



# Role of miRNA-181a in the treatment of oxaliplatin-induced neuropathy with asprosin: An examination in mouse dorsal root ganglia

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## Abstract

**Aim:** Neuropathic pain, a debilitating condition often resulting from chemotherapy treatments like oxaliplatin (OXA), poses significant treatment challenges. The molecular mechanisms underlying this condition remain incompletely understood, although microRNAs (miRNAs) have been implicated in its pathophysiology. This study focuses on miRNA-181a, known for its role in neuroinflammation and neuron signaling pathways, and investigates the therapeutic potential of asprosin, a novel hormone implicated in metabolic regulation, for modulating miRNA-181a expression in the context of OXA-induced neuropathy.

**Materials and Methods:** Mouse dorsal root ganglia (DRGs) were exposed to OXA to induce neuropathy, followed by treatment with asprosin. miRNA-181a expression levels were quantified and compared across healthy (control), OXA-treated, and asprosin-treated groups using qRT-PCR. Statistical significance was determined through ANOVA, with p-values less than 0.05 considered significant.

**Results:** Asprosin treatment did not significantly affect miRNA-181a expression in healthy mouse DRGs. However, OXA treatment resulted in a significant increase in miRNA-181a expression compared to controls ( $p < 0.0001$ ). Notably, asprosin administration significantly reduced miRNA-181a overexpression in OXA-treated DRGs ( $p < 0.0001$ ), suggesting a therapeutic effect of asprosin in modulating miRNA-181a expression under neuropathic conditions.

**Conclusion:** This study highlights the specific modulation of miRNA-181a by asprosin in OXA-induced neuropathic conditions, suggesting a novel therapeutic pathway for managing neuropathic pain. The findings underscore the potential of targeting miRNA pathways, particularly miRNA-181a, in developing treatments for chemotherapy-induced neuropathy. Further research is needed to elucidate the underlying mechanisms of asprosin's effect and its potential application in clinical settings.



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## Introduction

MicroRNAs (miRNAs), discovered in recent years, are important post-transcriptional regulators that regulate gene expression [1]. They modulate protein synthesis by binding to the 3'-untranslated regions of specific mRNAs. With over 2,500 mature miRNAs identified in the human genome, they play significant roles in various biological and pathological processes [2]. Particularly, miRNA-181a is part of a four-member miRNA family known to undertake critical roles in diverse biological functions [3-5].

Neuropathic pain is a prevalent and chronic health issue

caused by nerve damage and can be associated with various conditions, including cancer [6, 7]. Certain drugs used in cancer treatment, especially platinum-based anticancer drugs like oxaliplatin, can induce peripheral neuropathy leading to neuropathic pain [8, 9]. These drugs can cause damage to the peripheral parts of the nervous system, disrupting the processing of sensory signals and contributing to the emergence of chronic neuropathic pain.

Dorsal root ganglia (DRG) are essential components of the nervous system, playing a pivotal role in processing sensory signals and transmitting pain [10]. DRGs, located bilaterally along the spinal cord, house the cell bodies of sensory nerves. They play a crucial role in perceiving external stimuli and transmitting pain signals to the spinal

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cord. Recent studies have shown that exosomes secreted by satellite glial cells after in vitro oxaliplatin application have a pro-nociceptive effect on dorsal root ganglion neurons [11]. This provides a clue to the potential role of miRNA-181a in the pathophysiology of neuropathic pain. Asprosin, a recently discovered adipokine, plays a significant role in glucose regulation and insulin sensitivity [12, 13]. However, previous studies have suggested a possible association between asprosin and neuropathic pain [14]. It has been reported that asprosin has an analgesic effect and reduces pain sensitivity in an oxaliplatin-induced peripheral neuropathic animal model [15]. Specifically, the exact impact of asprosin on neuropathic pain via the regulation of miRNA-181a remains to be fully understood.

The main aim of this study is to investigate how the application of asprosin in dorsal root ganglia induced by oxaliplatin affects miRNA-181a expression. This investigation may contribute to a better understanding of neuropathic pain mechanisms and the development of potential treatment approaches.

## Materials and Methods

### Animals and experimental design

Mice were obtained from the Experimental Research Center of Firat University (FUDAM, Elazig, Turkey). In the experiments, a total of 28 male Balb/C mice weighing 25-30 g were used. The mice were housed under standard laboratory conditions with a constant temperature ( $23 \pm 2$  °C), humidity ( $60 \pm 5\%$ ), and a 12-hour light/dark cycle, with ad libitum access to food and water. After one week of acclimatization, the mice were randomly divided into four groups: Control, Asprosin (10 µg/kg), OXA, OXA+Asprosin (10 µg/kg), with 7 mice in each group. This study was approved by the Firat University Animal Experiments Local Ethics Committee (FUHADEK Decision No: 9, Protocol No: 2019/06). Care, housing, and use of animals were conducted following national and international criteria for laboratory animals [16].

