induction. Behavioral evaluations were conducted 30 minutes post-dosing on days 0, 7, 14, 21, and 28, following established procedures (Pottabathini et al., 2016),

5.5.1. Measurement of Physiological weight

5.5.1.1. Determination of Physiological weight

The percentage change in the initial Physiological weight of the animal was measured on day 0 and on the 35th day according to the specified formula.

$$\frac{\textbf{Physiological weight (day 35-day 1)}}{\textbf{Physiological weight 1}^{\textbf{st}}\textbf{ day}} \times \textbf{100}$$

5.5.2. Assessment of drug treatment on behavioural parameters

5.5.2.1 Thermosensitive test: heat hyperalgesia and heat allodynia test

For the induction of hyperalgesia (painful stimuli), rats were placed on Eddy's hot plate, and the nociceptive threshold was recorded in seconds by observing the licking of the hind paw. The temperature was maintained at 52.5 ± 0.5ºC with a cut-off time of 20 seconds for this evaluation on the 0, 7, 21 and 28th day. Similarly, for the assessment of allodynia (non-painful stimuli), the temperature was set at 45±0.5ºC with a cut-off time of 30 seconds. This evaluation measured the nociceptive threshold by observing the withdrawal response of the rats' left hind paw on the 0, 7, 21 and 28th day. (Ahlawat & Sharma, 2018; Melkani, 2019).

5.5.3.2. Cold test: Tail cold hyperalgesia test

To evaluate cold hyperalgesia, about 1 cm of the rat's tail was submerged in cold water maintained at 0-4°C. The withdrawal threshold was recorded, with a maximum exposure limit of 20 seconds. Measurements were conducted on days 0, 7, 21, and 28 following SNL induction (Ahlawat & Sharma, 2018; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019).

5.5.3.3. Paw cold-allodynia Test: Acetone drop test

Acetone Drop Test: Following the cold sensitivity assessment, the licking withdrawal response was evaluated by applying 100 µl of acetone to the plantar surface of the rat's hind paw. The cut-off time for this test was set at 20 seconds. Measurements were conducted on days 0, 7, 21, and 28 after SNL induction (Ahlawat & Sharma, 2018).

5.5.3.4 Grip muscles test using rota rod apparatus

Muscle strength was evaluated by recording the time taken for rats to fall from a rotating rod set at 25 rpm. Each trial lasted up to one minute. Assessments were conducted on days 0, 7, 21, and 28 following SNL induction (Ahlawat & Sharma, 2018; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019).

5.5.3.5 Mechanical hyperalgesia test: Pin-prick test

The mechanism of hyperalgesia was examined by observing the withdrawal response when a bent gauge needle, angled at 90° to the syringe, was gently applied to the skin without penetration. The cut-off time for this assessment was 20 seconds, with measurements taken on days 0, 7, 21, and 28 following SNL induction. (Ahlawat & Sharma, 2018; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019).

5.5.4. Tissue preparation: Dissection and homogenization

Following the completion of behavioral assessments, the animals were euthanized on day 28, and the spinal cord was promptly extracted from the L5-L6 region. For biochemical evaluations, a 10% (w/v) tissue homogenate was prepared using 0.1 M phosphate buffer (pH 7.4) with a Remi Motors homogenizer (India). The homogenate was then centrifuged at 10,000 g for 15 minutes at 4°C using a Sigma centrifuge (UK), and the resulting supernatant was collected for subsequent biochemical analyses (Dworkin et al., 2010; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019; Pottabathini et al., 2016).

5.5.4.1. Estimation of biochemical and oxidative biomarker

5.5.4.1.1. Estimation of total protein content

Protein concentration were be estimated according to the method of Lowry 1951, using Bovine serum albumin (BSA) as a standard. The absorbance were be determined spectrophotometrically at 750 nm (H. Kaur et al., 2014; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019).

5.5.4.1.2 Estimation of myeloperoxidase (MPO) activity

It were be estimated as per Grisham 1994. The absorbance were recorded spectrophotometrically at 460 nm. The results were be expressed as myeloperoxidase activity units per milligram of protein at one minute (H. Kaur et al., 2014; Muthuraman et al., 2011). Assessing MPO activity is crucial in NP studies as it provides insights into the extent of oxidative stress and inflammation at the site of nerve injury. Elevated MPO levels indicate enhanced immune cell activity and the production of reactive oxygen species, which contribute to nerve sensitization and pain progression. Monitoring MPO activity helps evaluate the severity of neuropathic conditions and assess the effectiveness of therapeutic interventions targeting inflammation and oxidative damage.

5.5.4.1.3 Estimation of reduced glutathione (GSH)

A 0.01 mL portion of the supernatant was mixed with 2 mL of phosphate buffer (pH 8.4). Then, 0.5 mL of 5,5′-dithiobis (2-nitrobenzoic acid) and 0.4 mL of double-distilled water were added. The mixture was vortexed and observed under UV light at 412nm, with the reaction completed within 15 minutes. The reduced GSH concentration was expressed in µg/mg of protein (Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019; Muthuraman et al., 2011)

5.5.4.1.4 Estimation of Catalase (CAT) enzyme activity

The degradation of hydrogen peroxide (H_2O_2) was measured in micromoles every 30 seconds for a duration of 2 minutes. This was done by mixing 3ml of H_2O_2 with phosphate buffer and 0.05ml of tissue supernatant. The mixture was then measured using a spectrophotometer at 240 nm (Choinski & Patterson, 1993; Dworkin et al., 2010; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019; Muthuraman et al., 2011).

5.5.4.1.5 Superoxide dismutase (SOD)

This test solution contains 0.1 mM EDTA, 50 mM sodium carbonate and 96 mM nitro blue tetrazolium. 2 mL of the above mixture, 0.05 mL of hydroxylamine and 0.05 mL of supernatant are added to the cuvette and the initial oxidation of hydroxylamine is measured at 30-second intervals for 2 minutes using a spectrophotometer with an absorbance of 560 nm.

(Himedia Laboratories, Mumbai, India) (Ahlawat & Sharma, 2018; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019)

5.5.4.1.6. Assessment of TNF-α, IL-6 and BDNF activity

Samples were homogenized and than kept into in centifugation appartaus at 10,000 g for 10 min at 14°C. The supernatant was collected, vortexed, centrifuged again and the resulting supernatant was aliquoted. TNF-α concentrations were measured using mouse TNF-α ELISA kit (E-EL-R2856). Similarly, IL-6 and caspase levels were estimated using mouse IL-6 ELISA kit (E-EL-R0015) and BDNF ELISA kit (E-EL-R1235) from ESSENCE LIFE SCIENCES, Chandigarh as per the manufacturer's instructions (Ahlawat & Sharma, 2018; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019; Muthuraman et al., 2011).

5.5.5. Histopathological studies

At the end of the experiment, the sciatic nerve was meticulously collected and fixed in 10% formalin for preservation. Staining with hematoxylin and eosin and examination of axonal degeneration under a light microscope (450X) (Ahlawat & Sharma, 2018; Melkani, Kumar, Panchal, Singh, Singh, Gulati, Gill, Jyoti, Pandey, Kumar, et al., 2019).

5.5.6. Statistical Analsis

The findings are presented as “mean±standard error of the mean (SEM)”. Behavioral data were evaluated using two-way ANOVA, followed by Bonferroni post hoc analysis for multiple comparisons. Biochemical, oxidative, and inflammatory parameters were evaluated through one-way ANOVA, with Bonferroni post hoc tests applied for further analysis.

CHAPTER 6
RESULTS

6. 1. Characteristics of the SD, FLX and LOVA

6.1.1. Physical description of the drugs

In the present investigation all the drugs were analysed for their physical characteristics and the details of their nature are elaborated below in Table17.

Table 17. Result for the SD, LOVA and FLX

Property	SD	FLX	LOVA
Chemical Formula	$C_{22}H_{30}N_6O_4S$ (Jendrzejewska et al.,2023)	$C_{17}H_{18}F_3NO$ (Drzewicz et al., 2019)	$C_{24}H_{36}O_5$ (Mulder et al., 2015)
Molecular Weight	474.6 g/mol	309.3 g/mol	404.6 g/mol
Appearance	Crystalline powder with the color white and off-white (KM & Chioma, 2023)	White to off-white (crystalline powder) (Okon, 2024)	White to off-white powder (Lakshmi & Abbulu, 2019)
Solubility	Soluble in DMSO, slightly soluble in water (Keizers et al., 2016)	Slightly water soluble freely ethanol soluble (Risley & Bopp, 1990)	Insoluble in water, soluble in methanol, DMSO and ethanol (Nti-Gyabaah et al., 2008)
Melting Point	187-189°C (Dale et al., 2000)	179-182°C (Yellamula et al., 2014)	174-176°C (Ugaonkar, 2008)
Odour	Odorless or slightly medicinal (Saputra et al., 2024)	Odorless to slightly amine-like (Nagabhusan, 2011)	Mild characteristic odor, slightly fungal or musty (Fergany et al., 2022)

6.2. FTIR analysis

The quality of the sample has been thoroughly examined, and no quality issues were detected. The results obtained from the tests indicate that the sample has successfully met the required standards, and there is no interaction of the drugs' sample was observed (Figure 16).

6.3. Determination of body weight

The Experimental Control (NP) group shows the highest body weight increase after the HFD+ low-dose of the STZ model in rats. The study analyzed the effects of various experimental conditions on the measured response values. The results, presented as mean ± SEM, provide insights into the performance under different treatments. The baseline (NORMAL) condition yielded a value of 4.69 ± 0.076, serving as a reference point for comparisons. Under the NP group, a notable increase was observed with a value of 15.98 ± 0.098, indicating the impact of introducing the base experimental condition. Further enhancements were examined by incorporating LOVA, SD, and FLX treatments, either individually or in combination (Figure 17).

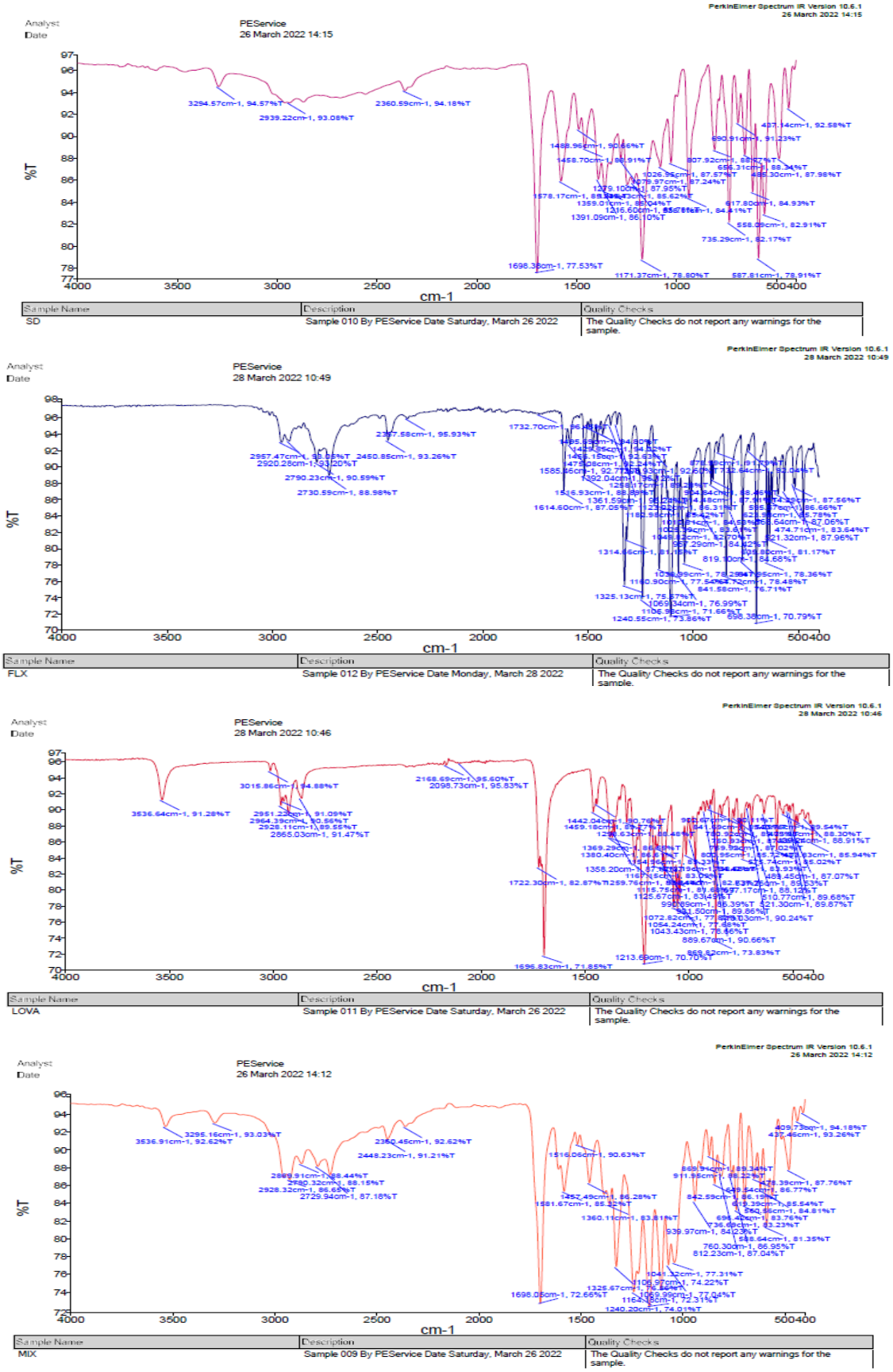


Figure 16. FTIR gram of sample A: SD labelled B: FLX Labelled C: LOVA Labelled D: Mix compound (SD, FLX and LOVA)

Among the single-treatment setups, the LOVA (L)+NP condition showed a value of 12.93 ± 0.11, while LOVA (H)+NP showed a 12.03 ± 0.12 percentage increase in body weight, suggesting a slight improvement with the administration of high doses of LOVA. The combination treatments exhibited varying effects in the improvement of body weight.

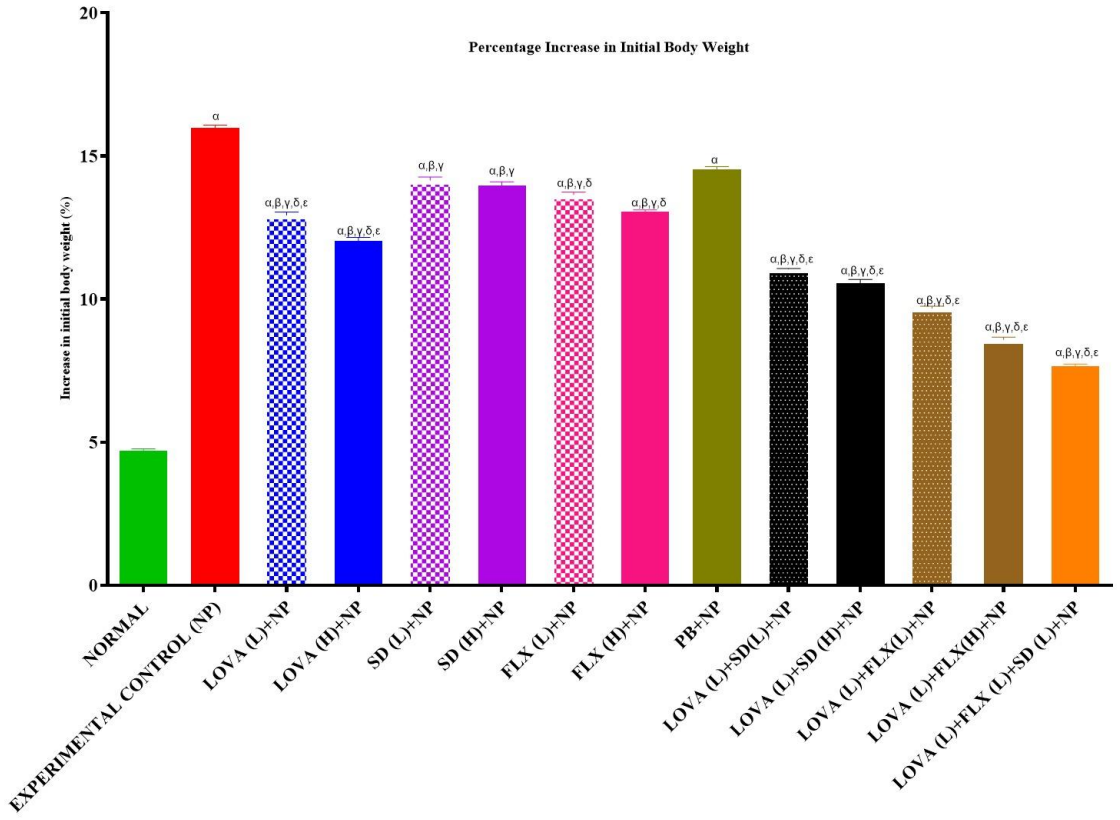


Figure 17. Body weight (% increase in starting weight): Effect of treatement on body weight in a HFD+STZ induced NP model in rats; The statistics presented as mean and standard error of mean (SEM), α=p<0.05 v/s normal control, β=p<0.05v/s Experimental control.The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

The group of rats that received LOVA (L)+SD (H)+NP achieved a value of 10.56 ± 0.12, demonstrating a more significant reduction compared to individual treatment of SD or LOVA in their respective group of rats. Similarly, the combination LOVA (L)+FLX (L)+SD (L)+NP recorded the lowest value of 7.65 ± 0.079, indicating the best outcome among all tested conditions. This combination showed significant protection against the rise in body weight. The superior performance of the LOVA (L)+FLX (L)+SD (L)+NP setup can be attributed to the synergistic effect of LOVA and FLX in combination with SD, enhancing the response mechanism and stabilizing the system. The earlier published scientific reports also showed that the combination of LOVA increases the antidepressant potential of FLX. It is well established that statins act on cholesterol biosynthesis, which results in a decrease in the level

of cholesterol. This effect of statins produces a pharmacological impact in the reduction of the risk of cardiovascular diseases and related comorbidities like obesity. Altered cholesterol is also responsible for an increase in antidepressant response. This result highlights the importance of integrating multi-treatment strategies for optimal outcomes. In summary, the study demonstrates that the combined application of LOVA (L)+FLX (L)+SD (L)+NP presents the most effective mechanism, yielding the best reduction with a value of 7.65 ± 0.079 units, outperforming both single and other multi-treatment setups. Hence, this altered cholesterol level can affect the pathway of the serotonergic system for neurotransmission, which might be responsible for the clinical efficacy of standard antidepressants.

6.4. Effect of blood glucose

The effect of SD, FLX, and LOVA, individually and in coadministration, on blood glucose levels was evaluated for 35 days. Initial measurements (Day 0) revealed blood glucose levels within the normal range for all groups, including the control (98.99 ± 0.3 mg/dL) and experimental groups (100 ± 1.32 mg/dL), with no significant differences.

Table 18. Effect of SD, FLX, and LOVA, and/or in combination, on glucose levels

S.N	Parameter	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
1	Normal	98.99 ± 0.3	97.56 ± 2.0	103 ± 1.1	104 ± 0.9	99.96 ± 2.09	102 ± 3.1
2	Experimental Control (NP)	100 ± 1.32	113 ± 0.87	130 ± 0.68	383 ± 1.32	400 ± 0.98	421 ± 1.44$^{\alpha\beta}$
3	LOV A (L)+NP	98.65 ± 0.19	115 ± 0.87	130 ± 0.68	385 ± 1.32	395 ± 0.87	400 ± 0.68$^{\alpha\beta}$
4	LOV A (H)+NP	101 ± 0.6	111 ± 0.8	132 ± 1.12	380 ± 0.5	389 ± 0.88	394 ± 0.88$^{\alpha\beta}$
5	SD (L)+NP	99.6 ± 0.7	111 ± 1.1	133 ± 1.21	375 ± 0.87	385 ± 0.98	393 ± 0.88$^{\alpha\beta}$
6	SD (H)+NP	100 ± 0.48	112 ± 0.99	129 ± 0.89	372 ± 0.65	385 ± 0.42	390 ± 0.98$^{\alpha\beta}$
7	FLX (L)+NP	100 ± 1.21	114 ± 0.98	130 ± 1.03	385 ± 0.87	395 ± 1.12	387 ± 1.11$^{\alpha\beta}$
8	FLX (H)+NP	98 ± 0.43	114 ± 0.85	130 ± 1.21	379 ± 1.11	380 ± 0.99	384 ± 0.99$^{\alpha\beta}$
9	PB + NP	99.4 ± 0.88	115 ± 1.32	134 ± 1.03	366 ± 0.98	382 ± 0.88	387 ± 0.88$^{\alpha\beta}$
10	LOV A (L)+SD (L)+NP	98.65 ± 0.19	115 ± 0.87	128 ± 1.32	382 ± 0.87	386 ± 0.68	386 ± 0.68$^{\alpha\beta}$
11	LOVA (L)+SD(H) +NP	104.3 ± 0.87	114 ± 0.68	130 ± 0.87	381 ± 0.87	382 ± 0.68	384 ± 1.32$^{\alpha\beta}$
12	LOV A (L)+FLX (L)+NP	101.6 ± 0.87	114 ± 0.68	130 ± 0.87	381 ± 0.87	384 ± 0.68	384 ± 0.68$^{\alpha\beta}$
13	LOV A (L)+FLX (H)+NP	104.3 ± 0.87	114 ± 0.89	131 ± 0.99	370 ± 0.54	380 ± 0.89	384 ± 1.32$^{\alpha\beta}$
14	LOV A (L)+FLX (L)+SD (L)+NP	102 ± 0.99	115 ± 1.21	134 ± 1.1	366 ± 0.54	378 ± 1.23	376 ± 1.23$^{\alpha\beta}$

The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

From Day 7 onward, a marked increase in glucose levels was observed across all the groups, and most prominently, its enhancement was recorded in the group of rats receiving HFD, i.e., EC groups, indicating the onset of hyperglycemia. In the EC group, glucose levels exhibited sustained elevation, reaching 421 ± 1.44 mg/dL by Day 35, indicating uncontrolled hyperglycemia. Among treatment groups, varying degrees of efficacy were observed. The group of rats that received LOVA (L)+NP showed a level of glucose 400 ± 0.68 mg/dL, SD (H) +NP-treated rats achieved a moderate reduction to 390 ± 0.98 mg/dL, and FLX (H)+NP demonstrated better regulation at 384 ± 0.99 mg/dL by Day 35. These drugs were not able to control the level of glucose till 35 days. But the group of rats received combination therapy LOVA (L) + FLX (L) + SD (L) + NP demonstrated the most effective glucose control, with blood glucose levels reduced to 376 ± 1.23 mg/dL by Day 35, compared to 421 ± 1.44 mg/dL in the NP group.

6.5. Effect on Oral Glucose Tolerance Test (OGTT)

The HFD + low-dose STZ-induced model demonstrated a significant increase in blood glucose levels following oral administration of 3 g/kg glucose, with measurements taken at 0, 30, 60, 90, and 120 minutes across all groups (Table 19). The drugs (single as well as combinations) did not show any significant effect in the blood glucose level.

Table 19. OGTT estimation on NP induced Model

S.No.	Parameter	0min	30min	60min	90min	120min
1.	Normal	83.67 ± 0.078	89.79± 0.07	94.91 ± 0.07	90.67 ± 0.08	85.87 ± 0.11
2.	Experimental Control (NP)	282.52 ± 0.04	387.78 ± 0.05	488.32 ± 0.06	394.23 ± 0.05	385.27 ± 0.04
3.	LOV A (L)+NP	282.23 ± 0.09	358.98 ± 0.07	454.23 ± 0.08	333.65 ± 0.05	321.44 ± 0.08
4.	LOV A (H)+NP	282.04 ± 0.09	344.87 ± 0.07	455.34 ± 0.06	314.27 ± 0.06	309.18 ± 0.06
5.	SD (L)+NP	283.98 ± 0.08	342.76 ± 0.08	429.28 ± 0.04	387.26 ± 0.05	382.14 ± 0.06
6.	SD (H)+NP	282.78 ± 0.04	336.87 ± 0.05	406.32 ± 0.04	375.25 ± 0.04	373.12 ± 0.05
7.	FLX (L)+NP	282.27 ± 0.13	308.89 ± 0.08	404.31 ± 0.08	399.35 ± 0.04	392.12 ± 0.98
8.	FLX (H)+NP	281.16 ± 0.05	392.87 ± 0.06	400.23 ± 0.04	396.21 ± 0.05	390.45 ± 0.03
9.	PB + NP	284.35 ± 0.07	339.64 ± 0.05	442.19 ± 0.08	308.18 ± 0.07	300.21 ± 0.05
10.	LOV A (L)+SD (L)+NP	282.16 ± 0.05	338.87 ± 0.06	410.17 ± 0.04	394.16 ± 0.05	386.45 ± 0.03
11.	LOVA (L)+SD(H)+NP	281.26 ± 0.05	305.87 ± 0.06	498.21 ± 0.04	393.18 ± 0.05	390.45 ± 0.03
12.	LOVA (L)+FLX (L)+NP	284.05 ± 0.08	390.12 ± 0.05	487.18 ± 0.08	382.13 ± 0.08	372.11 ± 0.06
13.	LOV A (L)+FLX (H)+NP	283.54 ± 0.04	387.16 ± 0.09	492.54 ± 0.04	388.65 ± 0.05	364.65 ± 0.04
14.	LOV A (L)+FLX (L)+SD (L)+NP	284.86 ± 0.66	385.09 ± 0.09	484.26 ± 0.04	367.21 ± 0.12	329.12 ± 0.08

Effect of treatment on OGTT test in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

OGTT was conducted to evaluate glucose regulation across all experimental groups following neuropathy induction. The normal group maintained consistent glucose levels,

starting at 83.67 ± 0.078 at 0 minutes, peaking at 94.91 ± 0.07 at 60 minutes, and gradually decreasing to 85.87 ± 0.11 at 120 minutes. In contrast, the neuropathy control group displayed significantly elevated values, beginning at 282.52 ± 0.04, reaching a maximum of 488.32 ± 0.06 at 60 minutes, and remaining high at 385.27 ± 0.04 after 120 minutes. Treatment groups receiving monotherapies such as LOVA (L), LOVA (H), SD (L), SD (H), FLX (L), and FLX (H) showed a similar trend of elevated glucose, with slight fluctuations over time. For instance, the LOVA (H)+NP group showed values ranging from 282.04 ± 0.09 at baseline to 309.18 ± 0.06 at 120 minutes, while the FLX (H)+NP group ranged from 281.16 ± 0.05 to 390.45 ± 0.03. Similarly, combination therapies including LOVA (L)+FLX (H) (283.54 ± 0.04 to 364.65 ± 0.04) and the triple therapy group LOVA (L)+FLX (L)+SD (L) (284.86 ± 0.66 to 329.12 ± 0.08) demonstrated elevated glucose levels across time points. However, none of these treatments resulted in a statistically significant improvement when compared to the neuropathy group. Overall, while minor variations were observed among the treatment groups, the interventions did not lead to a significant enhancement in glucose tolerance under OGTT conditions.

