# CURCUMIN AND CURCUMINOID EFFECTS MODULATING CHRONIC MECHANICAL SENSITIVITY IN SPINAL NERVE LIGATION MODEL REVERT MITOCHONDRIA DYSFUNCTION AND OXIDATIVE STRESS

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## ABSTRACT

**Objectives:** Studies have explored the role of curcumin in the mitigation of neuropathic pain (NP) due to curcumin's antioxidant and anti-inflammatory properties. We evaluated the effects of curcumin C3 complex ® (CUR) and bisdemethoxycurcumin (CMO) on the mRNA expression of oxidative stress and mitochondrial dysfunction in nervous tissues (amygdala and spinal cord) that are associated with pain processing in an NP model.

**Methods:** Twenty-three animals were randomly assigned to four groups: Sham, spinal cord ligation (SNL), SNL+100mg CUR/Kg BW, and SNL+50mg CMO/Kg BW for 4 weeks. Mechanical sensitivity was observed by the von Frey test weekly. The lumbosacral section of the spinal cord and the right amygdala were collected to determine the mRNA expression of genes related to oxidative stress (SOD1, NRF2, and UQCRC2) and mitochondrial function (MFN1, MFN2, OPA1 for mitochondrial fusion, and PINK1 for mitochondrial fission/mitophagy) utilizing qRT-PCR.

**Results:** Administration of CUR and CMO significantly decreased the mechanical hypersensitivity in SNL-operated groups, compared to SNL rats. The SNL procedure induced oxidative stress, shown by increased mRNA expression levels of NRF2 as well as decreased mRNA expression levels of SOD1 and UQCRC2. CUR and CMO administration mitigated the negative impacts of SNL on NRF2, SOD1, and UQCRC2 mRNA expression levels in the lumbosacral section of the spinal cord. Both CUR and CMO administration decreased the mRNA expression of MFN1 in the spinal cord and amygdala in SNL rats. CUR and CMO administration decreased OPA1 mRNA expression in the amygdala, while CMO administration increased OPA1 mRNA expression in the spinal cord of SNL rats. Moreover, compared to untreated SNL rats, CUR and CMO administration to SNL rats significantly decreased the mRNA expression of PINK1 in the spinal cord and amygdala.

**Conclusions:** CUR and CMO administration decreased mechanical hypersensitivity in SNL-operated rats. Based on the PCR results, CUR and CMO administration reduced SNL-induced oxidative stress and reverted mitochondrial dysfunction.

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## BACKGROUND/OBJECTIVES

Studies have explored the role of curcumin in the mitigation of neuropathic pain (NP) due to curcumin's antioxidant and anti-inflammatory properties. We evaluated the effects of curcumin C3 complex ® (CUR) and bisdemethoxycurcumin (CMO) on the mRNA expression of oxidative stress and mitochondrial dysfunction in nervous tissues (right amygdala and spinal cord) that are associated with pain processing in an NP model.

## HYPOTHESIS

Curcumin and Curcuminoid administration would decrease the mechanical sensitivity of SNL-treated rats, increase protection against oxidative stress, and reverse mitochondria dysfunction.

## METHODS

**Animal model of neuropathic pain**

Spinal nerve ligation (SNL) at lumbar level L5

**Treatments**

4 Groups of Male SD Rats (n=4-6 per group)

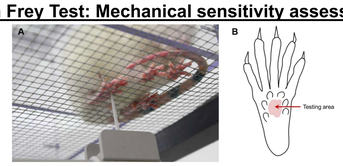
- Sham + vehicle (Sham)
- Spinal Nerve Ligation (SNL, pain model) + vehicle (SNL)
- SNL+ 100 mg/Kg BW Curcumin (SNL+100Curc)
- SNL+ 50 mg/Kg BW Curcuminoid (SNL+50CMO)
- Groups dosed daily for 4 weeks before the lower spinal cord and the right amygdala collection.

**Gene Expression**

Analyzed mRNA levels in the lower spinal cord and right amygdala using qRT-PCR

- Oxidative stress: SOD1 and NRF2
- Mitochondrial Function: UQCRC1 (Complex III), MFN1, MFN2, and OPA-1
- Mitophagy and Mitochondria Fission: PINK1

**Von Frey Test: Mechanical sensitivity assessment**

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**Statistical Analysis**

Data are presented as Mean ± SEM. * indicates p<0.05, # indicates 0.05<p<0.1 by One-way ANOVA and post hoc Tukey's test and T-test unpaired comparing Sham vs SNL, SNL vs SNL+100Cur, and SNL vs SNL vs SNL+50CMO.

