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Nitric oxide synthesis and TNF- $\alpha$  secretion  
in RAW 264.7 macrophages  
Mode of action of a fermented papaya preparation

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**Abstract**

Macrophage inducible nitric oxide synthase is able to generate massive amounts of nitric oxide (NO) which contributes to the host immune defense against viruses and bacteria. Monocyte-macrophages stimulated with the bacterial wall component lipopolysaccharide (LPS) and cytokines such as interferon- $\gamma$  (IFN- $\gamma$ ) express the inducible form of nitric oxide synthase (iNOS). Furthermore, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is one of the central regulatory cytokines in macrophage antimicrobial activity and synergizes with IFN- $\gamma$  in the induction of NO synthesis. Because of its pivotal role in both antimicrobial and tumoricidal activities of macrophages, a significant effort has focused on developing therapeutic agents that regulate NO production. In the present study fermented papaya preparation (FPP) is shown to exert both immunomodulatory and antioxidant activity in the macrophage cell line RAW 264.7. Interestingly, a low and a high molecular weight fraction (LMF and HMF, respectively) of FPP exhibited different activity patterns, FPP fractions alone did not affect NO production. However in the presence of IFN- $\gamma$ , both LMF and HMF significantly increased iNOS activity and nitrite as well as nitrate accumulation. NO radical formation measured in real-time by electron paramagnetic resonance spectroscopy was higher in the presence of LMF and IFN- $\gamma$ . On the contrary, iNOS mRNA levels were enhanced further with HMF than with LMF. Moreover, LMF displayed a stronger superoxide anion scavenging activity than HMF. In the presence of IFN- $\gamma$ , both FPP fractions stimulated TNF- $\alpha$  secretion. However in non-stimulated macrophages, TNF- $\alpha$  secretion was enhanced by HMF only. Since water-soluble FPP fractions contained no lipid A, present data indicate that FPP is a macrophage activator which

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augments nitric oxide synthesis and TNF- $\alpha$  secretion independently of lipopolysaccharides.

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