

Thymoquinone as a Possible Neuroprotective Agent Against Paclitaxel-Induced Peripheral Neurotoxicity

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01 BACKGROUND

Chemotherapy-induced peripheral neurotoxicity (CIPN) is a common and serious side effect associated with various chemotherapy agents, including paclitaxel (PTX) ⁽¹⁾. Unfortunately, current therapeutic approaches have shown limited efficacy in either preventing or mitigating neuropathic debilitating symptoms. Thymoquinone (TQ), the primary bioactive compound of *Nigella sativa* seeds, has exhibited notable potential in mitigating CIPN, particularly in acute preclinical models⁽²⁾. However, its effectiveness in addressing chronic CIPN remains unestablished.

02 OBJECTIVE

To fill this research void, this study aimed to evaluate the neuroprotective potential of TQ in mitigating peripheral neurotoxicity induced by chronic PTX administration, utilizing both an *in vivo* rat model and an *ex vivo* embryonic rat dorsal root ganglion (DRG) organotypic culture. Additionally, the study assessed whether TQ interferes with the anticancer efficacy of PTX *in vitro*.

03 MATERIALS & METHODS

In Vitro studies

To evaluate the neuroprotective effect of TQ, DRGs were collected from E15 embryos of a pregnant Sprague-Dawley rat. The DRGs were cultured on collagen-coated dishes (4 ganglia per dish) and treated with TQ, 5 μM or 10 μM, PTX (50 nM), or their combination for 24 or 48 hours. micrographs were taken using a light microscope, and the length of the longest neurite in each DRG was measured using ImageJ software (Figure 1A).

MTT assay was performed to evaluate whether TQ could interfere with the cytotoxicity of PTX when co-administrated for 24h and 48h (Figure 1B).

