PRETREATMENT WITH FUCOIDAN-RICH FRACTIONS FROM SELECTED SRI LANKAN *Sargassum* SPECIES MODULATES NITRITE PRODUCTION AND OXIDATIVE BURST ACTIVITY IN ACTIVATED RAW 264.7 MACROPHAGES

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Abstract

Marine fucoidans are sulfated polysaccharides that have been widely investigated for their immunomodulatory potential. This study investigated the ability of crude Fucoidan-rich fractions from Sri Lankan Brown algae, *Sargassum* species; *S. polycystum* (SP) and *S. ilicifolium* (SI) to modulate inflammatory mediator production in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages. Cell viability was assessed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl Tetrazolium Bromide (MTT) assay prior to Griess and Nitro blue Tetrazolium (NBT) assays, for nitrite production and oxidative burst activity, respectively. Fucoidan-rich fractions recovered at yields of 10.0% (SP) and 9.5% (SI) (w/w), with low residual protein contents of 0.759±0.318% (SP) and 1.948±0.429% (SI), and sulfate contents of 47.89% (SP) and 22.82% (SI) showed FTIR spectra of characteristic sulfate ester (1220–1260cm^{-1}) bands, consistent with sulfated polysaccharides. MTT analysis demonstrated macrophage viabilities ≥90.00% following 24-hour exposure to 50, 100 and 200µg/mL of the fractions. Both Fucoidan-rich fractions produced concentration-dependent reductions in LPS-induced nitrite accumulation. In *S. polycystum*, nitrite levels decreased from 100% in the LPS control to 35.0±1.3%, 26.7±2.9% and 3.8±2.5% at 50, 100 and 200 µg/mL, corresponding to 65.0%, 73.3% and 96.2% inhibition, respectively. Similarly, *S. ilicifolium* reduced nitrite accumulation to 51.9±3.6%, 40.6±2.3% and approximately 0% of the LPS control, corresponding to 48.1%, 59.4% and near- complete inhibition. NBT assays demonstrated considerable suppression of LPS-induced reactive oxygen species generation at higher concentrations. At 100µg/mL, LPS-associated oxidative response inhibition was 51.13±15.25% and 84.23±0.69% relative to controls by *S. polycystum* and *S. ilicifolium* fractions, respectively, while 62.92±4.60% and 97.82±0.15% of suppression occurred at 200µg/mL. These findings demonstrate that crude Fucoidan-rich fractions from Sri Lankan *Sargassum* species modulate LPS-induced nitrite production and reactive oxygen species generation in macrophages. The results support the potential anti-inflammatory activity of sulfated polysaccharide-rich extracts from *Sargassum* species and provide a basis for future studies involving Pretreatment models with purified Fucoidan fractions.

Keywords: Anti-inflammation**,** Fucoidan, Nitrite, RAW 264.7 macrophages, Reactive oxygen species