

## HEART FOR JAPAN

Although the O.R.I. is a rather small organization, we launched the "Heart for Japan" project using our global network. We would like to let the rest of the world know that life in Japan has been returning to normal since the March 11, 2011 earthquake. We would also like to announce our support for the educational needs of the children who lost their parents in the earthquake and subsequent tsunami.

### 〈Project Actions〉

#### Action1

June 10, 2011: Barcelona, Spain

SHA Resort Wellness Clinic ([www.shawellnessclinic.com](http://www.shawellnessclinic.com)). We held a press conference here under the chairmanship of Princess Beatrice, where Mr. Hayashi expressed his thanks for the international support of Japan, including Spain, and reported on the present conditions in Japan. He emphasized the importance of the economic benefit of diseases prevention as well as natural disaster preparedness.

Also in June, the SHA Wellness Clinic started a new treatment using FPP(Immun'Age) as one of their featured items.



Celebrities gather from all over the world at the SHA Resort Wellness Clinic on the beautiful Spanish coast.



Mr. Yuki Hayashi expresses thanks for the international support of Japan.



Dr. Pierre Mantello, Director of the Osato Research Institute, making a presentation about the mechanism of FPP and its clinical application.



Prof. Luc Montagnier presenting a lecture on the importance of preventative medicine, including FPP.

#### Action2

June 15 - 17, 2011: Paris, France

The Fourth Annual International Symposium for Nutrition, Oxygen Biology and Medicine was held at Campus des Cordeliers in Paris, where more than 200 doctors from around the world in both fundamental and clinical fields of medicine participated. At the request the symposium president, the Osato Research Institute produced a charity concert featuring Japanese Pianist, Yoshihiro OTA, on June 16th at Maison des Arts et Métiers benefitting victims of the March 11 earthquake and tsunami in Japan. Ms. Makiko Osato, an Osaka University School of Medicine graduate student, gave a speech in both French and English explaining the message of her father, Mr. Yuki Hayashi, President of the Osato Research Institute.



Prior to the concert: pianist, Yoshihiro OTA (second from left), a very happy-looking Dr. Lester Packer (far right) and his wife Anne (third from right)



Ms. Makiko Osato giving a speech in fluent French and English

### Action3

June 18-22, 2011: Tokyo, Japan

Even though there has been a decrease in the number of renowned scientists visiting Japan since the March 2011 earthquake, the Osato Research Institute, in cooperation with the French Embassy in Japan, invited two leading French scientists to a special conference in Tokyo: Prof. Luc Montagnier (2008 Nobel Prize winner in Physiology/Medicine) and Dr. Gerald Salama (a preventive medicine expert).

Following the lecture "Current Status of and Future Prospects in Anti-Aging Medicine", at the MAISON FRANCO-JAPONAISE in Tokyo on June 21, Prof. Luc Montagnier (acting as an O.R.I. Scientific Board Member), presented the Osato Research Institute's "HEART FOR JAPAN" funds of one million yen to the

'NPO Ashinaga'. Prof. Montagnier worries about the children who have lost their parents in the earthquake and tsunami. The 'NPO Ashinaga' will use the "HEART FOR JAPAN" funds to build a house to care for these children. At the completion of the conference, we were grateful to receive donations totaling 35,453 yen from the audience for 'NPO Ashinaga'.



Prof. Luc Montagnier lecturing Dr. Salama lecturing



Scene of the "Current Status of and Future Prospects in Anti-Aging Medicine" lecture

### Action4

June 25-26, 2011: Nürburgring, Germany

Aston Martin, the leading British auto maker with a proud 100 year long history and appearing in the latest Royal Wedding, entered the 24-hour race with its factory team supporting Japan with the roofs of their race cars painted with Japanese flags; the V12 "Zig", the V12 "Zag", and the V8 VantageN24. They all raced the Nürburgring circuit and made all the Japanese present there shed tears of appreciation for Aston Martin's thoughtfulness.

Dr. Bez, CEO of Aston Martin, handed a 100,000 pound check to Mr. Akio Toyoda, president of Toyota Motor Corporation to aid in Japan's recovery efforts. They shared the same pit for five years.



Aston Martin making a goal with a message for Japan — What an excellent performance for the 24 hours!



Aston Martin's racing car displayed the Japanese flag for the first time in history, to show their support for Japan.

### Action5

June 27, 2011: OSAKA, Japan

Prof. Rachmilewitz (senior director of the Osato Research Institute and personal physician of the Israeli prime minister) visited Japan to speak at the 4th MEET THE EXPERT in blood diseases at Osaka University Medical School sponsored by Pfizer Japan Inc.

