2nd International Conference of Multidisciplinary Approaches (iCMA), 2015
Faculty of Graduate Studies,
University of Sri Jayewardenepura,
Sri Lanka
ISSN: 2386 – 1509

ESSENTIAL OILS OF ALPITNIA CALCARATA ROSC. INHIBITS THE IN VITRO GENERATION OF REACTIVE OXYGEN SPECIES IN MOUSE MACROPHAGES

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Phagocytic cells, like macrophages, are known to be activated under oxidative conditions. The stimulated production of reactive oxygen species (ROS) by these phagocytic cells due to the increased consumption of oxygen results in the oxidative burst which causes tissue injury in chronic inflammatory conditions like rheumatoid arthritis (RA). Rhizomes of *Alpinia calcarata*, commonly known as *heen araththa* in Sinhala and *chittharaththa* in Tamil, are popularly used in herbal medicine for its reputed analgesic, antimicrobial, anti-inflammatory and anti-tumour properties. The herbal formulation, *Maha Rasnadhi Quathar* (MRQ), used by Sri Lankan ayurvedic practitioners in treating RA contains rhizomes of *A. calcarata* as a major ingredient. The present study, aimed to investigate the *in vitro* anti-inflammatory activity of essential oils (EO) of *A. calcarata* rhizome, leaf and stem sheath by a chemiluminescence based phagocytosis kinetic assay. Whole plants of *A. calcarata* were collected from Western province of Sri Lanka and the hydro-distillation using Clevenger apparatus yielded essential oils from rhizome, leaf and stem sheath. Gas chromatography-Mass Spectrometric (GC-MS) analysis was done to identify the components of the EO's. Murine macrophage RAW 264.7 cells were cultured in RPMI-1640 media supplemented with 5% Fetal bovine serum were activated by serum opsonized zymosan-A and changes in ROS production was determined by luminol based chemiluminescense. The cytotoxicity of EO's was determined by Tryphan blue exclusion assay (40, 20, 10, 5, 2.5 and, 1.25 µg/mL). The inhibitory effects on intracellular generation of ROS by essential oils of *A. calcarata* were studied. Results obtained showed that all essential oils at 40 µg/mL decreased ROS production and possess strong inhibitory activity in the range of 79.23±0.67, 80.45±0.63 and 79.45±0.34% for rhizome, leaf and stem sheath respectively. Aspirin (40 µg/mL) was used as a standard drug and it showed inhibition of 80.65±0.64%. A dose response relationship was observed in the inhibition of all EO's and the IC50 was found to be 21.97±0.84, 21.59±0.22, 23.27±1.58 µg/mL respectively. None of the EO's affected macrophage viability (90 -95%) upon 1 h incubation. Some of the major components by GC-MS analysis of EO's were 1,8- cineole, α-pinene, β-pinene, fenchyl acetate, borneol, camphor, carotol and, □-phellandrene. The emerging evidence in the use of EO's, which are commonly complex mixtures of volatile terpenes, as alternative medicine indicates the need of research and validation of the numerous health and wellness benefits of therapeutic grade EO's. The present study is the first report on intracellular ROS production inhibition by *A. calcarata* in murine macrophages by a novel cell based chemiluminescence assay. In

conclusion, essential oils of *A. calcarata* rhizome, leaf and stem sheath showed a significant inhibition of ROS production *in vitro* and could have a potential therapeutic effect on arthritis disease by inhibiting production of superoxide anions thus by preventing oxidative burst of macrophages.

Keywords: *Alpinia calcarata, ROS, Luminol, Zymosan, Anti-inflammatory*