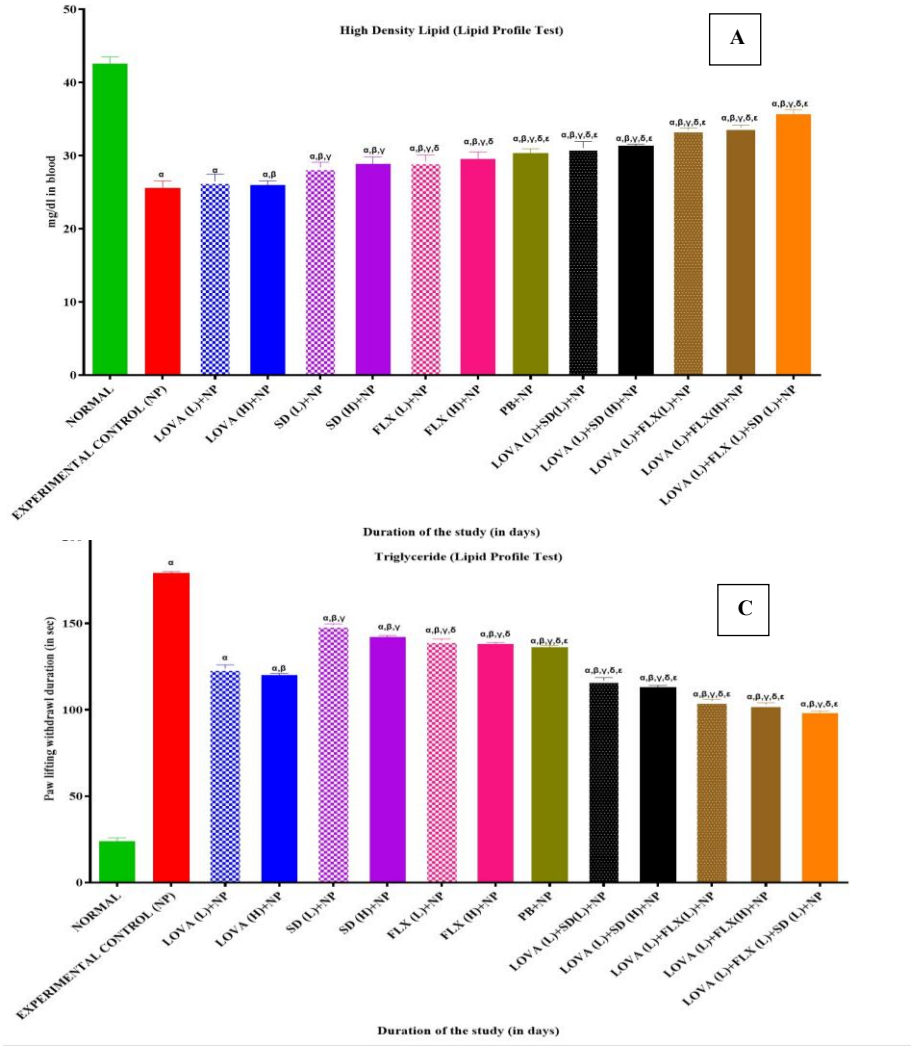
6.6. Lipid Profile Test

After induction of NP, the lipid profile parameters notably increase, except for HDL. The study demonstrated significant alterations in lipid profiles due to NP conditions, with various treatments yielding improvements. Level of LDL increase in the HFD+STZ group, rising to Figure 18, effect of treatment on the OGTT test. Single treatments resulted in dose-dependent reductions, with LOVA (H) and FLX (H) reducing LDL to 76mg/dl and 91.14 mg/dl, respectively. Double combinations offered further improvements, such as LOVA (L) + SD (H) lowering LDL to 71mg/dl, and LOVA (L) + FLX (H) reducing it to 65mg/dl. Remarkably, the triple combination of LOVA (L), FLX (L), and SD (L) achieved the most significant reduction, bringing LDL levels down to 55 mg/dl. HDL levels exhibited a pronounced decrease in the neuropathy group, falling to 25.55 ± 1 mg/dl compared to the normal group i.e. 42.56 ± 0.94 mg/dl. Single treatments yielded moderate improvements, with PB showing the highest increase at 29.56 ± 0.95 mg/dl. Double combination therapies enhanced HDL levels further, as seen with LOVA (L) with SD (H) at 31.33 ± 0.23 mg/dl and LOVA (L) + FLX (H) at 33.54 ± 0.24 mg/dl.

The triple combination produced the most remarkable increase, elevating HDL to 35.65 ± 0.65 mg/dl, nearing normal values. TG levels were significantly elevated in the neuropathic

group, reaching 179mg/dl compared to the normal group at 23.98mg/dl. Single treatments moderately reduced TG levels, with LOVA (H) and FLX (H) lowering levels to 120 and 138mg/dl, respectively. Combination therapies demonstrated greater effectiveness, with LOVA (L)+ SD (H) reducing TG to 113mg/dl, and LOVA (L) + FLX (H) further lowering it to 103 mg/dl. The triple combination achieved the most notable reduction, decreasing TG levels to 98mg/dl. Total cholesterol levels were also markedly increased in the neuropathic group. Single treatments yielded moderate reductions, with LOVA (H) and FLX (H) lowering cholesterol to 114mg/dl and 138mg/dl, respectively, while SD (H) reduced it to 147mg/dl. Double combinations provided enhanced improvements, with LOVA (L)+ SD (H) decreasing cholesterol to 102mg/dl and LOVA (L)+ FLX (H) bringing it down to 93mg/dl. The triple combination demonstrated the most prominent effect, reducing total cholesterol levels to 87mg/dl (Figure 18).

The triple combination demonstrated the most prominent effect, reducing total cholesterol levels to 87mg/dl. These findings collectively emphasize the remarkable potential of combination therapies, particularly the triple combination, in restoring lipid homeostasis and mitigating lipid profile disturbances associated with neuropathic conditions.



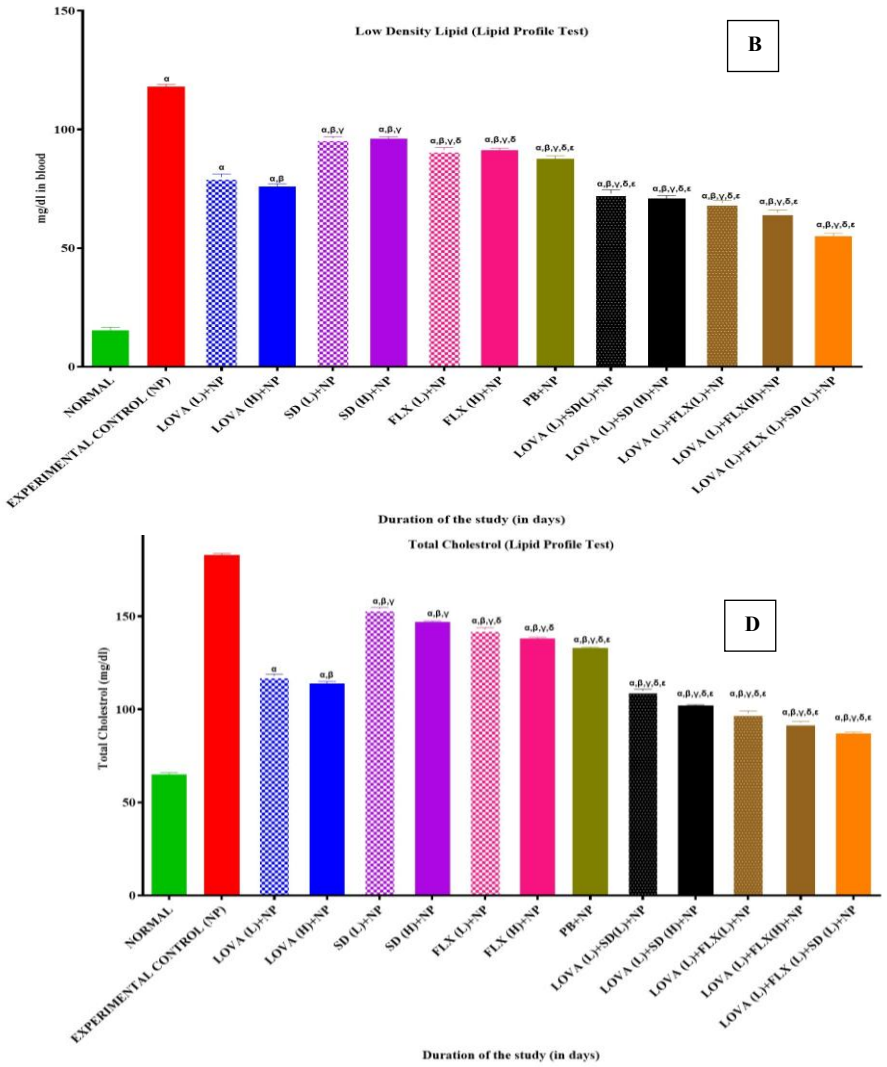


Figure 18. Effect of treatment on lipid profile test in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

6.7 Behavioural Parameter

6.7.1. Heat Hyperalgesia Test

The HFD + STZ induced NP model results in a decrease in hind paw withdrawal latency in heat allodynia, observed on days 0, 7, 14, 21, 28, and 35. The Normal group maintained stable and low values across all figures 19, ranging from 18.67 to 18.79s (healthy state). In contrast, the NP group exhibited a prominent decrease in values (pathological conditions). While treatments with SD, FLX, and LOVA do not completely restore the normal threshold values of pain, they do attenuate NP in a concentration-dependent manner. Initially, the degree of pain increases, but by the 28th day, these treatments help reduce nerve damage and metabolic disturbances associated with neuropathy, demonstrating partial efficacy in managing the condition.

Similarly, in the PB group, partial improvement was observed on day 35 to 12.29 ± 0.077s. The combination of FLX (low and high doses) with LOVA (L), as well as the combination of SD (low and high doses) with LOVA, prominent ($p < 0.05$) attenuated NP in diabetic reaching 4.13 ± 0.56s by day 35 (animals in a concentration dependent manner. A gradual improvement in pain attenuation was observed starting from day 21, with a notable increase becoming evident from day 28 onward. The maximum effect on pain relief was achieved by the 35th day of drug administration. The triple combination SD (L), FLX (L), and LOVA (L) demonstrated the most pronounced effect where group increase meaningfully, from 16.99 ± 0.12 s on day 21. This treatment began showing marked improvement as early as day 3, indicating rapid and sustained therapeutic action. At last, while single treatments and dual combinations provided some degree of attenuation, the triple combination therapy of SD and FLX with LOVA was the most effective. This therapy showed rapid improvement and sustained reduction in pathological values, making it a promising approach for mitigating the effects of HFD+STZ. Although SD, FLX, and LOVA alone cannot fully treat NP, they play a considerable role in alleviating some of the symptoms by the 28th day.

6.7.2. Heat Allodynia Test

HFD + STZ induced NP model results in a decrease in hind paw withdrawal latency in heat allodynia, observed on days 0, 7, 14, 21, 28, and 35. The Normal group maintained stable and low values across all figures 20, ranging from 22.65s to 22.69s (healthy state). In contrast, the EC group exhibited a considerable decrease in values, reaching 11.58 ± 0.023s by day 35 (pathological conditions).

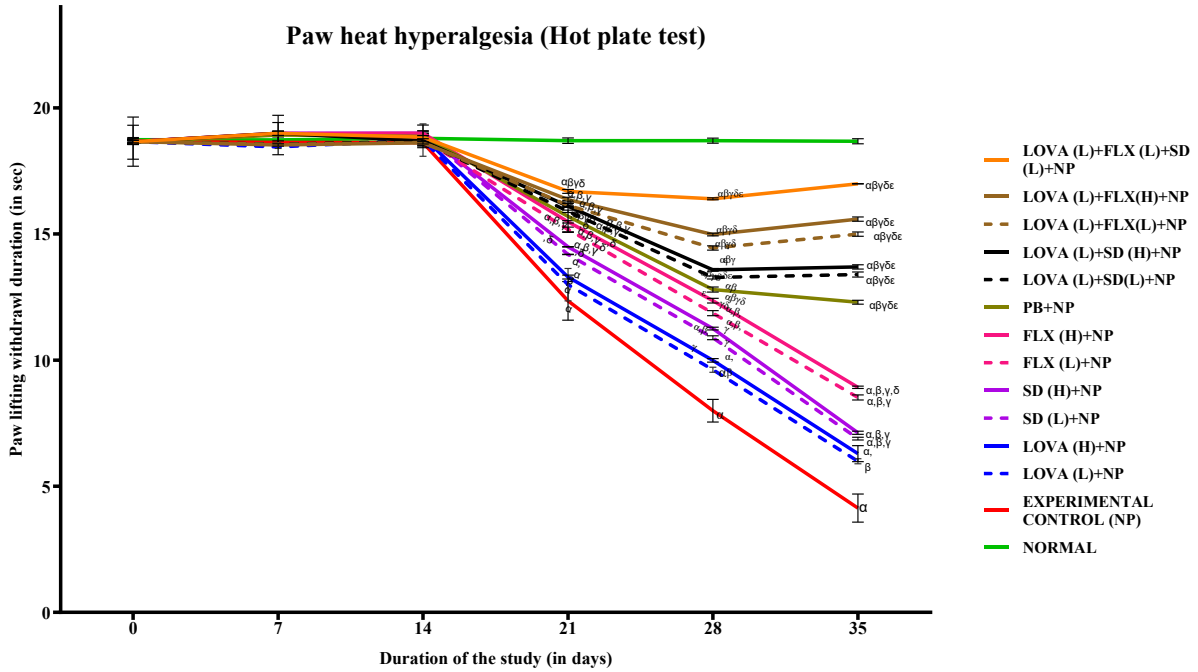


Figure 19. Paw Heat Hyperalgesia (Hot Plate Test): Effect of treatment on Paw Heat Hyperalgesia (Hot Plate Test) in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

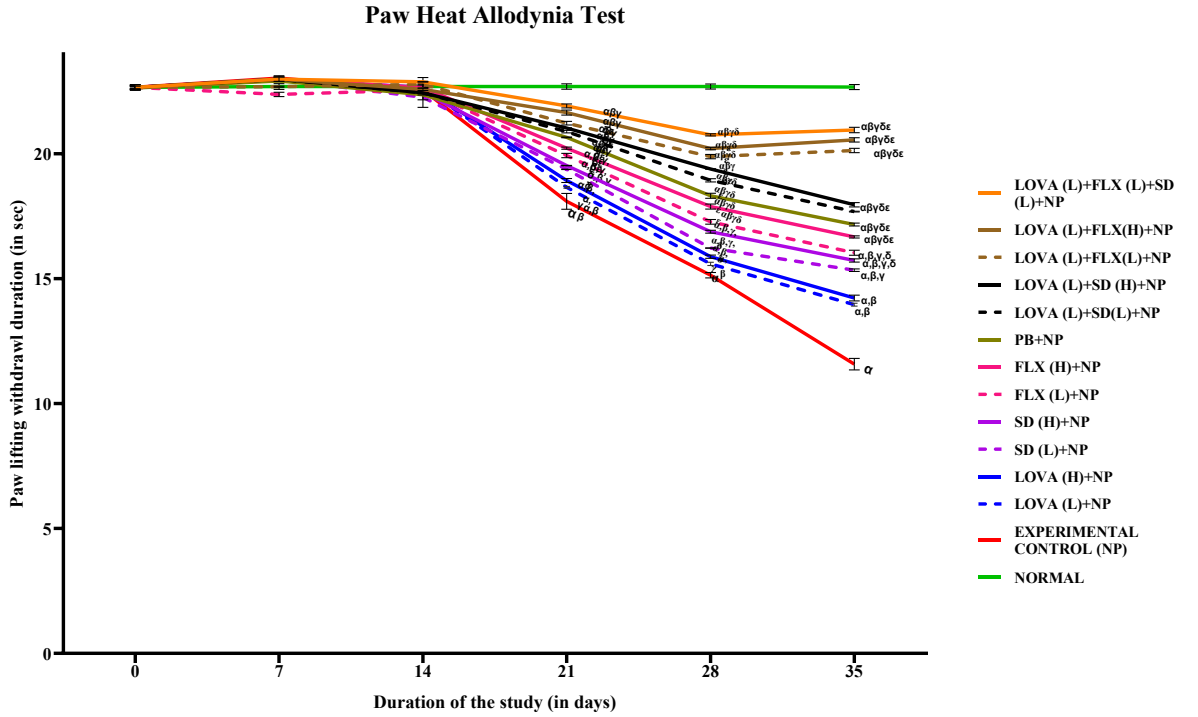


Figure 20. Paw Heat Allodynia Test (Heat Allodynia): Effect of treatment on Paw Heat Allodynia Test (Heat Allodynia) in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

While treatments with SD, FLX, and LOVA do not completely restore the normal threshold values of pain, they do attenuate NP in a concentration-dependent manner. Initially, the degree of pain increases, but by the 28th day, these treatments help reduce nerve damage and metabolic disturbances associated with neuropathy, demonstrating partial efficacy in managing the condition. Similarly, in the PB group, partial improvement was observed on day 35 to 17.17 ± 0.056s. The combination of FLX (low (L) and high (H) doses) with LOVA, as well as the combination of SD (low and high doses) with LOVA, substantially ($p < 0.05$) attenuated NP in diabetic animals in a concentration-dependent manner. A gradual improvement in pain attenuation was observed starting from day 21, with a notable increase becoming evident from day 28 onward. The maximum effect on pain relief was achieved by the 35th day of drug administration. The triple combination therapy SD_L, FLX_L, and $LOVA_L$ demonstrated the most pronounced effect, where the group showed a considerable increase in the value, from 20.95 ± 0.11 s on day 21. This treatment began showing marked improvement as early as day 3, indicating rapid and sustained therapeutic action. At last, while single treatments and dual combinations provided some degree of attenuation, the triple combination therapy of SD and FLX with LOVA was the most effective. This therapy showed rapid improvement and sustained reduction in pathological values, making it a promising approach for mitigating the effects of HFD+STZ. Although SD, FLX, and LOVA alone cannot fully treat NP, they play a considerable role in alleviating some of the symptoms by the 28th day (Figure 20).

6.7.3. Acetone Drop Test

The HFD + STZ induced NP model results in an increased hind paw withdrawal latency in cold chemical allodynia (acetone drop test), observed on days 0, 7, 14, 21, 28, and 35. The Normal group maintained stable and low values across values (Figure 21), ranging from 0.38 to 0.49. These values represent the baseline or healthy state, confirming the absence of pathological conditions. In contrast, the NP group exhibited a marked increase in values, reaching 7.49 ± 0.032s by day 35 (worsening condition in untreated HFD +STZ rats). While treatments with SD, FLX, and LOVA do not completely restore the normal threshold values of pain, they do attenuate NP in a concentration-dependent manner. Initially, the degree of pain increases, but by the 28th day, these treatments help reduce nerve damage and metabolic disturbances associated with neuropathy, demonstrating partial efficacy in managing the condition.

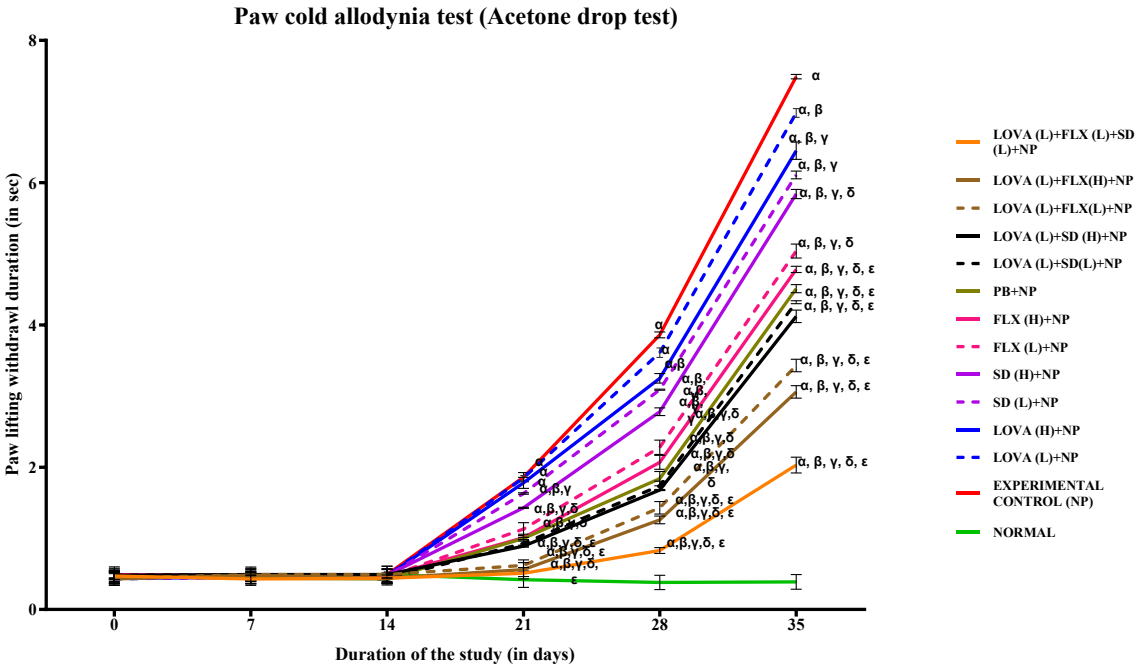


Figure 21. Paw Cold Sensitivity (Allodynia) Test (Acetone Drop Test): Effect of treatment on Paw Cold Sensitivity (Allodynia) Test (Acetone Drop Test) in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

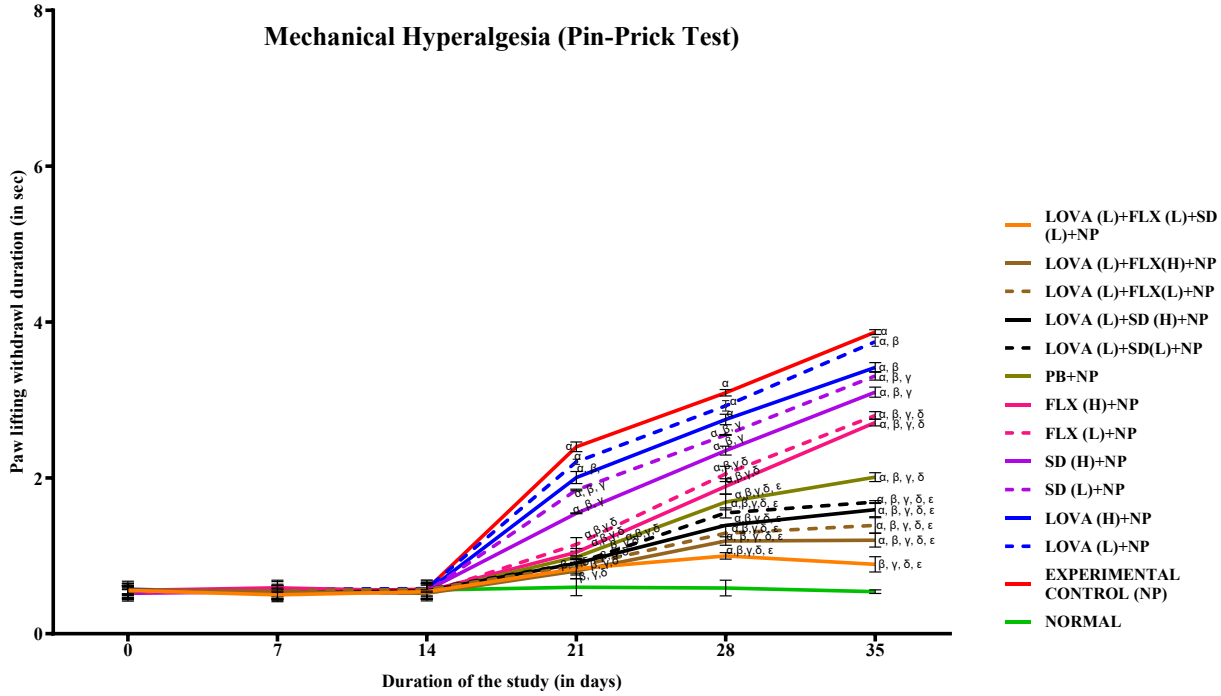


Figure 22. Paw Mechanical Hyperalgesia (Pin-Prick Test): Effect of treatment on Paw Mechanical Hyperalgesia (Pin-Prick Test) in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

Similarly, in the PB group, partial improvement was observed on day 35 to 4.51 ± 0.056s. The combination of FLX (low (L) and high (H) doses) with LOVA, as well as the combination of SD (low and high doses) with LOVA, markedly ($p < 0.05$) attenuated NP in diabetic animals in a concentration-dependent manner. A gradual improvement in pain attenuation was observed starting from day 21, with a notable reduction becoming evident from day 28 onward. The maximum effect on pain relief was achieved by the 35th day of drug administration. The triple combination therapy SD_L, FLX_L, and $LOVA_L$ demonstrated the most pronounced effect, where the group of rats declined markedly, from 0.47 ± 0.05 s on day 3 to 2.03 ± 0.11 s on day 21. This treatment began showing marked improvement as early as day 3, indicating rapid and sustained therapeutic action. At last, while single treatments and dual combinations provided some degree of attenuation, the triple combination therapy of SD and FLX with LOVA was the most effective. This therapy showed rapid improvement and sustained reduction in pathological values, making it a promising approach for mitigating the effects of HFD+STZ. Although SD, FLX, and LOVA alone cannot fully treat NP, they play a marked role in alleviating some of the symptoms by the 28th day.

6.7.4. Pin-prick Test

The HFD + STZ induced NP model results in an increased hind paw withdrawal latency in cold Mechanical Hyperalgesia (Pin-Prick Test) observed on days 0, 7, 14, 21, 28, and 35. The Normal group maintained stable and low values across all Values (Figure 22), ranging from 0.54 to 0.59. These values represent the baseline or healthy state, confirming the absence of pathological conditions. In contrast, the NP group exhibited a marked increase in values, reaching 3.87 ± 0.032s by day 35 (worsening condition in untreated HFD +STZ rats). While treatments with SD, FLX, and LOVA do not completely restore the normal threshold values of pain, they do attenuate NP in a concentration-dependent manner. Initially, the degree of pain increases, but by the 28th day, these treatments help reduce nerve damage and metabolic disturbances associated with neuropathy, demonstrating partial efficacy in managing the condition.

Similarly, in the PB group, partial improvement was observed on day 35 to 2.01 ± 0.056s. The combination of FLX (low (L) and high (H) doses) with LOVA, as well as the combination of SD (low and high doses) with LOVA, markedly ($p < 0.05$) attenuated NP in diabetic animals in a concentration-dependent manner. A gradual improvement in pain attenuation was observed starting from day 21, with a notable reduction becoming evident

from day 28 onward. The maximum effect on pain relief was achieved by the 35th day of drug administration. The triple combination therapy SD_L, FLX_L, and $LOVA_L$ demonstrated the most pronounced effect, where the effect on the group of rats declined markedly, from 0.89 ± 0.099s on day 35. This treatment began showing marked improvement as early as day 3, indicating rapid and sustained therapeutic action. At last, while single treatments and dual combinations provided some degree of attenuation, the triple combination therapy of SD and FLX with LOVA was the most effective. This therapy showed rapid improvement and sustained reduction in pathological values, making it a promising approach for mitigating the effects of HFD+STZ. Although SD, FLX, and LOVA alone cannot fully treat NP, they play a marked role in alleviating some of the symptoms by the 28th day.

6.7.5. Rota- Rod Test

The HFD + STZ induced NP model results in a decreased Motor Coordination Test (Rota-Rod Test), observed on days 0, 7, 14, 21, 28, and 35. The Normal group maintained stable and low values (Figure 23), ranging from 22.54 to 22.69s (baseline or healthy state, confirming the absence of pathological conditions). In contrast, the NP group exhibited a marked decrease in values, reaching 4.0 ± 0.032s by day 35 (worsening condition in untreated HFD +STZ rats). While treatments with SD, FLX, and LOVA do not completely restore the normal threshold values of pain, they do attenuate NP in a dose-dependently. Initially, the degree of pain increases, but by the 28th day, these treatments help reduce nerve damage and metabolic disturbances associated with neuropathy, demonstrating partial efficacy in managing the condition. Similarly, in the PB group, partial improvement was observed on day 35 to 14.79 ± 0.056s. The combination of FLX (low (L) and high (H) doses) with LOVA, as well as the combination of SD (low and high doses) with LOVA, markedly ($p < 0.05$) attenuated NP in diabetic animals in a concentration-dependent manner. A gradual improvement in pain attenuation was observed starting from day 21, with a notable increment becoming evident from day 28 onward. The maximum effect on pain relief was achieved by the 35th day of drug administration. The triple combination therapy SD_L, FLX_L and $LOVA_L$ demonstrated the most pronounced effect, where the group increased markedly 20.79973± 0.11 s on day 35.

