**ABSTRACT**

**Objectives:** Emerging evidence suggests that gut microbiota may serve at the intersection between microbiome-gut-brain and neuroinflammation in the development of neuropathic pain (NP). This study evaluated the effects of curcumin C3 Complex® (CUR) and bisdemethoxycurcumin (CMO), on the composition of gut microbiota and intestinal permeability- and neuroinflammation-associated gene expression in animals with NP.

**Methods:** 23 male rats were randomly divided into: sham, spinal nerve ligation (SNL group, pain model), SNL+100 mg CUR/kg BW (CUR group), and SNL+50 mg CMO/kg BW (CMO group) for 4 weeks. Fecal samples were collected for microbiota composition analysis using 16S rRNA gene sequencing. The mRNA expression level of tight junction proteins (Claudin 1, Occludin) and neuroinflammation (NF-kB) in the colon, amygdala, and spinal cord was analyzed by RT-PCR. Data were analyzed statistically.

**Results:** Using a beta-diversity weighted UniFrac distance metric, the microbiota profile of the CMO-treated group was significantly different than other groups (P<0.05). Regarding alpha-diversity, while most groups did not differ with respect to richness or evenness the CMO group improved microbiome evenness compared to the SNL group (P=0.0016). The relative abundance of several microbiome amplicon sequence variants (ASVs) changed with different treatments. The SNL group showed a depletion in Rotaia mexicana compared to the sham group (P<0.01). In contrast, Streptococcus and Clostridial ASVs (f. Oscillospiraceae g. UCMM-005) were enriched in the SNL group (P<0.01). CUR or CMO treatments induced changes in multiple species compared to SNL. CUR and CMO reversed the enrichment effect of SNL on Clostridial ASV (P<0.01). Compared to the sham group, the SNL group exhibited increased Claudin 1 mRNA expression levels in the amygdala. Relative to the SNL group, both CUR and CMO groups suppressed the mRNA gene expression of Claudin 1 (spinal cord, amygdala), Occludin (spinal cord, colon), and NF-kB (amygdala) in SNL-operated animals.

**Conclusions:** This study suggests CUR and CMO administration modifies multiple species of gut microbiome in an NP model. These effects may be associated with a reduction in SNL-induced intestinal permeability and neuroinflammation.

**Funding Sources.** Texas Tech University Health Sciences Center, Lubbock, TX.

**METHODS**

**Animals treatments:** 23 male SD rats (5-week-old) into 4 groups: Sham-vehicle (Sham group n=5), SNL-vehicle (SNL group n=6), SNL+100 mg CUR/kg BW CUR (CUR group n=6), and SNL+50 mg CUR/kg BW CMO (CMO group n=6) for 4 weeks. Gut microbiota composition in fecal ecles by 16S rRNA gene sequencing mRNA expression of claudin-1 and NF-kB in colon and amygdala by qRT-PCR.

**Statistical analysis:** mRNA expression levels were analyzed by one-way ANOVA followed by Tukey’s post-hoc analysis. Gut microbiota composition and diversity were analyzed by QIME2.

**RESULTS**

**Figure 1. Effect of CUR and CMO on beta-diversity.** Weighted UniFrac distance metrics for beta-diversity indicated that microbiome profiles changed significantly as a result of treatment (P = 0.032, PERMANOVA). Specifically, CMO group was structurally different than other groups, i.e., Sham (P = 0.013, pairwise PERMANOVA), SNL (P = 0.031, pairwise PERMANOVA), and CUR (P = 0.014, pairwise PERMANOVA). Distance between other groups were not statistically significant (P = 0.1, pairwise PERMANOVA).

**Figure 2. Effect of CUR and CMO on alpha-diversity.** Alpha-diversity was assessed with respect to species richness and evenness. First, in terms of species diversity or richness all groups were not different (P > 0.05, Faltis’ Phylogenetic Diversity). Second, while most groups didn’t differ with respect to evenness, CMO improved microbiome evenness in comparison to SNL (P = 0.016, Pielou’s Evenness), which suffered from a slight decrease that was not statically significant.

**CONCLUSIONS**

Our results suggest that the administration of the curcumin extracts CUR and CMO to rats with neuropathic pain: 1) modifies the composition of the gut microbiota; 2) alters the mRNA expression of the tight junction protein Claudin-1; and 3) suppresses the expression of the NF-kB1 marker for neuroinflammation.

**ACKNOWLEDGEMENTS**

This study was supported by Department of Pathology, Texas Tech University Health Sciences Center, Lubbock, TX.

**REFERENCES**

1. Pathology, Center of Excellence for Integrative Health, Texas Tech University Health Sciences Center, Lubbock, TX. 2. Department of Molecular Biology, Princeton University, Princeton, NJ.