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Commentary

# Applications and bioefficacy of the functional food supplement fermented papaya preparation

Okezie I. Aruoma[a,*], Yuki Hayashi[b], Francesco Marotta[c], Pierre Mantello[b], Eliezer Rachmilewitz[d], Luc Montagnier[e]

[a] Department of Pharmaceutical and Biomedical Sciences, Touro College of Pharmacy, New York, NY, USA
[b] Osato Research Institute, Gifu, Japan
[c] ReGenera Research Group for Aging Intervention, Milano, Italy
[d] Department of Hematology, The E. Wolfson Medical Center, Holon, Israel
[e] UNESCO, World Foundation AIDS Research and Prevention, UNESCO, Paris, France

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ABSTRACT

Fermented papaya preparation (FPP) (a product of yeast fermentation of *Carica papaya* Linn) is a food supplement. Studies in chronic and degenerative disease conditions (such as thalassemia, cirrhosis, diabetes and aging) and performance sports show that FPP favorably modulates immunological, hematological, inflammatory, vascular and oxidative stress damage parameters. Neuroprotective potential evaluated in an Alzheimer's disease cell model showed that the toxicity of the β-amyloid can be significantly modulated by FPP. Oxidative stress trigger apoptotic pathways such as the c-jun N-terminal kinase (JNK) and p38-mitogen activated protein kinase (MAPK) are preferentially activated by pro-inflammatory cytokines and oxidative stress resulting in cell differentiation and apoptosis. FPP modulated the $H_2O_2$-induced ERK, Akt and p38 activation with the reduction of p38 phosphorylation induced by $H_2O_2$. FPP reduces the extent of the $H_2O_2$-induced DNA damage, an outcome corroborated by similar effects obtained in the benzo[*a*]pyrene treated cells. No genotoxic effect was observed in experiments with FPP exposed to HepG2 cells nor was FPP toxic to the PC12 cells. Oxidative stress-induced cell damage and inflammation are implicated in a variety of cancers, diabetes, arthritis, cardiovascular dysfunctions, neurodegenerative disorders (such as stroke, Alzheimer's disease, and Parkinson's disease), exercise physiology (including performance sports) and aging. These conditions could potentially benefit from functional nutraceutical/food supplements (as illustrated here with fermented papaya preparation) exhibiting anti-inflammatory, antioxidant, immunostimulatory (at the level of the mucus membrane) and induction of antioxidant enzymes.



## 1. Introduction

The notion of functional foods has continued to represent a trend of opportunity for innovative solutions to address consumer health. The food and biotechnology industry sector develop, produce and present new products to the market in order to meet consumer's needs that are directed at positive effects on health. One such food supplement is fermented papaya preparation (FPP). FPP (ImmunAge®) is a product of yeast fermentation of *Carica papaya* Linn (Fig. 1). Many reports on papaya found in the literature are either based on the papaya fruit and the leaf (Kondo et al., 2005; Seigler et al., 2002; Hartmann-Schreier and Schreier, 1987; Canini et al., 2007; Mahattantawee et al., 2006; Lako et al., 2007; Simirgiotis et al., 2009), where reference to their phenolic, allosides and glucosides composition have been reported. Mahattantawee et al. (2006) report that HPLC–PDA–MS analyses of ripe and green papaya showed few candidate phenols, other than catechin conjugates. FPP is rich in amino acids and carbohydrates. The nature of the carbohydrates identified in FPP is the subject of ongoing research. FPP is made from non-genetically modified *Carica* papayas under strict quality control using specialized fermentation technology that has received ISO 9001:2000 (the international quality standard) and ISO 14001:2004 (the international environmental standard) certification and ISO 22000:2005 (the international food safety standard) certifications. The characteristic components of FPP are presented in Table 1.

## 2. Effect of fermented preparation on Fenton chemistry

Free radical generation occurs normally in the human body, and rates of free radical generation are probably increased in most diseases (Aruoma, 1998; Aruoma and Halliwell, 1998; Halliwell and

* Corresponding author. Tel.: +1 646 981 4741.
*E-mail address:* okezie.aruoma@touro.edu (O.I. Aruoma).

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**Fig. 1.** Papaya tree and fruit. The organically grown fruits are used in the manufacture of FPP.

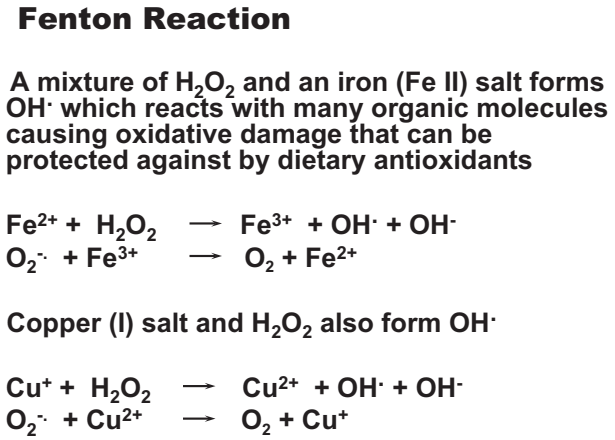


**Fig. 2.** Typical Fenton chemistry mechanism. As the data I Fig. 3 clearly shows, both superoxide anion and hydroxyl radical that may arise via the Fenton chemistry can be ameliorated by fermented papaya preparations (FPPs), as demonstrated by the interaction involving Aβ and copper ions.

Gutterdge, 1999; Valko et al., 2007). The toxicity of the superoxide radical ($O_2^{\bullet -}$) and $H_2O_2$ in living organisms is due to their conversion into $^{\bullet}OH$ and into reactive radical metal complexes via either the iron-catalyzed Haber-Weiss reaction or the superoxide-driven Fenton reaction (Fig. 2). The nature of the damage done by excess formation of $H_2O_2$ and $O_2^{\bullet -}$ is affected by the location and concentration of metal ion catalysts of reactions within the cells. This implies that if no catalytic metal ions are available, $O_2^{\bullet -}$ and $H_2O_2$ will have limited, if any, damaging effects. Redox cycling is a characteristic of transition metals including iron, which is centrally involved in the generation of reactive oxygen species. Nitrilotriacetic acid (NTA), a synthetic amino-tricarboxilic acid), forms water-soluble complexes with iron at neutral pH. This complex (a Fenton reaction catalyst, Fig. 2), is nephrotoxic and induces renal proximal tubular damage associated with oxidative damage that eventually leads to a high incidence of renal cell carcinoma in rodents after repeated administration (Toyokuni, 1996). Fe–NTA in the presence of $H_2O_2$ *in vitro*, also cause increased oxidative DNA damage measured by gas chromatography/mass spectrometry (Aruoma et al., 1989) and *in vivo* (Deiana et al., 2001). FPP protects supercoiled plasmid DNA against Fe–NTA plus $H_2O_2$ induced single and double strand breaks. Fe–NTA induces a dose dependent fragmentation of bovine serum albumin *in vitro* and depletes cellular GSH levels in lymphocytes both of which are 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 (Rimbach et al., 2000a). Thus, FPP when translated to an *in vivo* environment would be expected to exert antioxidant protection, a premise developed throughout this commentary paper.