This treatment began showing marked improvement as early as day 3, indicating rapid and sustained therapeutic action. At last, while single treatments and dual combinations provided some degree of attenuation, the triple combination therapy of SD and FLX with LOVA was

the most effective. This therapy showed rapid improvement and sustained reduction in pathological values, making it a promising approach for mitigating the effects of HFD+STZ. Although SD, FLX, and LOVA alone cannot fully treat NP, they play a marked role in alleviating some of the symptoms by the 28th day.

6.7.6. Tail immersion test

The tail immersion test was conducted over 35 days to evaluate the thermal nociceptive threshold in different experimental groups. At baseline (day 0), all groups exhibited similar reaction times, confirming the absence of significant pre-existing differences (Figure 24). By day 7 and day 14, no notable differences were observed in reaction times among the groups. However, starting from day 21, a significant decline in reaction time was observed in the NP group (8.34 ± 0.0641 s) compared to the normal group (17.66 ± 0.109 s), indicating heightened pain sensitivity due to neuropathy. Treatment with Lova (L) + NP and Lova (H) + NP resulted in moderate pain relief, with reaction times of 8.81 ± 0.078 s and 9.35 ± 0.01 s, respectively. SD administration produced a further improvement, where SD (L) + NP increased the reaction time to 10.05 ± 0.05 s, and SD (H) + NP to 10.39 ± 0.05 s, suggesting dose-dependent analgesic effects. FLX treatment also showed efficacy, with FLX (L) + NP reaching 10.89 ± 0.021 s and FLX (H) + NP increasing further to 10.99 ± 0.081 s. By day 28, the NP group continued to show a significant reduction (5.55 ± 0.1 s) in reaction time, indicating persistent pain hypersensitivity. However, treatment groups demonstrated further pain relief. Combination therapies, such as Lova (L) + SD (L) + NP (10.15 ± 0.1 s) and Lova (L) + FLX (L) + NP (11.54 ± 0.088 s), exhibited greater efficacy. The most significant improvement was observed in the Lova (L) + FLX (L) + SD (L) + NP group, which reached 14.32 ± 0.087 s, approaching the normal response threshold. By day 35, the NP group displayed the lowest reaction time (4.98 ± 0.098 s), confirming sustained thermal hyperalgesia. However, all treatment groups exhibited substantial improvements, with the most notable increases in combination therapy groups. The Lova (L) + FLX (L) + SD (L) + NP group demonstrated the highest reaction time (14.92 ± 0.091 s), suggesting a synergistic effect in reducing pain perception. Overall, these findings indicate that neuropathy significantly reduces reaction time in the tail immersion test, reflecting increased pain sensitivity. However, pharmacological interventions, particularly combination treatments, effectively increase reaction time, suggesting enhanced analgesic efficacy in NP management.

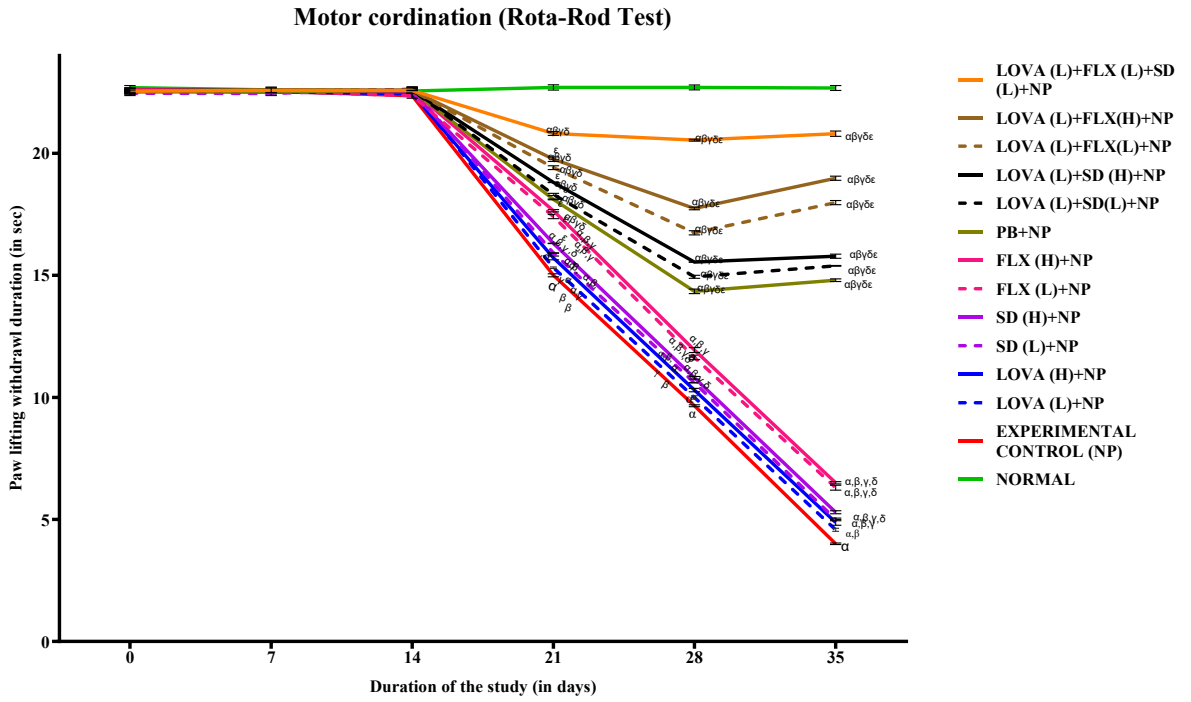


Figure 23. Motor Coordination Test (Rota-rod apparatus): Effect of treatment in Motor Coordination Test (Rota-rod apparatus) in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

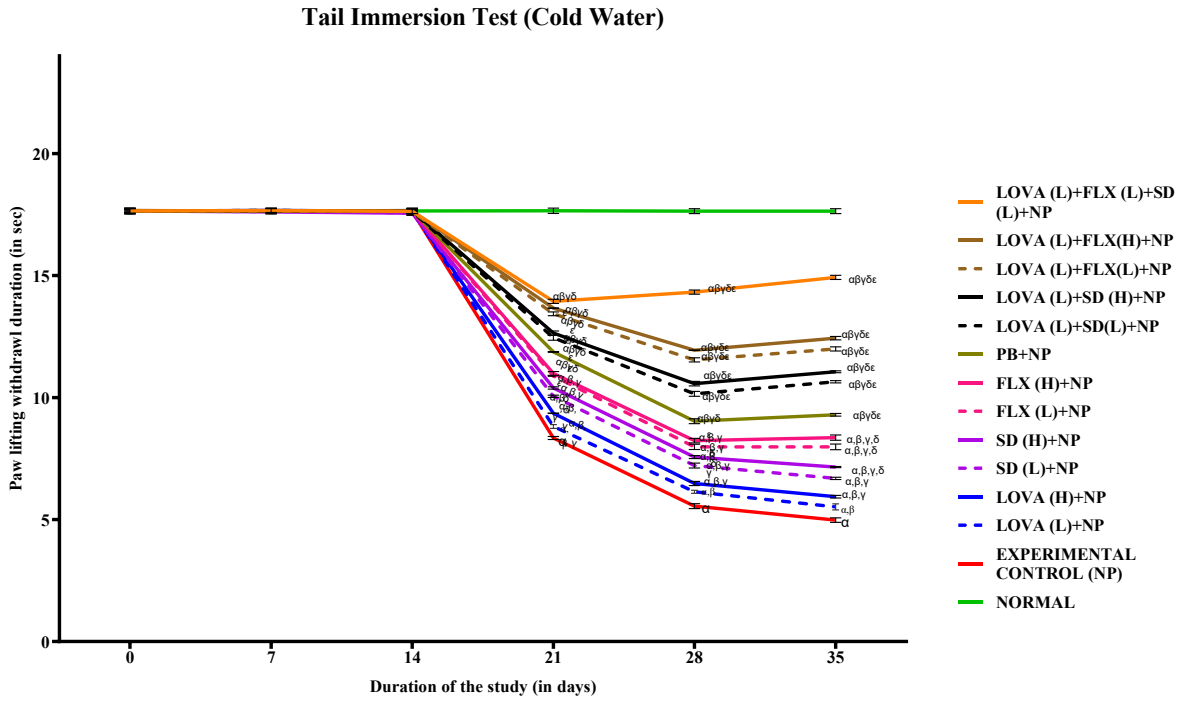


Figure 24. Tail Immersion Test (Cold Water): Effect of treatment on Tail Immersion Test (Cold Water) in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

6.8. Biochemical and Oxidative Biomarkers

6.8.1. Protein estimation

Combination for decreasing protein levels after HFD + STZ appears to be LOVA combined with FLX and SD at low doses, as this treatment resulted in the least elevation of protein levels, measuring 4.96 ± 0.066. This value is closest to the baseline protein level under normal conditions (Figure 25), indicating a marked reduction compared to other treatments The findings suggest that this combination may have a synergistic effect in mitigating protein elevation, making it the most effective option for controlling protein levels in the context of NP

6.8.2. Malondialdehyde (MDA)

The study revealed a marked increase in MDA levels in the NP group, 7.5 ± 0.067, compared to the normal group, 2.3 ± 0.054, indicating elevated oxidative stress. Various treatments showed a dose-dependent reduction in MDA levels (Figure 26). Double combination therapies further enhanced the reduction, with LOVA (L)+ FLX (H) showing a marked decrease to 2.87 ± 0.083. The triple combination of LOVA low dose, FLX low dose, and SD (L) demonstrated the most marked improvement, reducing MDA levels to 2.7 ± 0.056, approaching normal levels. These findings highlight the superior efficacy of combination therapies, particularly the triple combination, in mitigating oxidative stress by markedly lowering MDA levels. Among the single treatments, FLX (H) and PB were most effective, reducing MDA levels to 3.85 ± 0.071 and 3.42 ± 0.067, respectively.

6.8.3. Glutathione Test

The study revealed a substantial decline in GSH levels in the NP group, with levels dropping to 12.76 ± 0.5gm compared to the normal control of 49.55 ± 1.22 g. Various treatments demonstrated marked improvements in GSH levels in a concentration-dependent manner. Among single treatments, FLX (H), 24.05 ± 1.12 g and PB, 27.13 ± 0.86gm produced the most pronounced increases in GSH levels (Figure 27). Double combination therapies further enhanced GSH levels, with LOVA (L) + SD (H); 35.91 ± 0.88gm and LOVA (L) + FLX (H); 38.96 ± 0.66gm, showing remarkable efficacy. The triple combination of LOVA (L) + FLX (L) + SD (L) resulted in the highest restoration of GSH levels 44 ± 0.78gm, approaching near-normal levels.

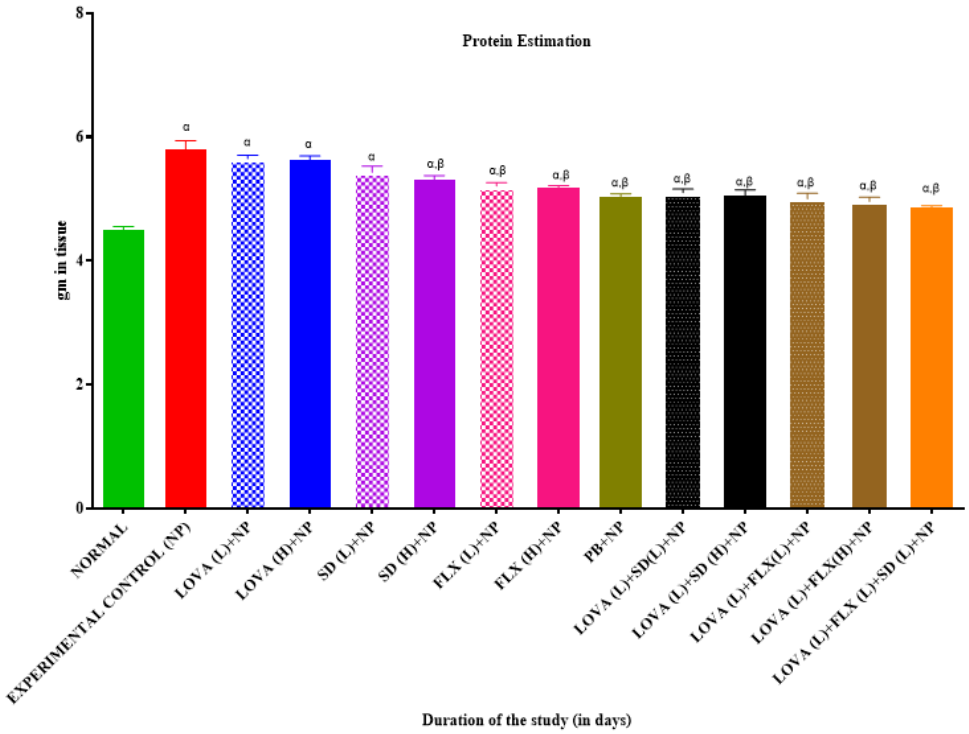


Figure 25. Protein (gm of tissues): Effect of treatment on Protein (gm of tissues) in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

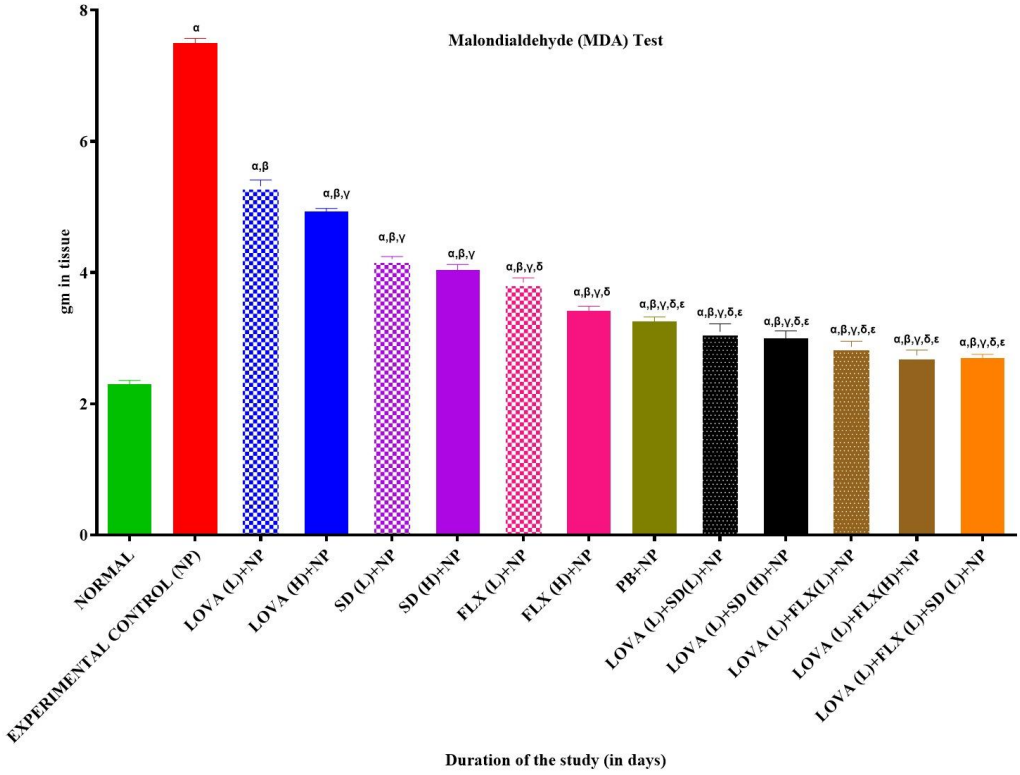


Figure 26. MDA Test (nM/mg of tissues): Effect of treatment on MDA Test (nM/mg of tissues) in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

These findings indicate that combination therapies, particularly the triple combination, are highly effective in restoring antioxidant defences, highlighting their potential in mitigating oxidative stress in neuropathic conditions (Figure 27).

6.8.4. Catalase Test

The study demonstrated that catalase levels, which were reduced in the NP group to 1.32 ± 0.065gm g, markedly improved following various treatments. Single treatments with low and high doses of LOVA, SD, and FLX, as well as PB, showed moderate increases in catalase levels, with FLX (H) and PB producing the highest elevations among the single groups, reaching 2.23±0.045 g and 2.53±0.038 g, respectively. Double combination therapies further amplified catalase levels, with LOVA (L)+ FLX (H) achieving the highest increase, reaching 3.39 ± 0.053 g among the double combinations. The most remarkable improvement was observed with the triple combination of LOVA (L)+ FLX (L)+ SD low dose, which resulted in a marked elevation of catalase levels to 4.59 ± 0.039 g, outperforming all single and double treatments. This highlights the synergistic effect of combined therapies in restoring antioxidant properties in neuropathy (Figure 28).

6.8.5. Superoxide dismutase (SOD) levels

The study demonstrated a marked reduction in SOD in the group of rats that received only HFD+low dose of STZ, i.e., 5.56 ± 0.26 U/mg tissue, while in the normal control group, the level of SOD was observed as 16.73 ± 0.23 U/mg Tissue, indicating impaired antioxidant defence in the experimental control group of rats. Various treatments produced a dose-dependent restoration of SOD levels (Figure 29). Among the group of rats received individual treatments, FLX (H), i.e., 12.65 ± 0.18 U/mg, but PB showed the highest improvements, i.e.. 12.76 ± 0.74 U/mg, while FLX (L) shows the SOD levels are 12.34 ± 0.16 U/mg. Double combination therapies showed enhanced SOD activity, with LOVA (L)+ SD (H) showing a marked increase to 13.87 ± 0.18 U/mg, while LOVA (L)+ FLX (H) restored SOD levels to 14.44 ± 0.088 U/mg. The triple combination of LOVA low dose, FLX low dose, and SD (L) yielded the most notable recovery, with SOD levels rising to 15.04 ± 0.056 U/mg, nearing the normal group levels. These results emphasize the superior effectiveness of combination therapies, particularly the triple combination, in restoring antioxidant capacity by enhancing SOD levels in neuropathic conditions.

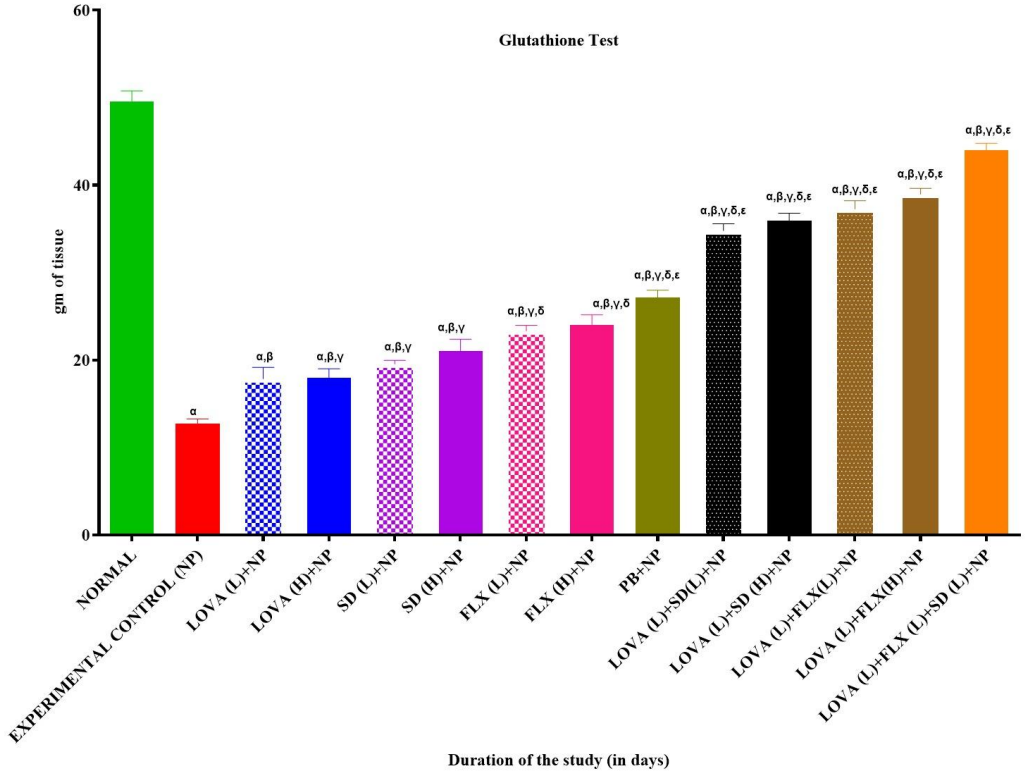


Figure 27. GSH Test (µmol/min/mg of tissues): Effect of treatment on GSH Test (µmol/min/mg of tissues) in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

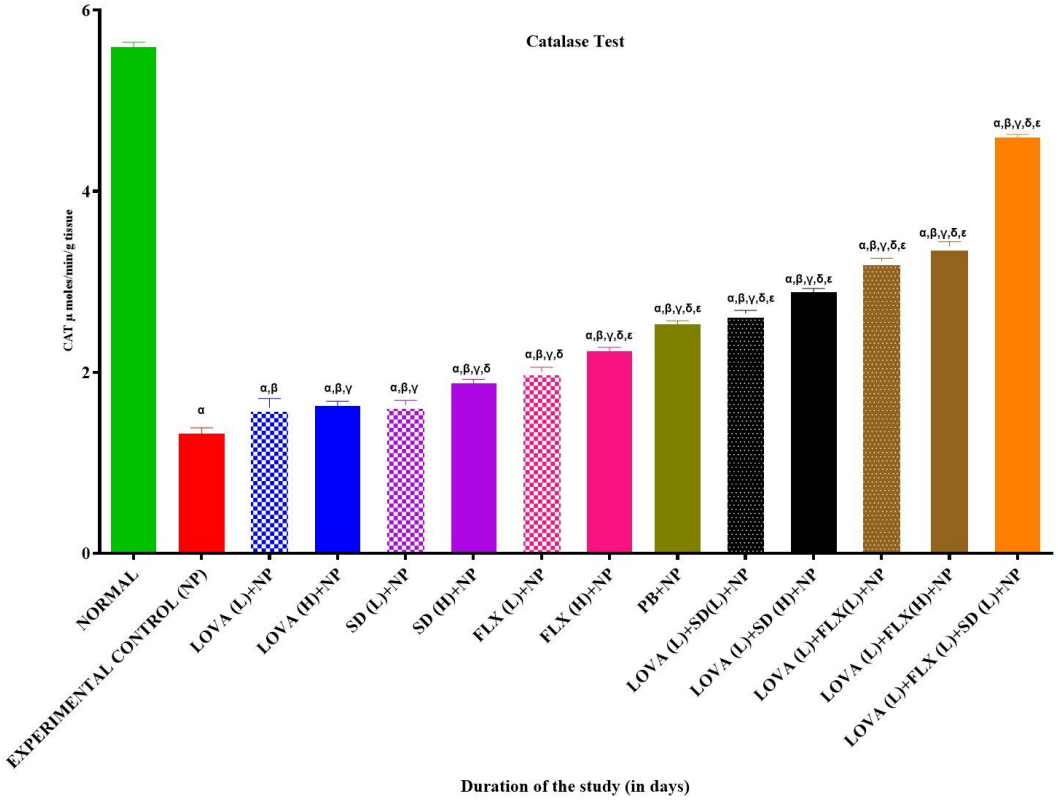


Figure 28. Catalase Test (µmoles/min/g of tissues): Effect of treatment on GSH Test Catalase Test (µmoles/min/g of tissues) in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

6.8.6. Tumour necrosis factor-alpha (TNF-α)

The study revealed a marked increase in TNF-α levels in the NP, i.e., 42.3 ± 0.85 pg/ml, which compared to the normal group i.e., 7.4 ± 0.76 pg/ml, indicating heightened inflammation in neuropathic conditions. Various treatments showed a dose-dependent reduction in TNF-α levels. Among single treatments, SD (H) i.e., 27.42 ± 1.077 pg/ml and FLX (H) give 25.65 ± 0.66 pg/ml, demonstrating the most pronounced reductions. Combination therapies further enhanced the anti-inflammatory effects, with LOVA (L)+ SD (H)reducing TNF-α levels to 18.21 ± 1.31 pg/ml and LOVA (L)+ FLX (H) lowering levels to 15.86 ± 0.95 pg/ml. The triple combination of LOVA low dose, FLX low dose, and SD (L)achieved the most remarkable decrease, reducing TNF-α levels to 12.88 ± 1.32 pg/ml, closely approaching normal levels. These findings underscore the superior efficacy of combination therapies, particularly the triple combination, in mitigating inflammation by markedly lowering TNF-α levels in neuropathic conditions (Figure 30).

6.8.7. Interleukin-6 (IL-6)

The interleukin test revealed a dose-dependent reduction in interleukin levels across all treatment groups when compared to the NP. Among the single treatments, LOVA high dose. demonstrated the highest IL-6; 35.76 ± 0.14 pg/ml, while PB showed a moderate decrease 16.23 ± 0.66 pg/ml. Combination therapies markedly enhanced the reduction in interleukin levels, with LOVA (L)+ SD (H) reducing levels to 14.04 ± 0.11 pg/ml, and LOVA (L)+ FLX (H) further lowering levels to 11.52 ± 0.042 pg/ml. The most remarkable improvement was observed with the triple combination of LOVA low dose, FLX low dose, and SD low dose which achieved the lowest interleukin level of 9.05 ± 0.092 pg/ml, closely followed by the group receiving LOVA (L), FLX (H), and SD (L) with a level of 9.35 ± 0.093 pg/ml. These results highlight the superior efficacy of combination therapies, particularly the triple combination, in markedly reducing interleukin levels, suggesting their potential for managing inflammatory responses in neuropathic conditions (Figure 31).