"Blood and oxidative stress" are emerging fields of research in Japan, so the professors at Osaka

University Medical School listened intently to the lecture.

The clinical study of Prof. Rahmilewitz was announced in the form of a treatise for FPP (Immun'Age) to control ROS (oxidative stress) in the blood of patients suffering from blood diseases and to increase the effect of GSH (glutathione), a useful enzyme or ferment for ROS simultaneously.



Active Questions and Answers

### Action6

December 17, 2011: Gifu, Japan

WFRP Charity Christmas Concert

Hiraga Marica Jazz Christmas

On December 17, 2011, the World Foundation Aids Research and Prevention Japan Office held a charity concert at the yma hall in the Osato Research Institute. The Jazz trio led by Marica Hiraga, known as one of the most proficient jazz vocalist, fascinated the audience with bouncy tempo and romantic singing voice. Proceeds and any other money raised from this concert were donated to NPO Ashinaga for children who have lost their parents in the earthquake and tsunami. This concert was co-sponsored by Ferrari Japan and Jaguar Land Rover Japan, as well as Osato Research Institute. We introduced the two other companies' activities to the

audience since they immediately started helping people in the regions affected by the earthquake and tsunami. This charity concert made us become more aware of the value of strong bonds between people.



Speech by Mr. Yuki Hayashi, president of O.R.I.



Wonderful vocal performance by Marica Hiraga

# *The Great News from Vatican*



January 17, 2012

## **Immun'Âge was presented to the Pope Benedict XVI**

On January 17, 2012, Immun'Âge was presented to the Pope Benedict XVI by OSATO team.

This audience with the Pope is the second time for us, to be our great honor, since Prof. Luc Montagnier (Nobel laureate in Physiology or Medicine) handed Immun'Âge to the late Pope John Paul II and recommended the treatment to aid in his recovery in June 2002.

The Pope Benedict was really delighted and expressed his gratitude.

Immun'Âge is sold at the Vatican Pharmacy, which is known as the busiest pharmacy in the world.



June 2002

## Marie Curie Museum Renovation

### Information on the achievement of Mrs. Marie Curie obtainable through a touch panel screen of Multi Media System

2011 was the 100th year since Mrs. Marie Curie received the Nobel Prize, and many events were held as part of its 100th anniversary celebrations both in France and USA. There is a museum called Marie Curie Museum within the Curie Institute in Paris, and Eve, the daughter of Mrs. Marie Curie, donated her inheritance to the Museum for the start of renovation, which was scheduled to be completed in 2012. In 2006, Osato Research Institute supported a project, scanning 1200 pages of newspaper articles about Mrs. Marie Curie's visit to USA during the year of 1921 in order to keep them forever on DVD and to show them to visitors from all over the world. This time, we helped the multi media system to get installed, so that visitors can get information on the career of Mrs. Marie Curie, as well as the events and results of her study, following her visit to USA in 1921, by use of a touch panel screen. (It was scheduled to be completed in May, 2011.) On December 7th, 2011, Marie Curie Charity

Gala Dinner was held at the French Embassy in Washington D.C. under the auspices of Prof. CLAUDE HURIET, the president of the Curie Institute.

We are told that, when Mrs. Marie Curie visited USA, some US women's organizations rose to give a present to her 1g of expensive radium to support her as a rare female great scientist at that time, which eventually opened the door for her to study radiation therapy for cancers.



1200 pages of more than 80-year-old newspaper article being well reserved



Mrs. Marie Curie and her two daughters, Eileen and Eve at their visit to USA in 1921



With Prof. CLAUDE HURIET, the president of the Curie Museum at French Embassy in Washington D.C.



With Prof. Maureen, the dean of SMITH COLLEGE, that provided an opportunity for the US women's organizations to give Marie Curie radium

## Mr. Yuki Hayashi, President of O.R.I., assuming the post of a trustee of Whittier College in USA

Mr. Yuki Hayashi, President of O.R.I., was recommended to be appointed as a trustee of Whittier College on February 18th, 2011. Whittier College was founded by members of the Quaker Society. It is the second oldest private college in California with the former President Nixon as one of its celebrated graduates.

In commemoration of his trustee appointment, O.R.I. promoted new courses of preventive medicine. They are for students of 6 faculties including science, social science, art and

human studies, to work on the problem of preventive health through the curriculums.



Watching a football game with President.....



A home coming game

## Aston Martin GT4 Challenge 2011

Dr. Pierre Mantello, the director of the Osato Research Institute, took part in Aston Martin GT4 Challenge 2011 as a driver to verify the effect of FPP for elder drivers, himself aged at 57.

