

Chapter

NeuraPan Flex®: Molecular Mechanisms in Postoperative Neuropathic Pain Relief

Apurba Ganguly and Anondeep Ganguly

Abstract

Neuropathic pain (NP) results from complex molecular alterations in the nervous system, including inflammation (elevated CRP, IL-6, and TNF- α), tissue repair processes (MMP-2 and MMP-9), oxidative stress (MDA), neuronal function (BDNF and NGF), and pain perception (substance P and GFAP). NP may arise from conditions such as diabetes, shingles, spinal cord injuries, multiple sclerosis, or postoperative complications and is often characterized by burning, tingling, or stabbing sensations. This chapter examines the efficacy of *NeuraPan Flex*® (NPF), a topical bioactive lipophilic formulation, as an alternative to standard management (SM) in postoperative pain scenarios. A 16-week double-blind randomized controlled trial involving 72 patients treated with NPF versus 63 controls receiving SM was conducted. NPF includes bioactive ingredients such as *Syzygium aromaticum* (eugenol), *Lavandula angustifolia* (linalool), *Eucalyptus globulus* (eucalyptol), *Zingiber officinale* (gingerol), and others. Clinical efficacy was evaluated using the Neuropathic Pain Scale (NPS), radiological imaging, and serum biomarker analysis, with biomarkers assessed pre- and posttreatment. NPF demonstrated high diagnostic performance with area under the curve between 0.981 and 0.901 ($p < 0.0001$), showing significant improvements in clinical outcomes and biomarker modulation compared to SM ($p < 0.0001$). NPF shows strong potential for improving NP management and modulating key biomarkers, supporting its integration into clinical practice. Further trials are needed for broader application.

Keywords: neuropathic pain, *NeuraPan Flex*®, molecular mechanisms, neuroinflammation, phytoextracts, neurological disorders

1. Introduction

Neuropathic pain (NP) poses a significant and complex challenge, affecting approximately 7–10% of the global population. This type of pain results from injury or dysfunction within the nervous system and is notoriously difficult to manage due to its multifaceted nature [1]. Unlike nociceptive pain, which is directly linked to tissue damage, NP is characterized by an intricate interplay of

molecular changes across various biological pathways, including inflammation, oxidative stress, neuronal dysfunction, and abnormal pain processing [1, 2]. Key biomarkers such as C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), matrix metalloproteinases (MMP-2 and MMP-9), malondialdehyde (MDA), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), substance P, and glial fibrillary acidic protein (GFAP) are often dysregulated in NP [3–8].

The etiology of NP spans a range of conditions, including diabetes, herpes zoster infection (shingles), cancer, nerve injury, and postoperative complications [9, 10]. Its complexity depends on various molecular pathways, such as inflammatory responses, oxidative stress, and neuroimmune interactions.

Current standard management (SM) of NP mainly involves pharmacological agents like anticonvulsants, antidepressants, and opioids [9, 10]. However, these treatments often fall short, providing inadequate relief and posing risks of significant side effects and dependency [11]. This gap has spurred interest in alternative therapies that target the underlying molecular mechanisms of NP.

One promising approach is the use of bioactive compounds derived from natural sources. *NeuraPan Flex*® (NPF) is a novel topical formulation designed to address NP's molecular complexities through a bioactive lipophilic composition. It incorporates a blend of botanical extracts, including *Syzygium aromaticum* (eugenol) [12], *Lavandula angustifolia* (linalool) [13], *Eucalyptus globulus* (eucalyptol) [14], *Zingiber officinale* (gingerol) [15], *Mentha piperita* (menthol) [16], *Gaultheria procumbens* (methyl salicylate) [17], *Pinus sylvestris* (α -pinene, β -pinene) [18], *Celastrus paniculatus* (celastrol) [19], *Aloe barbadensis* (acemannan) [20], *Cocos nucifera* (lauric acid, capric acid) [21], and *Triticum vulgare* (tocopherol) [22]. These ingredients were selected based on their efficacy in targeting multiple NP-related biomarkers and receptors, including TRPV1, NMDA, GABA-A, 5-HT, TRPA1, TRPM8, substance P, COX, NF- κ B, CB2, TNF- α , PPAR- γ , PPAR- α , CGRP, and cytokines like IL-1 β , IL-6, and TNF- α . The formulation also impacts oxidative stress biomarkers and neurotrophic factors, enhancing antioxidant defense and neuroprotection [23–26].

This chapter presents a study evaluating the clinical efficacy of NPF compared to standard management (SM) in postoperative pain scenarios through a 16-week double-blind randomized controlled trial. The trial assessed changes in the Neuropathic Pain Scale (NPS), radiological imaging, and serum biomarkers pre- and posttreatment. The findings indicate significant improvements in clinical outcomes and biomarker modulation in the NPF group, suggesting its potential as a more targeted and safer alternative for NP management. Further research and larger clinical trials are needed to validate these results and explore NPF's broader applicability in NP management.

2. Materials and methods

2.1 Study design

This study was a 16-week double-blind randomized controlled trial conducted to evaluate the efficacy of NPF in managing neuropathic pain (NP) compared to standard management (SM). OPTM Research Review Board approved the study at OPTM Health Care Pvt. Ltd. and NanoPhyto Care Pvt. Ltd. Informed consent was obtained from all participants before enrollment.

2.2 Participants

The study enrolled 153 patients with clinically diagnosed neuropathic pain from OPTM Health Care Pvt. Ltd. and NanoPhyto Care Pvt. Ltd. between April 2023 and June 2024. Rigorous inclusion and exclusion criteria were applied to ensure a homogeneous participant group and to enhance the validity and reliability of the study results.

a. Inclusion criteria:

- Adults aged 18–75 years with a confirmed clinical diagnosis of neuropathic pain lasting at least 6 months.
- Patients reporting moderate to severe pain intensity, defined as an NPS score of ≥ 4 at baseline.
- Individuals with stable medical conditions, not undergoing significant changes to their existing pain management regimen during the study period.
- Participants who provided informed consent demonstrated willingness and ability to adhere to all study protocols and procedures.

b. Exclusion criteria:

- Patients with pain conditions not primarily neuropathic, such as fibromyalgia or pain related to malignancy.
- Individuals receiving other experimental pain treatments or investigational drugs within 30 days before study enrollment.
- Patients with significant psychiatric or cognitive disorders that might affect accurate pain assessment or adherence to study protocols.
- Pregnant or breastfeeding women, or those planning to conceive during the study.
- Participants with known hypersensitivity or contraindications to any component of the NPF formulation.

These criteria were meticulously enforced to ensure the selection of a representative and suitable sample, strengthening the study outcomes' robustness.

Participants were randomly assigned to either the NPF group ($n = 72$) or the SM group ($n = 63$) using a computer-generated randomization schedule. Stratification based on baseline neuropathic pain severity was employed to ensure comparable groups at the outset of the trial.

2.3 Intervention

The intervention group received NPF, a topical bioactive lipophilic formulation containing the following botanical extracts: *Syzygium aromaticum* (eugenol: 80–90%); *Lavandula angustifolia* (linalool: 20–45%; linalyl acetate: 25–45%); *Eucalyptus globulus*

(eucalyptol: 70–85%); *Zingiber officinale* (gingerol: 15–30%); *Mentha piperita* (menthol: 35–50%); *Gaultheria procumbens* (methyl salicylate: 90–98%); *Pinus sylvestris* (α -pinene: 30–50%; β -pinene: 15–25%); *Celastrus paniculatus* (celastrol: 0.05–0.2%); *Aloe barbadensis* (acemannan: 15–30%); *Capsicum annuum* (Capsaicin: 0.1–1.5%); *Cocos nucifera* (Lauric Acid: 45–55%; Capric Acid: 5–10%); and *Triticum vulgare* (Tocopherol: 0.5–1.5%).