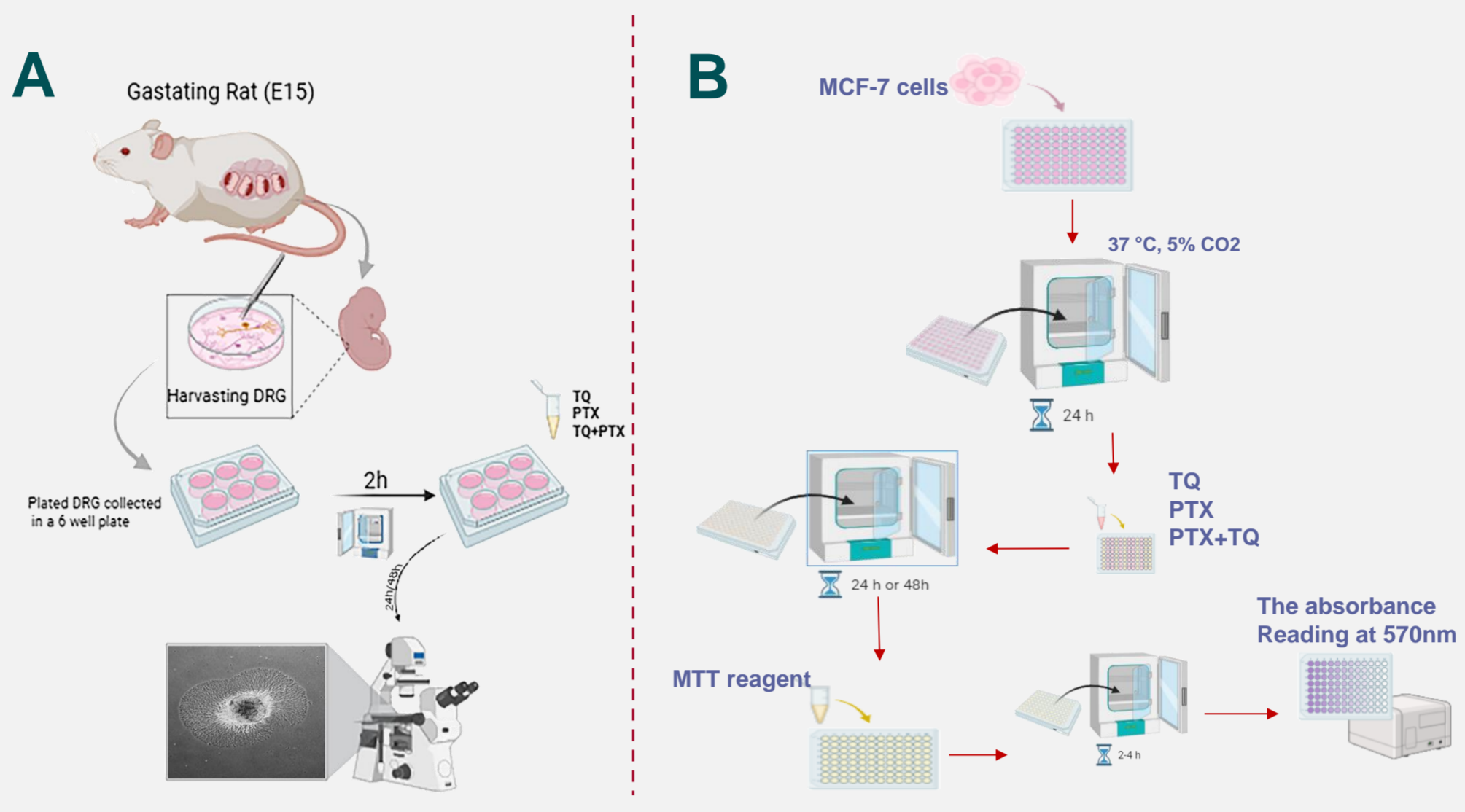


Figure1: Schematic representation of in Vitro Methods

In Vivo studies

Animals: A total of 60 rats were assigned to 5 experimental groups with 12 animals/groups based on their baseline responses to the behavioral test (Figure 2).

Behavioral Test: An aesthesiometer dynamic Test was performed at baseline, mid-treatment, and the end of treatment to assess mechanical allodynia. This parameter measure the development of neuropathic pain.

Intra Epidermal Nerve Fiber Density (IENF)
IENF density was quantified in the hind paw footpad in four animals/groups at mid-treatment and at the end of treatment to examine small unmyelinated peripheral nerve fiber damage. This parameter is correlated with CIPN.

Western Blot:
TLR4 protein expression was evaluated in DR which were collected from 4 animals/group at the end of the treatment period. This analysis aimed to assess neuroinflammatory signaling associated with CIPN.

