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Mode of Action of a Fermented Papaya Preparation on NO Synthesis and TNF-α Secretion in the Mouse Macrophages Cell Line RAW 264.7

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Bio-normalizer (BN), a nutraceutical from *Carica papaya* which is used as a health food supplement, has been recently reported to affect nitric oxide (NO) and H_2O_2 production in macrophages and neutrophils, respectively. Little is known which particular components of BN are mediating its cellular activity. In the present study we investigated whether a low (LBN) or high (HBN) molecular weight fraction of BN exhibit different mode of actions regarding NO synthesis and tumor necrosis factor-α (TNF-α) secretion in the mouse macrophage cell line RAW 264.7.

LBN and HBN fractions of BN were obtained by ultrafiltration of a BN solution through an anisotropic membrane (3000 MW cut-off). The efficacy of BN on NO synthesis was measured by nitrite accumulation into the medium by the Griess reaction. Real time NO radical formation was assayed by EPR using sodium N-methyl-D-glucamine iron, $(MGD)_2$-Fe^{+}_{2}, as a spin trap. The amount of TNF-α secreted by macrophages was determined by a capture ELISA. LBN and HBN were tested for lipopolysaccharide (LPS) using the colorimetric *Limulus* amoebocyte lysate assay.

Non-activated mouse RAW 264.7 macrophages did not produce NO nor TNF-α constitutively. However, a major increase of NO accumulation into the medium in the presence of IFN-γ (10 U/ml) was observed both after treatment with LBN and HBN. On the other hand, LBN alone did not induce secretion of TNF-α into the medium from macrophages. However, HBN alone induced secretion of TNF-α similar to the effect of LPS. In addition, LBN and HBN exhibited different kinetics in NO radical formation monitored after 8, 12 and 24 hours of stimulation. HBN contained considerable amounts of LPS reacting material, however no LPS was detectable in LBN.

In conclusion, the results clearly demonstrate that BN affects NO synthesis and proinflammatory cytokine TNF-α secretion in macrophages. Possibly the cellular activity of LBN is mainly mediated due its high glucose concentration, whereas HBN might partially meditate NO synthesis and TNF-α secretion due to LPS. Further studies are warranted to elucidate the effect of LBN and HBN on iNOS enzyme activity and gene expression.