### Oxaliplatin-induced neuropathy model

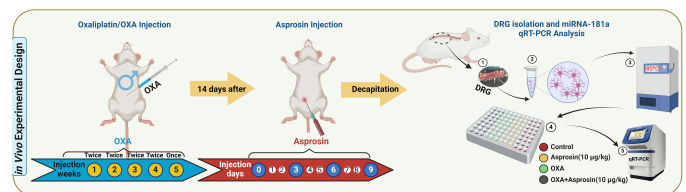
Oxaliplatin was prepared at a concentration of 3 mg/mL in 5% dextrose solution according to the weight of each animal and administered intraperitoneally (i.p.) at a dose of 3 mg/kg twice a week for 4.5 weeks. The Control group received injections of 5% dextrose solution. The development of peripheral neuropathy after oxaliplatin administration was followed for 2 weeks. Asprosin treatment was administered to the mice two weeks after the last injection of OXA.

### Asprosin treatment

Asprosin (Elabscience Company, USA) was dissolved in phosphate-buffered saline (PBS) (Sigma Aldrich, St. Louis, MO, USA). We evaluated the efficacy of asprosin (1, 10 and 30 µg/kg, ip) on pain behaviour in healthy animals. The effective dose of asprosin used in this study was calculated as 10 µg/kg by the LogEC50 method. Mice were injected intraperitoneally (i.p.) with asprosin at a dose of 10 µg/kg every three days for four times between 08:00 and 09:00, and then the mice were euthanized. The Control group received PBS injections only (i.p.).

### Preparation of DRG tissues and qRT-PCR analysis

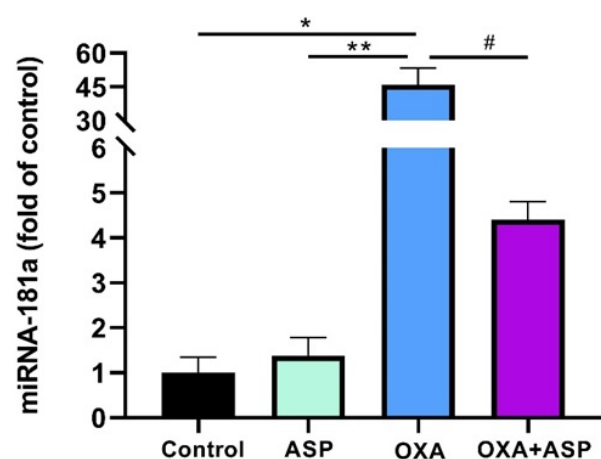
After euthanasia, DRGs were dissected from the lumbar 4 and 5 (L4,L5) of the mice and placed in RNase-free eppendorf tubes containing RNA later (Sigma-aldrich). Total RNA and miRNA were isolated from the DRG samples using the trizol extraction method. Concentration measurements were performed using the Qubit HS RNA Assay on a fluorometer device. Subsequently, cDNA synthesis was performed using the WizScript cDNA Synthesis kit with a starting concentration of 100 ng. During synthesis, RT primers specifically designed for SNO202 and miRNA-181a were used (Table 1). After cDNA synthesis, a qRT-PCR reaction was set up using BLIRT SimplyAmp SYBR Green Master Mix. Relative expression analysis was performed using the  $2^{-\Delta\Delta CT}$  method based on Ct values (Figure 1).



**Figure 1.** In vivo experimental design flowchart.

### Statistical analysis

All data were presented as mean and standard deviation (mean  $\pm$  SD). Statistical analyses and graphs were performed using GraphPad software (GraphPad Prism version 9.2.0 for macOS, GraphPad Software, San Diego, California USA), and the figures were prepared using biorender.com. One-way analysis of variance (One-Way ANOVA) and post-hoc Tukey tests were used for inter-group differences. A p-value of  $<0.05$  was considered statistically significant for all analyses.



**Figure 2.** The Effect of Asprosin on miRNA-181a Levels in the DRGs of Healthy and OXA-Treated Mice (n=7 for each group, \*p<0.0001; Control vs OXA, p<0.0001; ASP vs OXA, #p<0.0001; OXA vs OXA+Asprosin, one-way analysis of variance followed by a post-hoc Tukey HSD test. OXA– Oxaliplatin, ASP – Asprosin).

**Table 1.** Primer list for miRNAs used in qRT-PCR in the study.

	Primer sequences (5'-3')
SNO202	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACCATCAG
miRNA-181a	GTCGTATCCAGTGCCTGTCGTGGAGTCGGCAATTGCACTGGATACGACTCAC

## Results

### *The effect of asprosin on miRNA-181a expression in oxaliplatin-treated mouse dorsal root ganglia*

Compared to the control group, treatment with asprosin did not induce a significant change in miRNA-181a expression in the DRGs of healthy mice. However, when compared to the healthy group, mice in the OXA group showed a significant increase in miRNA-181a expression in their DRGs (\* $p < 0.0001$ ; Figure 2). The levels of miRNA-181a overexpressed in the DRGs of mice in the OXA group significantly decreased with treatment with asprosin (# $p < 0.0001$ ). Furthermore, miRNA-181a expression in the DRGs of mice in the OXA group significantly increased compared to the Asprosin group (\*\* $p < 0.0001$ ).