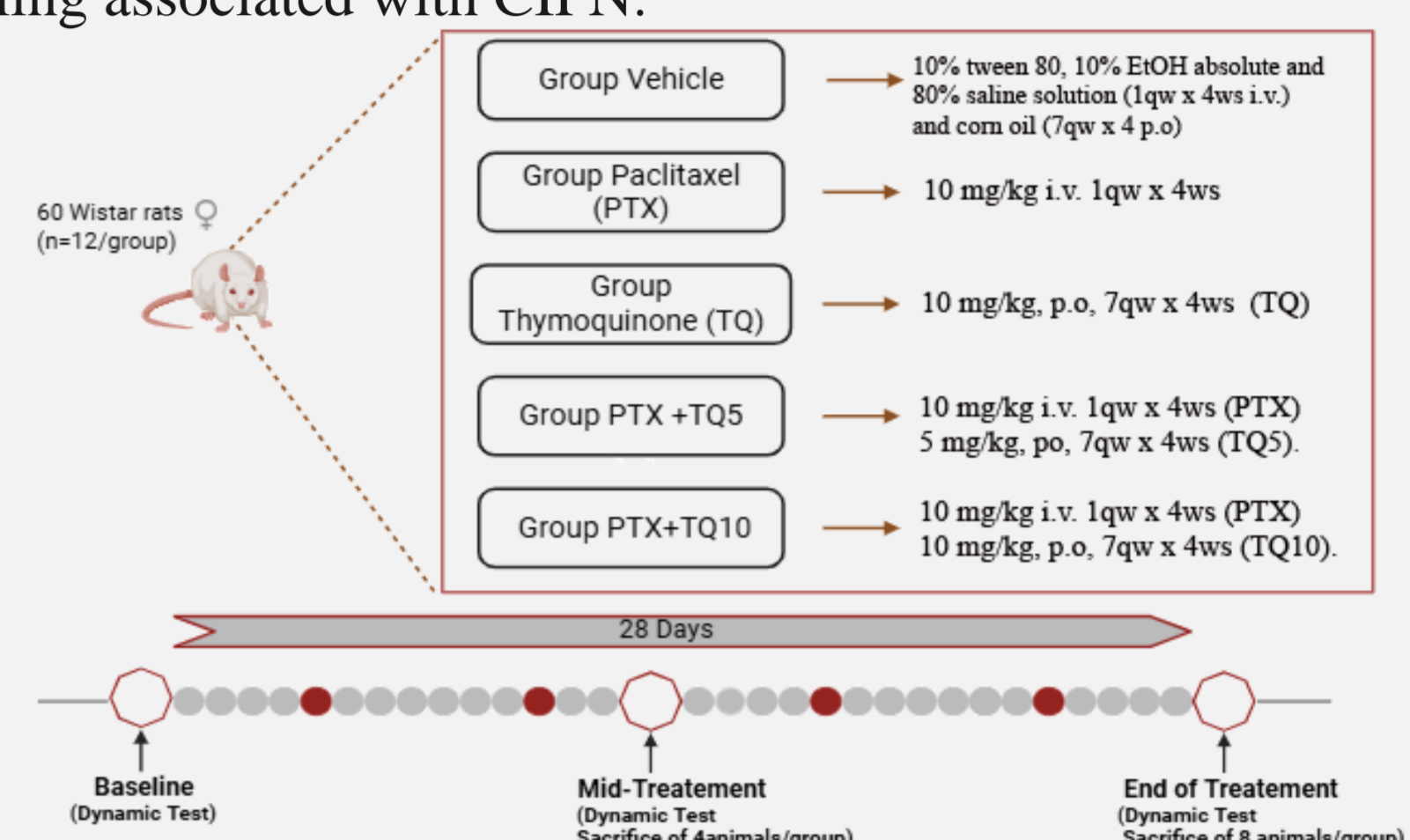


Figure 2: Experimental Design

04 RESULTS

In Vitro

Neurite Elongation Test

As expected, PTX (50 nM) significantly reduced neurite length compared to the CTRL group ($p < 0.0001$), confirming its strong neurotoxic effect. TQ alone (5 or 10 μM) did not alter neurite length, indicating no intrinsic toxicity. At these time points, co-treatment with PTX + TQ5 μM significantly prevented neurite impairment compared to PTX alone, demonstrating a consistent neuroprotective effect. In contrast, PTX + TQ10 μM failed to reverse the damage, suggesting that higher doses may not be effective (Figure 3).

