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Ferric Nitrilotriacetate Induced DNA and Protein Damage: Inhibitory Effect of a Fermented Papaya Preparation

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Abstract. *The carcinogen Fe-NTA catalyzes the hydrogen peroxide-derived production of free radicals and possibly acts through a mechanism involving oxidative stress. Fermented papaya preparation (FPP) has been reported as a natural antioxidant able to prevent lipid peroxidation in vitro and in vivo. However, little is known about the antioxidant properties of FPP regarding iron-mediated oxidative damage to DNA and proteins. In the present study FPP protected supercoiled plasmid DNA against Fe-NTA plus H_2O_2 induced single and double strand breaks. Similar protective effects of FPP were evident when human T-lymphocytes were challenged with Fe-NTA/H_20_2 and DNA damage was determined using the Comet assay. Fe-NTA/H_20_2 also induced fragmentation of bovine serum albumin (BSA) in vitro and depleted cellular GSH levels in lymphocytes. BSA fragmentation and GSH depletion were dose-dependently counteracted by FPP. EPR spin trapping studies demonstrated that antioxidant properties of FPP are related to both hydroxyl scavenging as well as iron chelating properties.*

Redox cycling is a characteristic of transition metals including iron, which is centrally involved in the generation of reactive oxygen species. Redox reactive iron is generally present in a weakly chelated form (1). Nitrilotriacetic acid (NTA) is a synthetic aminotricarboxlic acid, which forms water-soluble complexes with iron at neutral pH. NTA is a constituent of various domestic and hospital detergents and is a common water contaminant (2). A high incidence of renal cell carcinoma is prevalent in mice following repealed intra-peritoneal Fe-NTA injections (3). The carcinogenity of Fe-NTA seems to be associated with the interaction of NTA with Fe^{3+}, since no tumor formation was observed by admini

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stration of NTA alone (3). Fe-NTA has also been reported to induce severe hemochromatosis, diabetes as well as adenomocarcinoma in laboratory animals (4). Il is that the mechanism of Fe-NTA toxicity is mediated via the production of free radicals which in turn induce oxidative damage 10 lipids, proteins and DNA. Additionally, other reports have shown that Fe-NTA increases both hepatic ornithine decarboxylase activity and H thymidine incorporation possibly through the generation of oxidative stress (5, 6). It has been previously shown that Fe-NTA decreased antioxidant enzyme activities with the concomitant increase in the production of lipid peroxides and hydrogen peroxide (7). Antioxidants such as vitamin E and butylated hydroxyanisol, as well as the iron chelator desferrioxamine have been reported to partially prevent the toxic effect of Fe-NTA (8,9). There is a growing interest in the utilization of plant extracts as dietary food supplements. A wide spectrum of beneficial activity for the human health has been advocated for such supplements due, at least in part to their antioxidant activity (10). More recently the ability of antioxidant nutrients to affect cell response and gene expression has been reported *in vitro,* providing a novel and different mechanistic perspective underlying the biological activity of plant derived nutraceuticals (11, 12, 13). Fermented papaya preparation (FPP) is made by yeast fermentation of *Carica papaya Linn., Pennisetum purpeum Schum. and Sechium edule Swartz* and is used as a natural food health supplement in different parts of the world. FPP has been shown to upregulate phorbol ester or zymosan-induced superoxide production in rat peritoneal macrophages (14), natural killer cell activity (15), and the level of IFN-y in human blood (16). Recent studies of our laboratory demonstrated that FPP affects NO and hydrogen peroxide production as well as tumor necrosis factor alpha secretion in RAW 264.7 macrophages (17). Such evidence suggests a role of FPP as an immunomodulator. It has also been reported that FPP protects the brain of aged rodents *in vivo* challenged either by oxidative stress (18) or by ischemia-reperfusion injury (19), Furthermore, the accumulation of thiobarbituric acid reactive substances were found to be lower in heart homogenates from FPP supplemented rats exposed to peroxyl radicals as compared to non-supplemented

controls (20). From these reports it has been proposed that beside immuno-modulating FPP possess also antioxidant activities. However, the underlying mechanisms by which FFP acts as an antioxidant are largely unknown. In the present study both hydroxyl radical scavenging and iron delating properties of FPP have been evaluated. Furthermore, the ability of FPP to combat Fe-NTA oxidative damage to DNA and proteins has been investigated both *in vitro* as well as in cultured cells.