**Table 1**
General Composition of FPP®.

| Component | Level of component per 100 g FPP |
|---|---|
| Moisture [vacuum oven method] | 8.4 g |
| Protein[a] | 0.3 g |
| Fat | <0.1 g |
| Ash | <0.1 g |
| Carbohydrates[b] | 91.3 g |
| Energy[c] | 366 kcal |
| Sodium | 0.5 mg |
| *Amino acids* | |
| Arginine | 16 mg |
| Lysine | 6 mg |
| Histidine | 6 mg |
| Phenylalanine | 12 mg |
| Tyrosine | 8 mg |
| Leucine | 18 mg |
| Isoleucine | 10 mg |
| Methioneine | 5 mg |
| Valine | 14 mg |
| Alanine | 13 mg |
| Glycine | 11 mg |
| Proline | 12 mg |
| Glutamic acid | 40 mg |
| Serine | 11 mg |
| Threonine | 8 mg |
| Aspartic acid | 23 mg |
| Tryptophan | 2 mg |
| Cysteine | Not detected |

[a] The nitrogen to protein conversion factor was 6.25.
[b] The formula used was 100 – (moisture + protein + fat + ash); energy conversion factors were in accordance with Notification No. 176 (2003) Standards for Nutrition Labeling, Ministry of Health, Labour and Welfare, Japan.
[c] One sachet of FPP has a calorific value of 11 kcal, which is the equivalent of less than one tablespoon of cooked rice. Thus individuals with diabetes may take FPP without risks of raising level of sugar in the blood.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder whose pathogenesis includes extracellular deposition of β-amyloid, chronic inflammation and cellular insults resulting from free radicals reactions that may contribute to the neurotoxicity of AD (Vickers et al., 2000; Butterfield et al., 1999; Marchetti and Abbracchio, 2005). High concentrations of copper have been found within the amyloid deposits in AD brains (Lovell et al., 1998) and also in the postmortem brains of Parkinson disease patients (Spencer et al., 1994). Beta-amyloid precursor protein (APP) and β-amyloid both have a copper binding domain (Atwood et al., 1998) and the suggestion is that as a cofactor, interaction of $Cu^{2+}$ with β-amyloid peptides and APP protein could facilitate oxidative stress (Bossy-Wetzel et al., 2004; Huang et al., 1999). Zhang et al. (2006) found that supplementation of FPP to Alzheimer's disease cell model (β-amyloid precursor protein Swedish mutation (APPsw) stably overexpressed SH-SY5Y cells (APP cells)) was able to decrease the production of hydroxyl radicals and superoxide anion (Fig. 3) as well as decreasing nitric oxide accumulation and intracellular calcium ion. It is interesting that β-amyloid precursor protein (APP) and Aβ both have a copper binding domain and bound $Cu^{2+}$ is reduced to $Cu^+$, leading to reactive oxygen species (ROS) generation (Huang et al., 1999), presumably via mechanisms involving the Fenton reaction depicted in Fig. 2. The ROS generation catalyzed by $A\beta Cu^{2+}$ and $APPCu^{2+}$ is a major contributor to the oxidative stress in AD. Metal binding also can induce a β-sheet-like conformational change in Aβ, resulting in enhanced aggregation (Bossy-Wetzel et al., 2004). FPP was also shown to attenuate the apoptosis stress (Fig. 4) where upon the protein and mRNA level

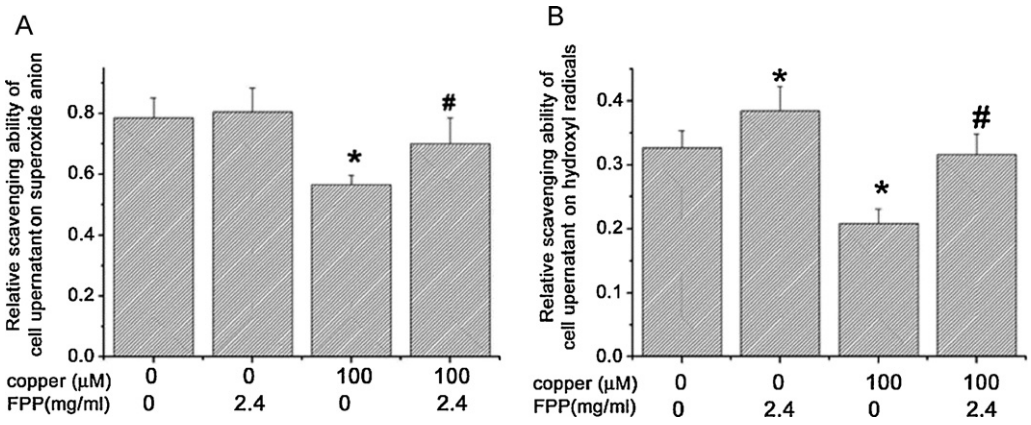


**Fig. 3.** The scavenging effect of the APPsw cells treated by copper and FPP on oxygen free radicals. The APPsw cells were treated with copper and FPP first then the scavenging ability of the cells on superoxide anion radical (A) and on hydroxyl radical (B) was measured by ESR spin trapping technique. The result is expressed in mean ± S.E.M., $n = 4$. Statistical analysis was done by ANOVA. $^{*}P < 0.05$ compared with [copper (0); FPP (0)]; $^{\#}P < 0.05$ compared with [copper (100); FPP (0)]. (Reproduced from Zhang et al. (2006), fermented papaya preparation attenuates β-amyloid precursor protein: β-amyloid–mediated copper neurotoxicity in β-amyloid precursor protein and β-amyloid precursor protein Swedish mutation over-expressing SH-SY5Y cells. Neuroscience 143, 63–72, with permission from Elsevier.)

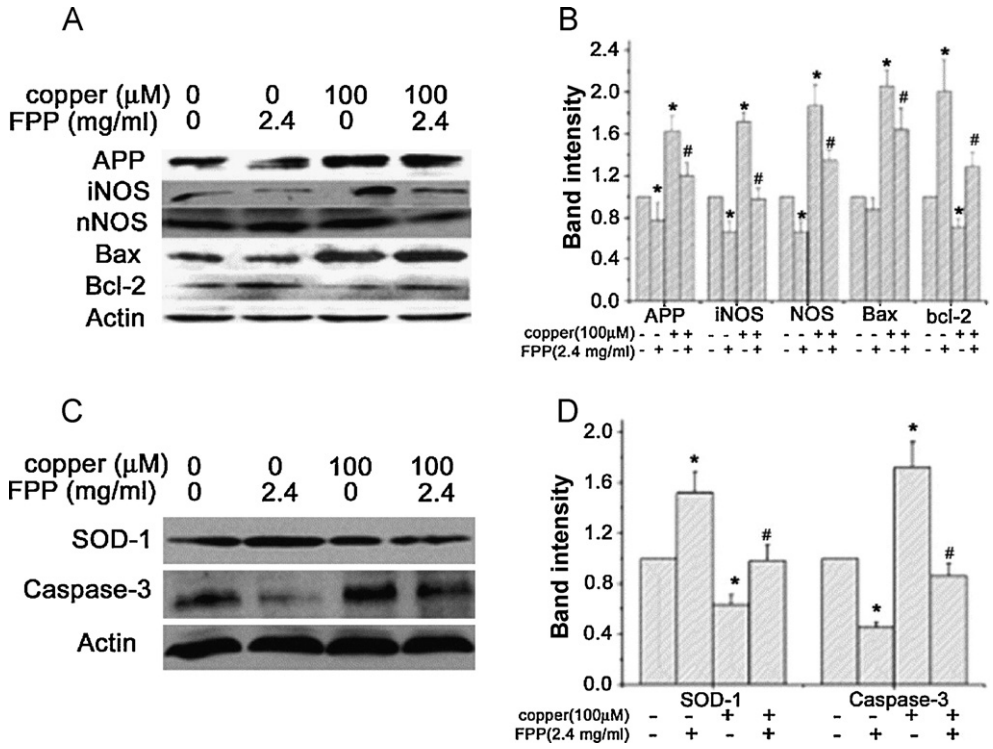


**Fig. 4.** Protein expressions in the APPsw cells treated with copper and FPP measured by Western blot. Panel A shows the APP, iNOS, nNOS, Bax and Bcl-2 expression in the APPsw cells received different treatment. Panel B depicts the statistical results. $^{*}P < 0.05$ compared with (copper 0; nicotine 0); $^{\#}P < 0.05$ compared with (copper 100; nicotine 0). The result is expressed in mean ± S.E.M., $n = 4$. Statistical analysis was done by ANOVA. (Reproduced from Zhang et al. (2006), fermented papaya preparation attenuates β-amyloid precursor protein: β-amyloid–mediated copper neurotoxicity in β-amyloid precursor protein and β-amyloid precursor protein Swedish mutation over-expressing SH-SY5Y cells. Neuroscience 143, 63–72, with permission from Elsevier.)