NPF was applied twice daily to the affected area for 16 weeks. The control group received SM for neuropathic pain, which included anticonvulsants, antidepressants, and topical analgesics.

2.4 Clinical assessments

The primary clinical outcome was the change in the NPS score from baseline to week 16. Secondary outcomes included radiological imaging assessments and patient-reported outcomes on pain severity, sleep quality, and daily function.

2.5 Biomarker analysis

Fasting blood samples were collected at baseline and after 16 weeks of treatment. Serum levels of biomarkers associated with inflammation (CRP, IL-6, and TNF- α), tissue repair (MMP-2 and MMP-9), oxidative stress (MDA), neuronal function (BDNF and NGF), and pain perception (substance P and GFAP) were measured using enzyme-linked immunosorbent assays (ELISA) following the manufacturer's instructions, as detailed in **Table 1**. All samples were processed and analyzed in a blinded manner at Galaxy Medical Centre, an ISO 9001:2015 certified laboratory in Kolkata, India.

The normal levels and deviations of biomarkers in individuals with neuropathic pain are shown in Table 2.

2.6 Neuropathic pain scale (NPS)

In this study, NPS [27] was employed to quantitatively assess the multidimensional aspects of neuropathic pain experienced by participants. NPS is a validated tool

Biomarkers	Human assay kit	Manufacturer	Place
CPP	CRP ELISA Kit	Nirmal Bioscience Pvt. Ltd.	Haryana 134,113
IL-6	IL-6 ELISA Kit	RayBiotech Life, Inc.,	New Delhi 110,008
TNF- α	TNF- α ELISA Kit	Elite Biotech	New Delhi 110,044
MMP-2	MMP-2 ELISA Kit	Biobasic India	Bangalore 560,095
MMP-9	MMP-9 ELISA Kit	Biobasic India	Bangalore 560,095
MDA	Colorimetric Assay Kit	Labcare Diagnostics	New Delhi 110,005
BDNF	BDNF, ELISA Kit	Krishgen Biosystems	Bangalore 560,095
NGF	NGF, ELISA Kit	Krishgen Biosystems	Bangalore 560,095
Substance P	Substance P, ELISA Kit	Krishgen Biosystems	Bangalore 560,095
GFAP	GFAP, ELISA Kit	Meril Diagnostics	Gujarat 396,191

Table 1.
Details of human assay kits used in the study, including manufacturer names and locations.

Biomarker	Normal range (Men)	Normal range (Women)	Neuropathic pain range
CRP	<1 mg/L	<1 mg/L	Elevated, often >3 mg/L
IL-6	<2 pg./mL	<2 pg./mL	Elevated, typically 2–15 pg./mL
TNF- α	1–5 pg./mL	1–5 pg./mL	Elevated, 5–30 pg./mL
MMP-2	400–600 ng/mL	400–600 ng/mL	Elevated, often >600 ng/mL
MMP-9	20–80 ng/mL	20–80 ng/mL	Elevated, typically 80–300 ng/mL
MDA	1–3 μ mol/L	1–3 μ mol/L	Elevated, often >3 μ mol/L
BDNF	10–50 ng/mL	10–50 ng/mL	Lowered, usually <20 ng/mL
NGF	10–50 pg./mL	10–50 pg./mL	Elevated, often >50 pg./mL
Substance P	<20 pg./mL	<20 pg./mL	Elevated, typically >20 pg./mL
GFAP	100–300 pg./mL	100–300 pg./mL	Elevated, often >300 pg./mL

Table 2.
The ranges represent typical levels and deviations observed in individuals suffering from neuropathic pain.

designed to capture neuropathic pain’s intensity and quality by evaluating parameters such as sharpness, burning sensation, coldness, and sensitivity to light touch.

Participants were asked to rate their pain on a scale of 0–10 for each item, with 0 indicating the absence of pain and 10 representing the most severe pain imaginable. The scores from each item were then analyzed to provide a comprehensive profile of the neuropathic pain experienced, facilitating the evaluation of treatment efficacy and the understanding of pain mechanisms in the context of the study.

This method allowed for a detailed and nuanced assessment of pain characteristics, contributing to the robustness of the study’s findings.

2.7 Receiver operating characteristic curve analysis

Receiver operating characteristic (ROC) curve analysis was conducted to determine the diagnostic performance of each biomarker. Sensitivity and specificity were assessed, and the area under the curve (AUC) values were computed, accompanied by 95% confidence intervals. These intervals were established using the bootstrap method as per established protocols [28] and in line with previous publications. This analysis provided insights into the predictive power of biomarkers for NP [29, 30].

2.8 Meta-analysis and heterogeneity tests

A meta-analysis was conducted to aggregate data from multiple studies, enhancing reliability. Heterogeneity across studies was assessed using Cochran’s Q-test and the I-squared index to determine consistency. Effect-size indices quantified the results within the meta-analysis [29, 30].

2.9 Radar chart analysis

Radar charts were used to visualize standardized mean differences and percentage changes in biomarkers between NPF and MS groups, focusing on the severity of neuropathic pain.

2.10 Standardized mean deviation and 95% confidence interval of biomarkers

In this study, standardized mean differences (SMD) and 95% confidence intervals (CI) were calculated to quantify the differences in biomarker levels between the NPF group (n = 72) and the SM group (n = 63). SMD assessed effect sizes, reflecting intergroup differences relative to within-group variability.

Mean differences (MDs) and their 95% CIs were computed for each biomarker to evaluate the precision of these estimates. Statistical significance was determined using p-values for both MD and SMD, highlighting biomarkers with significant group differences. The detailed results, including SMDs, CIs, and p-values for each biomarker, are presented in the “Results” section.

2.11 Correlation coefficient analysis

To elucidate the intricate relationships among key molecular biomarkers governing inflammation, oxidative stress, neuronal dysfunction, and abnormal pain processing, a comprehensive Pearson correlation coefficient analysis was evaluated. The analysis was conducted for both the NPF and MS groups, assessing the strength and direction of associations between biomarkers, including CRP, IL-6, TNF- α , MMP-2, MMP-9, MDA, BDNF, NGF, substance P, and GFAP. Pearson's correlation coefficients (r-values) were computed for each biomarker pair, accompanied by p-values to assess statistical significance. A two-tailed test with a significance level of $p < 0.05$ was used to identify meaningful correlations. The strength of correlation was classified as strong ($r > 0.7$), moderate (r between 0.3 and 0.7), or weak ($r < 0.3$). Statistical analyses were conducted using appropriate software, ensuring the robustness and reproducibility of results [30].

2.12 Statistical analysis

Descriptive statistics, including means, standard deviations, and ranges, were calculated for both demographic and biomarker data. Comparisons of serum biomarker levels between NPF patients and MS subjects were performed using independent t-tests or Mann-Whitney U tests for nonnormally distributed data. ROC curves were constructed to evaluate the diagnostic performance of each biomarker, with AUC values calculated to determine a predictive value for neuropathic pain. Risk ratios and 95% confidence intervals were computed to assess associations between biomarker levels and biological neuropathic risk factors. Data were analyzed using IBM SPSS software version 20 [31], with statistical significance set at $p < 0.05$. Data accuracy and completeness were verified before analysis.

