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Inhibition of pro-inflammatory cytokine secretion by human mononuclear cells and phagocytic activity of human neutrophils by aqueous leaf extracts of *Vitex negundo*

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The *in vivo* anti-inflammatory activity of the aqueous leaf extract (ALE) of *Vitex negundo* had been shown previously using the rat paw-oedema model. This *in vitro* study was carried out to investigate the specific *in vitro* inhibitory effects of ALE of *V. negundo* on human mononuclear cell and neutrophil functions. ALE concentrations of 31.3-500 µg/ml were selected based on having >80% viable human leukocytes in the *in vitro* cytotoxicity assay. Freeze-dried ALE inhibited the secretion of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) by LPS-stimulated human mononuclear cells in a dose-dependent manner ($r = 0.858$; $P = 0.007$ and $r = 0.995$; $P < 0.001$ respectively). ALE at 500 µg/ml showed the highest inhibition of IL-6 and TNF-α secretion (58.0% and 26.1% respectively). However, there was no significant inhibition of IL-1β secretion by ALE with all the concentrations used. ALE was tested *in vitro* for its inhibitory effect on neutrophil phagocytic activity, production of nitric oxide (NO) and reactive oxygen species (ROS). ALE showed the highest significant inhibition of yeast phagocytosis (55%) and ROS production (54%) at 500 µg/ml ($P<0.01$) and these inhibitory effects were dose-dependent ($r=0.94$; $P=0.15$ and $r=0.76$; $P=0.007$ respectively). ALE showed significant, dose-dependent inhibition of NO production by human neutrophils as measured by the nitrite and nitrate in culture supernatants ($r = 0.90$; $P < 0.001$ and $r = 88$; $P < 0.001$ respectively). The highest percent inhibition was observed at 500 µg/ml of ALE (91%) and this was comparable to that of the positive control, N-monomethyl-L-arginine (95%). Moreover, ALE possessed significant membrane stabilising activity with a highest activity of 91.4±1.00 % at 0.01 µg/ml ($P<0.001$). Inhibition of inflammatory mediators such as ROS, NO, IL-6, TNF-α and inhibition of phagocytic activity of neutrophils demonstrated that immunomodulation is one of the mechanisms by which ALE of *V. negundo* mediates anti-inflammatory effect.

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