of Bax that was increased significantly after the copper treatment was decreased following FPP administration. In the context of Bcl-2, FPP and copper play reversed roles compared with Bax. It is of interest to note that FPP up-regulated whilst copper down-regulated the level of bcl-2. The expression of caspase-3 in APPsw cells was increased by copper treatment and decreased by FPP treatment (Fig. 4) (Zhang et al., 2006).

## 3. Fermented papaya preparation and studies on diabetes

Type 2 diabetes accounts for approximately 90% of diabetes worldwide and is common in ethnic and, minority groups in developing and developed countries such as Africans, African Americans, Asians, Native Americans and Hispano-Latinos (Chan et al., 2009; Wild et al., 2004). Patients suffering from diabetes-caused oxidative stress have a greater risk of developing cardiovascular diseases, neuropathy, congenital malformations and retinopathy. Glycation products from excess glucose can chemically modify DNA causing mutations and cause complex DNA rearrangements. Advanced glycation end-products which play a role as proinflammatory mediators in gestational diabetes can accelerate vascular occlusion by quenching the vasodilating agent nitric oxide. Interaction with high-affinity receptors located on monocytes and macrophages can enhance oxidative stress, the secretion of tumor necrosis factor-α,

interleukin-1 and insulin-like growth factor I which can proliferate endothelial, mesangial and smooth muscle cells and hence contribute significantly to pathophysiology of the disease.

Oxidative stress is increased in diabetes because of glucose autoxidation and factors that include cellular redox imbalances and reduction in antioxidant defenses comprising decreased cellular antioxidant levels and a reduction in the activity of enzymes that remove free radicals (Aruoma et al., 2007; Rahimi et al., 2005). Nutritional recommendations for the prevention of diabetes have included modest weight loss, increased fiber intake (Rudkowska, 2009) and the incorporation of functional foods in the diet, including green tea catechins (Thielecke and Boschmann, 2009), pycnogenols (Zimbadi and Rohdewalde, 2008), legumes, fruits, vegetables, spices, condiments and beverages (Kaushik et al., 2008) that impact insulin action. In addition, supplementation with FPP has been suggested. Indeed, research have shown that that oral supplementation with FPP may cause a significant decrease in plasma sugar levels in both healthy subjects and type 2 diabetic patients (Danese et al., 2006). In a study conducted in the laboratory of Professor Sen and reported in Collard and Roy (2010), it was sought to establish if there is improved function of diabetic wound-site macrophages and accelerated wound closure in response to oral supplementation of FPP to diabetic db/db mice. It was found that FPP attenuated the gain in blood glucose (confirming the results of Danese et al., 2006) and improved the lipid profile after 8 weeks of oral supplementation but there was no influential weight gain during the supplementation period. FPP (0.2 g/kg body weight) supplementation for 8 weeks before wounding was effective in correcting wound closure. Studies on viable macrophages isolated from the wound site demonstrated that FPP supplementation improved respiratory-burst function as well as inducible NO production. NO availability in diabetic wounds is known to be compromised. Diabetic mice supplemented with FPP showed a higher abundance of CD68 as well as CD31 at the wound site, suggesting effective recruitment of monocytes and an improved pro-angiogenic response. Macrophages from diabetic mice produced higher levels of the pro-inflammatory cytokines TNF-α and IL-6 and lower anti-inflammatory cytokine IL-10 compared to non-diabetic controls and it can be envisaged that FPP could affect the genes for these proteins (Khanna et al., 2010; Wellen and Hotamisligil, 2005). Nevertheless, Collard and Roy (2010) concluded that diabetic-wound outcomes may benefit from FPP supplementation by specifically influencing the response of wound-site macrophages and the subsequent angiogenic response. The clinical significance of this finding is being addressed by Professor Sen and his colleagues. Pursuant of the same vein, a global clinical study of the effect of FPP and green tea on clinical markers of diabetes and the effect on cognitive function as well as ascertaining the potential effect of their supplementation on drug therapy (post-marketing research and compliance) is being instituted by Professor Aruoma (Touro College of Pharmacy, New York), North America perspective; Professor Wagner (University of Vienna, Austria), European perspective and by Professor Bahorun (University of Mauritius), African perspective. The outcome will extend the data of Collard and Roy (2010) and that of Danese et al. (2006) as well as data from studies discussed in this paper. It is interesting to note that the work of Velayudhan et al. (2010) reports that that individuals with mild cognitive impairment and diabetes are at increased risk of developing dementia after adjustment for sociodemographic factors, *APOE4*, premorbid IQ and other health conditions. Type-2 diabetes remains a clinical condition that has reached attained global health priority that has huge impact on the low and medium income countries as well as those of high economic status (Chan et al., 2009; Aruoma et al., 2007) which encapsulate the current need for a dietary supplement that can help towards the cost and burden of diabetes. The increasing burden of chronic diseases including diabetes continues to be highlighted as a major global risk predicted to cause substantial financial loss resulting from increased health care expenditure and lost productivity.

## 4. Role of FPP in inflammation and the immune system

Macrophage inducible nitric oxide synthase can generate nitric oxide (NO) which contributes the host immune defense against viruses and bacteria. Monocyte-macrophages stimulated with the bacterial wall component lipopolysaccharide (LPS) and cytokines such as interferon-γ (IFN-γ) express the inducible form of nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α) is one of the central regulatory cytokines in macrophage antimicrobial activity and synergy/ies with IFN-γ in the induction of NO synthesis. A study to assess the effect of FPP on nitric oxide synthesis and TNF-α secretion in RAW 267.7 macrophages indicated that it exerted both immunomodulatory and antioxidant activity (Rimbach et al., 2000b). FPP was found to up-regulate the IFN-γ-induced NO production in a dose-dependent manner (Kobuchi and Packer, 1997). The effect of FPP on NO production was not due to changes in the activity of iNOS and the levels of iNOS mRNA were augmented by treatment of the cells with FPP and IFN-γ. Interestingly, the ability of FPP to augment IFN-γ-induced iNOS mRNA expression was independent of any changes on the mRNA stability (Kobuchi and Packer, 1997). Tumor necrosis factor-α and interleukin-lβ are involved in the induction of iNOS gene as well as the immune system. The fact that FPP augmented the mRNA expression of these cytokines in the presence of IFN-γ suggests that FPP may not only be directly involved in the expression of iNOS, but shows synergistic interaction with IFN-γ to induce NO synthesis (Kobuchi and Packer, 1997).