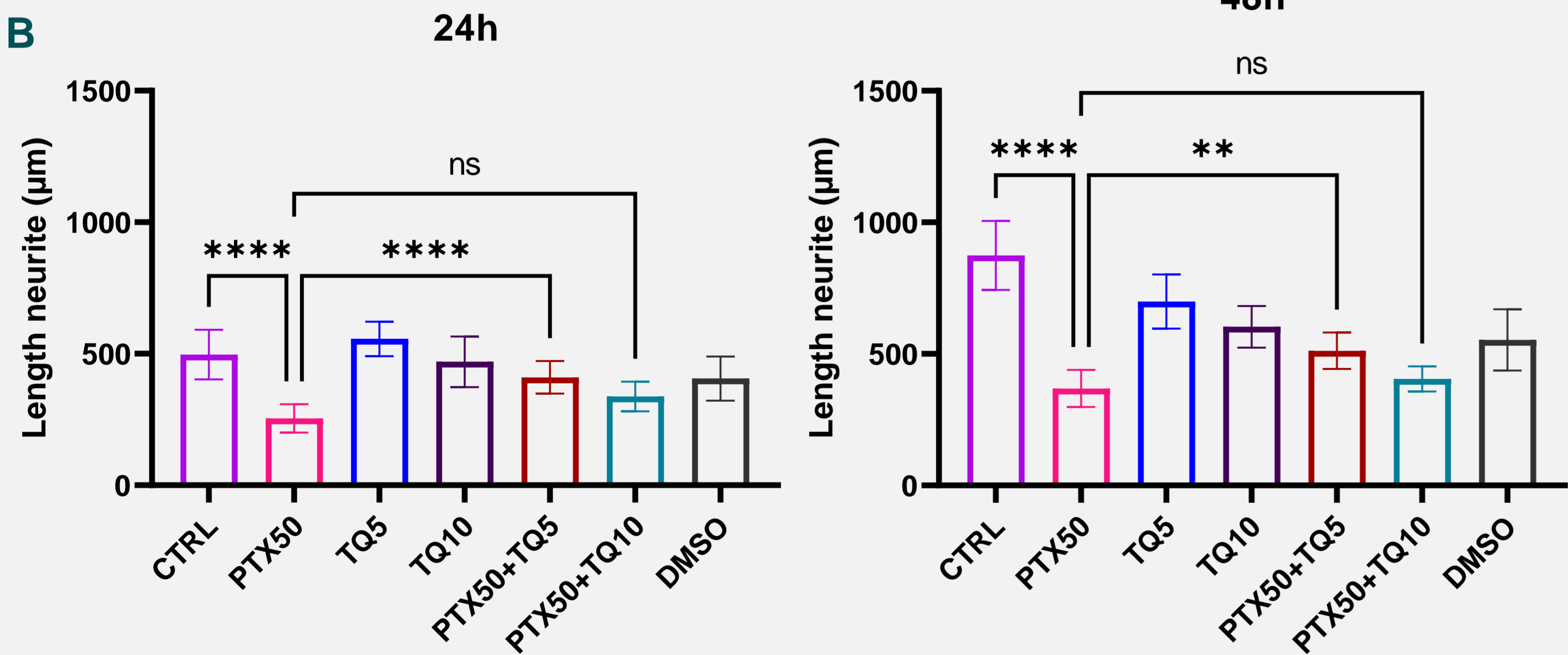
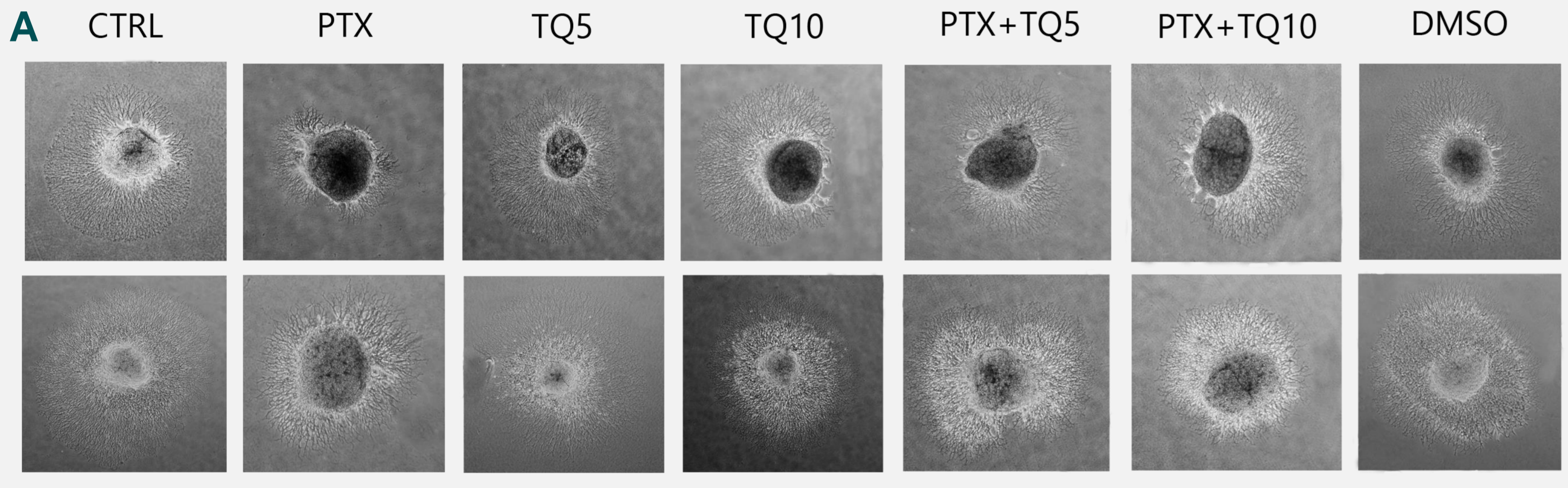


Figure 3: (A) Representative images of DRG treated with PTX, TQ, or its combination for 24 and 48 h.(B) Neurite outgrowth from DRG treated with PTX, TQ or its combination for 24 and 48 h.

