

Inhibition of the production of reactive oxygen and reactive nitrogen species in RAW 264.7 cells by aqueous extract of *Artocarpus heterophyllus*

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Reactive nitrogen species (RNS) and reactive oxygen species (ROS) are highly reactive molecules that are secreted by immune cells, during inflammatory responses. ROS and RNS are required for host defense and intracellular signaling. However, overproduction of these can cause damage to macromolecules such as lipids, proteins and DNA leading to cellular damage and dysfunction as well as leading to oxidative and nitrosative stress potentially resulting chronic inflammatory diseases. Medicinal plant-based natural products are rich in flavonoid and phenolic compounds and have gained attention for their potential to modulate ROS and RNS production and to inhibit inflammation. Aqueous preparations of different parts of *Artocarpus heterophyllus* Lam. (Moraceae) are traditionally used in Sri Lankan medicinal practices for treatment of various diseases including immune related diseases such as skin diseases and asthma. In this study, aqueous root extract (ARE) of *A. heterophyllus* was used to determine its effect on ROS and RNS production in stimulated RAW 264.7 murine macrophages. Using MTT assay, ARE concentrations of 62.5-2000 µg/mL were determined as non-toxic to RAW cells and suitable to perform the inhibition assays. Inhibitory effects of ARE on ROS and RNS production in RAW cells were assessed by quantitative nitro blue tetrazolium and Griess assays respectively. ARE treatment significantly inhibited phorbol 12-myristate 13-acetate-stimulated ROS production and lipopolysaccharide-stimulated NO production in dose-dependent manner ($r=0.724$; $p<0.001$ and $r=0.686$; $p<0.001$ respectively). The highest inhibition of ROS production was 71.2 ± 0.65 ($p<0.001$) at 2000 µg/mL of ARE with $IC_{50}=169.04$ µg/mL whereas highest inhibition of RNS production was 38.3 ± 4.9 ($p<0.001$) at 2000 µg/mL of ARE. Further, a strong positive correlation was observed between ROS and RNS inhibition by ARE ($r=0.972$; $p=0.001$). These results demonstrate that ARE can effectively suppress macrophage-derived ROS and RNS validating its anti-inflammatory potential and the use of *A. heterophyllus* in indigenous medicinal practices for immune related diseases.

Keywords: *Artocarpus heterophyllus*, Anti-inflammatory activity, Reactive nitrogen species and Nitric oxide, RAW macrophages

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