He participated in all the nine races from the 1st race at Silverstone circuit in April to the one at Dijon circuit in September, and finished overall the fourth place for his first challenge. On December 15th, he received "Spirit of the Challenge of Great Britain Award" in a ceremony held at the Head Quarters of Aston Martin. The effect of FPP and his further activities are expected in 2012.



Dr. Mantello, the 1st place in the race held in Silverstone



A hard fighting as an amateur driver among professional drivers



Japanese flag on the roof of Dr. Mantello's car for the "Heart for Japan" project

## ORI meeting 2010

On July 22, 2010, the ORI held a workshop on "Nutritional Approach to Preventive Medicine by FPP" at the UNESCO, Paris. Before starting the workshop, Mr. Hayashi, president of Osato Research Institute, welcomed the members of the ORI board with his message; "In Japan, more than 23 % of the population is over 65 years old, and so, Japan is now one of the super-aging societies. In the near future, the most of the country's tax revenue will have to cover the expanding medical cost. The only solution to avoid it, is disease prevention.

Especially, it is important to prevent chronic diseases. We have been researching the relation between intravital inflammation and chronic diseases. We have found that FPP has anti-inflammatory effect and believe that FPP has a big potential for disease prevention. Today, I thank you all to be here and would like to expect good research presentations, leading to reduce medical costs. Lastly, I appreciate that Prof. Montagnier, the 2008 Nobel Laureate, has accepted to serve as our chairman today."



Despite it was a summer holiday season, 8 (out of 10) members of the board attended this workshop at UNESCO.

## 2010 Annual Meeting of the Japanese Society of Applied Glycoscience

2010 Annual Meeting of the Japanese Society of Applied Glycoscience was held at Shizuoka Convention & Arts Center "GRANSHIP" from 15th to 17th of September, 2010. While we have been working together with internationally renowned scientists and doctors, and presented the results of the collaborative researches, we have been advancing our own research on carbohydrate which is a major component of FPP. On this

occasion, the O.R.I. researcher Keiko NISHIDA made a presentation at the session "Structure and Function of carbohydrate (polysaccharide)" to present the results of our research works. At the convivial party after the session, we had a chance to talk with many people who took interest in our presentation and got tips on further researches. We would like to continue to make this kind of presentation at other academic meetings successively.



On 15th of September, 2010, in front of the venue, from the right, Sachiko Okuda (ORI vice director), Keiko Nishida and Hiroshi Shimizu (ORI researchers)

### About the presentation

In the presentation entitled "Basic research of saccharides in Fermented Papaya Preparation (FPP) with anti-inflammatory effect (1)", after explaining on the previous clinical researches on FPP, we presented our basic researches on "Isolation and architectural analysis of saccharides in FPP" and "Effect of saliva on component of FPP"



Many audiences were listening tentatively to the presentation.

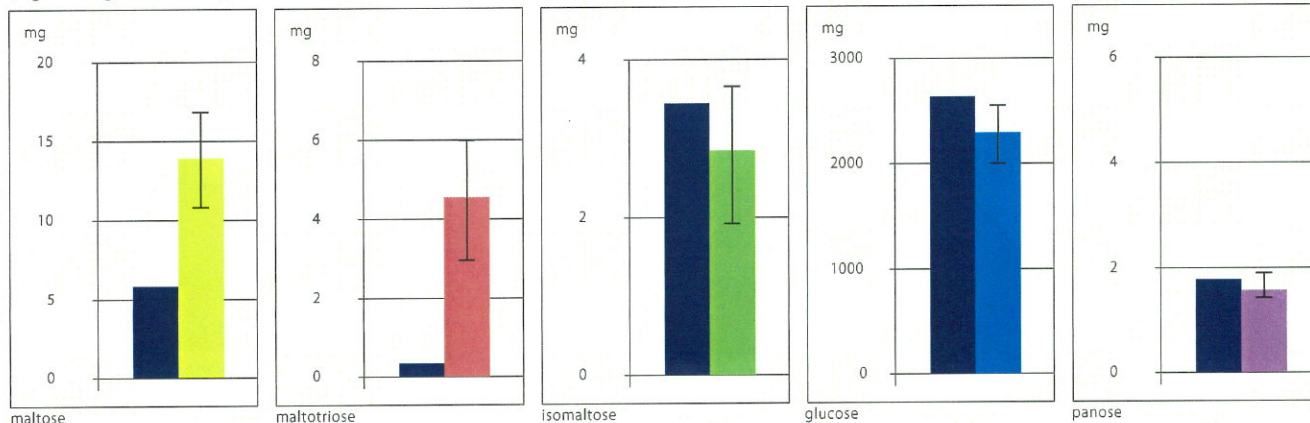
### Summary of "Basic research of the saccharides in Fermented Papaya Preparation (FPP) with anti-inflammatory effect (1)"