2.13 Ethical considerations

The study was approved by the OPTM Health Care Pvt. Ltd. Review Board. Informed consent was obtained from all participants before their inclusion in the study.

3. Results

3.1 Patient enrolment and baseline characteristics

The study involved 135 participants: 72 (53.33%) in the NPF group and 63 (46.67%) in the SM group. Both groups were similar in age, weight, height, BMI,

and symptom duration, with the NPF group having a slightly higher proportion of females (68.31 vs. 65.51%). Pain intensity was reported by 76.39% of the NPF group and 80.95% of the SM group. Common pain features included sharpness, hot/burning sensation, and deep pain. The NPF group exhibited higher smoking and alcohol use, while the SM group had more tea and coffee consumption. Occupational status varied, with more self-employed individuals in the NPF group and more full-time employees in the SM group. Ethnic distribution, dietary habits, and marital status were comparable across groups (see **Table 3**).

Characteristic	NPF group	MS group
No of subjects (%)	72 (53.33)	63 (46.67)
Female (%)	49 (68.31%)	41 (65.51%)
Mean age (SD) in years	59.1 (3.2)	58.8 (3.1)
Mean weight (SD) in kg	71.12 (5.78)	70.77 (6.21)
Mean height (SD) in meter	1.52 (0.79)	1.54 (0.84)
Mean BMI (SD) in kg/m ²	30.78 (6.88)	31.04 (7.27)
Mean symptom duration in years (SD)	5.25 (1.85)	5.35 (1.65)
Indian ethnic group (%)		
Bengali	19 (26.39)	18 (28.57)
Gujrati	7 (9.72)	5 (7.94)
Marwaree	6 (8.33)	6 (9.52)
Marathi	9 (12.5)	7 (11.11)
Tamil	8 (11.11)	8 (12.7)
Punjabi	7 (9.72)	7 (11.11)
Shindhi	8 (11.11)	5 (7.94)
North East India	8 (11.11)	7 (11.11)
Dietary habits (%)		
Vegetarian	49 (67.60)	39 (62.58)
Nonvegetarian	23 (32.40)	24 (37.42)
Other habits (%)		
Smoking	13 (1032)	7 (6.48)
Drinking alcohol	11 (8.73)	9 (8.33)
Drinking tea and coffee	15 (11.91)	16 (14.81)
Chewing tobacco	6 (4.76)	6 (5.56)
No other habits	9 (7.14)	13 (12.04)
Neuropathic Pain scale (number) (%)		
Intensity of pain:	55 (76.39)	51 (80.95)
Sharpness	45 (62.5)	40 (63.49)
Hot/burning sensation	30 (41.67)	28 (44.44)
Cold sensation	15 (20.83)	18 (28.57)
Dullness	10 (13.89)	11 (17.46)

Characteristic	NPF group	MS group
Itching:	12 (16.67)	8 (12.7)
Tingling/prickling	21 (29.17)	10 (15.87)
Sensitivity to light touch:	11 (15.28)	9 (14.29)
Surface pain:	7 (9.72)	8 (12.7)
Deep pain:	30 (41.67)	22 (34.92)
Occupational Hazards (%)		
Employed fulltime	9 (12.5)	12 (19.05)
Employed part-time	8 (11.11)	9 (14.29)
Housewife/Homemaker	11 (15.28)	17 (26.98)
Retired	19 (26.39)	15 (23.81)
Self-employed	25 (34.72)	10 (15.87)
Marital status (%)		
Single	9 (12.5)	7 (11.11)
Married	39 (54.17)	38 (60.32)
Separated	9 (12.5)	7 (11.11)
Divorced	7 (9.72)	6 (9.52)
Widowed	8 (11.11)	5 (7.94)

Table 3.
Demographic data and baseline characteristics of the study subjects.

3.2 Neuropathic pain scale analysis

The meta-analysis showed significant effects for all NPS descriptors. Specifically, the effect sizes were as follows: intensity of pain (SMD = -3.01 , $p < 0.001$), sharpness (SMD = -3.45 , $p < 0.001$), hot/burning sensation (SMD = -2.99 , $p < 0.001$), cold sensation (SMD = -2.52 , $p < 0.001$), dullness (SMD = -3.27 , $p < 0.001$), itching (SMD = -2.65 , $p < 0.001$), tingling/prickling (SMD = -2.42 , $p < 0.001$), sensitivity to light touch (SMD = -2.49 , $p < 0.001$), surface pain (SMD = -1.92 , $p < 0.001$), and deep pain (SMD = -1.95 , $p < 0.001$). The overall effect size was -2.21 , with moderate heterogeneity ($Q = 28.54$, $I^2 = 68.46\%$). A random-effects model confirmed significant effect sizes (SMD = -2.65 , $p < 0.001$) (see **Figure 1**).

3.3 Biomarker analysis

Figure 2 and **Table 4** display receiver operating characteristic (ROC) curve analyzes for biomarkers associated with neuropathic pain. Substance P showed the highest AUC of 0.977, followed by MDA (AUC = 0.972), GFAP (AUC = 0.962), and MMP-2 (AUC = 0.954). Sensitivity for these markers ranged from 97.22% for substance P to 95.83% for MDA, GFAP, and MMP-2, with corresponding specificities ranging from 82.54 to 71.43%.

CRP demonstrated a significant AUC of 0.965, with 93.06% sensitivity and 80.95% specificity, indicating its potential as a biomarker for pain processing abnormalities. IL-10 (AUC = 0.937), TNF- α (AUC = 0.913), and other biomarkers

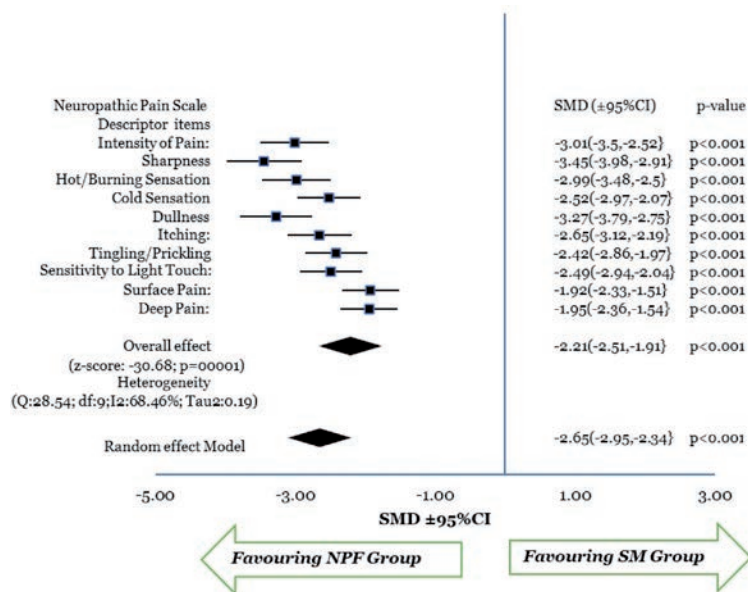


Figure 1.
Standardize mean difference of the items of neuropathic pain scale: NPS group versus SM group at 16 weeks.