## RESULTS

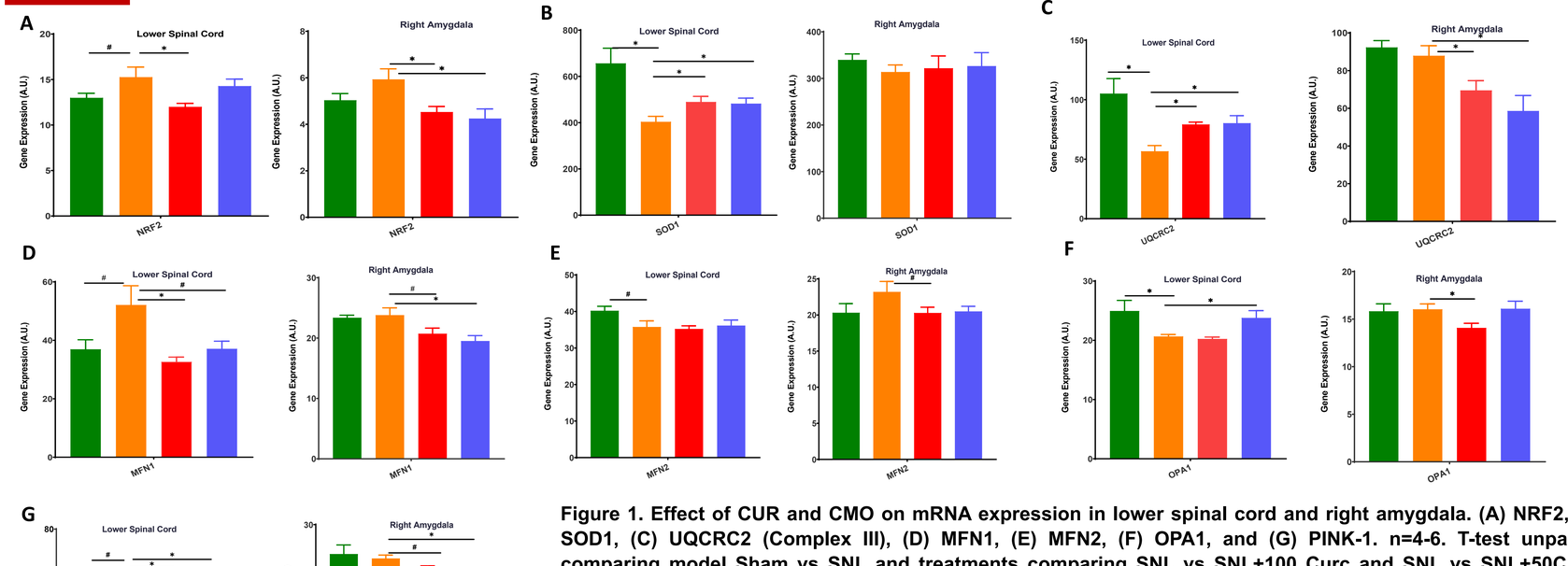


**Figure 1. Effect of CUR and CMO on mRNA expression in lower spinal cord and right amygdala. (A) NRF2, (B) SOD1, (C) UQCRC2 (Complex III), (D) MFN1, (E) MFN2, (F) OPA1, and (G) PINK-1. n=4-6. T-test unpaired comparing model Sham vs SNL and treatments comparing SNL vs SNL+100 Curc and SNL vs SNL+50CMO. *p<0.05, # 0.05<p<0.1.**

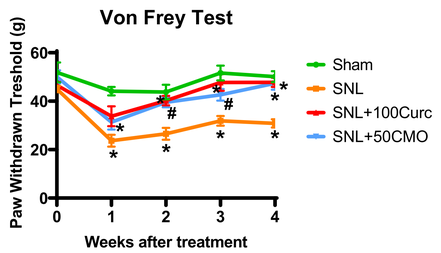


**Figure 2. Effect of CUR and CMO on mechanical sensitivity. n=6. One-way ANOVA and post hoc Tukey's test *p<0.05 and # 0.05<p<0.1.**

### Summary of genes expression

| | Lower Spinal Cord | | | Amygdala Right | | |
|---|---|---|---|---|---|---|
| Genes | SNL model | Curc 100 | CMO 50 | SNL model | Curc 100 | CMO 50 |
| NRF2 | ↑# | ↓* | - | - | ↓# | ↓* |
| SOD1 | ↓* | ↑* | ↑* | - | - | - |
| UQCRC2 | ↓* | ↑* | ↑* | - | ↓* | ↓* |
| MFN1 | ↑# | ↓* | ↓* | - | ↓# | ↓* |
| MFN2 | ↓# | - | - | - | ↓# | - |
| OPA-1 | ↓* | - | ↑* | - | ↓* | - |
| PINK-1 | ↑# | ↓* | ↓* | - | ↓# | ↓* |

## CONCLUSIONS

- Cur and CMO increased the expression of genes responsible for mitochondria function (Complex III) and for protection against oxidative stress (SOD1) in the lower spinal cord (site of the neuropathic model)
- Cur decreased the expression of NRF2 related to oxidative stress in both tissues lower spinal cord and right amygdala
- CMO also decreased the expression of NRF2 but only in the right amygdala
- Both Cur and CMO treatments decreased the mitochondria fusion genes in the lower spinal cord and the right amygdala
- In the lower spinal cord, the SNL model showed a decrease in OPA-1 gene expression (which regulates the shape of mitochondria and fusion) while CMO increased the OPA-1 expression reflecting a restoration of mitochondria function in lower spinal cord
- In the right amygdala, Cur decreased the expression of the OPA-1 gene
- Since the first week of treatment we have seen significant effects of Cur and CMO decreasing mechanical sensitivity and after 3-4 weeks the treated groups presented levels comparable to sham (control).

## ACKNOWLEDGMENTS

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