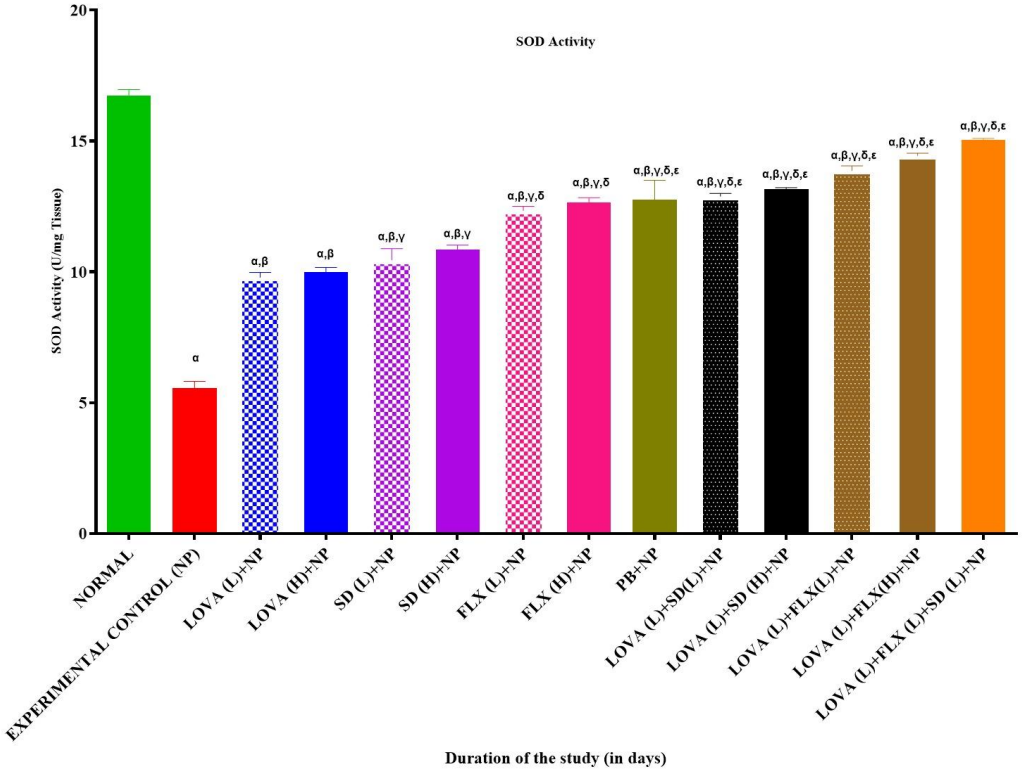


Figure 29. SOD Level (U/mg of Tissues): Effect of treatment on SOD Level (U/mg of Tissues) in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

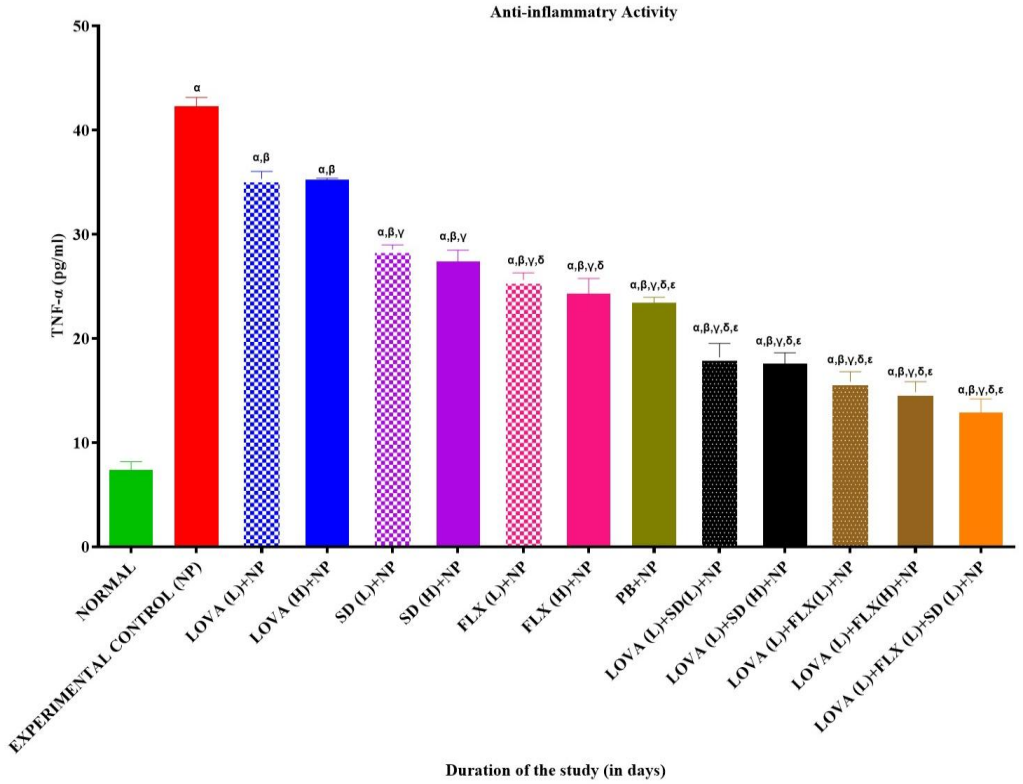


Figure 30. Determination of TnF-α Test (pg/ml of tissues): Effect of treatment on TnF-α Test (pg/ml of tissues) in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

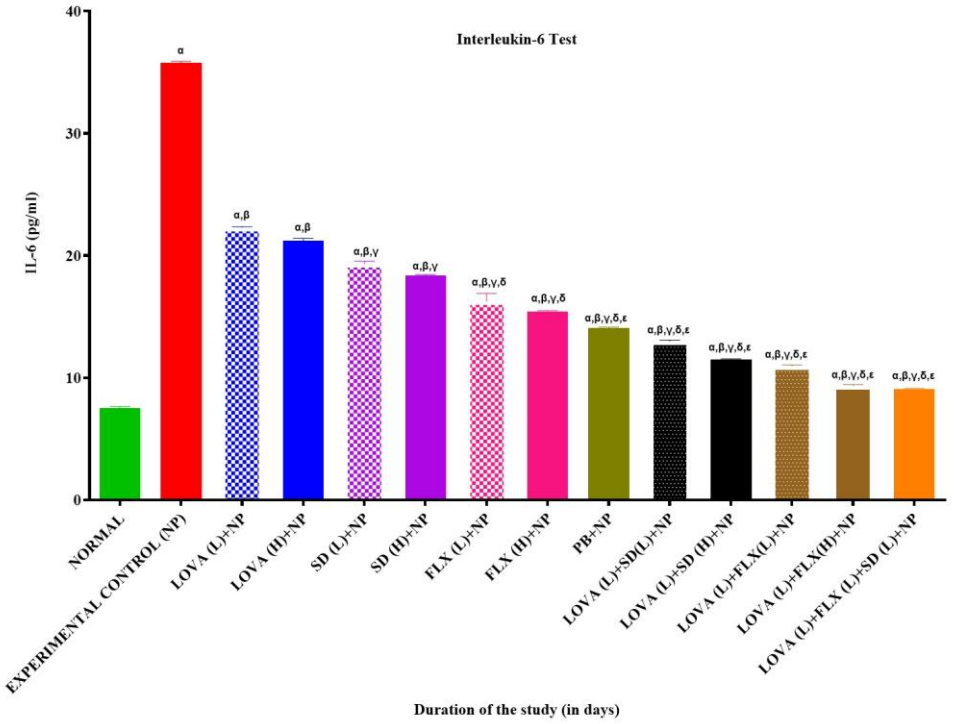


Figure 31. Determination of IL-6 Test (pg/ml of tissues): Effect of treatment on IL-6 Test (pg/ml of tissues) in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

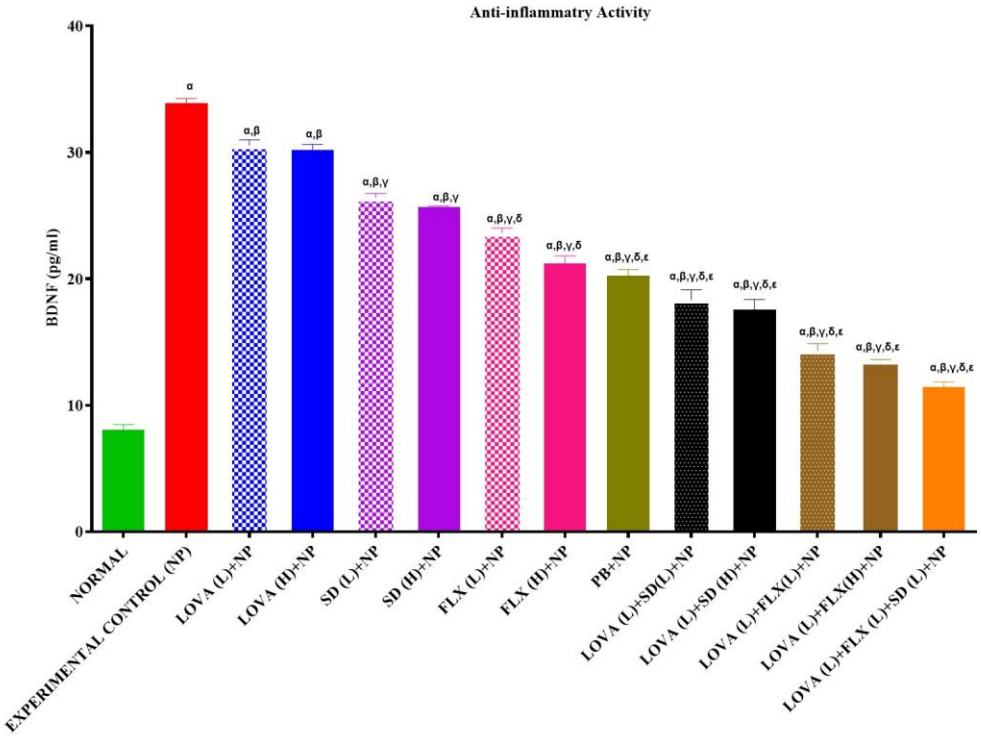


Figure 32. Determination of BDNF Level (pg/ml of tissues): Effect of treatment on BDNF Level (pg/ml of tissues) in a HFD and low dose of STZ induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s normal control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

6.8.8 BDNF level

BDNF levels were significantly elevated in the NP group (33.87 ± 0.39 pg/ml) compared to the normal group (8.04 ± 0.43 pg/ml). Treatment with LOVA (L) led to a reduction (30.54 ± 0.45 pg/ml), while a higher dose, LOVA (H), resulted in a further decrease (30.21 ± 0.42 pg/ml). Administration of SD also contributed to lowering BDNF levels, with SD (L) + NP at 26.43 ± 0.32 pg/ml and SD (H) + NP at 25.65 ± 0.097 pg/ml. A similar trend was observed with FLX, where FLX (L) + NP reduced BDNF levels to 23.64 ± 0.37 pg/ml, and FLX (H)+ NP further lowered it to 21.21 ± 0.59 pg/ml. The PB + NP group exhibited a decline in BDNF (Figure 32). The PB + NP group exhibited a decline in BDNF levels (20.23 ± 0.51 pg/ml). Combination treatments demonstrated an even greater reduction in BDNF levels. LOVA (L) + SD (L) + NP led to a decrease (18.34 ± 0.79 pg/ml), while LOVA (L) + SD (H) + NP showed a further reduction (17.56 ± 0.82 pg/ml). Co-administration of LOVA (L) + FLX (L) + NP resulted in a more pronounced decrease (14.32 ± 0.56 pg/ml), with LOVA (L) + FLX (H) + NP lowering it even further (13.54 ± 0.085 pg/ml). The most significant reduction was observed with LOVA (L) + FLX (L) + SD (L) + NP, which brought BDNF levels down to 11.43 ± 0.43 pg/ml, suggesting a strong synergistic effect of the combined treatments.

In neuropathic rats, levels of MDA, TNF-α, IL-6, BDNF, total protein, and LPO increased, while antioxidant markers like GSH, SOD, and catalase decreased compared to non-neuropathic controls. Treatments with SD, FLX, and LOVA, either individually or in combination, effectively modulated these oxidative biomarkers: SD reduced MDA & LPO levels and increased GSH & catalase activity. FLX showed similar effects, decreasing MDA & LPO levels and boosting GSH & catalase activity. LOVA also lowered MDA & LPO levels and enhanced GSH & catalase levels. Combined treatments produced greater reductions in MDA & LPO levels and more pronounced increases in GSH & catalase activity compared to individual treatments. The triple combination (SD + FLX + LOVA) showed the most remarkable outcomes by achieving the largest reductions in MDA & LPO levels and markedly enhancing GSH & catalase levels. These results highlight the superior effectiveness of the triple combination therapy in mitigating oxidative stress and restoring antioxidant defences in neuropathic rats.

6.9. Histopathological Studies

Histological Studies on Sciatic Nerve in HFD+STZ-Induced NP. NP induced by a combination of HFD and (STZ is a widely accepted model for studying metabolic syndrome-

related peripheral nerve damage. The sciatic nerve, as a key peripheral nerve, demonstrates marked structural and functional changes under neuropathic conditions. In this study, histological evaluations were conducted to understand the effects of various therapeutic interventions, including single and combination drug treatments, on the sciatic nerve in this neuropathy model. Normal Control microscopically showed well-preserved nerve fiber architecture with intact myelin sheaths, minimal inflammation, and no signs of axonal degeneration. This group serves as the baseline, representing a healthy physiological condition devoid of metabolic or oxidative stress. Experimental control showed, marked pathological alterations were observed, including disrupted nerve fiber alignment, loss of myelin sheath integrity. Increased inflammatory cell infiltration and evidence of axonal degeneration and vacuolation. These findings reflect severe oxidative stress, lipid peroxidation, and neuroinflammation associated with experimental control (Figure 33).

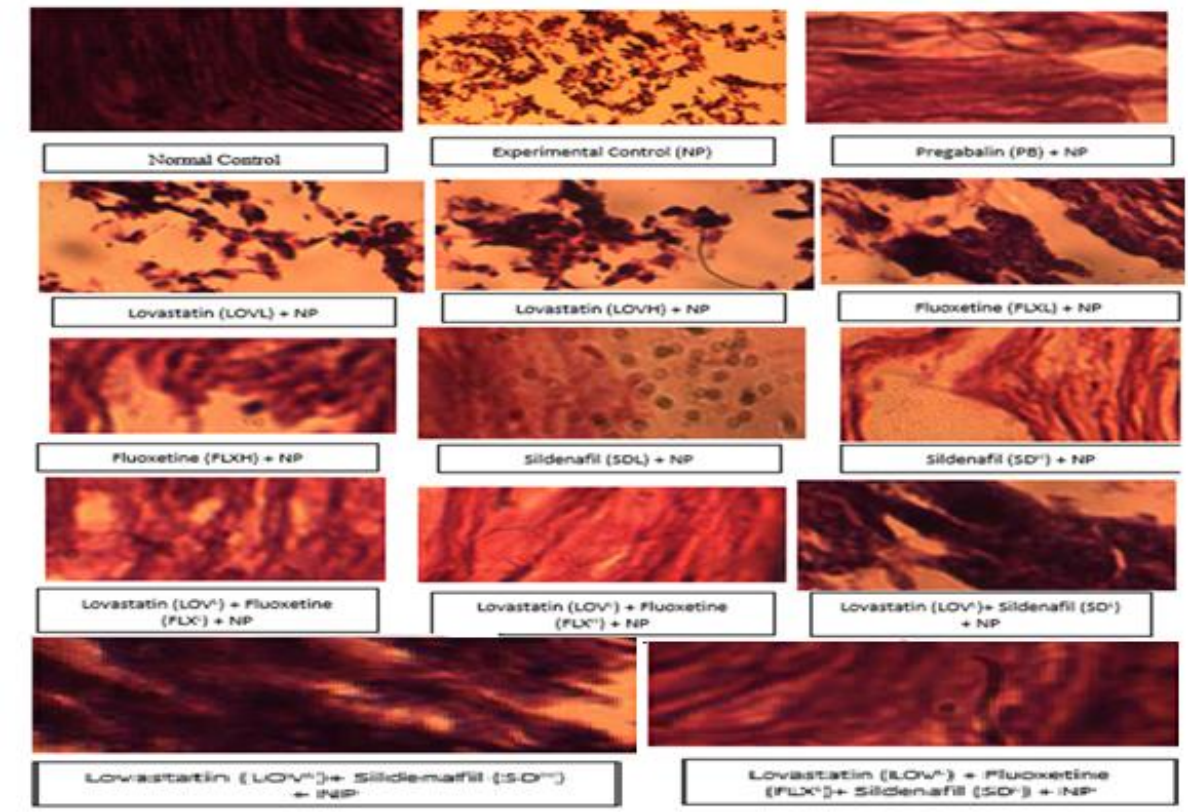


Figure 33: Histopathological study of the sciatic nerve in HFD+STZ induced NP model

The effect of standard drug PB resulted in moderate improvements in nerve histology, characterized by partial restoration of nerve fiber integrity and a reduction in inflammatory infiltration. While PB showed a neuroprotective effect, the repair was incomplete, indicating its limited efficacy as a monotherapy in metabolic neuropathy. LOVA, (L); minimal

improvement was noted, with slight reductions in inflammatory infiltrates and partial myelin sheath restoration, and (H); A dose-dependent improvement was observed, with better preservation of nerve fibers and reduced inflammation. Similarly, FLX, (L); modest recovery in nerve fiber organization and mild reduction in inflammatory markers were seen. (H); Marked restoration of nerve structure was evident, with better myelin sheath preservation, modulated oxidative stress, and neuroinflammation. SD; (L); Mild improvements in nerve morphology were observed, and (H); enhanced restoration of nerve structure, with reduced inflammation and better preservation of myelin integrity, suggesting a dose-dependent neuroprotective effect. Combination treatments demonstrated synergistic effects, outperforming single-drug therapies in nerve restoration. LOVA (L) + FLX (L) showed marked reduction in inflammation and better nerve fiber alignment compared to single treatments, and LOVA (L) + FLX (H) showed marked recovery, with near-normal nerve morphology, indicating enhanced efficacy with FLX (H) + LOVA (L) + SDL (L), moderate improvement in nerve histology, with reduced inflammatory infiltration, and LOVA (L) + SD (H) showed superior outcomes with well-preserved nerve fibers and minimal inflammation. At last, the Triple-Drug Combination SD (L) + FLX (L) + LOVA (L); showed remarkable recovery was observed in this group, with near-complete restoration of sciatic nerve histology. Inflammatory markers were markedly reduced, and the myelin sheath integrity was comparable to the normal control group. The synergistic neuroprotective and anti-inflammatory effects of combining SD, FLX, and LOVA. Histological studies demonstrate the devastating impact of HFD+STZ-induced neuropathy on sciatic nerve morphology. Single-drug treatments showed varying degrees of neuroprotection, with high-dose SD, FLX, and LOVA outperforming their low-dose counterparts. However, combination therapies, particularly the triple combination of SD (L) + FLX (L) + LOVA (L) yielded the most marked restoration of nerve structure, indicating their potential as an effective therapeutic strategy for metabolic neuropathy. These findings underscore the importance of multi-targeted approaches in treating complex neuropathic conditions and provide a basis for further clinical exploration of these drug combinations. The study also reinforces the role of histological analysis in elucidating the structural changes associated with neuropathy and evaluating the efficacy of therapeutic interventions. This research contributes to the growing body of knowledge on NP management and may serve as a foundation for future doctoral studies investigating combination therapies in metabolic and oxidative stress-related neuropathies.

The administration of a HFD + low dose of STZ successfully induced type II DNP, characterized by increased body weight, mild hyperglycemia, elevated triglyceride levels, and high cholesterol. The study findings revealed that the combination of low doses of SD, FLX, and LOVA resulted in a significant reduction in NP as early as the 21st day. In comparison, other combinations, such as SD with low-dose LOVA, FLX with low-dose LOVA, and PB, demonstrated NP reduction starting from the 28th day. Among these, the combination of FLX (high dose) with LOVA (L)proved to be superior, surpassing the effects of FLX (L)+ LOVA (low dose), SD (L)+ LOVA (low dose), and PB treatments. Furthermore, similar improvements were observed across biochemical parameters, including LDL, HDL, triglycerides (TG), and total cholesterol (TC). The drug treatments also played a key role in preserving oxidative biomarkers, such as MDA, SOD, GSH, and CAT, while modulating inflammatory markers TNF-α, IL-6, and BDNF levels.

6.10. Result of SNL

6.10.1. Heat Hyperalgesia Test

It indicate that different treatment groups produced varying degrees of improvement in heat hyperalgesia over the 28-day observation period. The most effective treatment combination was LOVA (L) + FLX (L) + SD (L) + NP, which demonstrated a progressive and sustained improvement, with values of 15.35 ± 0.21s on Day 7, 15.99 ± 0.19s on Day 14, 16.48 ± 0.22s on Day 21, and 16.89 ± 0.16s on Day 28. This group showed the highest level of recovery from hyperalgesia compared to other combinations. In contrast, the NP group exhibited a steady decline in response, with values dropping from 18.63 ± 0.12s on Day 0 to 4.32 ± 0.10s on Day 28, indicating severe hyperalgesia without intervention. Among single-agent therapies, high-dose SD (H) combined with LOVA (L) demonstrated notable improvement, reaching 13.23 ± 0.21s by Day 28. However, it was clear that multi-agent combinations, particularly involving SD, FLX, and LOVA, were the most effective strategies for mitigating heat hyperalgesia and promoting sustained recovery, confirming severe hyperalgesia. From these findings, it is evident that combination therapy, particularly incorporating SD, FLX, and LOVA at lower doses, exhibited superior pain modulation compared to monotherapy or other combinations (Figure 34). This suggests that such multi-drug regimens could offer a more effective approach to managing NP.

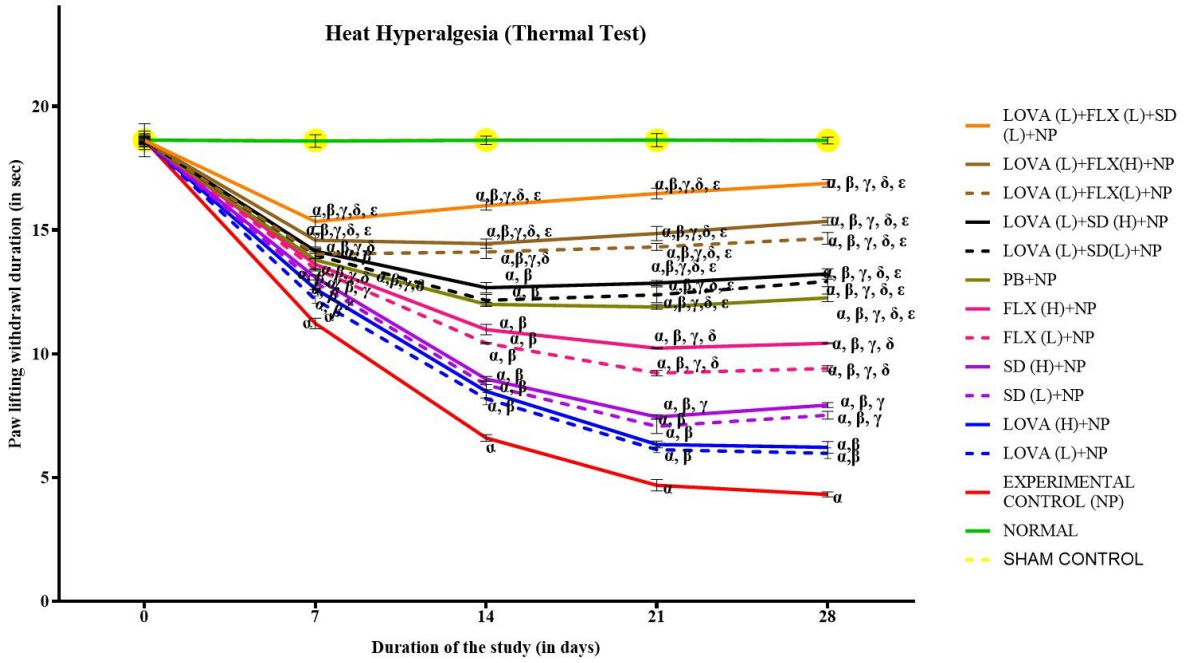


Figure 34. Paw Heat Hyperalgesia (Hot Plate Test): Effect of treatment on Paw Heat Hyperalgesia (Hot Plate Test) in a SNL induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s sham control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

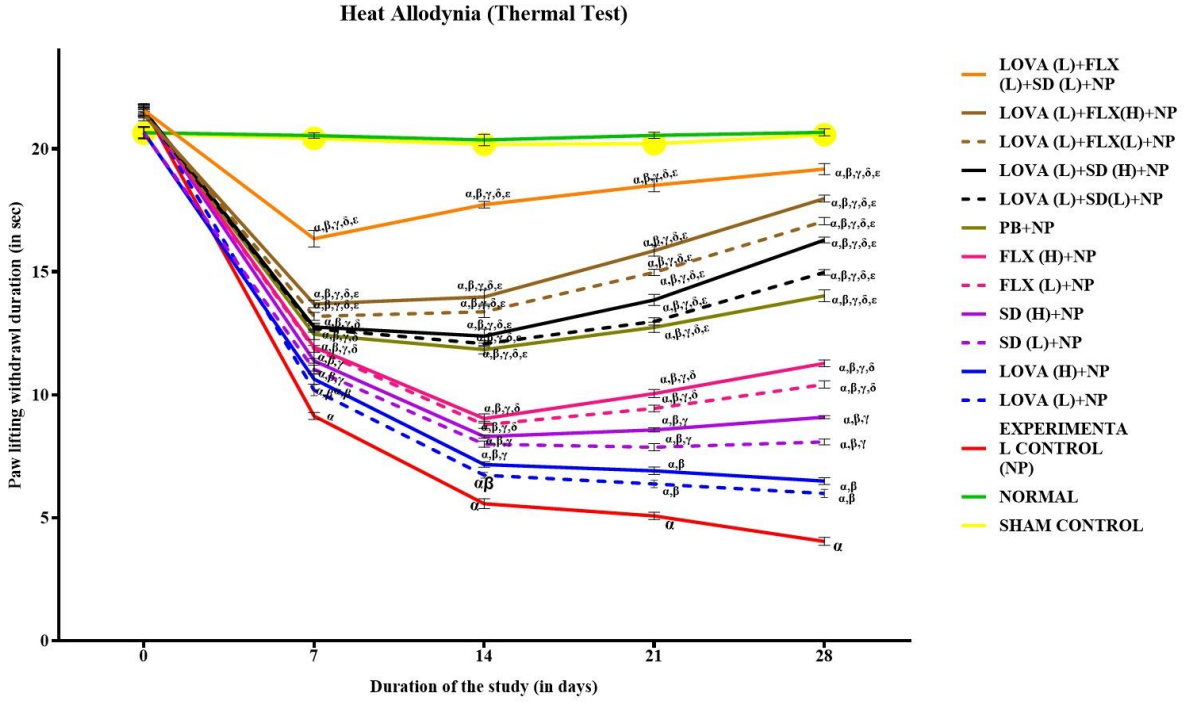


Figure 35. Paw heat allodynia (Hot plate test): Effect of treatment on Paw heat allodynia (Hot plate test) in a SNL induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s sham control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

6.10.2. Heat Allodynia Test

The assessment of mechanical allodynia in the NP model revealed marked differences across the treatment groups. The NP group exhibited a marked decrease in withdrawal threshold (4.04 ± 0.32s), confirming the successful induction of allodynia. Among the treatment groups, the combination therapy of LOVA (L) + FLX (L) + SD (L) + NP demonstrated the most marked improvement, with a final withdrawal threshold of 19.58 ± 0.23s, indicating its strong effectiveness in alleviating NP -induced allodynia. Other combination treatments, including LOVA (L) + FLX (H) + NP (17.98 ± 0.06s) and LOVA (L) + FLX (L) + NP (17.059 ± 0.06s), also showed notable improvements compared to monotherapies. Monotherapy treatments such as FLX (H) + NP (11.28 ± 0.06s) and SD (H) + NP (9.09 ± 0.06s) provided moderate pain relief, while LOVA (L) + NP (5.99 ± 0.06s) and LOVA (H) + NP (6.49 ± 0.06s) showed less pronounced effects (Figure 35). The normal and sham control groups maintained stable withdrawal thresholds of approximately 20.54±0.11s to 20.67±0.14s, validating the experiment's reliability. These results suggest that combination therapy, particularly SD, FLX and LOVA at lower doses, offers the most effective approach to mitigating NP -associated allodynia.

6.10.3. Acetone Drop Test

Over 28 days to assess the cold allodynia response in different experimental groups. At day 0, all groups displayed similar baseline withdrawal latencies, indicating no significant initial differences. By day 7, the NP group demonstrated a sharp decrease in withdrawal latency (4.456 ± 0.032s) compared to the normal group (0.496 ± 0.011s), indicating the development of cold hypersensitivity. Moreover, in the treatment with Lova (L) + NP (4.32 ± 0.01s) and Lova (H) + NP (4.02 ± 0.02s) significantly prolonged withdrawal latency, suggesting an analgesic effect. Further improvement was seen with SD and FLX treatments, with SD (H) + NP reaching 3.73 ± 0.04s, FLX (L) at 3.67 ± 0.03s, and FLX (H) + NP at 3.42 ± 0.09s, indicating a dose-dependent effect. By day 14, a further increase in withdrawal latency was observed in treatment groups, with Lova (H) + NP (5.24 ± 0.04s) and SD (H) + NP (4.73 ± 0.032s) showing continued effectiveness. The NP group remained highly sensitive resistance to cold stimuli (5.74 ± 0.06s), whereas combination therapies such as Lova (L) + SD (H) + NP (3.28 ± 0.03s) demonstrated superior pain reduction. By day 21, the NP group (6.15 ± 0.09s) still exhibited severe cold hypersensitivity.