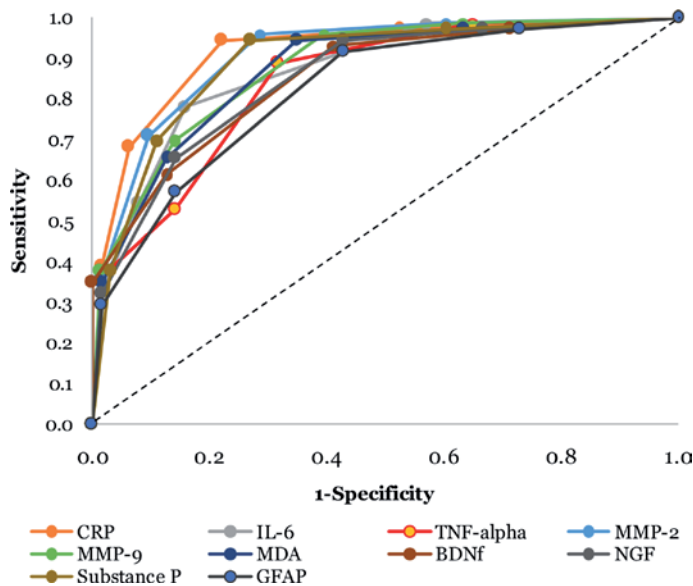


Figure 2.
ROC curves for biomarkers of biological risk factors of neuropathic pain: Experimental group ($n = 72$) versus control group ($n = 63$).

also demonstrated clinical relevance based on cutoff values (e.g., CRP: 1.5 mg/L, IL-10: 1.9 pg./mL, TNF- α : 10.6 pg./ml, MMP-2: 450 ng/ml, MMP-9: 81 ng/ml, MDA: 3.1 μ mol/, BDNF: 55 ng/ml, NGF: 199 pg./ml, substance P: 232 pg./ml, and GFAP: <10 ng/ml).

Biomarkers	AUC (95%CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Cutoff value
CRP	0.965 (0.94,0.99)	93.06	80.95	96	71.76	80.74	1.5 mg/L
IL-10	0.937 (0.9,0.97)	77.78	84.13	88.64	63.74	71.85	1.9 pg./ml
TNF-alpha	0.913 (0.87,0.95)	88.89	69.84	96.3	57.41	65.19	6 pg./ml
MMP-2	0.954 (0.93,0.98)	95.83	71.43	89.47	73.08	80	450 ng/mL
MMP-9	0.949 (0.92,0.98)	95.83	63.65	87.72	71.29	78.52	81 ng/ml
MDA	0.972 (0.95,0.99)	95.83	84.13	96	71.76	80.74	3.1 µmol/L
BDNF	0.922 (0.88,0.96)	93.06	58.73	84.62	66.27	73.33	55 ng/ml
NGF	0.923 (0.89,0.96)	94.44	57.14	83.93	68.35	74.81	199 pg./ml
Substance P	0.977 (0.96,1)	97.22	82.54	96.43	77.22	85.19	232 pg./ml
GFAP	0.962 (0.94,0.99)	95.83	68.25	95.65	68.54	77.78	<10 ng/m

Table 4.

Analysis of important parameters receiver operating characteristic curves of biomarkers for the biological risk factors of neuropathy pain: NPF group versus SM group at 16 weeks.

3.4 Meta-analysis of biomarker reductions

The meta-analysis identified significant reductions in key biomarkers associated with neuropathic pain. Inflammatory markers such as CRP (RR = 0.19, 95% CI: 0.10–0.29) and IL-10 (RR = 0.21, 95% CI: 0.11–0.32) were reduced significantly ($p < 0.0001$). Similarly, reductions were seen for TNF- α (RR = 0.26, 95% CI: 0.15–0.37, $p < 0.0001$), MMP-2 (RR = 0.32, 95% CI: 0.19–0.45), and oxidative stress marker MDA (RR = 0.14, 95% CI: 0.07–0.21).

Neurotrophic markers, such as BDNF (RR = 0.15, 95% CI: 0.08–0.22), NGF (RR = 0.13, 95% CI: 0.07–0.20), and pain-related markers such as substance P (RR = 0.11, 95% CI: 0.05–0.16), were also significantly modulated ($p < 0.0001$). The overall effect size was significant, and no heterogeneity was detected (see **Figure 3**).

3.5 Radar chart comparison of NPF and SM groups

- Figure 4** illustrates that NPF demonstrated superior efficacy over SM in alleviating pain across various metrics.

Intensity of pain: NPF achieved a 73.97% reduction compared to 13.52% with SM (60.45% more effective). Sharpness: NPF saw a 69.82% reduction versus 16.35% for SM (53.47% more effective). Hot/burning sensation: NPF led to a 70.70% decrease compared to 14.68% with SM (56.02% more effective). Cold sensation: NPF resulted in a 73.85% reduction versus 4.81% for SM (69.04% more effective).

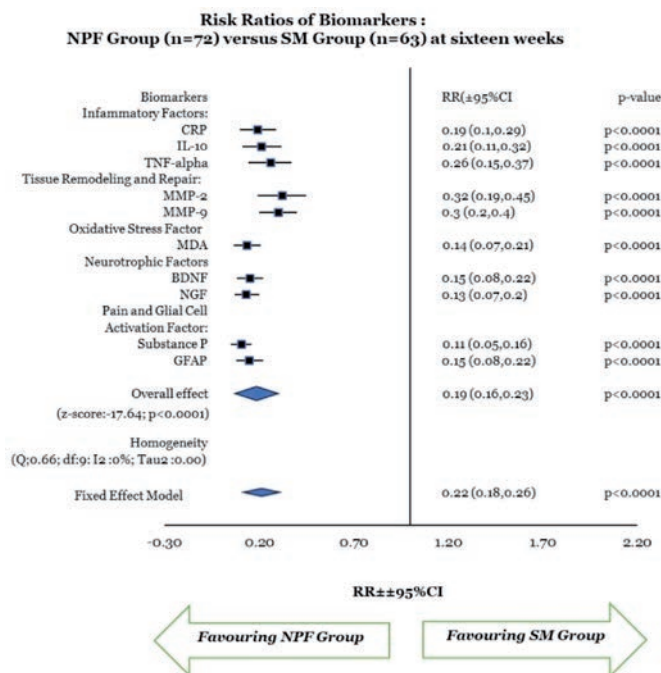


Figure 3.
Risk ratio of biomarkers: NPF group versus SM group at 16 weeks.

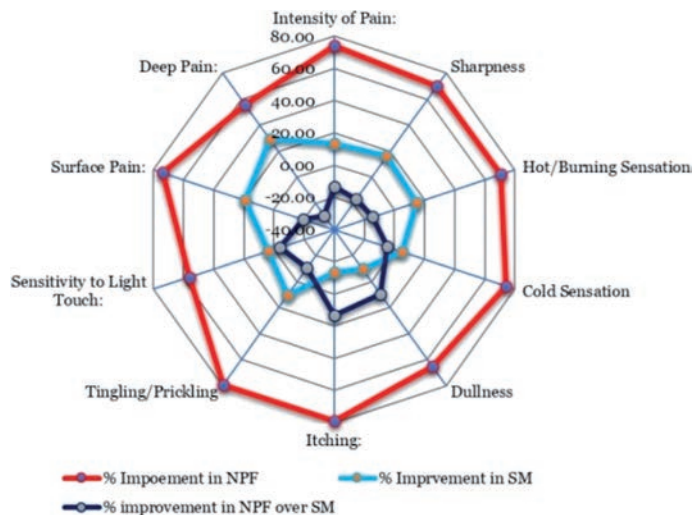


Figure 4.
Percentage of improvement of standardized mean of descriptors of NPS: NPF group, SM group, and NPF group over SM group at 16 weeks.