The effect of FPP on the ethanol-related gastric mucosal damage and cyanocobalamin absorption abnormality in alcoholics has been assessed (Marotta et al., 1999). For the gastric mucosa damage double blind study, twenty-two healthy teetotal volunteers underwent gastroscopy during which biopsy samples from the antrum and body were taken for chemiluminescence assay, routine histology, and for malonyldialdehyde (MDA), xanthine oxidase and glutathione determination with subjects were given either 9g of FPP at bedtime and 3 h prior to blood examination, or flavored sugar placebo. Subjects on FPP had reduced gastric mucosal damage at endoscopy and the histological level. The net effect of ethanol administration was reflected by the significant increase in the luminol-amplified chemiluminescence response in gastric mucosa as well as increased xanthine oxidase activity and MDA together with a decreased glutathione concentration. FPP supplementation was effective in protecting against ethanol-induced gastric mucosal damage (Marotta et al., 1999).

Marotta et al. (2006) examined the effects of FPP on redox status and DNA damage in healthy elderly individuals and relationship with GSTM1 genotype in a 3 months placebo controlled cross-over study (involving 54 randomly selected elderly patients without major invalidating diseases. Supplemented group consumed FPP at 9 g/day followed by a 6-week washout period). The glutathione-S transferase M1 (GSTM1) genotype was null (−) in 40% and 46% of groups A and B, respectively. GSTM1 (−) smokers had a significantly higher level of plasma DNA adducts and leukocytes level of 8-OHdG than their GSTM1 (+) counterparts. The weak correlation between cigarettes smoked/day and DNA adduct ($r$: 0.61, $P < 0.05$) was correlated with antioxidant concentrations, but only in GSTM1 (−) smokers ($P < 0.01$) (Marotta et al., 2006). The FPP-supplemented group showed a significant enhancement of protection ($P < 0.01$ vs. $A$) within the subgroups with GSTM1 (−) and of plasma DNA adduct, irrespective of the GSTM1 genotype. Interestingly, only the FPP-treated GSTM1 (−) subgroup had an increase in their lymphocyte 8-OHdG ($P < 0.01$), suggesting that

FPP can improve antioxidant-defense in elderly patients even without any overt antioxidant-deficiency state (Marotta et al., 2003, 2006, 2007). FPP appeared to exert protective effects on leukocyte DNA adducts formation, irrespective of genotype profile, while also enhancing DNA repair mechanisms against the highly mutagenic base modification, but only in GSTM1-null genotype participants.

In an extension of the idea of nutrigenomics study relating to FPP efficacy, Marotta et al. (2010) found that the genes for antioxidant enzymes catalase, superoxide dismutase and glutathione peroxidase as well as the gene for the DNA repair enzyme *hOGG1* were up-regulated by FPP in humans. Indeed, the effect of diet on gene expression and chromosomal structure, and the extent to which genetic differences between individuals affect response to a specific dietary intake, functional food or supplement in terms of a defined health outcome are pivotal to the debate as to whether supplemental antioxidants are beneficial (Herrera et al., 2009). Elevated serum levels of Hsp70 have been reported in patients suffering from a trauma, colon cancer and in elderly persons suffering heat-induced injury. The increased levels of proinflammatory cytokines may be inversely correlated with Hsp70 an index that can be modulated by FPP (Marotta et al., 2006, 2007). High serum levels of these markers may predict functional disability and an increased mortality rate in older individuals.

It is to be that Professor Montagnier commented on the potential application of FPP toward the management of infection, typically as with the influenza virus H1 N1. Indeed the mucus membranes of mouth, pharynx, throat, nose, are fragile, especially in a cold season and these are partly protected from invading viruses and bacteria (by the mucus and saliva). From the foregoing discussions, the anti-inflammatory, immunostimulatory (particularly at the level of mucus membranes) and potential induction of antioxidant enzymes can be deduced. Thus improving the body's immune system using immunostimulants of natural origin such as suggested for FPP constitutes a preventive measure for influenza. The levels of the *in vivo* antioxidant glutathione (GSH) which is involved in conjugation reactions during phase II drug metabolism reactions to facilitate elimination of toxic wastes and drug metabolites, may be decreased in infective stages and supplementation of both FPP and GSH could be synergistic. It must be remembered that some viral strains (H5 N1, not H1 N1 so far), possess a virulence gene that targets the mitochondria, thereby increasing oxidative burden to pulmonary tissues which could benefit from such complementary supplementation.

## 5. Effect of fermented papaya preparation in congenital and acquired hemolytic anemias

Thalassemia syndrome is a group of inherited diseases of the blood that affect a person's ability to produce hemoglobin, resulting in anemia and is associated with considerable morbidity and motality (see Rund and Rachmilewitz, 2005). About 100,000 babies worldwide are born with severe forms of thalassemia each year. Thalassemia occurs most frequently in people of Italian, Greek, Middle Eastern and Southern Asian Ancestry. The two main types of thalassemia are called "alpha" and "beta", according to the protein chain which is lacking in the red blood cells. Both types of thalassemia are inherited in the same manner. The disease is passed to children by parents who carry the mutated thalassemia gene. A child who inherits one mutated gene is a carrier, which is defined as "thalassemia trait". Most carriers lead completely normal, healthy lives. Fig. 5 illustrates the current status of the management of thalassemia and treatment-related complications (Rund and Rachmilewitz, 2005). The anemia in the homozygote forms of thalassemia is severe and is accompanied by ineffective erythropoiesis, with bone expansion and extramedullary hematopoiesis in the liver, spleen, and other sites, such as paravertebral masses. Transfusion therapy, which is the mainstay of treatment, allows for normal growth and development and suppresses ineffective erythropoiesis. Transfusion-transmitted infections (primarily hepatitis B and C) are an important cause of death in countries where proper testing is not available. Iron overload results both from transfusional hemosiderosis and excess gastrointestinal iron absorption.

Iron deposition in the heart, liver, and multiple endocrine glands results in severe damage to these organs, with variable endocrine organ failure. The endocrinopathies can be treated with hormone replacement. However, the most serious result of iron overload is life-threatening cardiotoxicity, for which chelation therapy is required. Iron overload in the heart is a life-threatening complication in transfusion-dependent patients with thalassaemia major and to a lesser extent in sickle cell disease, while no data are available in patients with sickle/β°-thalassaemia. Ghoti et al. (2009) assessed iron deposition in the heart, liver and pancreas was assessed using T2* MRI sequences, free iron species assays (non-transferrin bound iron (NTBI), labile plasma iron (LPI), serum ferritin, percentage transferrin saturation and serum hepcidin, in 10 multitransfused patients (>30 years) with sickle/β°-thalassaemia. Magnetic resonance utilizes the paramagnetic properties of tissue iron allowing direct, non-invasive iron content evaluation. T2* multi-echo gradient echo is a robust reproducible method allowing accurate iron content assessment in the heart, liver and pancreas (Ooi et al., 2009; Tanner et al., 2006). None of the patients had iron deposition in the heart. Three patients had mild, one had moderate, and two had severe liver IO. Two patients had mild iron deposition in the pancreas. In all the patients, serum hepcidin levels were normal with no detection of NTBI and LPI (Ghoti et al., 2009).