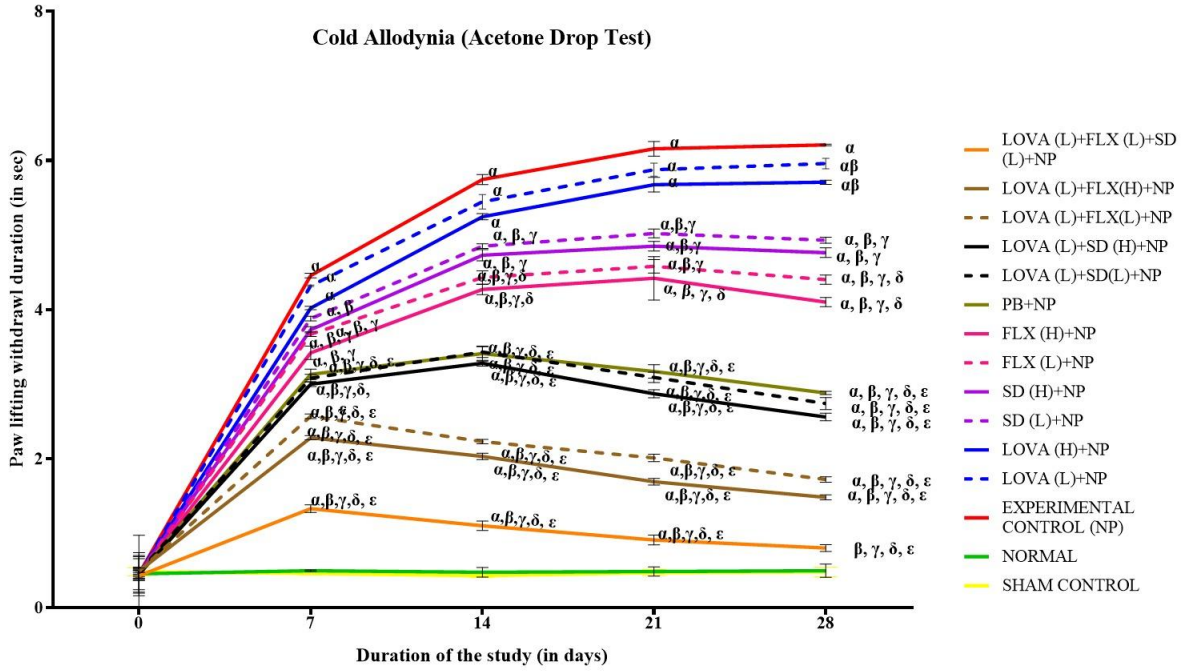


Figure 36. Paw Cold Sensitivity (Allodynia) Test (Acetone Drop Test): Effect of treatment on Paw Cold Sensitivity (Allodynia) Test (Acetone Drop Test) in a SNL induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s sham control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

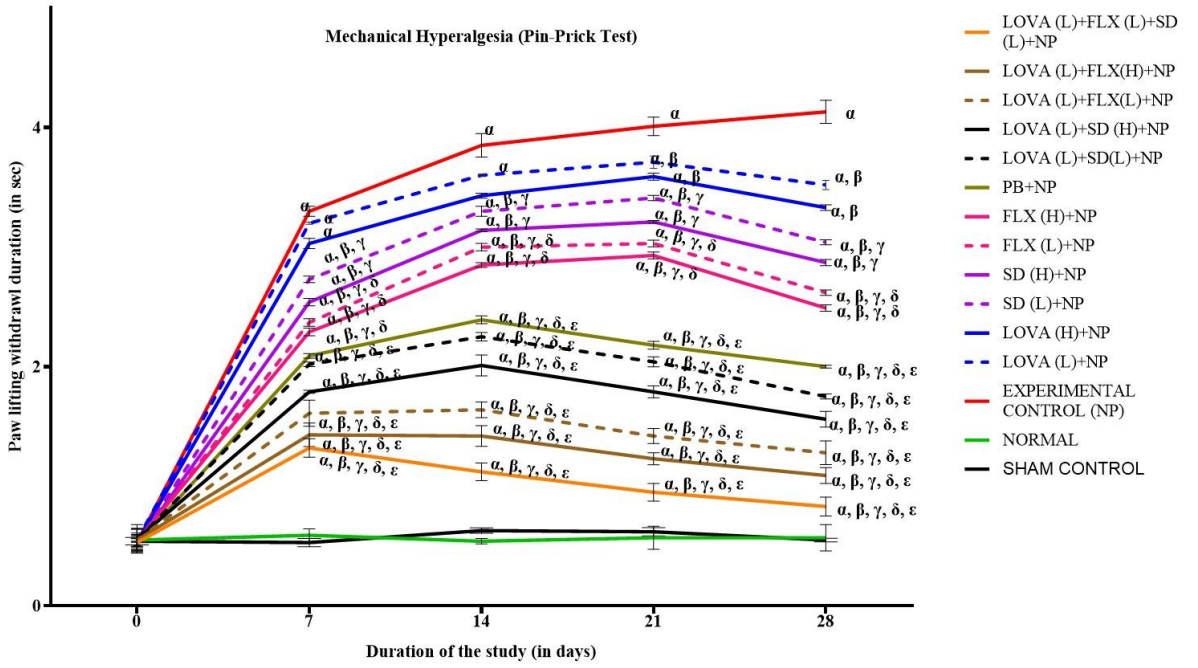


Figure 37. Paw Mechanical Hyperalgesia (Pin-Prick Test): Effect of treatment on Paw Cold Sensitivity (Allodynia) Test (Acetone Drop Test) in a SNL induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s sham control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

However, the combination of Lova (L) + NP with SD and FLX significantly increased withdrawal latencies, with Lova (L) + FLX (H) + NP reaching 1.69 ± 0.045 s and Lova (L) + FLX (L) + SD (L) + NP at 0.91 ± 0.069 s, suggesting a synergistic analgesic effect. By day 28, the NP group continued to exhibit the lowest latency (6.21 ± 0.01 s), confirming sustained cold hypersensitivity. However, all treatment groups showed further improvements, with Lova (H) + NP (5.71 ± 0.02 s) and SD (H) + NP (4.76 ± 0.065 s) maintaining significant pain relief. Combination therapies Lova (L) + FLX (H) + NP (1.48 ± 0.034 s) and Lova (L) + FLX (L) + SD (L) + NP (0.80 ± 0.047 s) demonstrated the highest efficacy, suggesting that multi-drug approaches are more effective in reducing cold allodynia in NP conditions (Figure 36). Overall, the results indicate that neuropathy leads to heightened cold sensitivity, as seen in the NP group. However, pharmacological interventions, particularly combination treatments, significantly increase withdrawal latency, suggesting a potent analgesic effect in managing cold allodynia in NP.

6.10.4. Pin-Prick test

The study revealed marked differences in the response values across various treatment groups. The NP group showed elevated values of 3.3±0.042ss, indicating the negative impact of the experimental condition. Single compound treatments such as SD (L) i.e. 2.73±0.028s and low-dose FLX (L), 2.37±0.03s demonstrated partial improvements. LOVA (L) + NP yielded a moderate response of 3.2±0.01s (Figure 37). Combination therapies, however, demonstrated superior results. The group receiving LOVA (L) with low-dose SD (L) exhibited an enhanced response value of 2.04±0.043, while LOVA (L) combined with FLX (L) further improved the response to 1.79±0.05 on 21st day. The most marked improvement was observed in the group treated with SD (L), FLX (L) and LOVA (L), achieving a value of 0.83±0.079s on 28th day. These findings suggest potential synergistic effects among the compounds, with SD enhancing nitric oxide availability, FLX modulating serotonergic pathways, and LOVA providing endothelial protection. This combination therapy demonstrated superior efficacy compared to single treatments, presenting a promising therapeutic strategy.

6.10.5. Rota-Rod test

The study results highlighted marked variations across treatment groups over time (days 0, 7, 14, 21, and 28). On day 0, all groups exhibited stable baseline values with no remarkable differences. By day 7, the NP group showed a notable decline (11.21±0.31), emphasizing the

adverse impact of the induced condition, compared to the Normal Control (22.69±0.21) and Sham Control (22.13±0.02) (Figure 38).

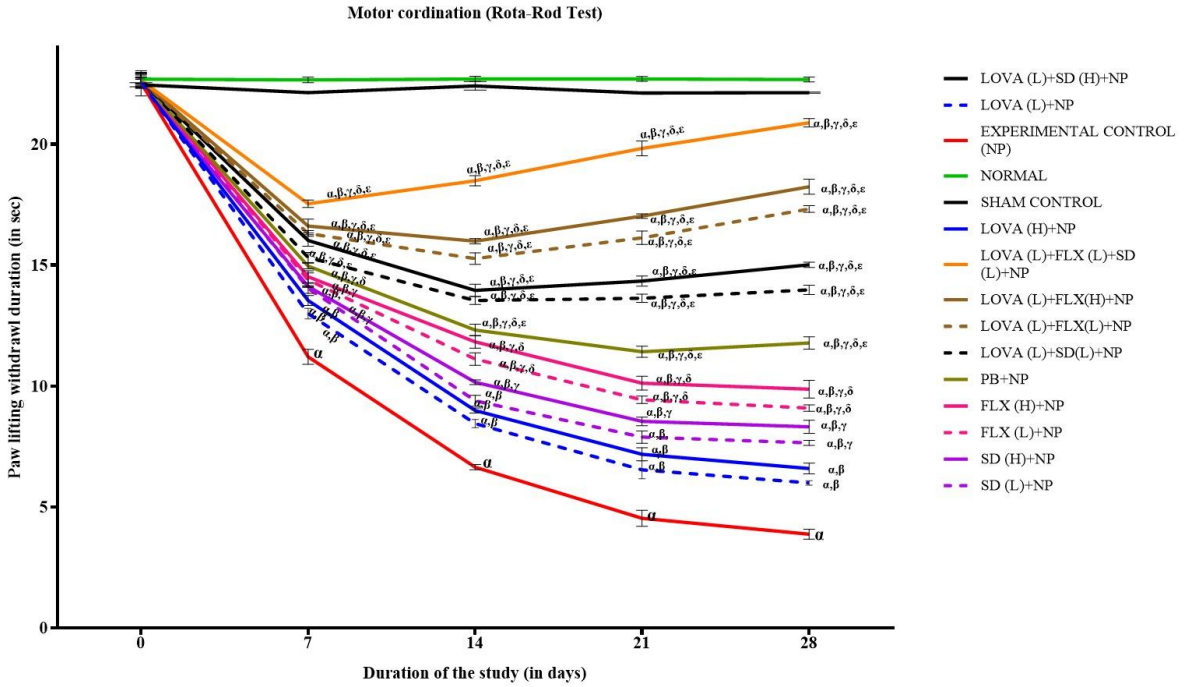


Figure 38. Motor Coordination Test (Rota-Rod Apparatus): Effect of treatment on Motor Coordination Test (Rota-Rod Apparatus) in a SNL induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s sham control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

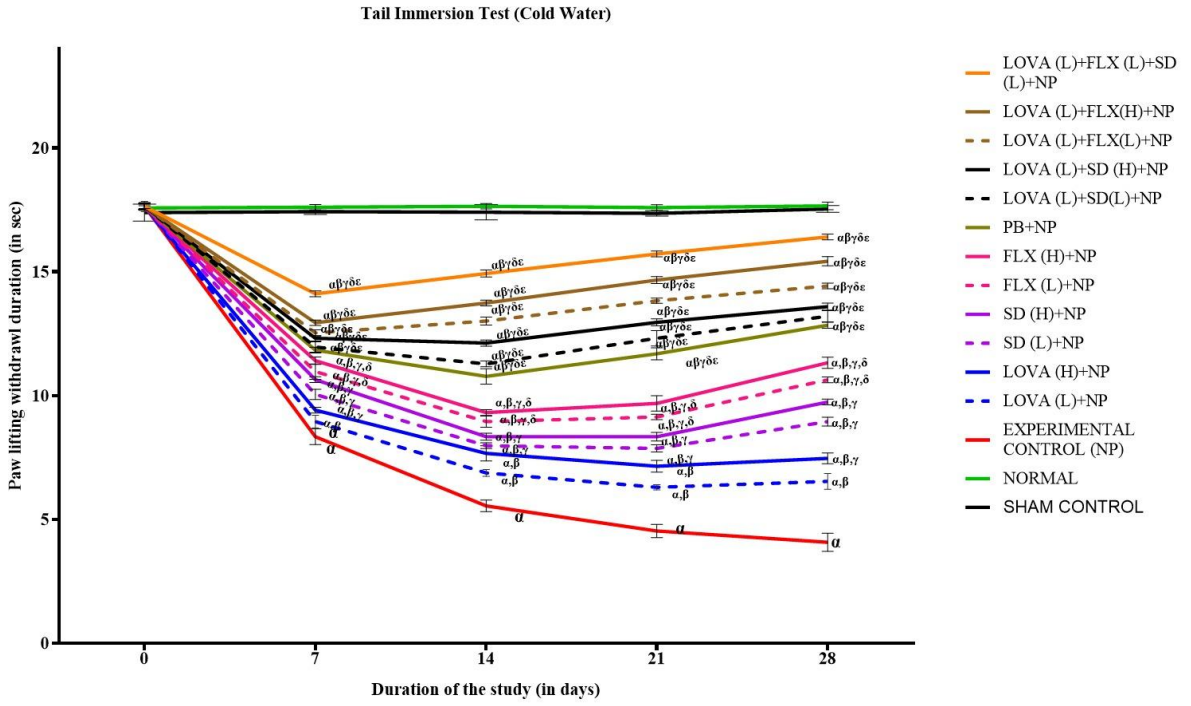


Figure 39. Tail Immersion Test (Cold Water): Effect of treatment on Tail Immersion Test (Cold Water) in a SNL induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s sham control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

Low-dose single treatments, such as LOVA (13±0.22), SDL (14.04±0.21), and FLX (14.39±0.29), provided partial recovery, though not markedly restoring the normal state. On day 14, the effects of treatment became more pronounced. The NP group continued to deteriorate (6.65±0.11), while combination therapies began to show better outcomes. Notably, LOVA combined with low-dose SD and low-dose FLX markedly improved the condition (15.99±0.11), compared to the experimental control and single-drug treatments. By day 21, the therapeutic potential of combination therapies was further evident. The triple combination group (LOVA+SD+FLX) exhibited substantial improvement (17.03±0.10), outperforming other combinations and single-agent treatments such as SD (H) (8.54±0.18) and FLX (H) (10.12±0.29). On day 28, the most remarkable recovery was observed in the low dose of LOVA+SD+FLX group, achieving a value of 20.89±0.17, closely approaching the normal control value (22.68±0.11). This marked recovery demonstrates the synergistic effects of the combination therapy, likely driven by SD's vasodilatory action, FLX's serotonergic modulation, and LOVA's endothelial protective properties. These findings suggest that the combination therapy provided a superior and sustained therapeutic effect, making it a promising strategy for the management of the experimental condition.

6.10.6. Tail immersion test

The tail immersion test was conducted over five time points (0, 7, 14, 21, and 28 days) to evaluate the analgesic efficacy of different treatments in SNL (Spinal Nerve Ligation) model rats. Initially, at day 0, no marked differences were observed between groups, with values ranging around 17.58±0.1 across all treatments, indicating a baseline response. By day 7, the experimental control group exhibited a significant decline in tail withdrawal latency to 8.34±0.32, indicating pronounced thermal hyperalgesia resulting from nerve injury. In contrast, treatment with single compounds led to some improvements: low-dose LOVA yielded 8.95±0.26, while high-dose SD reached 10.65±0.12. Notably, combination therapies produced superior results, with the low-dose combination of LOVA and SD achieving a latency value of 14.11±0.12, representing the highest improvement at this stage. On day 14, single-agent treatments showed continued recovery, with high doses of FLX and SD reaching 9.32±0.12 and 8.34±0.14, respectively (Figure 39).

Combination therapies demonstrated enhanced efficacy, with the low-dose combination of LOVA, SD, and NP reaching 14.93±0.14, highlighting a synergistic effect. By day 21, experimental control values plateaued at 4.54±0.27, while combination groups continued to

show progressive improvement. The triple combination therapy (low-dose SD, FLX and LOVA) produced remarkable results, recording latency values of 15.72±0.12, indicating near-restoration of normal pain thresholds. On day 28, tail withdrawal latency in the experimental control group remained persistently low at 4.08±0.36. Single-compound treatments such as high-dose FLX and SD reached 11.33±0.23 and 9.74±0.12, respectively. However, combination therapies maintained the best outcomes, with the triple combination achieving the highest latency at 16.41±0.12, suggesting near-complete reversal of hyperalgesia symptoms. These findings confirm that multi-drug regimens, particularly the triple combination of low doses of SD, FLX and LOVA, provided the most effective analgesic effects, likely through mechanisms involving enhanced serotonin levels, endothelial protection, and improved nitric oxide signaling pathways.

6.11. Biochemical and Oxidative Biomarkers

6.11.1. Protein Estimation

In the present study, the normal group exhibited the lowest tissue protein level, measuring 4.50 ± 0.21 g, reflecting the baseline status under healthy physiological conditions. Interestingly, the sham control group demonstrated a comparable protein level of 4.30 ± 0.14 g, indicating no significant alteration due to surgical intervention alone. This similarity confirms the appropriateness of the sham control as a reference for evaluating experimental manipulations, as it closely mirrors the normal physiological state without the influence of induced pathology. The NP group increased in protein levels to 7.2 ± 0.14 g/tissue, indicating heightened protein expression or accumulation by SNL. In the LOVA groups, treatment with low-dose LOVA reduced protein levels to 6.32 ± 0.053 g in tissues, while high-dose LOVA further decreased it slightly to 6.04 ± 0.073 g in tissues, demonstrating a dose-dependent improvement in maintaining protein balance under stress conditions. The SD groups displayed a greater reduction in protein levels compared to LOVA. Low-dose SD decreased protein levels to 5.89 ± 0.098 g in tissues, while high-dose SD reduced it further to 5.74 ± 0.067 g in tissues, suggesting SD's effectiveness in mitigating protein dysregulation caused by SNL ligation.

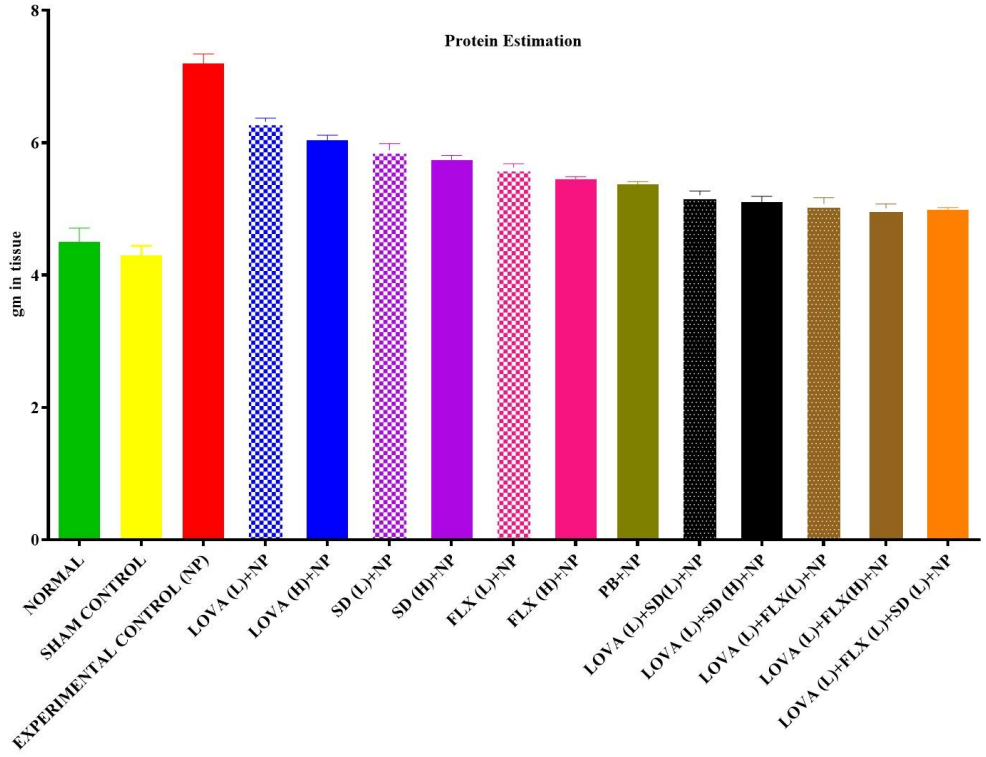


Figure 40. Protein Estimation (gm in Tissue): Effect of treatment on Protein Estimation (gm in Tissue) in a SNL induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s Sham Control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

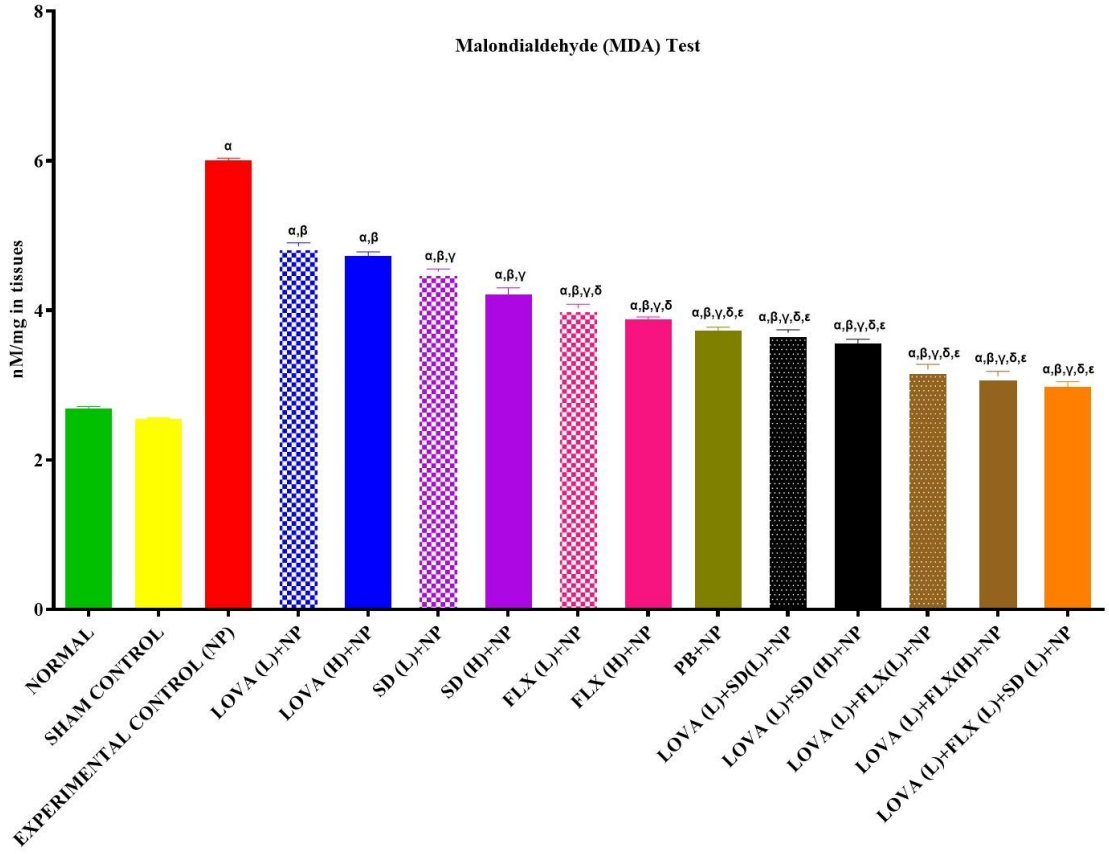


Figure 41. MDA Test (nM/mg of tissues): Effect of treatment on MDA Test (nM/mg of tissues) in a SNL induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s sham control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

The FLX groups achieved better results than both LOVA and SD treatments. Low-dose FLX reduced protein levels to 5.63 ± 0.053 gm in tissues, and high-dose FLX brought it down further to 5.45 ± 0.034 gm in tissues, indicating a clear dose-dependent effect of FLX in reducing excessive protein expression. The PB+NP group showed a further reduction in protein levels to 5.37 ± 0.043 gm in tissues, indicating PB's comparable efficacy in improving protein regulation. The combination therapies demonstrated the most reductions in protein levels. The combination of low-dose LOVA and low-dose SD reduced protein levels to 5.21 ± 0.061 gm in tissues, while combining low-dose LOVA with high-dose SD lowered it further to 5.1 ± 0.088 gm in tissues. Adding FLX to LOVA resulted in even greater improvements, with the combination of low-dose LOVA and low-dose FLX reducing protein levels to 5.08 ± 0.091 gm in tissues, and the high-dose FLX combination achieving a level of 5.01 ± 0.066 gm in tissues. The most reduction in protein levels was observed in the group receiving low-dose LOVA, low-dose FLX, and low-dose SD, which achieved the lowest protein level of 4.98 ± 0.041 gm in tissues, indicating the strongest synergistic effect among all treatments (Figure 40).

6.11.2. MDA

The observed MDA levels provide important insights into the oxidative stress induced by SNL ligation and the impact of various treatments. The normal group exhibited the lowest MDA level at 2.69 ± 0.023 nM/mg, representing the baseline oxidative stress under healthy conditions. In contrast, The NP group showed an increase MDA levels rising to 6.00 ± 0.035 nM/mg, indicating a substantial elevation in lipid peroxidation caused by SNL ligation. In the LOVA groups, low-dose LOVA reduced MDA levels to 4.86 ± 0.043 nM/mg, while high-dose LOVA further decreased it slightly to 4.73 ± 0.051 nM/mg, demonstrating a modest dose-dependent reduction in oxidative stress. The SD groups showed a greater ability to lower MDA levels compared to LOVA. Low-dose SD decreased MDA to 4.52 ± 0.032 nM/mg, while high-dose SD further reduced it to 4.21 ± 0.091 nM/mg, highlighting SD's superior efficacy in mitigating lipid peroxidation. Similarly, Low-dose FLX lowered MDA levels to 4.03 ± 0.051 nM/mg, and high-dose FLX decreased it further to 3.88 ± 0.029 nM/mg, indicating a clear dose-dependent reduction in oxidative stress. The PB group showed a further reduction in MDA levels to 3.73 ± 0.042 nM/mg, suggesting that PB provides comparable oxidative stress mitigation. The combination of low-dose LOVA and low-dose SD reduced MDA to 3.70 ± 0.039 nM/mg, while combining low-dose LOVA with high-dose

SD brought it down further to 3.56 ± 0.054 nM/mg . Adding FLX to LOVA resulted in even greater improvements, with the combination of low-dose LOVA and low-dose FLX reducing MDA to 3.21 ± 0.069 nM/mg, and the high-dose FLX combination achieving a level of 3.12 ± 0.063 nM/mg. The most remarkable reduction in MDA was observed in the group receiving low-dose LOVA, low-dose FLX, and low-dose SD, which achieved the lowest MDA level of 2.98 ± 0.065 nM/mg, indicating the strongest synergistic effect in reducing oxidative stress.

These findings suggest that combination therapies, particularly involving SD, FLX and LOVA are the most effective in lowering MDA levels, mitigating oxidative stress, and reducing lipid peroxidation after SNL ligation (Figure 41).

6.11.3. Glutathione activity

The normal group showed a GSH level of 50.21±1.22 µmol/min/mg, representing the baseline. The NP group displayed a reduction in GSH to 10.88±0.5 µmol/min/mg due to oxidative stress caused by SNL ligation. In the LOVA-treated groups, low-dose LOVA restored GSH to 20.43±1.31 µmol/min/mg, while high-dose LOVA increased it further to 21.08±0.99 it to 24.05 µmol/min/mg, showing a dose-dependent effect. The SD-treated groups showed higher GSH restoration, with low-dose SD increasing GSH to 23.54±0.43 µmol/min/mg and high-dose SD further enhancing it to 24.05±1.41 µmol/min/mg. In the FLX-treated groups, low-dose FLX increased GSH to 29.05±0.65 µmol/min/mg, while high-dose FLX raised it to 30.44±1.12 µmol/min/mg. The PB group showed a GSH level of 30.89±0.86 µmol/min/mg, slightly higher than FLX alone. Combination therapies demonstrated the greatest improvement. Low-dose LOVA and low-dose SD increased GSH to 34.32±0.78 µmol/min/mg, while low-dose LOVA and high-dose SD further raised it to 35.41 ± 0.88 µmol/min/mg. Adding FLX resulted in greater improvement, with low-dose LOVA and low-dose FLX achieving 38.23±0.93 µmol/min/mg, and low-dose LOVA with high-dose FLX reaching 40.32±0.66 µmol/min/mg. The highest GSH level of 43.42±0.78 µmol/min/mg was observed in the combination of low-dose LOVA, low-dose FLX, and low-dose SD, showing the strongest synergistic effect (Figure 42).