After extracting FPP using various solvent extractions, the method of isolation and purification of the chromatography extract was studied. Structural analysis of the obtained fractions was made by NMR. The result of the analysis showed that oligosaccharide is included in the saccharide contents in FPP, and we found out that this oligosaccharide in FPP is a kind of malt oligosaccharides, which is known to be good for improvement of enteral environment, immune enhancement and inhibition of streptococcus mutant.

Also, we analyzed FPP mixed with saliva in mouth using 18 adults of both sexes. The result of the analysis showed that malt oligosaccharides in the specimens increased significantly, up to 13.1 times more (see diagram below). Thus, it is suggested that FPP is activated in the mouth, which is the very first place for the food taken by ourselves, and we can expect the effect of malt oligosaccharides by mixing FPP with saliva.

Accordingly, we recommend to mix FPP with saliva well before swallowing. We are now analyzing the structure of minor components of FPP and investigating as to why malt oligosaccharides in FPP increase when it is mixed with saliva, as well as what effect can be expected by this increase.

(Fig. Change in malt oligosaccharides content being mixed with saliva)



## O.R.I. Scholarship to Alice Powell – supporting the dream of a young driver

Alice Powell is 18 years old, with participation in the scholarship of the Osato Research Institute since March 2010. Her ambition is to become the first successful female driver in Formula 1. She was introduced to motor sport by her grandfather, and learned to drive a car at the age of six and started go-carting at eight. In 2007/2008, she achieved eight podiums in the Ginetta and BRDC Stars of Tomorrow Championships (excerpted from the Official Website of Alice Powell). She is always fighting against experienced, elder drivers under the circumstances easy to suffer from a great stress both physically and mentally. It is important for her to maintain good conditions, and she uses

FPP everyday to reduce stress hurting her body.

On May 9, 2010, she became the first female champion in the Formula Renault BARC Championship at Silverstone.

Alice Powell Racing Official Website  
<http://www.alice-powell.com/>



The third place on an honor platform for the Donington Park Championship



Victory in the Graduate Cup in August 2011



Lord Wakefield Trophy 2010, with which she was honored as the best female driver of 2010 in the Motorsport World!

## Commended as International Hospitality Business Enterprise

Hospitality Business Enterprise is "an enterprise which has a business model to upgrade its own quality by keeping delivery of desired value to its customers"

\* The International Hospitality Research Center Inc. was founded in the year of 2000 to develop and research Hospitality Business, and certified six enterprises as the first Hospitality Business Enterprises. And the ORI was chosen as one of the above six enterprises among a number of companies, and awarded the fourth prize in Japan. The reason of this certification was due to "the fact that the ORI has been delivering their technology and study results to contribute to human health, directly from Japan to the rest of the

world". Other companies, which were chosen as Hospitality Business Enterprise, are all powerhouse enterprises such as Toppan Printing Co., Ltd., Sugamo Shinkin Bank, Pola Inc., The Ritz-Carlton OSAKA, and May Bach/Daimler AG. We feel honored to receive this commendation of Hospitality Business Enterprise.

\* based on the principle of International Hospitality Research Center, Inc.



Mr. Hayashi, president of ORI, expressing his gratitude for this prestigious award



The Certificate Plate being awarded by Mr. Gwenael Nicolas (designer)



Representatives of the certified six enterprises

# Autumn has come...

Let us have a breath...,  
And enjoy the Autumn of  
O.R.I. with us, please.



O.R.I. standing in the autumnal scenery,  
ablaze with red and yellow tints



Autumnal coloring different each year  
Winter in Gifu, all these leaves gone...

## More New Research Equipments in the laboratory at ORI

On January 13, 2012, we installed a new biomedical cooler at the Osato Research Institute. It is designed to meet the dimensional and temperature requirements for laboratory use to keep many varieties of reagents or specimens at low temperature in store. The capacity of this biomedical cooler makes it possible for us to conduct our low-temperature tests more efficiently, since we have now more than 3 times bigger low-temperature storage space

than before. Three days later, on January 16, 2012, we installed a new high-speed cooling centrifuge. The Maximum Speed is 20,000 rpm and Maximum RCF is 48,000xg, and it spins at constant temperature within the range of -20°C to 40°C to avoid heat-induced alteration. With these functions, we can separate fractions of FPP by infinitesimal weight difference.