Thalassemia can be cured by bone marrow transplantation. Experimental therapies to ameliorate the anemia that have been or are currently under investigation include fetal hemoglobin modifiers and antioxidants (Rund and Rachmilewitz, 2005). Many aspects of the pathology in β-hemoglobinopathies (β-thalassemia and sickle cell anemia) are mediated by oxidative stress. Rachmilewitz and colleagues (Amer et al., 2008) tested FPP for its antioxidant effects spectrofluorometrically in a cell-free system using 2′-7′-dichlorofluorescin-diacetate (DCF) and found that both spontaneous and $H_2O_2$-induced DCF oxidations were decreased by FPP in a dose-dependent fashion. Treatment of blood cells from β-thalassemic patients with FPP increased the glutathione content of red blood cells, platelets and polymorphonuclear (PMN) leukocytes, and reduced their reactive oxygen species, membrane lipid peroxidation and externalization of phosphatidylserine. These effects result in (a) reduced thalassemic RBC sensitivity to hemolysis and phagocytosis by macrophages; (b) improved PMN ability to generate oxidative burst—an intracellular mechanism of bacteriolysis, and (c) reduced platelet tendency to undergo activation, as reflected by fewer platelets carrying external phosphatidylserine. Oral administration of FPP to β-thalassemic mice (50 mg/mouse/day for 3 months) and to patients (3 g × 3 times/day for 3 months), reduced all the above mentioned parameters of oxidative stress ($P < 0.001$ in mice and $P < 0.005$ in patients) (Amer et al., 2008).

Hereditary spherocytosis (HS) is a genetic disorder of the RBC skeleton with primary deficiency in spectrin, ankyrin-1, band 3 or protein 4.2 associated with chronic hemolytic anemia. Given that secondary protein deficiencies resulting from oxidative stress are often observed and may be involved in the clinical manifestations of the disease, Ghoti et al. (in press) explored the oxidative status of HS-RBC and its contribution to hemolysis and evaluated the effects of FPP. RBC from the HS patients generates more reactive oxygen species, membrane lipid peroxides, and less reduced glutathione, than normal RBC. The oxidative stress markers were significantly reduced following supplementation with FPP. There was a decreased tendency to undergo hemolysis in the HS patients.

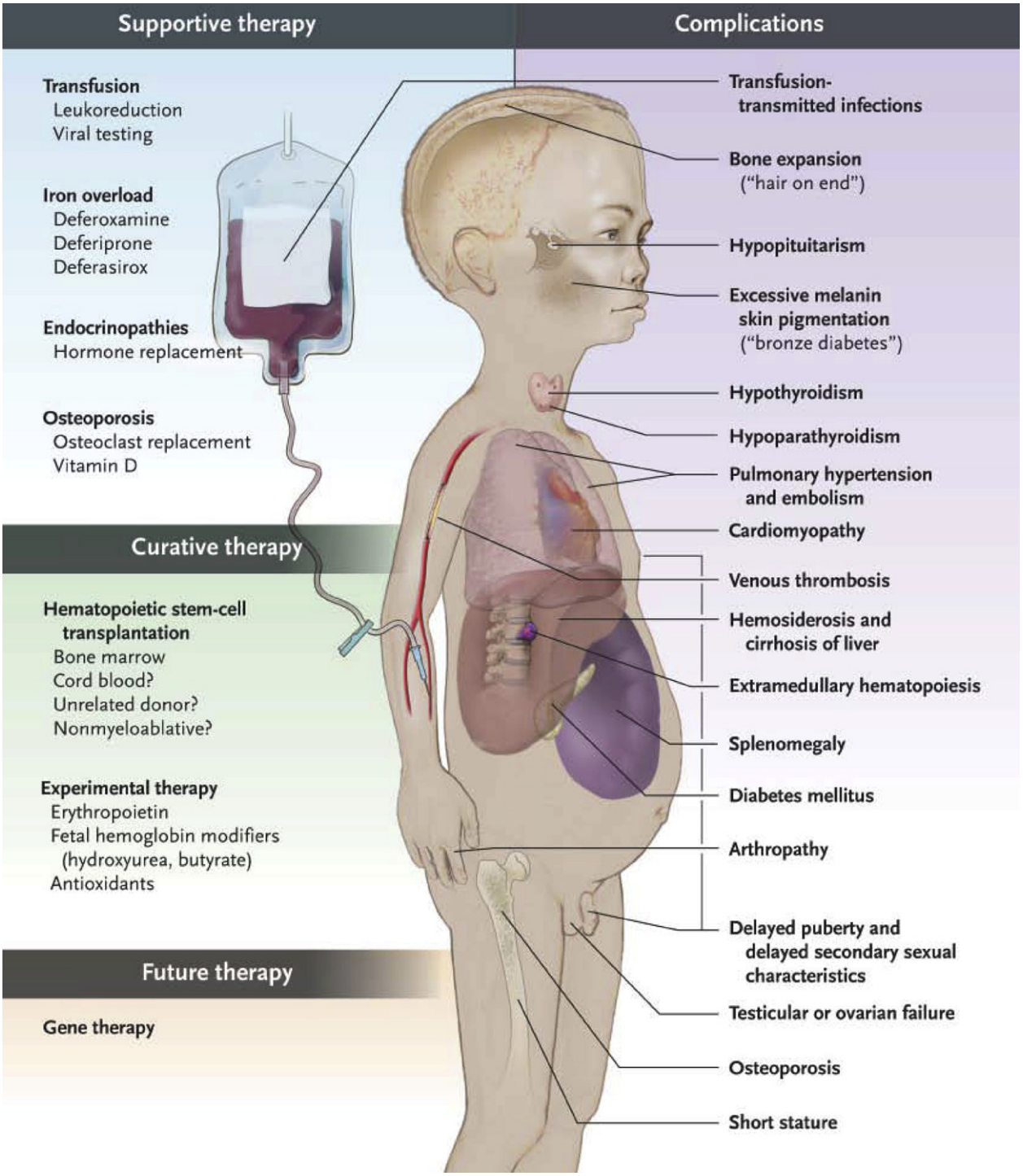


**Fig. 5.** Management of thalassemia and treatment-related complications. The anemia that is associated with thalassemia may be severe and is accompanied by ineffective erythropoiesis, with bone expansion and extramedullary hematopoiesis in the liver, spleen, and other sites, such as paravertebral masses. Transfusion therapy, which is the mainstay of treatment, allows for normal growth and development and suppresses ineffective erythropoiesis. Transfusion-transmitted infections (primarily hepatitis B and C) are an important cause of death in countries where proper testing is not available. Iron overload results both from transfusional hemosiderosis and excess gastrointestinal iron absorption. Iron deposition in the heart, liver, and multiple endocrine glands results in severe damage to these organs, with variable endocrine organ failure. The endocrinopathies can be treated with hormone replacement. However, the most serious result of iron overload is life-threatening cardiotoxicity, for which chelation therapy is required. Thalassemia can be cured by bone marrow transplantation. Experimental therapies to ameliorate the anemia that have been or are currently under investigation include fetal hemoglobin modifiers and antioxidants (such as afforded by fermented papaya preparation). In the future, gene therapy or other molecular methods may be feasible. (Reproduced from Rund and Rachmilewitz (2005), New England Journal of Medicine 353, 1135–1146, with permission from the Publishing Division of the Massachusetts Medical Society.)

The hemoglobin levels increased by >1 g/dl, MCHC decreased by >1g/dl and the reticulocyte count decreased by 0.93%. Concomitantly, lactic dehydrogenase decreased by 17% and indirect bilirubin by 50%. A significant decrease in malonyldialdehyde was also detected. The data of Ghoti et al. (2010) indicate that oxidative stress plays an important role in the pathophysiology of HS which can be ameliorated by antioxidants as illustrated with FPP.