6.11.4. Catalase Activity

On the terminal day the low dose of triple combination (SD, FLX and LOVA) showed elevation in catalase activity i.e 3.98 ± 0.039.

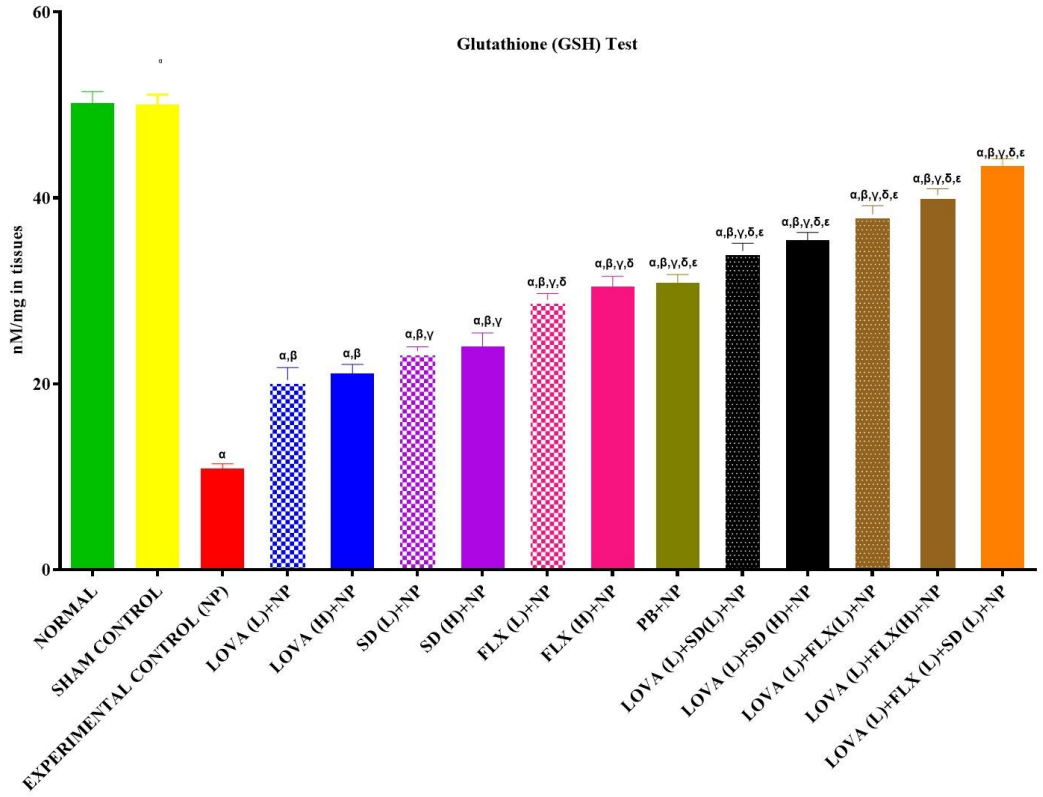


Figure 42. GSH Test (µmol/min/mg of tissues): Effect of treatment on GSH Test (µmol/min/mg of tissues) in a SNL induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s sham control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

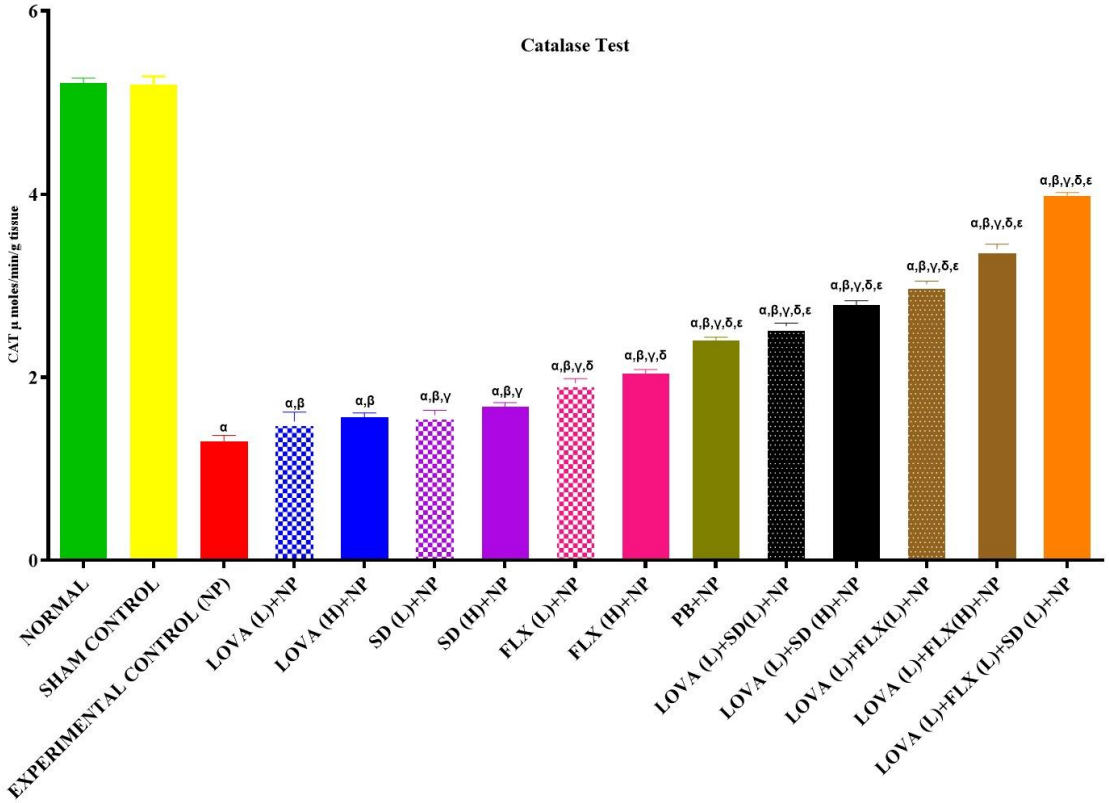


Figure 43. Catalase Test (µmoles/min/g of tissues): Effect of treatment on Catalase Test (µmoles/min/g of tissues) in a SNL induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s sham control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

Catalase activity, measured in µmoles/min/g tissue, was highest in the normal group (5.21 ± 0.057 µmoles/min/g tissue), reflecting optimal enzymatic function. A minor reduction was noted in the NP group (5.20 ± 0.087 µmoles/min/g tissue), suggesting oxidative stress. A significant decline was observed following LOVA administration, with activity levels dropping to 1.30 ± 0.065 µmoles/min/g tissue (Lova (L) + NP) and 1.52 ± 0.1 µmoles/min/g tissue (Lova (H) + NP). SD treatment similarly reduced catalase levels (1.56 ± 0.052 µmoles/min/g tissue for SD (L) + NP, 1.59 ± 0.051 µmoles/min/g tissue for SD (H) + NP)). FLX treatment mitigated this decline (FLX (L) + NP: 1.68 ± 0.043 µmoles/min/g tissue, FLX (H) + NP: 1.94 ± 0.045 µmoles/min/g tissue), while PB + NP (2.04 ± 0.045 µmoles/min/g tissue) exhibited additional improvement. Combination therapies showed superior efficacy, as demonstrated by LOVA (L) + SD (L) + NP (2.40 ± 0.038 µmoles/min/g tissue) and LOVA (L) + SD (H) + NP (2.56 ± 0.032 µmoles/min/g tissue). Further gains were seen with LOVA (L) + FLX (L) + NP (2.79 ± 0.045 µmoles/min/g tissue) and LOVA (L) + FLX (H) + NP (3.02 ± 0.029 µmoles/min/g tissue). The most substantial increase was recorded in LOVA (L) + FLX (L) + SD (L) + NP (3.98 ± 0.039 µmoles/min/g tissue), indicating a synergistic effect. These findings emphasize the role of oxidative stress and suggest that combination therapies enhance enzymatic recovery, potentially offering better neuroprotection (Figure 43).

6.11.5. SOD Activity

The observed SOD levels highlight marked changes across the experimental groups following SNL ligation. The normal group exhibited the highest SOD level at 17.11±0.23, representing the baseline (healthy conditions). In contrast, the NP group showed a substantial decrease, with SOD levels dropping to 5.39 ± 0.26 U/mg, reflecting the severe oxidative stress caused by SNL ligation. With the dose of LOVA groups, low-dose LOVA (L) increased SOD to 9.78 ± 0.19 U/mg, while high-dose LOVA (H) showed a marginal increase to 9.89 ± 0.18 U/mg. These results indicate that LOVA helps in restoring SOD activity, albeit with a limited dose-dependent effect. The SD groups demonstrated a greater recovery in SOD activity compared to LOVA. Low-dose SD (L) increased SOD levels to 11.32 ± 0.43 U/mg, while high-dose SD (H) further enhanced it to 11.83 ± 0.16 U/mg, suggesting that SD is more effective in improving antioxidant activity after SNL ligation.

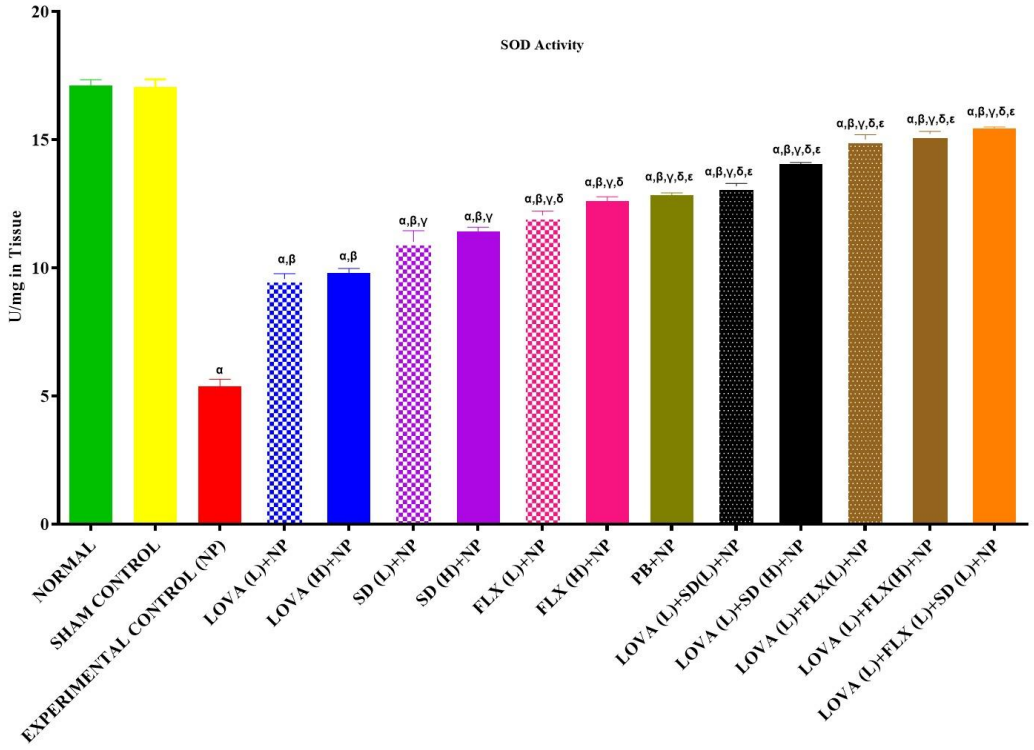


Figure 44. SOD Level (U/mg of Tissues): Effect of treatment on SOD Level (U/mg of Tissues): in a SNL induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s sham control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

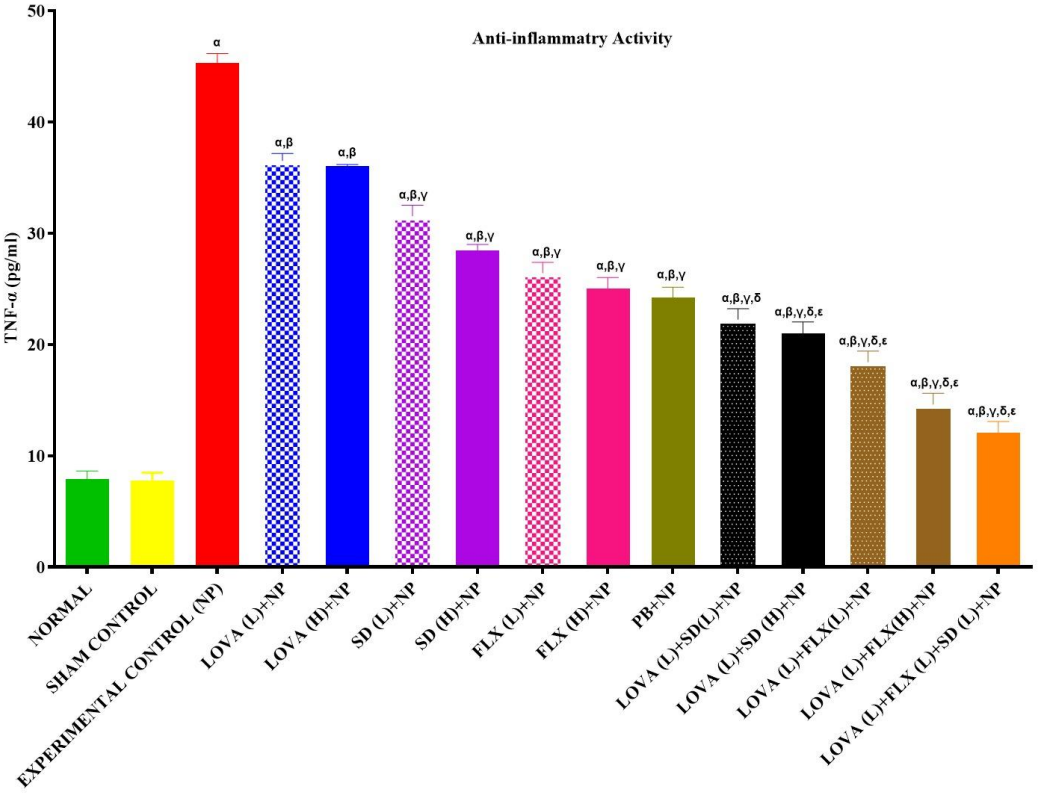


Figure 45. Determination of TnF-α Test (pg/ml of tissues): Effect of treatment on TnF-α Test (pg/ml of tissues) in a SNL induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s sham control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

Moreover, the FLX groups showed even better results. Low-dose FLX (L) raised SOD levels to 12.05 ± 0.16 U/mg, and high-dose FLX (H) increased it further to 12.59 ± 0.18 U/mg, indicating a clear dose-dependent improvement in SOD activity with FLX treatment. U/mg, The PB group exhibited SOD levels of 12.82 ± 0.74 U/mg, slightly higher than the FLX groups, suggesting that PB treatment. The combination of low-dose LOVA and SD achieved SOD levels of 12.62 ± 0.098 U/mg, while combining low-dose LOVA with high-dose raised it further to 12.89 ± 0.065 U/mg. Adding FLX to LOVA produced even greater improvements, with the combination of low-dose LOVA and FLX increasing SOD to 14.3 ± 0.18 U/mg, and the high-dose FLX combination reached 14.61 ± 0.088 U/mg. The highest SOD level was observed in the group receiving low-dose SD, FLX, and LOVA, which achieved a level of 15.34 ± 0.056 U/mg, demonstrating the strongest synergistic effect among all treatments (Figure 44).

6.11.6. TnF-α level

TNF-α levels were markedly elevated in the NP group (45.3 ± 0.95 pg/mL) compared to the normal group (7.87 ± 0.67 pg/mL), indicating a strong inflammatory response associated with NP. Treatment with Lova (L) + NP led to a reduction (36.52 ± 0.65 pg/mL), while Lova (H) + NP produced a further slight decline (36.01 ± 0.18 pg/mL). Administration of SD also contributed to decreasing TNF-α levels, with SD (L) + NP reducing levels to 31.54 ± 0.98 pg/mL and SD (H) + NP to 28.43 ± 0.055 pg/mL, suggesting a dose-dependent anti-inflammatory effect. A similar trend was observed with FLX, where FLX (L) + NP lowered TNF-α to 26.44 ± 0.94 pg/mL, and FLX (H) + NP further decreased it to 25.02 ± 0.99 pg/mL. The PB + NP group also showed a reduction (24.22 ± 0.92s pg/mL), reinforcing the role of pharmacological intervention in mitigating TNF-α levels. Combination treatments produced a more pronounced anti-inflammatory effect. Lova (L) + SD (L) + NP resulted in a reduction to 22.26 ± 0.95 pg/mL, while Lova (L) + SD (H) + NP further decreased TNF-α levels to 21.01 ± 1.02 pg/mL. The co-administration of Lova (L) + FLX (L) + NP led to a further decline (18.43 ± 0.98 pg/mL), whereas Lova (L) + FLX (H) + NP resulted in a more significant reduction (14.63 ± 1.00 pg/mL). The most substantial decrease was observed in the Lova (L) + FLX (L) + SD (L) + NP group, where TNF-α levels dropped to 12.06 ± 1.01 pg/mL, indicating a strong synergistic effect of combination therapy. These findings suggest that TNF-α is significantly elevated in NP, reinforcing its role in neuroinflammation (Figure 45). However, pharmacological interventions, particularly combination treatments, effectively

attenuate TNF-α expression, potentially providing an effective therapeutic approach to reducing inflammation and pain associated with neuropathy.

6.11.7. IL6

IL-6 levels showed a slight low in the diseased (7.41 ± 0.75 pg/mL) compared to the normal group (7.54 ± 0.65 pg/mL). However, treatment with Lova (L) + NP resulted in a substantial increase (35.76 ± 0.076 pg/mL), while a higher dose of Lova (H) + NP led to a marked reduction (22.29 ± 0.097 pg/mL), suggesting dose-dependent modulation of IL-6 expression. SD also demonstrated a suppressive effect on IL-6 levels, with SD (L) + NP reducing IL-6 to 21.23 ± 0.065 pg/mL and SD (H) + NP further lowering it to 19.32 ± 0.058 pg/mL. Similarly, FLX treatment resulted in 18.36 ± 0.043 pg/mL for FLX (L) + NP and 16.23 ± 0.054 pg/mL for FLX (H) + NP, indicating a progressive decline with higher doses. The PB + NP group exhibited a moderate reduction, with IL-6 levels at 15.44 ± 0.039 pg/mL. Combination treatments produced a more pronounced decline in IL-6 levels (Figure 46). Lova (L) + SD (L) + NP led to a reduction (14.04 ± 0.054 pg/mL), while Lova (L) + SD (H) + NP further lowered IL-6 to 12.99 ± 0.087 pg/mL. The co-administration of Lova (L) + FLX (L) + NP resulted in IL-6 levels of 11.52 ± 0.049 pg/mL, while Lova (L) + FLX (H) + NP reduced it to 10.97 ± 0.059 pg/mL. The most significant suppression was observed in Lova (L) + FLX (L) + SD (L) + NP (9.35 ± 0.039 pg/mL) and Lova (L) + FLX (L) + SD (H) + NP (9.05 ± 0.26 pg/mL). These findings indicate that IL-6 levels increased under neuropathic conditions, but treatment with Lova, SD, and FLX helped mitigate this effect. Notably, combination therapies exhibited a more profound suppressive impact on IL-6 expression, suggesting a potential synergistic mechanism in reducing neuroinflammation associated with NP (Figure 46).

6.11.8. BDNF level

BDNF levels were notably elevated in the NP group (5.76 ± 0.43 pg/ml) compared to the normal group (5.1 ± 0.34 pg/ml). Administration of Lova (L) + NP led to a substantial increase (62 ± 0.23 pg/ml), while Lova (H) + NP significantly lowered BDNF levels (42 ± 0.43 pg/ml). Similarly, treatment with SD (L) + NP and SD (H) + NP resulted in a progressive decline in BDNF levels, measuring 40 ± 0.42 pg/ml and 35 ± 0.33 pg/ml, respectively.

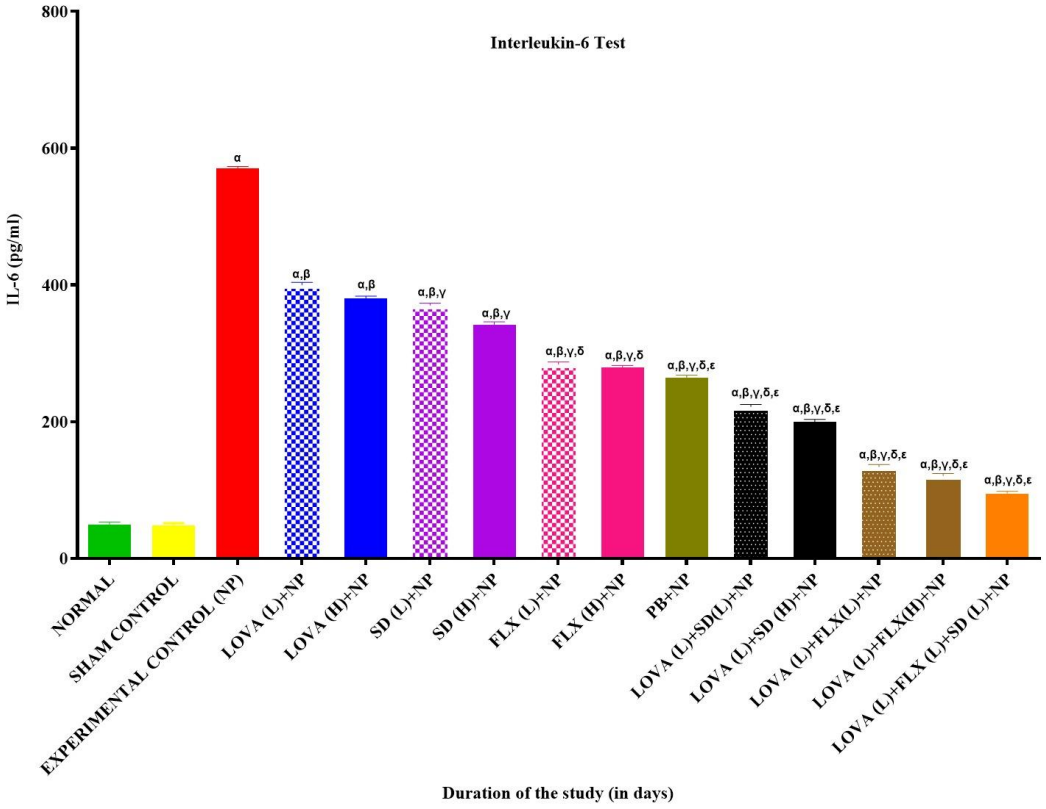


Figure 46. Determination of IL-6 Test (pg/ml of tissues): Effect of treatment on IL-6 Test (pg/ml of tissues) in a SNL induced NP model in rats. The statistics presented as mean and SEM, rats, α=p<0.05v/s sham control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

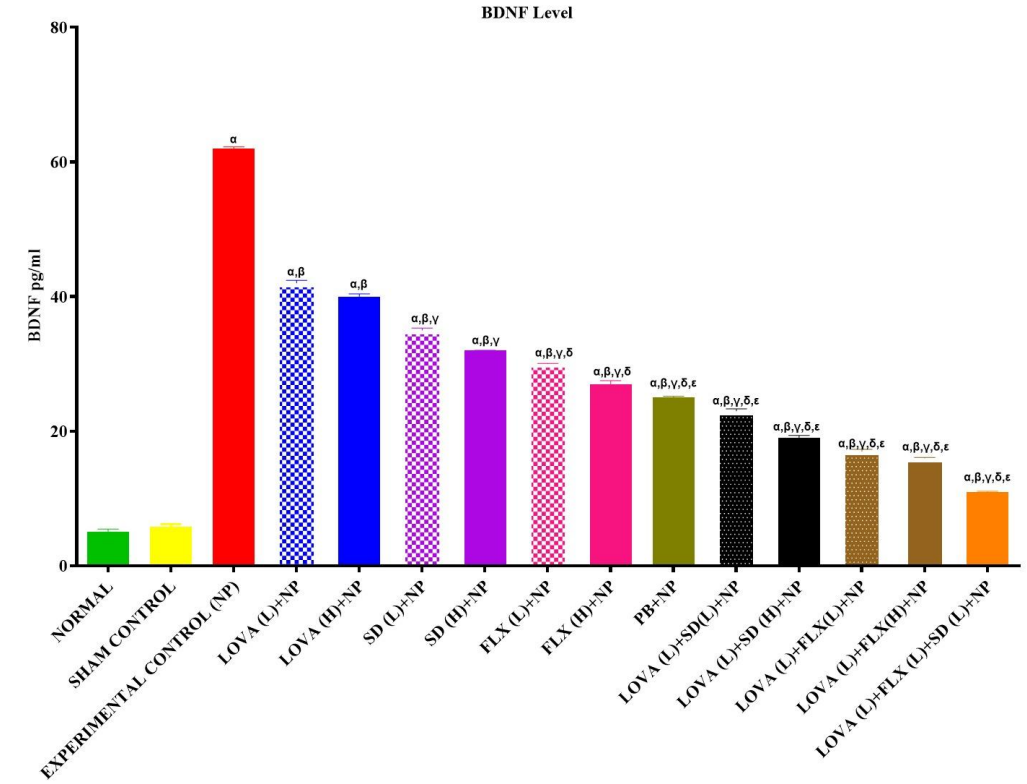


Figure 47. Determination of BDNF Level (pg/ml of Tissues): Effect of treatment on BDNF Level (pg/ml of Tissues) in **a SNL induced NP model in rats.** The statistics presented as mean and SEM, rats, α=p<0.05v/s sham control, β=p<0.05v/s EC, γ=p<0.05v/s $LOVA_L$, δ=p<0.05v/s SD_L, ε=p<0.05v/s FLX_L

Further reduction were observed following FLX (L) + NP (32 ± 0.044 pg/ml) and FLX (H) + NP (30 ± 0.041 pg/ml) treatments. The PB + NP group exhibited a decline to 27 ± 0.52 pg/ml, demonstrating a moderate effect. Combination therapies showed more pronounced reductions, with Lova (L) + SD (L) + NP at 25 ± 0.21 pg/ml, and Lova (L) + SD (H) + NP further lowering BDNF levels to 23 ± 0.31 pg/ml. A greater reduction was observed in Lova (L) + FLX (L) + NP (19 ± 0.37 pg/ml) and Lova (L) + FLX (H) + NP (17 ± 0.32 pg/ml) groups. The most substantial decrease in BDNF levels was seen in Lova (L) + FLX (L) + SD (L) + NP (11 ± 0.084 pg/ml), indicating a synergistic effect of combined drug therapies in modulating BDNF expression in neuropathic conditions. This data suggests that while monotherapies with Lova, SD, and FLX contribute to lowering BDNF levels, their combination results in a more profound reduction, potentially offering a more effective therapeutic strategy for mitigating NP-related neuroinflammation.

Adding FLX to LOVA resulted in even greater reductions, with the combination of low-dose LOVA and decreasing levels to 178 ± 3.0 pg/ml, and the high-dose FLX combination lowering it to 164 ± 3.41 pg/ml. The most reduction observed in the combination of low-dose of SD, FLX and LOVA, which achieved the lowest level at 111 ± 3.78 pg/ml, demonstrating the strongest synergistic effect among all treatments in reducing inflammation (Figure 47).