MTT Analysis

Treatment with TQ (25μM) led to approximately 50% cell viability at 24h, which significantly improved by 48h, indicating a time-dependent toxicity.

At both time, Co-treatment with PTX at different doses significantly reduced viability compared to single treatments ($p < 0.01$).

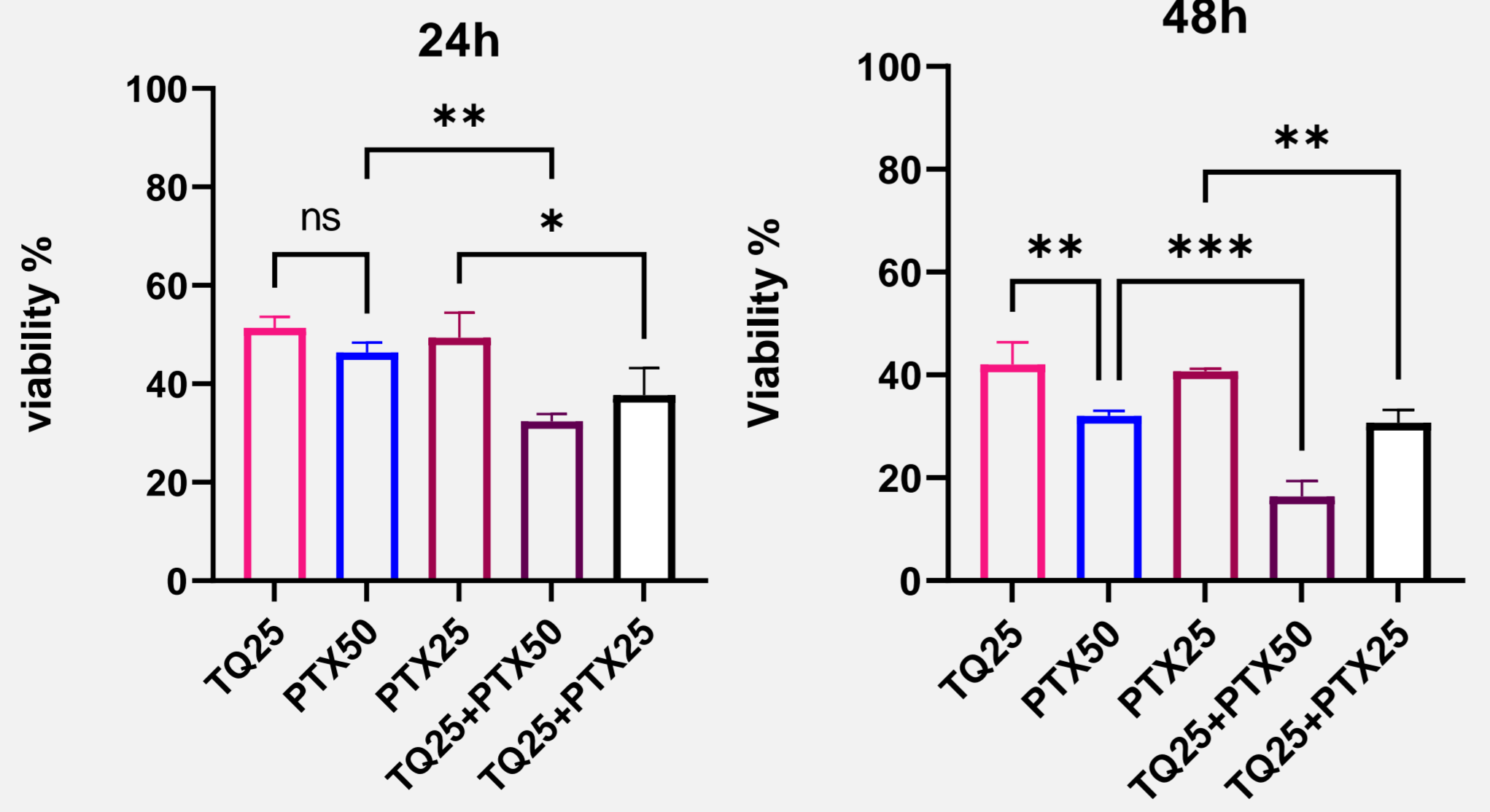


Figure 4: Cell viability Test

Body Weight Analysis

Throughout the experiment, the daily oral administration of TQ and the weekly intravenous administration of PTX, whether given alone or in combination, were well tolerated. A highly significant change in body weight over time was observed within groups ($p < 0.0001$), however, no significant differences were found between groups during the experiment ($p > 0.05$) (Figure 5).