Further, Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal hematopoietic stem cell disease (HSC), characterized by intravascular hemolysis due to inactivating mutation of the X-linked PIG-A gene in an HSC that is essential for the synthesis of glycosylphosphatidylinositol (GPI) anchor molecules. The surface proteins CD55 and CD59, which regulate complement activation on the cell surface are GPI-linked and their deficiency may explain the hypersusceptibility of PNH red blood cells to complement-mediated lysis, intravascular hemolysis, and release of free hemoglobin. Oxidative stress has been reported in the pathological clone with a CD55–CD59 phenotype and hence oxidative burden may play a significant role in the pathophysiology of PNH suggesting the potential benefit of FPP supplementation. Indeed, FPP supplementation in one case of PNH led to increased hemoglobin level and improvement of hemolytic parameters (Ghoti et al., in press). These series of results suggest that FPP, as a potent antioxidant, might alleviate symptoms (Fig. 5) associated with oxidative stress in both congenital and acquired hemolytic anemias (Amer et al., 2008; Fibach et al., 2010; Ghoti et al., 2010, in press; Rund and Rachmilewitz, 2005) and hence benefit the inherent pathologies and complications.

## 6. Fermented papaya preparation and related molecular studies

Nitroxyl radicals are very useful as exogenous spin probes for measuring free radical distribution, oxygen concentration, and redox metabolism by *in vivo* ESR in biological systems. Given that the nitroxyl radicals lose their paramagnetism through a redox reaction when exposed to a reducing agent in biological systems, the signal decay rate of the nitroxyl radical gives evidence of free radical generation and changes of redox status in biological systems. This has led to the description of the technique involving the blood brain barrier permeable nitroxyl spin probe MC-PROXYL (3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl) for the assessment of oxidative stress in the brain (Miura et al., 1997; Miura and Ozawa, 2000; Lee et al., 2004). The spontaneously hypertensive rat (SHR), a model of essential hypertension, has several characteristics of increased oxidative stress in the rats brain. The effect of FPP on SHR-induced oxidative stress in the brain was investigated using the spin probe MC-PROXYL with the resulting spectra analyzed with an L-band ESR spectrometer. Supplementation of SHR rats with FPP significantly inhibited the increased decay rate constants of MC-PROXYL in the isolated SHR brain, suggesting that FPP reduced the oxidative stress in the SHR brain (Aruoma et al., 2006; Yoshino et al., 2009). Oxidative stress occurs as a result of decreased antioxidant systems (prevented by the over-expression of SOD-1) and an increase in nigral iron content. FPP reduces the extent of oxidative stress in the brain of spontaneously hypertensive rats thus supporting the argument that FPP could have an important prophylactic potentials in neurodegenerative diseases and in particular diseases of overt inflammation (Aruoma et al., 2006; Zhang et al., 2006), implying that conditions that expose the human body to oxidative stress can be harmonized by FPP. Supplementation with FPP significantly inhibited the increased decay rate constant of the MC-PROXYL (a blood brain barrier permeable nitroxyl spin probe) ESR signal in the spontaneously hypertensive rat brain suggesting modulation of oxidative stress. These studies indicate that FPP can modulate oxidative injury supporting the view that prophylactic potentials in neurodegenerative diseases could be facilitated by FPP (Aruoma et al., 2006). The over-activation of *N*-methyl-D-aspartate receptor-operated channels can result in excitotoxicity causing excessive influx of $Ca^{2+}$. The entry of calcium into the cytoplasm of cells at concentrations that can activate oxidative enzymes such as phospholipase $A_2$ and xanthine oxidase, deplete cells of cysteine and glutathione, cause mitochondrial release of cytochrome *C*, increased oxidative stress resulting in cell death and neuronal injury. That oxidative stress may influence the P13-kinase/AKT pathway and the transcription factor nuclear factor kappa B in acute neuronal injury of stroke and that p38 MAP kinase pathway may be an important therapeutic pathway in cardiovascular disease combine to suggest either upstream oxidative stress or downstream MAP kinase-mediated signaling cascade could be targeted in pharmacological antioxidant and/or anti-inflammatory interventions. So inflammatory responses at the molecular level can exert their deleterious effects by (i) causing DNA damage, (ii) alter cell signaling pathways (MAPK, NFκB, AP-1, etc.) and (iii) modulate gene expression (proto-oncogene, tumor suppressor gene). Establishing the relationship to the presence of particular genetic polymorphism and modulation of the complex cell signalling cascades involving gene trancsription remain a major scientific challenge. Dementia is a syndromic manifestation typical but not exclusive of aging, characterized by memory loss and impairment of at least another cognitive function at such an extent to significantly affect daily life style, with a progressive loss of autonomy and the social role. In association to cognitive functions impairment, psychopathological and behavioral disorders often referred to as behavioral and psychological symptoms in dementia can occur before or after clinical manifestation of the cognitive disorder. FPP can act as a macrophage activator as a result of its augmentation of nitric oxide synthesis and the secretion of TNF-α (a central regulatory cytokine in macrophage antimicrobial activity), and can modulate atrophic and metaplastic changes of gastric mucosa in chronic atrophic gastritis patients. Oxidative stress can trigger apoptotic pathways such as the c-jun N-terminal kinase (JNK) and p38-mitogen activated protein kinase (MAPK) (the JNK and p38 MAPK are preferentially activated by pro-inflammatory cytokines and oxidative stress resulting in cell differentiation and apoptosis). FPP modulated the $H_2O_2$-induced ERK, Akt and p38 activation with the reduction of p38 phosphorylation induced by $H_2O_2$ (Fig. 6A and B) (Aruoma et al., 2006 and citations therein). These data correlate well with the finding of Zhang et al. (2006).

## 7. Effect of FPP on benzo[*a*]pyrene and $H_2O_2$-mediated toxicity on human HepG2 hepatoma cells

The genotoxic and antigenotoxic (chemopreventive potency) of FPP was assessed by examining its potency to induce DNA damage in HepG2 hepatoma cell using the DNA migration a biological endpoint in the alkaline single cell gell electophoresis (SCGE) assay and contrasted with its ability to modulate the benzo[*a*]pyrene (BaP)-dependent DNA damage in human hepatoma (HepG2) cells (Kassie et al., 2003). No genotoxic effect was observed in experiments with only FPP exposed HepG2 cells in a concentration range of 5–100 μg/ml. Fig. 7A and B show the OTM of FPP/B[*a*]P and FPP/$H_2O_2$ co-treated cells as a measure of DNA damage in the SCGE/HepG2 model. In Fig. 7A, the OTM of B[*a*]P-treated (positive control, 50 μM) HepG2 cells was $5.11 \pm 0.74$; the corresponding OTM of cells exposed to the solvent control was $1.28 \pm 0.54$. A significant ($P \leq 0.05$) reduction of DNA migration in co-treated cells could be observed in concentrations ≥50 μg/ml FPP compared to only B[*a*]P treated cells. A dose of 100 μg/ml FPP reduced the DNA damage 2-fold compared to only B[*a*]P-treated cells. The $H_2O_2$ (50 μM) exposed HepG2 cells showed an OTM of $10.56 \pm 1.44$ compared to $1.37 \pm 0.29$ of the solvent control (Fig. 7B). A significant reduction ($P \leq 0.05$) of DNA damage was observed in concentrations ≥10 μg/ml FPP. A concentration of 50 μg/ml FPP reduced the genotoxic effect of $H_2O_2$ about 1.5-fold compared to only $H_2O_2$ exposed cells (Aruoma et al., 2006). The FPP concentrations tested exhibited no relevant cytotoxicity and cell viability was always ≥80%,