Dullness: NPF achieved a 65.22% reduction compared to -9.66% with SM (74.88% more effective). Itching: NPF showed a 79.04% improvement versus -13.35% with SM (92.39% more effective). Tingling/prickling: NPF resulted in a 79.25% decrease compared to 10.39% with SM (68.86% more effective).

Sensitivity to light touch: NPF saw a 56.21% reduction versus 3.49% for SM (52.72% more effective). Surface pain: NPF achieved a 73.80% reduction compared to 19.21% with SM (54.59% more effective). Deep pain: NPF resulted in a 55.77% decrease versus 29.13% for SM (26.64% more effective).

2. **Figure 5** illustrates substantial biomarker improvements in the NPF group relative to the SM group after 16 weeks. Notably, there were significant increases in several biomarkers, indicating enhanced therapeutic effects of NPF.

CRP levels increased by 107.41%, reflecting a marked reduction in inflammation. IL-10 showed a 112.02% increase, suggesting improved anti-inflammatory responses. TNF- α levels rose by 105.29%, indicating reduced pro-inflammatory activity. MMP-2 increased by 103.00%, highlighting enhanced tissue remodeling. MMP-9 saw a dramatic 329.55% rise, suggesting significant modulation of matrix degradation processes. MDA levels increased by 215.97%, demonstrating improved oxidative stress management. BDNF levels surged by 985.00%, reflecting substantial neuroprotective effects. NGF levels rose by 1278.13%, indicating significant neurogenic benefits. Substance P increased by 1037.50%, pointing to enhanced modulation of pain pathways. GFAP levels rose by 451.25%, suggesting improved glial cell activation and response.

3.6 Comparative analysis of biomarker differences

A comparative analysis of MD, SMD, and their respective 95% CIs between the NPF and SM groups at 16 weeks demonstrated significant variations across key biomarkers. Notably, the NPF group exhibited markedly lower levels of CRP, IL-6, TNF- α , MMP-2, and MDA compared to the SM group. For instance, MD for CRP was 9.83 (95% CI: 7.97 to 11.69, $p < 0.0001$) with an SMD of 1.91 (95% CI: 1.50 to 2.32, $p < 0.0001$), indicating a substantial reduction in the NPF group.

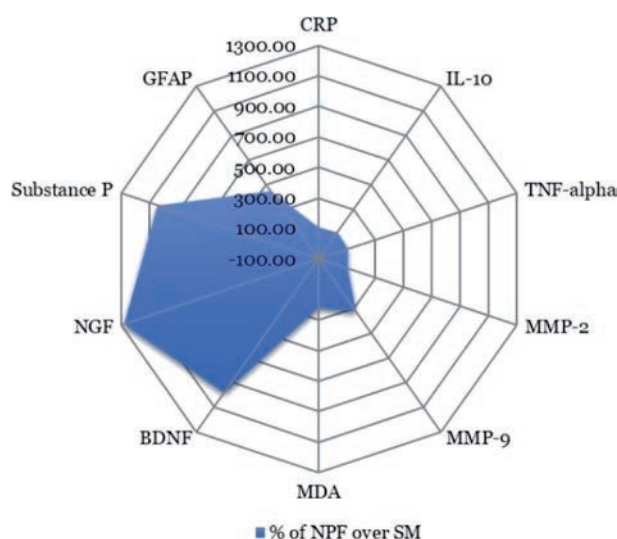


Figure 5.
Percentage of biomarkers of healthy cohorts of NPF group over SM group at 16 weeks.

Similar trends were observed for TNF- α , where MD was 19.45 (95% CI: 15.38 to 23.52, $p < 0.0001$) and SMD was 1.72 (95% CI: 1.33 to 2.12, $p < 0.0001$). In contrast, biomarkers like BDNF and NGF were significantly higher in the NPF group, with negative MDs and SMDs, highlighting their inverse relationship with the inflammatory markers.

These findings underscore the effectiveness of the NPF regimen in modulating key biomarkers associated with neuropathic pain, demonstrating significant improvements in inflammatory and neurotrophic factors when compared to the SM group (see **Table 5**).

3.7 Correlation coefficient

The Pearson correlation analysis revealed significant associations between key biomarkers in both the NPF and SM groups after 16 weeks. In the NPF group, strong positive correlations were observed between CRP and IL-6 ($r = 0.782$, $p < 0.001$),

Biomarker	NPF group (n = 72)		SM group (n = 63)		MD (95% CI)	p-value (MD)	SMD (95% CI)	p-value (SMD)
	Mean	SD	Mean	SD				
CRP	5.89	0.9	15.72	7.47	9.83 (7.97, 11.69)	<0.0001	1.91 (1.5, 2.32)	<0.0001
IL-6	4.91	1.2	8.27	5.73	3.36 (1.92, 4.8)	<0.0001	0.84 (0.49, 1.19)	<0.0001
TNF-alpha	8.88	2.7	28.33	16.3	19.45 (15.38, 23.52)	<0.0001	1.72 (1.33, 2.12)	<0.0001
MMP-2	101.45	12.45	275.5	64.28	174.05 (157.92, 190.18)	<0.0001	3.88 (3.31, 4.46)	<0.0001
MMP-9	148.41	23.65	221.4	117.08	72.99 (43.57, 102.41)	<0.0001	0.89 (0.54, 1.25)	<0.0001
MDA	2.41	1.01	7.32	2.92	4.91 (4.15, 5.67)	0.0042	2.31 (1.87, 2.75)	0.0002
BDNF	21.28	2.4	15.21	4.76	-6.07 (-7.37, -4.77)	0.339	-1.64 (-2.03, -1.25)	0.0004
NGF	36.45	6.7	15.24	9.78	-21.21 (-24.08, -18.34)	0.1423	-2.56 (-3.02, -2.11)	0.0006
Sub. P	14.45	7.2	22.32	10.98	7.87 (4.69, 11.05)	0.1278	0.86 (0.51, 1.21)	0.0025
GFAP	9.87	2.9	15.04	9.15	5.17 (2.81, 7.53)	0.4808	0.78 (0.43, 1.13)	0.0013

Table 5.
 Comparative study of mean difference, standardized mean difference, and their 95% CI and p-values between NPF group and SM group at 16 weeks.

CRP and TNF- α ($r = 0.762$, $p < 0.001$), and CRP and MMP-9 ($r = 0.722$, $p < 0.001$). Negative correlations were noted between CRP and BDNF ($r = -0.533$, $p < 0.001$) and CRP and NGF ($r = -0.437$, $p < 0.001$).

Similarly, in the SM group, significant correlations were found, particularly between IL-6 and TNF- α ($r = 0.994$, $p < 0.0001$) and IL-6 and MMP-9 ($r = 0.992$, $p < 0.0001$). The SM group also exhibited strong negative correlations between CRP and BDNF ($r = -0.378$, $p < 0.0001$) and between IL-6 and BDNF ($r = -0.367$, $p < 0.0001$).

Additionally, markers like MDA, which were significantly correlated with MMP-9 ($r = 0.764$, $p < 0.001$) and IL-6 ($r = 0.811$, $p < 0.001$) in the NPF group, showed weaker associations in the SM group ($r = 0.235$, $p = 0.0074$ and $r = 0.258$, $p = 0.0034$, respectively).

These findings highlight the differential biomarker interactions in the two treatment groups and underscore the importance of these biomarkers in the context of neuropathic pain (see **Table 6**).