In the SNL-induced neuropathy model, this study identified notable oxidative stress and inflammation, reflected in altered levels of catalase, SOD, MDA, protein, IL-6, and TNF-α. The findings demonstrate that SD, FLX and LOVA especially in combination, can effectively mitigate these pathological changes. LOVA, showed moderate improvements in oxidative stress and inflammation, with limited dose-dependent effects, indicating its role as a supportive therapy. SD, outperformed LOVA in reducing oxidative damage and inflammation, highlighting its efficacy as a primary therapeutic agent. FLX, was the most effective single treatment, showing consequential improvements in both oxidative stress and inflammatory markers. Combination therapy involving SD, FLX and LOVA yielded the best results, with notable reductions in oxidative stress and inflammation and improved protein integrity. The low-dose combination of these drugs exhibited the strongest synergistic effects, offering enhanced neuroprotection compared to individual treatments. This study underscores the significance of multi-targeted therapy in managing neuropathy lies in its combined antioxidant, anti-inflammatory, and neuroprotective effects, which form a strong foundation for developing effective combination treatments. Notably, the amelioration of NP was more pronounced in rats that received low-dose statin and FLX (both high and low doses) from day

14 onwards. Additionally, a low-dose combination of FLX, SD, and LOVA demonstrated earlier improvements, with noticeable pain relief observed from day 7. This suggests that lower drug concentrations provided a stronger response during the early stages of pain development. Furthermore, rats that received low-dose LOVA and low-dose SD showed a greater therapeutic effect compared to the PB-treated group, particularly from day 21 onward. In contrast, individual drug treatments only began to show marked ameliorative effects by day 28. Among the individual treatments, FLX at both its doses exhibited superior pain reduction compared to other monotherapies. These findings highlight the enhanced efficacy of low-dose combination therapies in early-stage neuropathy while also demonstrating that FLX monotherapy outperforms other individual treatments in reducing NP symptoms.

6.12. Histopathological studies

The histopathological outcome of the present study revealed significant nerve damage in the experimental control group subjected to SNL, characterized by extensive axonal degeneration, inflammatory cell infiltration, fibrosis, and edema. In contrast, the normal control group exhibited intact and well-organized nerve fibers without any pathological changes. Similarly, the sham control group showed no major structural abnormalities, confirming that the observed damage was due to nerve injury rather than the surgical procedure itself (Figure 48). Treatment with pregabalin demonstrated moderate nerve protection, with reduced inflammatory infiltration and partial structural recovery, indicating its neuroprotective effect against SNL-induced injury. Monotherapies with SD, FLX and LOVA at both low and high doses showed varying degrees of histopathological improvements. LOVA effectively reduced inflammatory infiltration and axonal degeneration, particularly at higher doses, by inhibiting pro-inflammatory cytokine production. FLX treatment demonstrated moderate recovery, reducing nerve edema and inflammatory changes, likely through its serotonergic pathway modulation and inhibition of glial cell activation. LOVA showed protective effects by mitigating nerve damage and promoting structural recovery, attributed to enhanced NO availability and improved peripheral blood flow. Combination therapies yielded superior outcomes compared to individual treatments. Dual therapies involving low doses and high doses of LOVA (L) and FLX (L and H), as well as LOVA (L) and SD (L and H), displayed better nerve fiber organization and reduced inflammatory markers. Notably, the triple combination of low-dose SD, FLX and LOVA provided the most significant protection against SNL-induced histopathological changes.

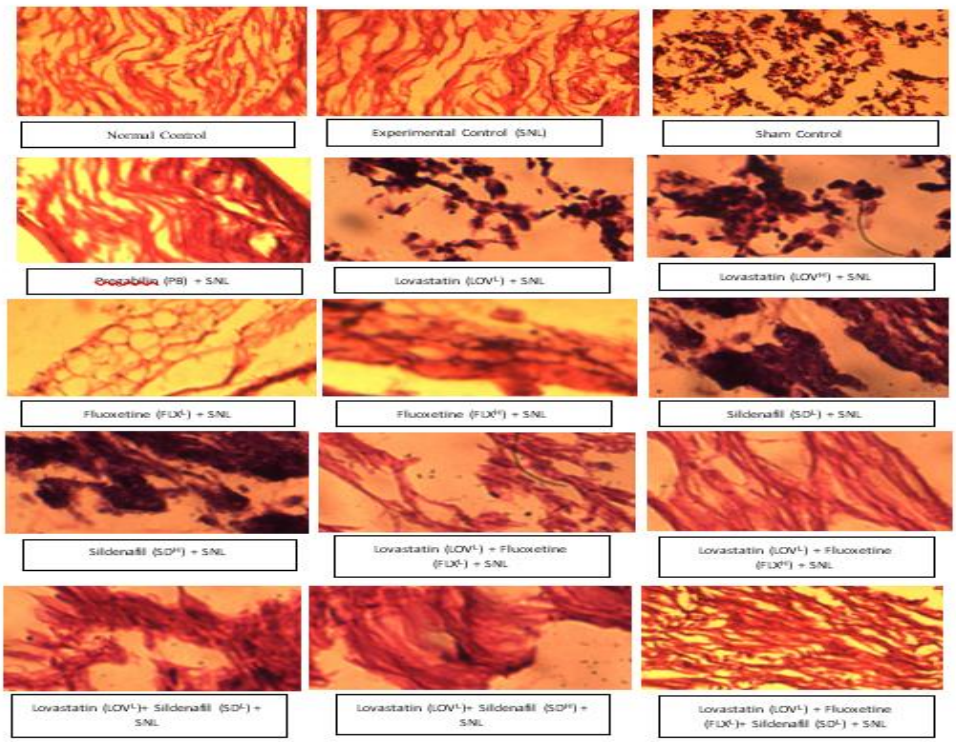


Figure 48. Histopathological study of the sciatic nerve SNL induced NP model: Effect of SD, FLX and LOVA alone and/or in combination

This combination resulted in markedly reduced axonal degeneration, improved nerve structure, and minimal inflammatory cell infiltration, indicating a synergistic effect among the drugs. The enhanced efficacy of the triple therapy likely stems from complementary mechanisms, including reduced oxidative stress and inflammation, serotonergic modulation, and vasodilatory effects. These findings underscore the potential of multi-target pharmacological approaches in mitigating nerve injury and NP. It is noteworthy that significant alleviation of NP (NP) was observed in rats treated with the combinations of LOVA (L)+ FLX (high dose) as well as LOVA (L)+ FLX (L) from the 14th day onward. Remarkably, the combination of SD (L)+ FLX (L)+ LOVA (L) demonstrated therapeutic improvements as early as the 7th day, indicating that low-dose combinations are more effective in preventing pain at an early stage. The group receiving SD (L)+ FLX (L)+ LOVA (L)exhibited superior outcomes compared to the PB-treated group. Additionally, SD at both low and high doses, when combined with low-dose LOVA, demonstrated more pronounced benefits than PB treatment, starting from the 21st day. In contrast, individual treatments required a longer duration, with noticeable improvements emerging around the 28th day. Among the single-drug treatments, FLX at both low and high doses yielded better pain relief compared to individual treatments with either SD or LOVA at their respective doses.

Furthermore, drug interventions played a protective role in maintaining oxidative biomarkers, such as MDA, SOD, GSH, and CAT, while also modulating inflammatory markers, including TNF-α and IL-6, along with BDNF levels.

Additionally, the LOVA, an HMG-CoA reductase inhibitor, contributes to neuroprotection by stabilizing neuronal membranes and improving synaptic function through cholesterol regulation. It promotes anti-apoptotic signaling and supports eNOS activation for enhanced NO production and vasodilation by activating the PI3K/Akt pathway. LOVA also downregulates inflammatory pathways mediated by NF-κB and TLR4, thereby reducing level of TNF-α and IL-6. Additionally, it strengthens antioxidant systems by increasing SOD and catalase levels while decreasing lipid peroxidation. The combined actions of these drugs effectively inhibit microglial and astrocytic activation, suppressing inflammation and oxidative stress. FLX further regulates TRPV and AMPA receptor activities, stabilizing synaptic transmission, while SD and LOVA maintain ionic balance through the Na+/Ca2+ exchanger, preventing cellular injury. Together, their multifaceted mechanisms synergistically provide robust neuroprotection, offering an effective strategy for managing NP and fostering nerve repair.

CHAPTER 8

SUMMARY AND CONCLUSION

8.1. Summery and Conclusion

This study demonstrates the remarkable therapeutic potential of combination drug therapies in managing NP, particularly the synergistic effects of low-dose combinations of SD, FLX and LOVA. The findings highlight a clear efficacy hierarchy, where combination therapies consistently outperformed individual treatments, providing superior pain relief and neuroprotection. The research employed two distinct experimental models: the SNL model and the HFD combined with a low-dose STZ model. These models were selected for their ability to replicate different aspects of NP pathology. The SNL model mimicked traumatic nerve injury, characterized by heightened pain sensitivity, impaired motor coordination, and severe neuroinflammation. In contrast, the HFD + STZ model simulated metabolic dysfunction-induced neuropathy, which exacerbated neuropathic symptoms and biochemical imbalances, including oxidative stress and inflammatory marker elevations. In untreated SNL models, animals exhibited severe nerve damage, heightened pain sensitivity, impaired motor coordination, and significant biochemical imbalances, including elevated pro-inflammatory markers and oxidative stress. The metabolic dysfunction model (HFD + STZ) demonstrated even more pronounced neuropathic impairments due to the combined impact of diet-induced metabolic stress and low-dose STZ toxicity.

PB offered moderate neuroprotection but did not achieve the same level of improvement as more advanced combination strategies (combination of SD, FLX and LOVA). Monotherapies with low doses of LOVA, FLX, or SD demonstrated some therapeutic benefits, with higher doses yielding more pronounced effects on pain relief and oxidative stress reduction. However, these individual treatments required longer durations to achieve meaningful improvements. Dual combinations of low-dose treatments, such as SD, FLX and LOVA, this regimen achieved near-complete normalization of behavioral responses, substantial reductions in pro-inflammatory cytokines (TNF-α, IL-6), and restoration of antioxidant defences (SOD, GSH). The triple combination’s ability to target multiple pathways simultaneously, including neuroprotective, anti-inflammatory, serotonergic, and vasodilatory mechanisms underscores its unparalleled efficacy in alleviating NP symptoms. Additionally, the study highlighted the detrimental impact of an HFD combined with low-dose STZ, which exacerbated neuropathic symptoms and biochemical dysfunctions. This finding emphasizes the importances of lifestyle factors in modulating NP severity.

In conclusion, the results of this research underscore the therapeutic superiority of multi-targeted pharmacological approaches for NP management. The inclusion of both traumatic and metabolic dysfunction models provided a comprehensive understanding of NP pathophysiology and treatment responses. The triple low-dose combination of SD, FLX and LOVA stands out as a promising strategy, offering earlier and more sustained pain relief compared to conventional treatments. These findings provide a strong foundation for future clinical studies aimed at optimizing combination therapies for patients with NP, ultimately advancing more effective and holistic pain management solutions.

This study highlights the significant therapeutic potential of low-dose combination drug therapies in managing neuropathic pain (NP). Notably, the combination of SD (L) + FLX (L) + LOVA (L) demonstrated the earliest improvement, with a pronounced reduction in NP symptoms by day 7. This suggests the effectiveness of multi-targeted interventions during the early stages of pain development. Additionally, the combinations of LOVA (L) + FLX (H) and LOVA (L) + FLX (L) showed substantial amelioration from day 14, emphasizing the role of these drug pairings in providing timely relief. In contrast, SD (L) and SD (H) combined with low-dose LOVA exhibited better effects compared to PB, with notable improvements observed by day 21. Individual drug treatments, including SD at both doses, required longer durations, showing measurable effects only by day 28. Among the monotherapies, FLX provided superior results compared to SD and LOVA in alleviating NP symptoms. In the diabetic neuropathy model induced by HFD and low-dose STZ, type II diabetes was characterized by increased body weight, mild hyperglycaemia, hypertriglyceridemia, and elevated cholesterol. The combination of SD (L) + FLX (L) + LOVA (L) delivered significant symptom reduction by day 21, whereas the combinations of SD + LOVA, FLX + LOVA, and PB showed improvements only by day 28. FLX (H) + LOVA (L) proved superior compared to FLX (L) + LOVA (L), SD (L) + LOVA (L), and PB treatments.

Further, improvements were observed across biochemical parameters, such as LDL, HDL, triglycerides (TG), and total cholesterol (TC). The drug treatments effectively protected oxidative biomarkers, including MDA, SOD, GSH, and CAT, while also modulating inflammatory markers TNF-α, IL-6, and BDNF. In summary, the findings underscore the importance of early intervention with low-dose combination therapies for superior NP management and highlight their superiority over traditional treatments like PB, offering promising avenues for future research and clinical applications.

8.2. Future recommendations

The combination of SD, FLX, and LOVA represents a promising and novel approach to NP treatment. By targeting key factors such as vascular health, inflammation, oxidative stress, and pain perception, it offers a well-rounded solution to address the complex nature of this condition. Additionally, using lower doses of each drug in combination reduces the likelihood of side effects, enhancing the safety and tolerability of the therapy for patients. This groundbreaking combination has the potential to transform the management of NP, bringing hope to those who have not experienced relief from conventional treatments. With further clinical research and validation, it could set a new benchmark for treating this challenging and debilitating condition.

There are several models for the development of NP and each model emphasizes different pathological aspects of development of NP. In the present investigation individual as well as co-administration of SD, FLX and LOVA were administered to investigate their impact in amelioration of NP. In reliance on preventive approach of the treatment, therefore should be more as compared to that on curative approach.

Therefore, further extensive molecular studies as well as completely designed prospective preclinical and clinical trials are warranted to establish any therapeutic role for SD, FLX and LOVA in NP in different models. Furthermore, there is requirement to understand the formulations prospective to provide sufficient relief from NP in humans.

CHAPTER 7
DISCUSSION

7.1. Interpretation of the study

A high-fat diet causes rats to become obese and insulin resistant over time. A low dose of streptozotocin (STZ), usually 30–35 mg/kg, causes partial damage to but does not totally destroy pancreatic β-cells (Ahlawat & Sharma, 2018). Type 2 Diabetes Mellitus (T2DM), which is typified by hyperglycemia brought on by decreased insulin production and increased insulin resistance, is mimicked by this combination. Nerve injury is caused by oxidative stress, the production of advanced glycation end products, and the activation of inflammatory pathways brought on by prolonged hyperglycemia (Nguyen et al., 2012; Schreiber, 2015). Because neuropathic pain is caused by altered pain signaling pathways and degeneration of nerve fibers, it presents as hypersensitivity to mechanical and thermal stimuli. Damage to peripheral nerves is the source of diabetes-induced neuropathic pain (DNP) (Fuchs et al., 2010), a chronic consequence of diabetes. Hyperalgesia, or a heightened sensitivity to pain, and allodynia, or discomfort from non-painful stimuli, are some of its symptoms. Numbness and burning feelings, frequently in the extremities are another consequences of DNP (Mitsikostas et al., 2022). DNP impairs quality of life and is brought on by chronic hyperglycemia that results in oxidative stress, nerve ischemia, and microvascular problems. The development of diabetic neuropathic pain involves several interconnected mechanisms:

1. Oxidative Stress: Hyperglycemia increases the production of reactive oxygen species (ROS), damaging neurons and Schwann cells (Bhatti et al., 2022).
2. Inflammation: Elevated levels of proinflammatory cytokines (e.g., TNF-α, IL-6) activate inflammatory cascades in nerve tissues (Kany et al., 2019).
3. Advanced Glycation End Products (AGEs): Hyperglycemia accelerates AGE formation, impairing nerve conduction and causing structural damage (Rungratanawanich et al., 2021).
4. Polyol Pathway Activation: Excess glucose is shunted into the polyol pathway, leading to sorbitol accumulation, osmotic stress, and reduced nerve cell function (Berrone et al., 2006).
5. Impaired Neurovascular Function: Reduced blood flow to nerves causes ischemic damage (Hu et al., 2017).
6. Neurochemical Alterations: Imbalances in neurotransmitters like glutamate lead to abnormal pain signaling (Teleanu et al., 2022).

On the other hand, Several inflammatory chemicals stimulate the glial cells (microglia and astrocytes) in the spinal dorsal horn after nerve damage, and primary afferent terminals discharge inflammatory mediators into the spinal cord (Liang et al., 2019; Sommer et al., 2018). NP results from glial activation, which causes pro-inflammatory reactions with pathological consequences include neuronal hyperexcitability, neurotoxicity, and chronic inflammation (Authier et al., 2000; Castro-Lopes et al., 1993; de Santana Nunes et al., 2016; Wang et al., 2018). The spinal nerve ligation (SNL) paradigm produces symptoms of hyperalgesia and chronic pain (Kumar et al., 2018; Pottabathini et al., 2016). The SNL model, which was first presented by Kim et al. in 1992, was created by securely tying the L5 or L5 and L6 spinal nerves together (S. H. Kim et al., 1993; LaBuda & Little, 2005). Like the CCI and PSL models, the SNL model induces partial denervation of the sciatic nerve, affecting afferent axons of all sizes uniformly. However, unlike the PSL model, the SNL model involves injury at the spinal nerve level (L5 and L6) (K. J. Kim et al., 1997), At the point where the dorsal and ventral roots merge, located beyond the dorsal root ganglia but before the lumbar plexus, spinal nerves are organized into different peripheral nerves. Most afferent axons within the sciatic nerve extend their central branches into the fourth and fifth lumbar dorsal roots (Fried et al., 1993; Polomano et al., 2001). Earlier investigation of the SNL model showed a considerable increase in the number of microglia, the major immune cells in the central nervous system (CNS), within the L5 spinal dorsal horn. Activated microglia can release a variety of inflammatory mediators, such as TNF-α and IL-1β, by activating relevant inflammatory signaling pathways like nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) (Liang et al., 2019; Zhao et al., 2017). These mediators support neuronal hyperexcitability and play a role in the development and course of NP. The main characteristics of NP were allodynia, hyperpathia, and spontaneous pain; behavioral tests related to pain were used as a means of gauging the degree and course of pain (Melkani et al., 2019). According to studies, rats in NP models including CCI, SNL, and sparing nerve injury (SNI) exhibit notable spontaneous pain responses such elevating and licking their feet on their own, as well as notable reductions in the thresholds for mechanical pain and cold allodynia (Dupuis et al., 2017; Melkani et al., 2019; Shankarappa et al., 2012).

One of the main benefits of the SNL model is that the fourth lumbar dorsal root provides access to the sciatic nerve's undamaged afferent axons (Dupuis et al., 2017; Schäfers & Cain, n.d.).

Tests for motor coordination, paw heat-hyperalgesia (hot plate test), paw cold-allodynia (acetone drop test), mechanical hyperalgesia (pin prick test), and tail cold hyperalgesia (tail immersion test) were among the factors assessed in this study (Kaur et al., 2017; Melkani et al., 2019). The Pin Prick Test measures the withdrawal reaction to a harsh stimulation in order to evaluate mechanical hyperalgesia. shows the degree of sensory sensitivity and nerve damage (Kaur et al., 2017; Melkani et al., 2019). Through the monitoring of tail withdrawal latency from cold water, the Tail Immersion Test aids in the measurement of thermal nociception aids in measuring cold hyperalgesia in neuropathic disorders (Ling et al., 2007; Pottabathini et al., 2016). The Rota Rod Test aids in determining balance and motor coordination. utilized to assess neuropathy-related motor impairments and nerve function (Kaur et al., 2017; Leonard et al., 2016). Conversely, the Heat Hyperalgesia and Heat Allodynia Tests use a hot plate to test thermal sensitivity, which indicates altered pain threshold and injury to peripheral nerves. The results of this study revealed that diabetic rats exhibited lower response in mechanical sensitivity tests, which indicated neuropathic pain, decreased reaction times in heat sensitivity tests, which indicated hyperalgesia, and impaired motor function. All of these changes in behavior are the result of nerve damage brought on by inflammatory and oxidative stress. Obesity and hyperglycemia cause motor impairments and a changed perception of pain. When animals are exposed to a cold stimuli in the acetone drop test, their paw withdrawal latency indicates their response to allodynia. It provides important information about identifying irregularities in the senses. By calculating the latency of paw withdrawal from a warm surface, the Hot Plate Test detects thermal hyperalgesia. It is recognized as a sign of heat sensitivity and pain threshold (Melkani et al., 2019).

7.2. SD

Acts as a phosphodiesterase type 5 (PDE5) inhibitor, increasing intracellular cyclic guanosine monophosphate (cGMP) levels, which helps reduce nociception by modulating calcium channel activity. SD is known to alleviates oxidative stress and inflammation, both of which are key contributors to neuropathic pain. It helps in enhancement of neuroprotection by restoring nerve conduction and reducing nerve damage. As per the previous published reports by raising cyclic guanosine monophosphate (cGMP), sildenafil decreases nociception and modifies calcium influx. It has demonstrated efficacy in reducing allodynia and hyperalgesia by reestablishing nerve and vascular function. It enhances neurotransmitter activation, which enhances the effects of fluoxetine.

7.3. FLX

It is also a well known drug for the treatment of neuropathic pain. It functions as a selective serotonin reuptake inhibitor (SSRI), increasing serotonin levels and modulating central pain pathways. It demonstrates anti-inflammatory and antioxidant effects, contributing to pain relief. It also improves behavioral and sensory responses in neuropathic pain models. By modifying serotonin levels in central pain pathways, fluoxetine lessens central sensitization and hyperalgesia. Literature review also suggests that by lowering cytokine release and microglial activation, fluoxetine has anti-inflammatory properties. Patients with neuropathic pain, who frequently suffer from co-occurring anxiety or depression, benefit from its antidepressant and mood-stabilizing qualities.

7.4. LOVA

helped in the treatment diabetes-induced neuropathic pain (DNP). It has been demonstrated that lovastatin lowers pro-inflammatory cytokine levels, including IL-6 and TNF-α. These cytokines encourage nerve inflammation and destruction, which is a major factor in the development of diabetic neuropathic pain. Oxidative stress brought on by hyperglycemia is a factor in diabetes-induced neuropathy. By enhancing antioxidant defense mechanisms, such as raising superoxide dismutase (SOD) activity and lowering malondialdehyde (MDA) levels, lovastatin can lessen oxidative damage. These effects lessen the symptoms of neuropathic pain and protect nerve integrity. In diabetic animals, lovastatin may increase nerve fiber density and restore nerve conduction velocity (NCV). This helps to lessen neuropathic pain and sensory deficiencies. By altering pain signaling pathways, lovastatin can reduce hyperalgesia and allodynia. This involves the suppression of excitatory pathways that are triggered by oxidative damage and chronic hyperglycemia. A common complication of diabetes, dyslipidemia, makes nerve injury in DNP worse. By lowering serum cholesterol and triglyceride levels, lovastatin's lipid-lowering properties indirectly prevent neuropathy from worsening.

According to earlier research, lovastatin's synergistic anti-inflammatory and antioxidant properties help fluoxetine's pharmacological action in treating neuropathic pain. While fluoxetine affects serotonin levels, lowering central sensitization and enhancing pain perception, lovastatin lowers oxidative stress and enhances vascular function, preserving peripheral neurons. When combined, they increase motor capabilities, reduce hyperalgesia

and allodynia, and strengthen neuroprotection. By increasing neuronal perfusion, lovastatin also promotes improved neurotransmitter activity. Together, they offer a thorough strategy for reducing diabetic neuropathic pain. Combination therapy leverages the strengths of multiple mechanisms, providing a holistic approach to managing neuropathic pain. This not only improves efficacy but also ensures better patient outcomes with reduced adverse effects, making it superior to individual therapies. Here in our present investigation, the combination therapy demonstrates additive or synergistic effects**,** resulting in faster and more pronounced attenuation of neuropathic pain compared to individual drugs. This is particularly evident in behavioral parameters, such as reduced hyperalgesia and allodynia, observed earlier in combination-treated groups.

Statins are widely used not only for treating dyslipidemia but also in managing conditions like coronary artery disease, diabetes, stroke, hypertension, and chronic kidney disease, even in the absence of abnormal lipid levels. Beyond lowering cholesterol, statins offer various additional benefits, known as pleiotropic effects, such as reducing inflammation and oxidative stress, modulating the immune response, regulating the cell cycle, and influencing cell growth and programmed cell death (Liao & Laufs, 2005). Statins, originally derived from fungi, act as potent cholesterol-lowering agents by blocking the enzyme HMG-CoA reductase, which plays a crucial role in the biosynthesis of sterols, including cholesterol (Melkani et al., 2024). Statins are categorized into two types—lipophilic and hydrophilic—both exhibiting pleiotropic properties. Lipophilic statins, such as lovastatin, simvastatin, fluvastatin, atorvastatin, and pitavastatin, enter cells through passive diffusion and show low specificity toward liver tissue. In contrast, hydrophilic statins like rosuvastatin and pravastatin depend on active, carrier-mediated transport to enter cells, making them more liver-selective. In many of the studies, statins were used as adjuvant therapy. Adjuvants are the contributing factor that enhances the efficacy of other drugs, or in another way, it can be stated as an agent added to other therapies to boost their effectiveness. The following is the evidence that it enhances immune reaction, development of germinal center,

1. Lovastatin serves as an effective adjuvant to boost the immune response to the H1N1 influenza vaccine. When used alongside the vaccine, lovastatin not only increases the production of H1N1-specific antibodies but also stimulates T-cell activity and cytokine release, indicating a strong mixed Th1/Th2 immune response. Furthermore,

this evidence also provides strong evidence that it helps to enhance the whole immune system due to the development of the germinal centre (Song et al., 2025).

2. It is well known that this drug is commonly used to lower the level of cholesterol; it may enhance immune responses when combined with vaccines and could also have potential benefits in addressing certain mental health disorders (Khatiwada & Hong, 2024).
3. This 6-week randomized placebo-controlled clinical trial was conducted to evaluate the efficacy and safety of lovastatin as an adjuvant agent with fluoxetine in the treatment of major depressive disorder (MDD), and it was reported that lovastatin as an adjuvant treatment may be effective for treating patients with MDD in association with fluoxetine (Ghanizadeh & Hedayati, 2013; Vital et al., 2025).
4. Statins, including lovastatin, are the primary treatment for lowering LDL cholesterol and reducing cardiovascular risk. Lovastatin may also be used in combination with other agents like niacin, fibrates, or intestinal cholesterol absorption inhibitors to enhance lipid control, highlighting its value as part of an adjunctive therapeutic approach in dyslipidemia management (Sorrentino, 2012).
5. Statins, including lovastatin, have shown potential adjunctive benefits in managing neuropathic pain in preclinical models, primarily through cholesterol-independent mechanisms such as anti-inflammatory, antioxidant, and neuromodulatory effects. These findings suggest lovastatin may help alleviate neuropathic symptoms when used alongside standard treatments. However, clinical data reveal a contrasting picture, with some reports linking statin use to the development of neuropathy, highlighting a paradoxical and context-dependent role (Bhalla et al., 2014).
6. In a study by Ghanizadeh and colleagues, 20 mg/day lovastatin was used for eight weeks as adjunctive therapy in the treatment of schizophrenia. However, no changes were observed in the Positive and Negative Syndrome Scale (PANSS) score between the intervention and placebo groups. In this study, no serious adverse events were observed in either group (Ghanizadeh et al., 2014).
7. In many literature, it was reported that statins cause NP, indicating its paradox in the treatment of NP (Attardo et al., 2022). Statins have also been associated with the potential to induce certain adverse effects, a peripheral neuropathy, although this risk is quite low, with an incidence of approximately 12 per 100,000 persons per year, or with a prevalence of 6 per 10,000 persons, or 1 in 10,000 patients treated for 1 year

(Menahem & Shvartzman, 2011; Sikka et al., 2011). Patients on statin therapy may develop a peripheral neuropathy, complaining of numbness, tingling, pain, and tremor in hands or feet, as well as unsteadiness during walking. All these symptoms are usually generated by a long-term therapy (>1 year) (Attardo et al., 2022). Moreover, the incidence of polyneuropathy has been reported more frequently with atorvastatin than with fluvastatin (Al-Kuraishy et al., 2019). This is very important to note that the exact mechanism by which statins can cause neuropathy is still undefined. A possible theory that is sustained in most of the research papers is that statins, inhibiting cholesterol synthesis, may impair the function of nerve membranes (De Langen & Van Puijenbroek, 2006).

7.5. Current study

This combination demonstrated improved attenuation against neuropathic pain in both models, in accordance with the documented pharmacological actions of separate medications.