In Vivo

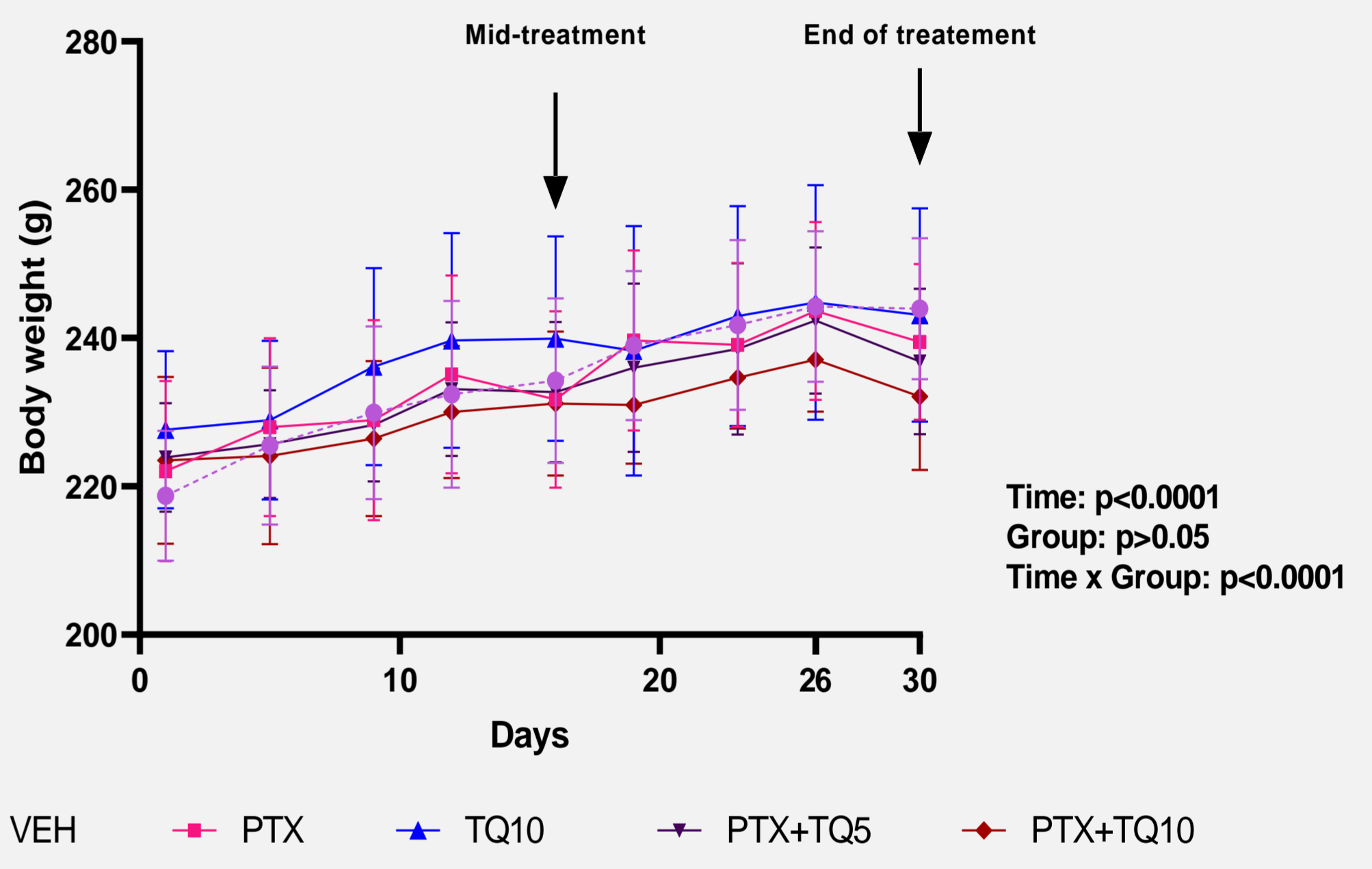


Figure 5 : Rat's Body weight growth curve during the experimental period

Mechanical allodynia

At the mid-treatment, PTX and PTX+TQ5 groups demonstrated a significant decrease in the mechanical threshold compared to the VEH group ($p < 0.05$ and $p < 0.01$, respectively; Figure 6A), indicating the development of mechanical allodynia. Furthermore, no significant difference in the effect of TQ (10mg/Kg) alone or in combination with PTX compared to the VEH group ($p > 0.05$; Figure 6A), suggesting that TQ (10mg/Kg) was able to prevent the mechanical allodynia induced by PTX. At the end of treatment, Both TQ5 and TQ10 groups were able to prevent PTX induced mechanical allodynia (Figure 6A). Furthermore, the quantitative analysis of IENF revealed