A SULFATED POLYSACCHARIDIC FRACTION FROM THE GREEN SEAWEED CAULERPA CUPRESSOIDES ATTENUATES TEMPOROMANDIBULAR JOINT INFLAMMATORY HYPERNOCICEPTION IN RATS

INTRODUCTION. Temporomandibular disorders represent a heterogeneous, often idiopathic, group of inflammatory pain conditions involving masticatory muscles or the temporomandibular-joints (TMJ). Experimentally-induced-TMJ-inflammation in rat has becomes screening-tool for studies that search for potential-therapeutic-substitutes. Caulerpa cupressoides (Chlorophyta) contains three-sulfated-polysaccharidic-fractions (Cc-SP1, Cc-SP2 and Cc-SP3). Our group has shown both antinociceptive and anti-inflammatory-effects of Cc-SP2 in preclinical-trials. OBIEJCTIVE. This study analyzed Cc-SP2 efficacy in zymosan-(Zy)-induced TMJ inflammatory hypernociception in rats. MATERIALS AND METHODS. Cc-crude-SP was extracted by proteolytic-digestion (60°C,6h), and further fractionated by DEAE-cellulose-column applying a NaCl-gradient-(0→1M, with 0.25M of intervals). Quantitative-determination of sulfate-(S), hexose-(HEX) and uronic-acid-(UA) of the fractions were carried out using spectrophotometric-analysis. SPs in the fractions were verified by 0.5%-agarose-gel-electrophoresis in comparison to standards-HEP, chondroitin-4-sulfate-(40kDa) and chondroitin-6-sulfate-(60kDa), and their respective-molecular-masses estimated by 6%-polyacrylamide-gel-electrophoresis (PAGE) employing the same standards and dextran-sulfate-(8kDa). Rats (200-240g) were pretreated subcutaneously with Cc-SP2 1h before Zy-injection into the left TMJ. Von Frey test was used to evaluate inflammatory-hypernociception. 6h after Zy, synovial lavage was collected for leukocyte counting and myeloperoxidase (MPO) activity, and joint tissue for histopathological analysis (H&E). RESULTS AND DISCUSSION. Fractionation of Cc-SPs by DEAE-cellulose yielded Cc-SP1-(0.5M-NaCl), Cc-SP2-(0.75M-NaCl) and Cc-SP3-(1M-NaCl) containing differences among the relative-proportions of S-(10.99-18.38%), HEX-(34.92-49.73%) and UA-(6-72.2%). Agarose-gel showed polydisperse-bands and differences in charge-density among the fractions. Regarding the PAGE-analysis, the average-molecular-weights of four-different-SPs-(SP-1, SP-2, SP-3 and SP-4)-subfractions from the Cc-SPs-fractions ranged from 8 to >100kDa. These procedures, associated with the use of Stains-All, also revealed different-molecular-components on gels and the presence of UA in the fractions. Cc-SP2 (1, 3 or 9mg/kg) attenuated mechanical-hypernociception (52.82, 89.13 and 97.95%, respectively, p<0.05), and inhibited the leukocyte-influx (54.65, 72.94 and 82.99%, respectively, p<0.05), being confirmed by MPO-activity-
(p<0.05). In respect to histopathological-examination, Cc-SP2 (9mg/kg) reduced (p<0.05) the cellular-infiltration, but tissue-damage was observed at low-dose. Cc-SP2 interfered on TMJ-inflammatory-parameters in rats. **CONCLUSION.** Cc-SP2 demonstrates antinociceptive and anti-inflammatory efficacies in Zy-induced TMJ inflammatory hypernociception in rats.

**Keywords:** inflammation, polysulfated, nociception.

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