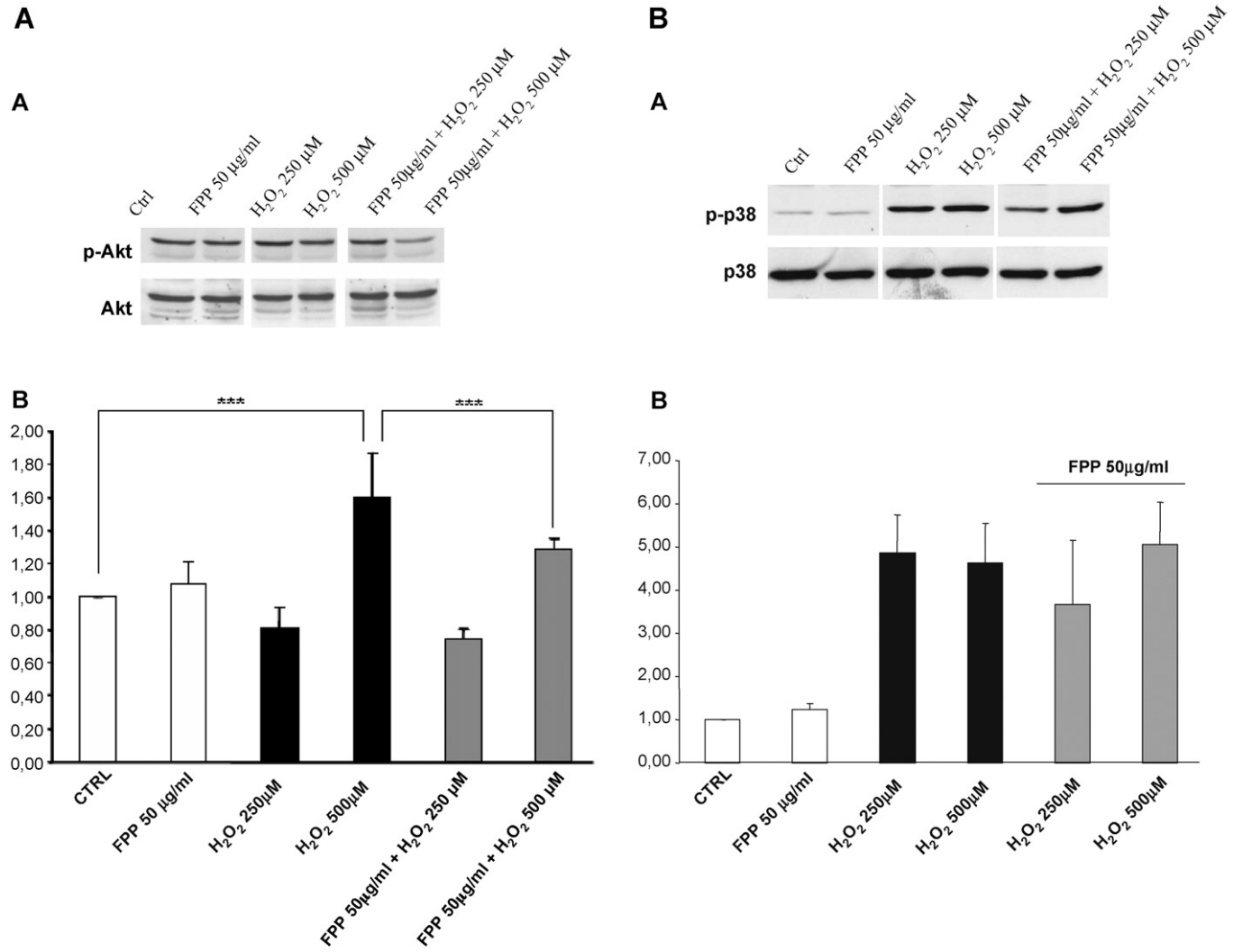


**Fig. 6. A**. Western blot analysis of Akt phosphorylation. (A) Western blots of Akt phosphorylation. (B) Graphical representation of the inhibitory effect of a 23h pre-treatment with FPP (50 μg/ml) followed by a 1 h $H_2O_2$ (250 μM and 500 μM) induced on Akt phosphorylation. **B**. Western blot analysis of p38 phosphorylation. (A) Western blots of p38 phosphorylation. (B) Graphical representation of the inhibitory effect of a 23h pre-treatment with FPP (50 μg/ml) followed by a 1 h $H_2O_2$ (250 μM and 500 μM) treatment on p38 phosphorylation. Data represent the O.D. normalized to control of $n = 4$ independent experiments. Values are expressed by means ± S.E.M. (Reproduced from Aruoma et al. (2006), Molecular effects of fermented papaya preparation on oxidative damage, MAP Kinase activation and modulation of the benzo[*a*]pyrene mediated genotoxicity. Biofactors 26, 147–159, with permission from John Wiley and Sons.)

data consistent with the observations in studies with the PC12 cells. Indeed, attack of •OH radicals on DNA lead to fragmentation, base loss, and strand breakage with a terminal sugar residue fragment (Halliwell and Aruoma, 1991). That FPP was able to reduce the extent of the $H_2O_2$-induced DNA damage was corroborated by similar effects obtained in the B[*a*]P-treated cells. No genotoxic effect was observed in experiments with only FPP exposed to HepG2 cells nor was FPP toxic to the PC12 cells (Aruoma et al., 2006). Indeed the $LD_{50}$ for FPP has been estimated to be 29.24 ± 0.64 g/kg of adult body mass. An acute toxicity testing for FPP on Himedaka fish (measuring 1.9 cm and weighing 0.2 g) where the fish swam for 96 h in 1 g/l FPP water showed no sign of adverse effects, attesting to the safety of FPP (Aruoma et al., 2006).

## 8. Effect of fermented papaya preparation on performance sport

The interest in the role of functional foods and nutrition in physical exercise and performance sports is equally gaining impetus. Motor car racing is representative of concentrative sporting activities involving mental-concentrative and psycho-emotional stress (which predominates with lower intensity, but longer duration). The autonomic nervous system plays an important role in the regulation of many cardiovascular and pulmonary functions and metabolic processes during such performance period. The adaptation of heart rates and $O_2$ consumption during physical exercise is regulated by the central nervous system, the autonomic nervous system, withdrawal of the vagal tone (and the activation of the sympathetic nervous system), humoral influences, and by local mechanisms (Hristova and Aloe, 2006; Praagman et al., 2006).

Exercise is generally advocated to be beneficial for the prevention of hypertension, heart disease, type 2 diabetes, depression and osteoporosis (Di Pietro, 2001; Pratt et al., 2009; Van Praag, 2009). Booth and Roberts in their seminal review observed that “higher aerobic capacity and often higher skeletal muscle strength are associated with a lower prevalence of most chronic diseases” and that “maintenance of aerobic capacity and skeletal muscle strength by lifelong physical activity delays the biological ageing in most organ systems, therefore delaying premature death”. The question as to whether associations between high aerobic capacity and muscle