Biomarker pair	NPF group (n = 72)		SM group (n = 63)	
	Pearson's r	p-value	Pearson's r	p-value
CRP and IL-6	0.782	<0.001	0.683	<0.0001
CRP and TNF-alpha	0.762	<0.001	0.681	<0.0001
CRP and MMP-2	0.758	<0.001	0.488	<0.0001
CRP and MMP-9	0.722	<0.001	0.782	<0.0001
CRP and MDA	0.719	<0.001	0.246	0.0051
CRP and BDNF	-0.533	<0.001	-0.378	<0.0001
CRP and NGF	-0.437	<0.001	0.698	<0.0001
CRP and Sub. P	0.761	<0.001	-0.43	<0.0001
CRP and GFAP	0.489	<0.001	0.542	<0.0001
IL-6 and TNF-alpha	0.876	<0.001	0.994	<0.0001
IL-6 and MMP-2	0.748	<0.001	0.496	<0.0001
IL-6 and MMP-9	0.802	<0.001	0.992	<0.0001
IL-6 and MDA	0.811	<0.001	0.258	0.0034
IL-6 and BDNF	-0.646	<0.001	-0.367	<0.0001
IL-6 and NGF	-0.447	<0.001	0.982	<0.0001
IL-6 and Sub. P	0.733	<0.001	-0.433	<0.0001
IL-6 and GFAP	0.513	<0.001	0.545	<0.0001
TNF-alpha and MMP-2	0.706	<0.001	0.48	<0.0001
TNF-alpha and MMP-9	0.742	<0.001	0.544	<0.0001
TNF-alpha and MDA	0.753	<0.001	0.258	0.0033
TNF-alpha and BDNF	-0.618	<0.001	-0.36	<0.0001
TNF-alpha and NGF	-0.486	<0.001	0.612	<0.0001
TNF-alpha and Sub. P	0.699	<0.001	-0.431	<0.0001
TNF-alpha and GFAP	0.518	<0.001	0.942	<0.0001

Biomarker pair	NPF group (n = 72)		SM group (n = 63)	
	Pearson's r	p-value	Pearson's r	p-value
MMP-2 and MMP-9	0.761	<0.001	0.479	<0.0001
MMP-2 and MDA	0.69	<0.001	0.165	0.0704
MMP-2 and BDNF	-0.523	<0.001	-0.027	0.826
MMP-2 and NGF	-0.415	<0.001	0.487	<0.0001
MMP-2 and Sub. P	0.657	<0.001	0.077	0.467
MMP-2 and GFAP	0.466	<0.001	0.32	<0.0001
MMP-9 and MDA	0.764	<0.001	0.235	0.0074
MMP-9 and BDNF	-0.621	<0.001	-0.387	<0.0001
MMP-9 and NGF	-0.458	<0.001	0.555	<0.0001
MMP-9 and Sub. P	0.711	<0.001	-0.426	<0.0001
MMP-9 and GFAP	0.487	<0.001	0.944	<0.0001
MDA and BDNF	-0.566	<0.001	0.111	0.3007
MDA and NGF	-0.408	<0.001	0.234	0.0077
MDA and Sub. P	0.692	<0.001	0.148	0.102
MDA and GFAP	0.445	<0.001	0.07	0.5104
BDNF and NGF	0.294	0.019	-0.353	<0.0001
BDNF and Sub. P	-0.567	<0.001	0.169	0.062
BDNF and GFAP	-0.583	<0.001	-0.245	0.0052
NGF and Sub. P	0.364	0.001	-0.437	<0.0001
NGF and GFAP	0.361	0.001	0.741	<0.0001
Sub. P and GFAP	0.436	<0.001	-0.453	<0.0001

Table 6.

The Pearson correlation coefficients and p-values for each pair of biomarkers of NPF group (n = 72) and SM group (n = 63) at 16 weeks.

3.8 Bioactive compounds and biomarker modulation in NPF formulation

The analysis of bioactive compounds and their associated biomarker modulation reveals a comprehensive approach to managing neuropathic and muscular pain through the NPF formulation. The key bioactive ingredients, including clove oil, lavender oil, eucalyptus oil, ginger oil, and others, were assessed for their impact on various biomarkers such as CRP, IL-6, TNF- α , MMP-9, MMP-2, MDA, BDNF, NGF, substance P, and GFAP.

Each bioactive compound targets specific biomarkers through distinct receptors and mechanisms. For instance, eugenol in clove oil modulates inflammatory markers like CRP, IL-6, and TNF- α by inhibiting the TLR4 and NF- κ B pathways. Similarly, linalool in lavender oil exhibits antioxidant activity, reducing oxidative stress and promoting neurogenesis by interacting with TrkB receptors.

The modulation of these biomarkers by various bioactive compounds demonstrates the potential efficacy of the NPF formulation in addressing the multifaceted nature of neuropathic and muscular pain. This table encapsulates the interplay between these compounds and biomarkers, providing insight into their therapeutic roles (see **Table 7**).

Ingredient	Bioactive compounds	Target biomarkers	Receptors/Mechanisms
Clove oil	Eugenol	CRP, IL-6, TNF-alpha	TLR4, NF-kB pathway, COX-2 inhibition
		MMP-9, MMP-2	MMP inhibition, ECM remodeling
		MDA	Antioxidant activity, lipid peroxidation reduction
		BDNF, NGF	TrkB receptors, neuroprotective pathways
		Substance P	NK1 receptor modulation
		GFAP	Glial cell activation pathways
Lavender oil	Linalool, linalyl acetate	CRP, IL-6, TNF-alpha	TLR2, NF-kB pathway, COX-2 inhibition
		MMP-9, MMP-2	MMP inhibition, tissue repair
		MDA	Antioxidant activity, oxidative stress reduction
		BDNF, NGF	TrkB receptors, neurogenesis
		Substance P	NK1 receptor modulation
		GFAP	Glial cell response modulation
Eucalyptus oil	Eucalyptol (1,8-cineole)	CRP, IL-6, TNF-alpha	TLR4, NF-kB pathway, COX-2 inhibition
		MMP-9, MMP-2	MMP modulation, ECM remodeling
		MDA	Antioxidant effects, lipid peroxidation reduction
		BDNF, NGF	TrkB receptors, neuroprotective effects
		Substance P	NK1 receptor modulation
		GFAP	Glial cell activity modulation
Ginger oil	Gingerol	CRP, IL-6, TNF-alpha	TLR4, NF-kB pathway, COX-2 inhibition
		MMP-9, MMP-2	MMP inhibition, tissue repair
		MDA	Antioxidant activity, oxidative stress reduction
		BDNF, NGF	TrkB receptors, neurogenesis
		Substance P	NK1 receptor modulation
		GFAP	Glial cell activation pathways
Peppermint oil	Menthol, menthone	CRP, IL-6, TNF- α	TLR4, NF-kB pathway, COX-2 inhibition
		MMP-9, MMP-2	MMP modulation, ECM remodeling
		MDA	Antioxidant effects, lipid peroxidation reduction
		BDNF, NGF	TrkB receptors, neuroprotective pathways
		Substance P	NK1 receptor modulation
		GFAP	Glial cell response modulation