## Discussion

This study investigated the effect of asprosin on miRNA-181a expression in DRG of mice treated with OXA. The findings reveal that asprosin administration does not significantly alter miRNA-181a expression in the DRGs of unaffected, healthy mice. However, a marked escalation in miRNA-181a expression was noted in the DRGs of the OXA-treated relative to the control group, an increase that was significantly abated following asprosin treatment. Moreover, the level of miRNA-181a expression within the DRGs of the OXA-treated group was substantially higher than that observed in the asprosin-treated group. This study sheds light on the complex interaction between asprosin and miRNA-181a expression in the context of OXA-induced neuropathy, suggesting novel therapeutic potentials for neuropathic pain—a frequently encountered and debilitating consequence of chemotherapy. Traditionally associated with metabolic roles, asprosin's capacity to modulate miRNA-181a expression under neuropathic conditions signifies a broader and more profound biological significance.

The demonstrated capability of asprosin to significantly mitigate the overexpression of miRNA-181a induced by OXA treatment not only highlights its potential as an efficacious therapeutic agent for neuropathic pain management but also broadens the scope of its medical applications. This aligns with a substantial body of research on oxaliplatin-induced peripheral neuropathy, which explore the clinical manifestations, underlying mechanisms, and approaches for prevention and treatment of this condition [17, 18]. The findings from this current study further underscore the pivotal regulatory role of miRNA-181a in the context of oxaliplatin-induced peripheral neuropathy, emphasizing the potential for targeted therapeutic interventions in this area. This adds an important dimension to our understanding of neuropathic pain's molecular basis and opens avenues for novel treatment strategies that could significantly enhance patient outcomes in the face of chemotherapy-induced neuropathic conditions.

The importance of miRNAs, including miRNA-181a, as critical regulators of gene expression post-transcription, is well-documented across various disease states, especially those involving neurodegenerative conditions and the side effects of cancer therapies [19-22]. Context of OXA-induced neuropathy prompts a closer examination of the molecular pathways involved. Asprosin's known functions include influencing glucose homeostasis through cyclic AMP (cAMP) signaling pathways [23-25], which are also implicated in neuronal function and pain perception. It is plausible that asprosin's modulation of miRNA-181a involves cAMP signaling pathways [26], leading to changes in gene expression that mitigate neuropathic pain. Future research could explore this hypothesis through targeted gene expression analyses and the use of pathway inhibitors to delineate the specific signaling cascades involved. This study's alignment with existing literature further cements miRNA-181a's pivotal role in the pathophysiology of OXA-induced neuropathy and underscores the therapeutic potential of asprosin in modulating this miRNA's expression. Such modulation could represent a novel strategy for addressing the complex and multifaceted manifestations of neuropathy, providing relief where traditional pain management approaches may falter.

Moreover, the implications of asprosin's regulatory effect on miRNA-181a extend well beyond the confines of chemotherapy-induced neuropathy. By influencing the pathways governed by miRNA-181a, asprosin may hold the key to unlocking new therapeutic interventions for a variety of conditions marked by dysregulated miRNA expression. This includes diseases where miRNA-181a plays a role in inflammation, immune responses, and beyond, hinting at asprosin's potential applicability in treating autoimmune diseases, cancers, and cardiovascular disorders. Such cross-disciplinary potential emphasizes the need for deeper investigation into the molecular mechanisms driving asprosin's effects, potentially unveiling a host of new therapeutic targets.

This foundational study not only highlights asprosin's role in modulating miRNA-181a expression against the backdrop of OXA-induced neuropathy but also sets the stage for expansive research into asprosin's therapeutic capabilities. Future endeavors should aim to translate these preliminary findings into clinical applications, exploring optimal dosing, potential side effects, and efficacy in human patients. Navigating the journey from bench to bedside presents numerous challenges, yet the prospect of novel treatments that offer solace to those battling neuropathic pain is a compelling motivation. Through continued research, the insights gleaned from this study may eventually translate into significant benefits for patients, heralding new advances in combating chronic pain and related disorders.

## Conclusion

In conclusion, this study demonstrates the regulatory effect of asprosin on miRNA-181a in the OXA-induced neuropathy model. These findings provide significant evidence that asprosin could play a potential therapeutic role in the management of neuropathic pain. However, further research is needed on the mechanism of asprosin's effect and the specific roles of miRNA-181a in the pathophysiology of neuropathy. The results of this study represent an important step in developing new targets and strategies for treating neuropathic pain.

## Ethical approval

This study was approved by the Firat University Animal Experiments Local Ethics Committee (FUHADEK Decision No: 9, Protocol No: 2019/06).

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