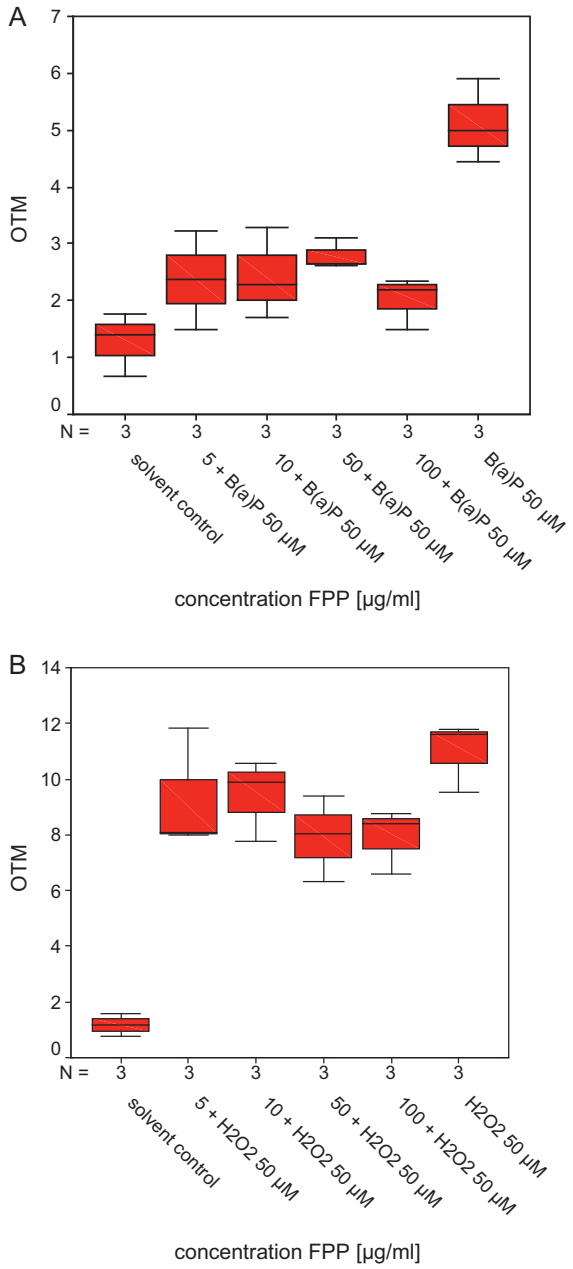


**Fig. 7. A**. OTM of HepG2 cells exposed to FPP (5–100 μg/ml) for 24 h and benzo[*a*]pyrene (50 μM) for another 24 h (solvent control = double distilled and sterilized water). **B**. OTM of HepG2 cells exposed to FPP (5–100 μg/ml) for 24 h and $H_2O_2$ (50 μM) for another 30 min (solvent control = double distilled and sterilized water). (Reproduced from Aruoma et al. (2006), Molecular effects of fermented papaya preparation on oxidative damage, MAP Kinase activation and modulation of the benzo[*a*]pyrene mediated genotoxicity. Biofactors 26, 147–159, with permission from John Wiley and Sons.)

strength are causally or associatively related to either metabolic health or elite performance was then raised (see Booth and Roberts, 2008, and references therein). Cotman et al. (2007) and Hillman et al. (2008) have argued that physical activity might improve cognition and hence delay age-related memory decline. Van Praag (2009) has argued that "the potential synergy between diet and exercise could involve common cellular pathways important for neurogenesis, cell survival, synaptic plasticity and vascular function" Strategies for such intervention and prevention require an understanding of the basic molecular mechanism(s) of prophylactic agents (dietary antioxidant factors from food plants and medicinal plants in this context) that may potentially prevent or reverse the promotion or progression of the diseases.

The management of wellbeing during sports and exercise in general led to the question "can the functional food fermented papaya preparation to modulate indices of oxidative stress during performance sport"? In a proof of concept non-blinded study involving 3 elite motor racing drivers during the 2007 Le-Mans race in France, the influence of fermented papaya preparation (FPP) on urinary markers of oxidative stress, malondialdehyde (MDA) and protein carbonyls was assessed. The drivers with the Aston Martin Racing team, Prodrive, UK were aged 33–42 years and weighing 68–72 kg. FPP was consumed 14 days (9 g/day) before the race. The drivers were not controlled for their diet however they maintained their routine dietary habit consisting of the consumption of pasta, rice, chicken, meats, vegetables, cereals and bread with consumption of adequate water and carbonated soda. During the race, drivers were supplemented with FPP (6 g) at 8 h intervals (maximum consumption of 18 g) during the 24 h race. The drivers consumed water during the race period as needed and had light food following each on the race stints. Although the MDA values were similar at the beginning of the race and at the 12 h period, there were notable differences in the values at 24 h following the race. The non-supplemented driver had the highest level of MDA and protein carbonyl with values of 1.26 μM and 0.411 μmol/g protein respectively. The corresponding values for the FPP-supplemented drivers were 0.714 μM and 0.115 μmol/g protein respectively. The Le Mans 24 h race is a very demanding and challenging sporting event for the drivers who have to negotiate the race-circuit at speeds at well over 300 km/h. This level of concentration invokes a variety of psychoemotional stress activities that are associated with catecholamine release, increased number of isometric contractions and oxygen consumption.

The benefits of exercise and the mechanisms of tissue injury in humans are very complex and defining the role played by oxidative stress and nutrition in the process is an area of increasing interest (Aruoma, 1994). However data are beginning to emerge to show that optimal pro-oxidant status can serve to up-regulate antioxidant defenses (as reviewed in Ji, 2008, and citations therein). Humans are complex in their anticipated responses during exercise. There is however a need to balance training optimization (and the actual event performance) with nutrition (Aruoma, 1994; Ramel et al., 2004; Watson et al., 2005; Kreider et al., 2004). Kreider et al. (2004) have addressed not only the scientific merits of nutritional supplements but the general nutritional strategies to optimize performance and enhance recovery. The net effect of antioxidant supplementation on oxidative stress environment is to spare the utilization of endogenous antioxidants such as glutathione and to modulate gene expression whereupon the levels of the antioxidant enzymes glutathione peroxidase, superoxide dismutase and catalase as well as the integrity of protein thiols are modulated (Dalle-Donne et al., 2005), principally contributing to the homeostatic balance *in vivo*.

The wellbeing of the drivers is of critical importance given the psycho-emotional pressures in performance sports. The FPP-supplemented drivers reported improved recovery rate following the race stints of about 2 h, feeling less fatigued, being less emotional drained and feeling alert during the more difficult night driving sessions during the 24 h race. The non-supplemented driver however felt both physically and mentally tired and began to prominently notice the tiresomeness towards the early morning hours even with 8–9 h more to the end. The FPP-supplemented drivers who would normally be exhausted a day after the race, reported feeling less mentally drained and well rested after a night's sleep and were able to recover more quickly. These are important observations. The implication for the elite driver is that the higher fatigue and more muscle's oxidative attack (stiffness, cramps) due

to the exhaustive exercise could be modulated by supplementation with FPP. It will be important to define the contribution of functional foods on biological adaptation mediating the up-regulation of antioxidant defense systems (and hence homeostasis) in elite performance sports. The result of such a study will have a public health implication given that large majority of the general population drive a motor vehicle and the psychoemotive stress and wellbeing endured during a driving bout can be adequately compensated by dietary management.

In conclusion, extracts from food plants and medicinal plants continue to be used in herbal medicine practice for the treatment of many chronic or acute diseases, viral pathologies and as immune modulators. The studies alluded to in this commentary paper indicate that fermented papaya preparation can modulate oxidative injury as well as injury due to inflammation and improve the immune function. This supports the context that functional nutraceutical and/or food supplements (as exemplified with fermented papaya preparation) exhibiting anti-inflammatory, antioxidant, immunostimulatory (at the level of the mucus membrane) and induction of antioxidant enzymes, may have beneficial prophylactic potential in the management of chronic diseases with overt inflammation.

## Conflict of interest

YH and PM are affiliated with the Osato Research Institute (not for profit organization focused on biomedical research involving FPP). OIA, ER, FM and LM are actively involved in biomedical research involving fermented papaya preparation. FPP is produced by the Osato International Inc., Japan.

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