Ingredient	Bioactive compounds	Target biomarkers	Receptors/Mechanisms
Wintergreen oil	Methyl salicylate	CRP, IL-6, TNF- α	TLR4, NF-kB pathway, COX-2 inhibition
		MMP-9, MMP-2	MMP inhibition, tissue repair
		MDA	Antioxidant effects, lipid peroxidation reduction
		BDNF, NGF	TrkB receptors, neuroprotective effects
		Substance P	NK1 receptor modulation
		GFAP	Glial cell activation pathways
Pine oil	α -pinene, β -pinene	CRP, IL-6, TNF- α	TLR4, NF-kB pathway, COX-2 inhibition
		MMP-9, MMP-2	MMP modulation, ECM remodeling
		MDA	Antioxidant activity, oxidative stress reduction
		BDNF, NGF	TrkB receptors, neuroprotective pathways
		Substance P	NK1 receptor modulation
		GFAP	Glial cell response modulation
Malkangni oil	Celastrol, celastrene	CRP, IL-6, TNF- α	TLR4, NF-kB pathway, COX-2 inhibition
		MMP-9, MMP-2	MMP modulation, tissue repair
		MDA	Antioxidant effects, oxidative stress reduction
		BDNF, NGF	TrkB receptors, neurogenesis
		Substance P	NK1 receptor modulation
		GFAP	Glial cell activation pathways
Aloe vera extract	Aloectin, acemannan	CRP, IL-6, TNF- α	TLR4, NF-kB pathway, COX-2 inhibition
		MMP-9, MMP-2	MMP modulation, ECM remodeling
		MDA	Antioxidant effects, lipid peroxidation reduction
		BDNF, NGF	TrkB receptors, neuroprotective effects
		Substance P	NK1 receptor modulation
		GFAP	Glial cell response modulation
Coconut oil	Lauric acid, capric acid	CRP, IL-6, TNF- α	TLR4, NF-kB pathway, COX-2 inhibition
		MMP-9, MMP-2	MMP modulation, ECM remodeling
		MDA	Antioxidant effects, lipid peroxidation reduction
		BDNF, NGF	TrkB receptors, neuroprotective effects
		Substance P	NK1 receptor modulation
		GFAP	Glial cell response modulation
Wheat germ oil	Tocopherols, squalene	CRP, IL-6, TNF- α	TLR4, NF-kB pathway, COX-2 inhibition
		MMP-9, MMP-2	MMP modulation, ECM remodeling
		MDA	Antioxidant activity, oxidative stress reduction
		BDNF, NGF	TrkB receptors, neuroprotective effects
		Substance P	NK1 receptor modulation
		GFAP	Glial cell response modulation

Table 7.
Bioactive compounds and biomarker modulation.

Table 8 elaborates on the roles of various bioactive compounds in targeting specific biological risk factors through their synergistic effects on the biomarkers involved in neuropathic pain.

Biomarker	Description	Bioactive compounds of lipophilic botanical extracts
1. Inflammatory and immune response factor:		
CRP	A marker of systemic inflammation	Eugenol, linalool, eucalyptol, gingerol, menthol, methyl salicylate, alpha-pinene, celastrol, aloectin, lauric acid, vitamin E
IL-6	A cytokine involved in inflammation	Eugenol, linalool, eucalyptol, gingerol, menthol, methyl salicylate, alpha-pinene, celastrol, aloectin, lauric acid, vitamin E
TNF- α	A pro-inflammatory cytokine	Eugenol, linalool, eucalyptol, gingerol, menthol, methyl salicylate, alpha-pinene, celastrol, aloectin, lauric acid, vitamin E
2. Tissue repair and remodeling factor:		
MMP-2	Involved in ECM remodeling	Eugenol, linalool, eucalyptol, gingerol, menthol, methyl salicylate, alpha-pinene, celastrol, aloectin, lauric acid, vitamin E
MMP-9	Involved in ECM remodeling	Eugenol, linalool, eucalyptol, gingerol, menthol, methyl salicylate, alpha-pinene, celastrol, aloectin, lauric acid, vitamin E
3. Oxidative damage and cellular stress factors:		
MDA	A marker of lipid peroxidation	Eugenol, linalool, eucalyptol, gingerol, menthol, methyl salicylate, alpha-pinene, celastrol, aloectin, lauric acid, vitamin E
4. Neurotrophic factors:		
BDNF	Supports neuron survival and growth	Eugenol, linalool, eucalyptol, gingerol, menthol, methyl salicylate, alpha-pinene, celastrol, aloectin, lauric acid, vitamin E
NGF	Essential for nerve cell survival and growth	Eugenol, linalool, eucalyptol, gingerol, menthol, methyl salicylate, alpha-pinene, celastrol, aloectin, lauric acid, vitamin E
5. Pain perception and glial cell activation factors:		
Substance P	A neuropeptide involved in pain perception and inflammation	Eugenol, linalool, eucalyptol, gingerol, menthol, methyl salicylate, alpha-pinene, celastrol, aloectin, lauric acid, vitamin E
GFAP	An indicator of glial cell activation	Eugenol, linalool, eucalyptol, gingerol, menthol, methyl salicylate, alpha-pinene, celastrol, aloectin, lauric acid, vitamin E
<i>Eugenol: (clove - Syzygium aromaticum), linalool: (lavender - Lavandula angustifolia), eucalyptol: (eucalyptus - Eucalyptus globulus), gingerol: (ginger - Zingiber officinale), menthol: (peppermint - Mentha piperita), methyl salicylate: (wintergreen - Gaultheria procumbens), α-pinene: (pine - Pinus species), celastrol: (malkangni - Celastrus paniculatus), aloectin: (aloe vera - Aloe barbadensis), lauric acid: (coconut - Cocos nucifera), vitamin E: (wheat germ - Triticum vulgare).</i>		

Table 8.
Summary of biomarkers of lipophilic botanical extracts synergistically affecting the biological risk factors of neurological pain.

3.9 Safety and cost evaluation

The safety, tolerability, and comparative cost of using topical NPF-lipophilic phytochemicals were evaluated. There were no problems with NPF oil and no significant safety problems requiring the suspension of the product or altering compliance to the supplementation plan were observed. The use of other medicines, including physiotherapy, and painkillers, was completely suspended for the subjects in the NPF group compared to subjects in the control group (SM) ($p < 0.05$).

The average management cost was evaluated for the subjects using NPF oil. This cost was defined as an average of 100% (for the 16 weeks of management) including treatment, diagnostic, and loss of working days, and these were reduced to 94% with a range of 78–98 ($p < 0.05$) compared to using the best standard management for neuropathic pain.

4. Discussion

The investigation of the topical formulation NPF underscores its comprehensive approach to managing postoperative neuropathic and muscular pain through a precisely formulated synergy of essential oils and botanical extracts, each selected for its potent bioactive properties. These components target key pain-related biomarkers, central to modulating inflammatory and neurogenic pathways. The efficacy of NPF is demonstrated by its ability to influence biomarkers closely linked to neuropathic and muscular pain, including C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), matrix metalloproteinases (MMP-2 and MMP-9), malondialdehyde (MDA), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), substance P, and glial fibrillary acidic protein (GFAP), thus providing targeted pain relief in the postoperative context.

4.1 Neuropathic pain scale analysis

A meta-analysis of NPF's effects on the NPS demonstrates significant improvements across all pain descriptors. The results show SMDs that indicate notable reductions in pain intensity (-3.01), sharpness (-3.45), and hot/burning sensations (SMDs ranging from -2.42 to -2.99). All findings showed strong statistical significance (p -values < 0.001), with the overall effect size recorded at -2.21 . The random effects model further confirmed a significant reduction in pain with an effect size of -2.65 ($p < 0.001$), despite some heterogeneity ($Q = 28.54$, $I^2 = 68.46\%$) (see **Figure 1**).