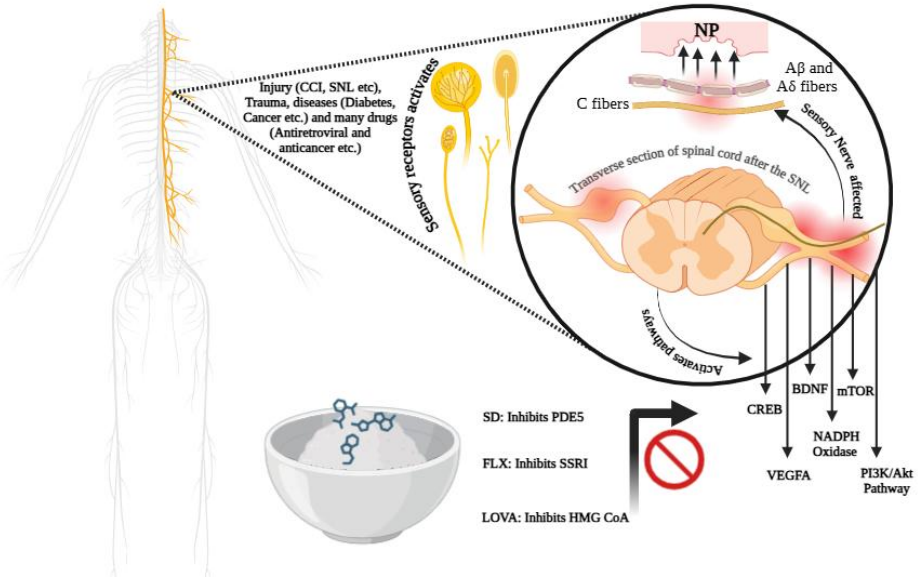


Figure 49s. Effect of combination (SD, FLX and LOVA) of drugs on the NP induced model

This could be because they improve neurochemical regulation, lessen calcium overload, and offer long-lasting neuropathic pain relief. In addition, fluoxetine's control over central pain pathways, sildenafil's enhancement of vascular and nerve function, and lovastatin's combat of inflammation and oxidative stress may be the cause of this exceptional defense against neuropathic pain. Together, these medications provide a comprehensive therapeutic approach by addressing the complex nature of neuropathic pain.

We have not received any findings related to the development of NP by Lovastatin at a dose of 2 and 4mg/kg in HFD with a low dose of STZ-induced NP model in rats. According to Lehrer et al., statins combined with niacin (vitamin B3) may reduce the risk of peripheral neuropathy (Lehrer & H. Rheinstein, 2020). However, early detection of peripheral neuropathy and changing hypercholesterolemia treatment may prevent permanent nerve damage. Similarly, here we are coating a result that the combination of sildenafil, fluoxetine, and lovastatin in concomitant administration to the rats provides better attenuation in both models of neuropathic pain. The administration of combination of drugs or its concomittant traetment ameliorate the pain in the early days as well as bring down the pain almost closer to the normal group of rats.

CHAPTER 9
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APPENDIX

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Dated: 25 Jun 2020

Km Indu Melkani
VID: 41900351
Programme Name: Doctor of Philosophy (Pharmacology)

Subject: Letter of Candidacy for Ph.D.

Dear Candidate,

We are very pleased to inform you that the Department Doctoral Board has approved your candidacy for the Ph.D. Programme on 25 Jun 2020 by accepting your research proposal entitled: "Pharmacological impact of cholesterol-lowering drug as adjuncts to fluoxetine and sildenafil in attenuation of neuropathic pain in rats "

As a Ph.D. candidate you are required to abide by the conditions, rules and regulations laid down for Ph.D. Programme of the University, and amendments, if any, made from time to time.

We wish you the very best!!

In case you have any query related to your programme, please contact Centre of Research Degree Programmes.

Head

Centre for Research Degree Programmes

Note:-This is a computer generated certificate and no signature is required. Please use the reference number generated on this certificate for future conversations.

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Research Article

DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR THE ESTIMATION OF SILDENAFIL, FLUOXETINE, AND LOVASTATIN

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ABSTRACT

Objective: Neuropathic pain (NP) arises from trauma to the somatosensory nervous system and can be managed using selective serotonin reuptake inhibitors, such as fluoxetine (FLX) and phosphodiesterase inhibitors, such as sildenafil (SD), and cholesterol-lowering agents such as lovastatin (LOVA). The present study aimed to develop and validate an analytical method for the simultaneous estimation of these drugs (SD, FLX, and LOVA [SFL]) using reverse-phase high-performance liquid chromatography (RP-HPLC).

Methods: An RP-HPLC method was developed and validated for the quantification of SFL. Chromatographic separation was achieved using a C-18 reverse-phase ODS column with a mobile phase consisting of acetonitrile and 0.2 M ammonium acetate buffer (55:45) in gradient elution mode. The flow rate was maintained at 1 mL/min, and detection was carried out at 228 nm. The method was validated following the ICH Q2 (R2) guidelines, assessing parameters such as linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ).

Results: The developed method exhibited linearity within the concentration range of 20–100 µg/mL, with a regression coefficient (r^2) of 0.9992. Retention times for FLX, SD, and LOVA were recorded at 6.481, 4.238, and 19.778 min, respectively. Recovery studies demonstrated an accuracy range of 94.61–110.44%, with a relative standard deviation of 0.06–2.00%, confirming the precision of the method. The LOD values for FLX, SD, and LOVA were found to be 12.77 µg/mL, 14.81 µg/mL, and 13.28 µg/mL, respectively, while the LOQ values were 45.16 µg/mL, 42.33 µg/mL, and 38.71 µg/mL.

Conclusion: The validated RP-HPLC method met all required validation criteria and demonstrated suitability for the accurate quantification of FLX, SD, and LOVA in pharmaceutical formulations. These findings support the potential use of these drugs as an alternative therapeutic strategy for NP.

Keywords: RP-HPLC, Neuropathic pain, Elution, Analytical method, Validation.

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INTRODUCTION

Chemically, sildenafil (SD), fluoxetine (FLX), and lovastatin (LOVA) are 5-(2-ethoxy-5-((4-methylpiperazin-1-yl)sulfonyl)phenyl)-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d] pyrimidin-7-one 2-hydroxypropane-1,2,3-tricarboxylate [1], N-methyl-3-phenyl-3-(4-trifluoromethyl) phenox propane -1-aminene hydrochloride [2], (1S,3R,7S,8S,8aR)-8-{2-((2S,4R)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl)ethyl} -3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (S2-methyl butanoate, respectively) [3] (Figs. 1 and 2.) SD, a drug for erectile dysfunction [4], alleviated neurotransmission and pain signaling [5] and treated the neuropathic pain (NP) [6]. FLX, an antidepressant [7,8], enhanced serotonin availability, prevented agonizing stimuli, and elevated mood. LOVA, a prodrug, is rendered inactive when administered orally. Statins have been shown in numerous studies to be beneficial in treating a range of neurological conditions. According to published research, statins may help treat neurodegenerative conditions, such as multiple sclerosis (MS) [9], Parkinson's disease [10], and Alzheimer's disease (AD) [11]. According to some research, statins can also lessen the effects of traumatic brain and spinal cord injuries [12,13].

Neuronal injury, direct nervous system damage, or somatosensory nervous system diseases are the causes of NP [6,14]. Paresthesia, hyperalgesia, and allodynia are its hallmarks [6,15]. The complicated process of NP development deteriorates as patients get inadequate treatment. Patients with HIV, rheumatoid arthritis, diabetes, cancer, MS, and brain and spinal cord traumas are at risk for a worsened state of NP [16-20]. As a result of numerous molecular, cellular, and systemic alterations throughout time, the nervous system developed maladaptive reactions and changed nerve function [21]. Neuronal hyperexcitability, cerebral sensitization, immunological activation, and modifications to neurotransmitter systems were among the factors behind this long-term development (Fig. 1a) [19,22]. The peripheral and central nervous systems were affected by these disorders, which resulted in abnormalities in the functioning of sodium and calcium channels as well as imbalances in substances, such as GABA, NMDA, NE/5HT, adrenoreceptors, cytokines, TRPV1, and AMPA/KA. These disturbances led to aberrant signaling pathways, which aided in the emergence of NP [23-27]. Over time, these changes affected neuronal excitability and pain modulation, perpetuating chronic pain and leading to sensory abnormalities (Fig. 1b) [22,28]. These disruptions affected the functioning of pain fibers and contributed to the degeneration of the peripheral and central nervous systems (Fig. 1c) [29]. Treating NP requires various medications, including anticonvulsants, antidepressants, and opioids [6,30,31]. A single medication alone could not effectively treat NP; rather, a combination of drugs was needed to manage the condition. In rats given a high-fat diet and a low dose of streptozotocin [32,33], as well as spinal nerve ligation [34,35], these NP symptoms are evident. Many research studies reported that the individual effect of a drug in treating the disease could not provide sufficient relief, hence the combination of these drugs offered a multi-faceted approach for treatment, potentially resolving the complications of the disease. Abdelshakour *et al.* developed a high-performance liquid

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Article

Mechanism and Preclinical Models of Neuropathic Pain: An Update

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Abstract: Neuropathic pain is a debilitating condition that is a product of nervous system damage or dysfunction. Since the drugs prescribed by the physician provide partial pain relief to the patients. Hence, current updates for its treatment are required. It is a global concern as neuropathic pain happens in many diseased conditions like cancer, trauma, surgery and diabetics, etc. Developed as well as developing countries are both trying to find suitable medicine. Understanding the mechanisms behind it can be crucial for the effective treatment and management of neuropathic pain. Central sensitization in the spinal cord and brain amplifies pain signals, increasing pain sensitivity even without tissue damage. Peripheral sensitization, at the injury site, sensitizes peripheral nerves, lowering pain thresholds. Recognizing and studying these sensitizations are vital for understanding and managing chronic neuropathic pain and improving patients' quality of life. The present manuscript encompasses a mechanism and model for neuropathic pain in animals with its advantages and disadvantages.

Keywords: Peripheral Sensitization; Central Sensitization; Neuropathic Pain; Pain Signals

1. Introduction

A painful condition that appears due to abnormalities in the somatosensory nervous system is called neuropathic pain (NP), which can be recognized by dysesthesias and paresthesias-like conditions [1]. Allodynia (pain in response to a stimulus that does not usually provoke pain) and hyperalgesia (an increased response to painful stimuli that do not occur normally) are identified as hallmarks of NP (Figure 1). The exact population of patients with NP is still unknown, but various published reports put an estimate between 100–560 million people globally [2]. NP is highly associated with patients with long-standing diabetes, stroke, cancer, AIDS, herpes virus infection, multiple sclerosis, and traumatic nerve injury. It is often associated with conditions like post-herniorrhaphy, syringomyelia, post-mastectomy, and Fabry neuropathy. Hence it is a state that occurs as a result of a multifactorial pathological condition, and now it becomes a global challenge for medical sciences [3].

Currently used medications like non-steroidal anti-inflammatory drugs (NSAIDs) and opiates have attained limited effectiveness or no response to treat NP [4]. Therefore, new treatment methods need to be investigated. It is a bit difficult to evaluate NP in humans. The probable reason may be the use of stimuli, which causes irreversible damage to the individual. In addition, it is also very difficult to select a large number of individuals for the reduction of subject variability. Henceforth, to broaden the underlying mechanisms and find novel treatments for NP, an ideal

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RESEARCH ARTICLE | FEBRUARY 20 2024

Neuropathic pain and diabetes: A complicated clinical condition

Indu Melkani; Gagandeep Kaur; Sukhanpreet Kaur; Ruhi Rana; Bimlesh Kumar ✉; Shubham Kumar; Narendra Kumar Pandey; Kardam Joshi; Dhara Patel; Omji Porwal

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One of the diseases that is increasingly prevalent worldwide is neuropathic pain (NP), which affects the somatosensory nerve system directly and causes an imbalance between excitation and inhibitory activities. There are numerous illnesses that cause NP, however, the precise pathophysiology is yet unknown. Diabetes is a leading cause of NP development, which can harm the entire nervous system and cause glial cell malfunction. Due to extensive destruction both centrally and in the periphery, NP worsens quality of life. Using a CT scanner or magnetic resonance imaging is a good way to examine the nervous system. The lack of a precise NP treatment is increasingly emphasizing research and development in the realm of pharmaceuticals. The main goal of treatment is to lessen nerve sensitivity, perception, and numbness. The best way to treat NP is to connect it directly to both myelinated and non-myelinated functions.

Topics
Pharmaceuticals, Sensory nervous system, Nerve cells, Magnetic resonance imaging, Pathophysiology

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ORIGINAL ARTICLE

Pharmacokinetic and pharmacodynamic evaluation of Solid self-nanoemulsifying delivery system (SSNEDDS) loaded with curcumin and duloxetine in attenuation of neuropathic pain in rats

Bimlesh Kumar[1] · Sachin Kumar Singh[1] · T. Prakash[2] · Amit Bhatia[3] · Monica Gulati[1] · Varun Garg[1] · Narendra Kumar Pandey[1] · Saurabh Singh[1] · Indu Melkani[1]

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Abstract

The present investigation is focused on improving oral bioavailability of poorly soluble and lipophilic drugs, curcumin (CRM) and duloxetine (DXH), through the solid self-nanoemulsifying drug delivery system (S-SNEDDS) and identifying their potential against attenuation of NP in chronic constriction injury (CCI)–induced rats through the solid self-nanoemulsifying drug delivery system (S-SNEDDS). The optimized batch of S-SNEDDS reported was containing CRM and DXH (30 mg each), castor oil (20% w/w), tween-80 (40% w/w), transcutol-P (40% w/w), and syloid 244 FP (1 g). The high dose of each of naïve CRM (NCH), naïve DXH (NDH), physical mixture of DXH and CRM (C-NCM-DXH), S-SNEDDS-CRM (SCH), S-SNEDDS-DXH (SDH), and S-SNEDDS-CRM-DXH (C-SCH-SDH) was subjected for MTT assay. The developed formulations were subjected to pharmacokinetic studies and results showed about 8 to 11.06 and 2-fold improvement in oral bioavailability of CRM and DXH through S-SNEDDS. Furthermore, CCI-induced male Wistar rats were treated with SSNEDDS containing CRM and DXH, S-SNEDDS containing individual drug, individual naïve forms, and their combination from the day of surgery for 14 days and evaluated for behavioral at pre-determined time intervals. On the terminal day, animals were sacrificed to assess tissue myeloperoxidase, superoxide anion, protein, tumor necrosis factor-α, total calcium levels, and histopathological changes. Pronounced effect was observed in rats treated with S-SNEDDS containing both drugs with respect to rats receiving any of other treatments owing to enhanced oral bioavailability through S-SNEDDS. Therefore, it can be concluded that S-SNEDDS of both drugs and their coadministration can accelerate the prevention of NP.

Keywords Curcumin · Duloxetine · Chronic constriction injury · TNF-α · Calcium inhibition

Introduction

Abnormal sensory processing in peripheral as well as central nervous systems develops a chronic painful condition known as neuropathic pain (NP) which is characterized by allodynia and hyperalgesia [1]. Conventional therapies like non-steroidal anti-inflammatory drugs (NSAIDs), opioid analgesics, tricyclic antidepressants (TCA), and anticonvulsants have been extensively reported in the treatment of NP; however, they have several side effects such as dry mouth, orthostatic hypotension, constipation, and urinary retention [2–5]. There are many etiologies and mechanisms that cause NP; hence, combination therapy with agents that act at different sites and mechanisms may provide better alternative for NP [1, 5, 6]. Chronic

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This is to certify that Prof./Dr./Mr./Ms. INDU MELKANI has successfully participated as Delegate & Presented Poster/ Oral Presentation on AN ACCEPTABLE OUTCOME: HOW PAIN FIBERS AFFECT NEUROPATHIC PAIN ? in the 3rd **International Conference of Pharmacy (ICP-2022)** on the **Theme of "Practice, Promotion & Publication of Innovation : A Way of Transforming Health"** held on **09th & 10th November 2022** organized by School of Pharmaceutical Sciences in a collaboration with Indian Pharmaceutical Association (IPA) at Lovely Professional University, Punjab.

Mr. Suresh Khanna
National Hon. Gen. Secretary, IPA

Dr. Bimlesh Kumar
Organizing Secretary

Dr. T.V. Naryana
National President IPA

Dr. Monica Gulati
LOC Chairperson

Sri Herbasia Biotech

Tishk International University, Iraq

26TH ANNUAL NATIONAL CONVENTION

PSIT Kanpur

APTICON 2023

Fiesta of Innovation, Research and Collaboration: Thriving towards excellence in Pharmacy Teaching

ASSOCIATION OF PHARMACEUTICAL TEACHERS OF INDIA (APTI)
AND
PSIT-PRANVEER SINGH INSTITUTE OF TECHNOLOGY (PHARMACY), KANPUR

CERTIFICATE
OF APPRECIATION

This is to certify that

Prof./Dr./Mr./Ms. Indu Mehani

From Lovely Professional University, Punjab.

has secured **FIRST/SECOND/THIRD** position in **POSTER PRESENTATION** on the topic entitled

Synergistic Pharmacological Approaches for Neuropathic Pain Management: Unveiling Multidimensional Solution to a multifaceted disorder

in the **26th Annual National Convention** of the **Association of Pharmaceutical Teachers of India -2023** held at **PSIT- Pranveer Singh Institute of Technology (Pharmacy), Kanpur (India)** from 2nd to 3rd September 2023.

Dr. Pranay Wal	Dr. A K Rai	Dr. Deependra Singh	Dr. Milind J. Umekar
Organising secretary	Head of the Institute	LOC Chairman	APTI president

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GST TAX INVOICE "ORIGINAL"

INVOICE NO.: 1165 Date: 16/09/2021

To,
Indu Mulkani M. Pharm (Pharmacology)
Pharmacy Block , Animal House,
Lovely Professional University,
Jalandhar - Delhi G.T. Road,
Phagwara, Punjab – 144411

Mobile: +91-9872584056

Order No.: ikashifamulkani@gmail.com
DATE: Sep 13, 2021, 2:25 PM
Dispatched through SPEED POST
MUMBAI TO JALANDHAR
L.R. NO.: DATE: 16/09/2021
Payment Terms ADVANCE
UPI/T2566920/1163/NA/9872584056@paytm/Stat
11470 RECD. ON 13/09/2021

S. No.	Description	HSN	GST	Packing	Unit Rate	Discount	Net	Nos.	AMOUNT
1	Pregabalin 148553-50-8	996339	18%	10 g	972.03	783.53	188.50	1	2966.10
2	Lovastatin 75330-75-5	996332	18%	5 g	940.80	1685.56	1754.34	1	6754.24
									9720.34
	Central Tax (IGST) @ 18%								1749.66
								Rup	
					TOTAL INVOICE VALUE Rs.				11470.00

Invoice Value (in words) Rupees Eleven Thousand Four Hundred Seventy Only

Terms & Conditions:-
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2.Any Chemical item sold in this bill is not for medical use
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4.Payment to be made in favour of "YARROW CHEM PRODUCTS" by A/c payee
Cheque / DD payable at Mumbai Or
Payment of this invoice can be deposit with ICICI BANK LTD Nearest to your area branch/anywhere Banking in our
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Email:-yarrowchemproducts@gmail.com, www.yarrowpharma.com
GSTIN: 27ACHPT7360F1ZA , PAN ACHPT7360F

GST TAX INVOICE

(Rule 1 / Section 31 of GST)

INVOICE NO:-1067

Date:-27/09/2017

To,
The Head,
Department of Pharmaceutical Science,
Lovely Professional University,
Jalandhar Delhi, G.T. Road, Block -3A
PHAGWARA-144401. (PANJAB)
GSTIN:03AAATL1797K1ZI
Tel. No.= 018104642686 Ms.Sakshi Panchal

Order No.:-panchalsakshi20@gmail.com
DATE:-Fri, Sep 22, 2017 at 9:14 PM
Dispatched through:SPEED POST
MUMBAI TO KAPURTHALA
L.R NO.: 27/09/2017
Payment Terms:ADVANCE
AMT. DEPOSITED IN ICICI BANK A/C RS. 2100/-
ON 26/09/2017

S. No.	Description	Packing	Unit Price	Discount	Net	Nos	AMOUNT
1	Sildenafil citrate	20 g	2224.58	444.92	1779.66	1	1779.66
	Total Taxable Value			444.92			1779.66
	Central Tax (IGST) @ 18%						320.3
	Round Off						Round Off
			TOTAL INVOICE VALUE Rs.				2100.0

Delivwery Address:-
Ms.Sakshi Panchal
H.No.305-B ,TYPE 3 ,
RAIL COACH FACTORY
KAPURTHALA-144602 (PUNJAB)
MOBILE NO.9041176036

Invoice Value (in words) Rupees Two Thousand One Hundred Only

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Authorised Signatory

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Current A/c No. 000 [illegible] Dombivali (E) Branch-421 202 IFSC Code-ICIC0000950

YARROW CHEM PRODUCTS

208, Uma Mahesh Society, Ramnagar, Shivmandir Road
DOMBIVALI (EAST) MUMBAI-421 201
Cell:- +91 98202 21877, +91 87795 94616, +91 97023 12195
Email:-yarrowchemproducts@gmail.com, www.yarrowpharma.com
GSTIN. 27ACHPT7360F1ZA , PAN:ACHPT7360F

GST TAX INVOICE

(Rule 1 / Section 31 of 501)

INVOICE NO:-1024 Date:-07/08/2017

To,	Order No.:-ikshitamelkani@gmail.com
The Head,	DATE:-Sat, Aug 5, 2017 at 6:23 PM
Department of Pharmaceutical Science,	Dispatched through:SPEED POST
Lovely Professional University,	MUMBAI TO JHANSI
Jalandhar Delhi, G.T. Road, Block -3A	L.R NO.: 7/8/2017
PHAGWARA-144401.	Payment Terms:ADVANCE
Punjab (INDIA)	AMT. DEPOSITED IN ICICI BANK A/C RS. 2400/-
Tel. No.= 018104642886	ON 05/08/2017

S. No.	Description	Packing	Unit Price	Discount	Net	Nos.	AMOUNT
1	Fluoxetine HCl	50 g	2542.35	508.47	2033.88	1	2033.88
	Total Taxable Value			508.47			2033.88
	Delivery Address:- Ms. INDU MELKANI Lovely professional University Near chaheru village, Jalandhar, Girl Hostel 4, Block 12, PUNJAB- 144411 Cell:-9872584050						
	Central Tax (CGST) @ 18%						366.10
	Round Off					Round Off	0.02
						TOTAL INVOICE VALUE Rs.	2400.00

Invoice Value (In words) Rupees Two Thousand Four Hundred Only

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2.Any Chemical item sold in this bill is not for medical use.
3.All Goods are dispatched on buyer's risk
4.Payment to be made in favour of "YARROW CHEM PRODUCTS" by A/c
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Current A/c No. 095 00 55 00 069 Dombivali (E) Branch 421 202 IFSC Code-ICIC0000950

For Yarrow Chem Products
Authorised Signatory

NATIONAL INSTITUTE OF PHARMACEUTICAL EDUCATION & RESEARCH

राष्ट्रीय औषधीय शिक्षा एवं अनुसंधान संस्थान (नाईपर)

Sector 67, S.A.S.Nagar, Mohali - 160 062 Pb. (INDIA), Tel. : +91-(0)172-2214682-87, Fax: +91-(0)172-2214692

RECEIPT/रसीद

Receipt No./रसीद सं.	Receipt Date/ रसीद तिथि	Amount (Rs.)/राशि (रुपये में)
17347	29-10-2021	33,600/-

Received with thanks from / धन्यवाद सहित प्राप्त	The sum of Rupees/ कुल राशि
Ms Indu Melkani L.P.U. Phagwara P.B.	Thirty three thousand six hundred only

VIDE- DD/CHQ/CASH द्वारा-डीडी/चैक/नकद / NEFT/RTGS एनईएफटी/आरटीजीएस	Number/संख्या	Dated/तिथि	Drawn on/के नाम	In Part/Full/Advance/Payment of आंश./पूर्ण/अग्रिम/भुगतान का
	982240	25-10-21	SBI, Mohali	Sale of Animals (112 x 300)

Payments by Cheque are acknowledged subject to realization

For NIPER/ नाईपर के लिए

29/10/21

Authorised Signatory/प्राधिकृत हस्ताक्षरकर्ता

NATIONAL INSTITUTE OF PHARMACEUTICAL EDUCATION & RESEARCH (NIPER) (Ministry of Chemicals & Fertilizers, Govt. of India)
Sector 67, S.A.S. Nagar (Punjab) - 160 062, Web : www.niper.gov.in
Tel. No. : 0172-2214682-87, Extn. (2078, 2143) Fax No. : 0172-2214692

S.A.S. Nagar

Central Animal Facility

INVOICE CUM GATE PASS

No. 1529

Name & Address of the Indenter: Ms Indu Melkani, Lovely Professional University Phagwara Punjab

Lovely Professional University, Phagwara Punjab Pin-144411
GRN No. 101803
DATE 29-10-2021
SIGN.
UIC 31366

Order No./Date (If any): LPU/SOP/SS/2021025/ISCU/021/27/10/21

Date: 29/10/2021
Received Rs. 33600/-
Receipt No./Date 173/(?) Dt. 29/10/21
Stock Register Page No. 06
CAF Issue Voucher No. 178/21
Qty. Ordered (A) 112-0/0
Qty. Transferred (B) 112-0/0
Balance Qty. (A-B) NIL

S. No.	Particulars	Qty. Transferred	Rate (Rs.) /Unit	Amount (Rs.)	Remarks
1	Rats SD Male	112-0/0	300/-	33600/-	Sold for research purpose only
	Total		Rs=	33600/-	

Rs. Thirty Three Thousand Six hundred only

NIPER Central Animal Facility

For DIRECTOR NIPER

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Phone : 9216340111

GST No. **04ARDPK8471D1ZY** **Email : info@essencels.com**

D.L. No **9151-2022/BW**

D.L. No 2 **9150-2022/OBW**

Invoice No. **ELS/2022-23/082** **State Code:** **04** **Dated** **04-07-2022**

Details of Receipient (Billed To)
Indu Melkani
Deptt. Of Pharmacy
Lovely Professional University
Delhi G.T. Road , Phagwara.,
Jalandhar, Punjab, 144411
Phone No. 9872584050

Details of consignee (Delivary at) (If other than bill to)

GSTIN State Code: 3 GSTIN State Code:

ORDER NO email **dated : 08-06-2022**

Sr.	HSN/ SAC	Description	Qty	Rate	Disc %	Taxable Value	UT GST %	UT GST Amt.	C GST %	C GST Amt.	IGST %	IGST Amt.	Amount
1	3822	E-EL-R2856 - Rat TNF-α(Tumor Necrosis Factor Alpha) ELISA Kit - 24T	1	₹ 7,000.00		₹ 7,000.00					5%	₹ 350.00	₹ 7,000.00
2	3822	E-EL-R0015- IL-6 ELISA Kit - 24T	1	₹ 7,000.00		₹ 7,000.00					5%	₹ 350.00	₹ 7,000.00
3	3822	E-EL-R1235 - BDNF elisa kit :: Rat BDNF ELISA Kit 24-T	1	₹ 7,000.00		₹ 7,000.00					5%	₹ 350.00	₹ 7,000.00
		Totals:	3			21000.00						1050.00	₹ 21,000.00

Total	₹ 21,000.00
IGST 5% on ₹ 21,000.00	₹ 1,050.00
Total Value	**₹ 22,050.00**
IGST = ₹ 1,050.00	
Grand Total	₹ 22,050.00
Net Amount	**₹ 22,050.00**

Rupees : **Twenty Two Thousand and Fifty Only**

Bank Details : Canara Bank : Branch - 31D Chandigarh, A/c No. 2478201000464, (Current A/c) RTGS/IFS Code: CNRB0002478

1 Goods once sold will not be taken back
2 Interest @ 24% p.a. on payments not received within 30 days
3 we do not own any responsibility after goods leaves our premises
4 Subject to CHANDIGARH Jurisdiction E. & O. E.

For M/s ESSENCE LIFE SCIENCES

Authorised Signatory