a significant reduction in the density of small unmyelinated fibers in the PTX group which was prevented by PTX+TQ10 only at mid-treatment ($p > 0.05$ compared to the CTRL, Figure 6B).

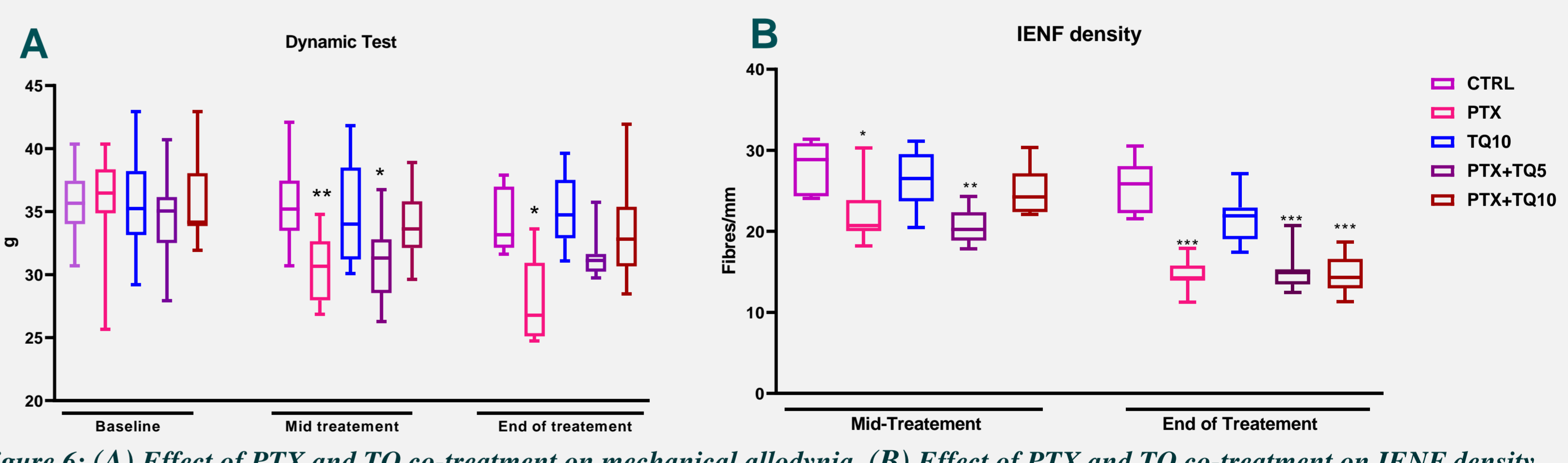


Figure 6: (A) Effect of PTX and TQ co-treatment on mechanical allodynia. (B) Effect of PTX and TQ co-treatment on IENF density.

Western Blot analysis

Western blot analysis revealed a significant upregulation of TLR4 protein expression in DRG following 4 weeks of PTX treatment Compared to the VEH group, indicating activation of inflammatory pathways. These findings support the role of TLR4-mediated neuroinflammation in PTX-induced peripheral neuropathy.

Analysis of the PTX + TQ co-treatment groups is currently in progress to evaluate potential modulation of TLR4.