4.2 Overview of bioactive compounds and their molecular targets

NPF harnesses diverse bioactive compounds from essential oils and botanical extracts, each targeting molecular biomarkers related to inflammation, oxidative stress, tissue repair, and neuroprotection. The formulation's multitargeted approach modulates critical biomarkers like CRP, IL-6, TNF- α , MMP-2, MMP-9, and MDA, playing a pivotal role in reducing inflammation and oxidative damage, while promoting neuronal health via modulation of BDNF, NGF, and substance P. These targets are crucial in both the onset and progression of neuropathic and muscular pain.

4.3 Anti-inflammatory effects and cytokine modulation

Bioactive compounds such as eugenol from clove oil, linalool from lavender oil, and eucalyptol from eucalyptus oil demonstrate significant anti-inflammatory properties. These compounds inhibit key pro-inflammatory cytokines, including CRP, IL-6, and TNF- α , by modulating the NF- κ B and TLR4 signaling pathways. This reduction in systemic inflammation is particularly critical in mitigating chronic pain conditions [32].

4.4 Matrix metalloproteinases (MMPs) inhibition and tissue repair

Key components like gingerol from ginger oil and methyl salicylate from wintergreen oil inhibit MMP-2 and MMP-9, enzymes critical in the degradation of the extracellular matrix. By inhibiting these MMPs, NPF promotes tissue repair and prevents excessive tissue breakdown, a common issue in chronic inflammation and musculoskeletal disorders [33].

4.5 Antioxidant activity and oxidative stress reduction

Bioactive compounds in pine oil (α -pinene, β -pinene) and coconut oil (lauric acid and capric acid) contribute significant antioxidant properties. These compounds reduce oxidative stress by neutralizing free radicals, as reflected by the decrease in MDA levels, a marker for oxidative damage. This antioxidant activity is crucial for mitigating the oxidative damage linked to chronic pain conditions [34].

4.6 Neuroprotective pathways and pain modulation

The neuroprotective effects of NPF are mediated by compounds like eugenol from clove oil and linalool from lavender oil, which target neurotrophic factors BDNF and NGF. These factors are essential for maintaining neuronal health and modulating pain pathways. Moreover, bioactive compounds like menthol from peppermint oil and celastrol from malkangni oil modulate substance P, further enhancing pain relief and neuroprotection [35].

4.7 Glial cell modulation and neurological impact

Glial fibrillary acidic protein (GFAP), a marker for glial cell activation, is influenced by components like aloe vera extract (aloectin and acemannan) and wheat germ oil (vitamin E). Modulation of GFAP is significant for chronic pain management, as glial cell activation is associated with persistent pain states [36].

Recent research underscores the critical involvement of inflammatory biomarkers such as CRP, IL-6, and TNF- α in NP. Elevated levels of these biomarkers in NP patients reflect their significant roles in neuroinflammation and central sensitization. Notably, studies by Tuttle et al. [37] and Ramer et al. [38] highlight IL-6 as a pivotal player in both peripheral and central mechanisms of NP. Inhibition of IL-6 has been shown to reduce pain symptoms in animal models, positioning it as a potential therapeutic target. Similarly, TNF- α is known to amplify inflammatory responses in chronic pain conditions such as diabetic neuropathy and chemotherapy-induced peripheral neuropathy, further solidifying its role in exacerbating NP.

In the context of oxidative stress, MDA has been closely linked to neuronal damage and the chronicity of NP. Uceyler et al. [39] identified this connection, reinforcing the understanding that oxidative stress contributes to the persistence of neuropathic conditions. Neurotrophic factors, particularly BDNF, are equally important in regulating pain responses. Obata and Noguchi [40] demonstrated that BDNF plays a crucial role in chronic pain and neuronal plasticity, making it an attractive target for novel therapies aimed at mitigating pain-related neuroplastic changes.

Glial cell activation is another key contributor to the progression of acute pain to chronic NP. Ji et al. [41] conceptualized NP as a “gliopathy,” wherein microglia and astrocytes, once activated, release pro-inflammatory cytokines like TNF- α and IL-1 β , sustaining the pain state through central sensitization. This cellular activation amplifies neuroinflammatory responses, driving the chronicity of NP.

Moreover, the neuropeptide substance P (SP) has been shown to play a significant role in amplifying pain signaling pathways, particularly through its interaction with neurokinin 1 (NK1) receptors. Xu et al. [42] detailed how SP facilitates synaptic transmission, contributing to neuroplastic changes such as sprouting and dendritic spine alterations, which are critical for the persistence of NP. The maladaptive responses driven by SP underscore its importance in pain modulation, particularly after nerve or tissue injury.

Overall, the interplay among inflammatory biomarkers, oxidative stress, neurotrophic factors, and glial activation offers a comprehensive view of the molecular mechanisms underlying NP. This multifaceted understanding provides a foundation for developing innovative therapeutic strategies that target these molecular pathways, potentially improving pain management outcomes.

Table 4 and **Figure 2** detail the ROC curve analysis, showing high diagnostic sensitivity for biomarkers such as substance P and MDA. **Figure 3** highlights significant reductions in inflammatory and oxidative stress markers, while **Figure 4** demonstrates superior pain alleviation with NPF compared to standard management. **Table 5** and **Table 6** provide detailed insights into biomarker differences and correlations, underscoring the formulation’s impact on inflammatory and neurotrophic factors.

4.8 Broader implications and clinical relevance

The comprehensive bioactive profile of NPF suggests its potential utility beyond pain management. By modulating neurotransmitters and supporting neuroprotective pathways, the formulation may also benefit cognitive function and mood stabilization, addressing broader aspects of patient well-being. Botanical extracts such as *Syzygium aromaticum* (clove), *Lavandula angustifolia* (lavender), *Eucalyptus globulus* (eucalyptus), and *Zingiber officinale* (ginger) contribute to its anti-inflammatory, analgesic, and antioxidant properties, enhancing overall efficacy.

4.9 Study limitations and future directions

While the findings are promising, this study has some limitations, notably the duration of the trial and the patient population sample size. Future studies should focus on larger, more diverse populations, with longer follow-up periods to fully validate the efficacy and long-term benefits of NPF.

5. Conclusion

NPF presents a promising and innovative therapeutic strategy for the management of neuropathic pain, addressing both symptomatic relief and the underlying pathophysiological mechanisms. Through the targeted modulation of critical biomarkers—including nociceptive receptors, pro-inflammatory cytokines, oxidative stress indicators, and neurotrophic factors—NPF emerges as a potential adjunct or alternative to conventional pain management therapies, with particular relevance for addressing postoperative complications. Preliminary findings are optimistic, but additional clinical trials and research must validate its efficacy and define its place within standard pain management protocols. Its potential to improve the quality of life, especially for patients suffering from postoperative pain, warrants further exploration.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper. Ganguly Apurba and Ganguly Anondeep are affiliated with Nano Phyto Care Pvt. Ltd., which produces *NeuraPan Flex*®. This affiliation did not influence the design, data collection, analysis, or interpretation of the study outcomes.

Author details

Apurba Ganguly^{1,2,3*} and Anondeep Ganguly^{4,5,6}

1 Nano Phyto Care Pvt. Ltd., Kolkata, India

2 OPTM Health Care Pvt. Ltd, Kolkata, India

3 Biochemistry of Techno India University, Kolkata, India

4 Nano Phyto Care Pvt Ltd, A Premier Phytomedicine Manufacturing and Marketing Company, Kolkata, India

5 Oxford Brook University, UK

6 DigitalHealth, Harvard Medical School, USA

*Address all correspondence to: apurbaganguly15@gmail.com

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