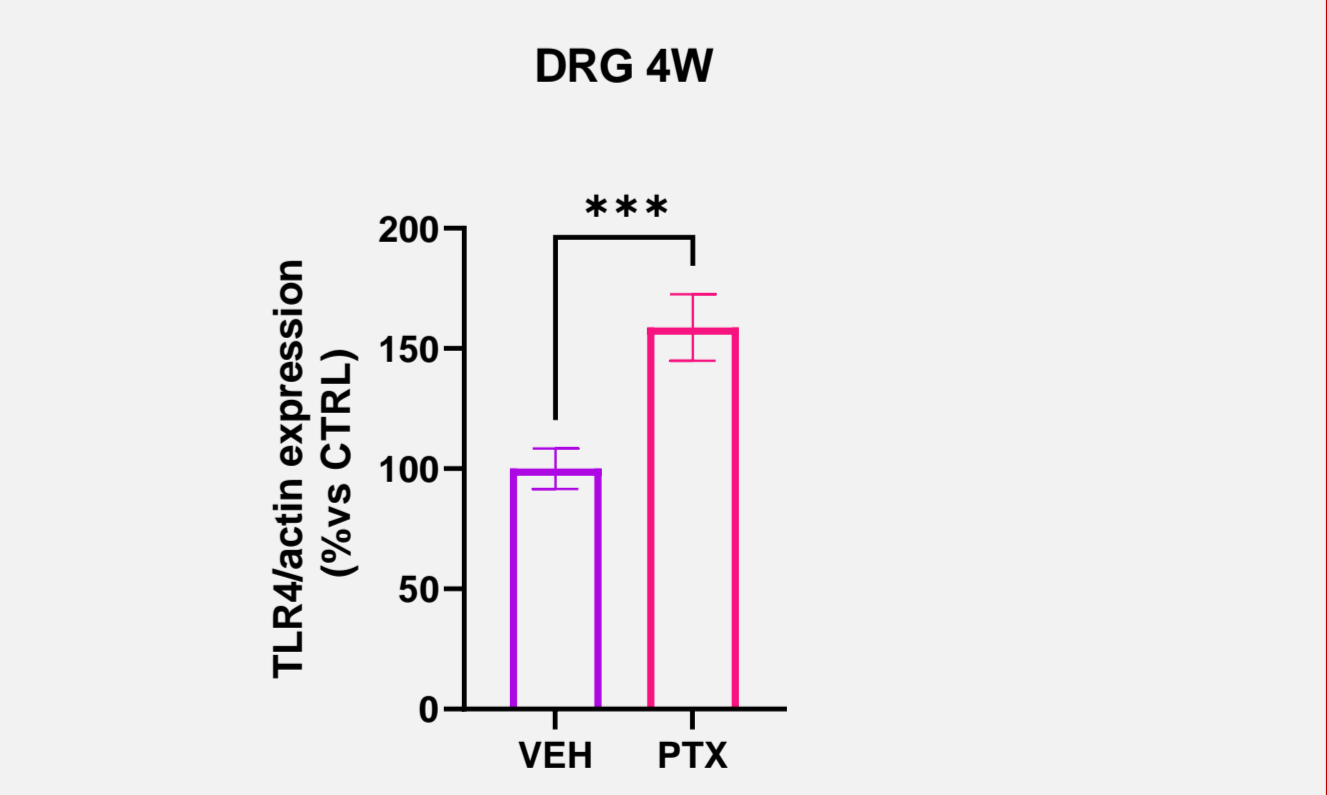


Figure 7:Western blot profiling of the expression of TLR4 in DRG

05 CONCLUSION & PERSPECTIVE

In *In vitro part*, TQ prevented PTX-induced neurite impairment supporting its neuroprotective potential. Importantly, TQ did not alter the anticancer activity of PTX, maintaining its therapeutic efficacy.
In *in vivo part*, TQ significantly prevented PTX induced mechanical allodynia at both mid-treatment and end of treatment and preserved from IENF loss at mid treatment.
Ongoing molecular analyses, including TLR4 and TRPV1 expression in DRG, will provide further insight into the anti-inflammatory and desensitizing mechanisms of TQ.
Future work will assess long-term outcomes, dose optimization, and explore combination strategies to enhance therapeutic benefit.

These preliminary findings suggest that TQ may serve as a safe, multitargeted adjunct therapy to prevent or mitigate CIPN without compromising anticancer efficacy.

References

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