Both of these new equipment produce non chlorofluorocarbonic gas with

zero-ODP refrigerant. Moreover, they are much more energy-saving than before and use 30% less electricity, which can achieve more CO2 reductions. We are looking forward to advancing our researches, using this new environmental-friendly equipment to show you more findings about FPP in the new fields, commencing with the research of saccharides.



Use of the new white medical cooler next to the old one



Spacious enough inside with useful movable dividers



Good appearance of cooling centrifuge, looking unlike a research machine



Putting specimen in a white bottle in center and spinning it with the lid on

## Symbol Rose of Osato Research Institute called "Rose ORI" in bloom

Ono-town where we are situated is famous as top producer of rose seedlings in Japan. Thanks to a rose specialist at a shop named "Sun Rose", we succeeded in developing a new variety of rose called Rose ORI (Osato Research Institute). In May, 2011, Rose ORI was in bloom and an application for variety registration was filed.

Rose ORI does not just name after Osato Research Institute, but also means the

ending of oxidative stress or ROS, which is closely related to the theme of ORI's study such as preventive medicine, that is

Reactive Oxygen Species End by Osato Research Institute.

Sales of Rose ORI were started in 2012. If you have any questions on Rose ORI, please let us know.



The deep red of Rose ORI in the beginning of bloom dazzling all who see it



Smelling fresh like a natural rose perfume



Changing of colors from red to reddish violet, and pale violet in the end, as time goes by - charming

### Oxidative-Inflammatory Damage in Cirrhosis : Effect of Vitamin E and a Fermented Papaya Preparation

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【Journal】 Journal of Gastroenterology and Hepatology 22 : 697-703 (2007).

Oxidative DNA damage occurs as an early event in hepatitis C virus (HCV) infection and is an indication of the potential for carcinogenesis. The aim of this study was to test a novel antioxidant/immunomodulator in patients with HCV-related cirrhosis.

The study group consisted of 50 patients with HCV-related cirrhosis with transaminase values less than twofold increased (ALT<80 IU/L). Patients underwent a standardized food-vitamin composition assessment and were assessed for dietary intake, nutritional status and iron level. Patients were randomly allocated into 2 groups and then given either  $\alpha$ -tocopherol 900 IU/day or 9 g/day of a fermented papaya preparation (FPP, Osato Research Institute, Gifu, Japan) at bedtime for 6 months. Ten healthy subjects served as controls. Patients were checked monthly for: routine tests, redox status (reduced glutathione(GSH), glutathione peroxidase(GSH-Px), oxidized glutathione(GSSG), malondialdehyde(MDA)),

plasma  $\alpha$ -tocopherol, 8-OHdG level in circulating leukocyte DNA and serum levels of cytokines.

Patients with cirrhosis showed a significant imbalance of redox status (low antioxidants/high oxidative stress markers) ( $p<0.005$  vs controls). Neither treatment regimen affected transaminases as a whole. However, vitamin E supplementation almost normalized ALT only in the limited vitamin-E-deficient subgroup. A significant improvement of redox status was obtained by both regimens. However, only FPP significantly decreased 8-OHdG and the improvement of cytokine balance with FPP was significantly better than with vitamin E treatment ( $p<0.05$ ). Although the present data seem to suggest a potential supportive role of antioxidants / immunomodulators as FPP in HCV patients, more studies are needed to substantiate their effect on the natural history of the disease.

Table 1 : Redox status in patients with HCV - related cirrhosis : effect of vitamin E or FPP supplementation

Parameter	Control	Vitamin E supplement		FPP supplement	
		Baseline	6 months	Baseline	6 months
GSH ( $\mu$ mol/L)	972 $\pm$ 52	561 $\pm$ 34	786 $\pm$ 69*	521 $\pm$ 54	713 $\pm$ 72*
GSSG ( $\mu$ mol/L)	29 $\pm$ 3	26 $\pm$ 4	27 $\pm$ 5*	29 $\pm$ 4	27 $\pm$ 8*
GSH/GSSG ratio	33.5	21.6	29.1*	18.0	26.4*
MDA ( $\mu$ mol/L)	1.1 $\pm$ 0.3	3.2 $\pm$ 0.6	1.7 $\pm$ 0.2*	3.4 $\pm$ 0.5	1.9 $\pm$ 0.2*
$\alpha$ -tocopherol ( $\mu$ mol/L)	29.2 $\pm$ 1.2	14.4 $\pm$ 0.7	23.8 $\pm$ 0.3*	15.6 $\pm$ 0.6	15.9 $\pm$ 0.8*

\* $p<0.05$  vs vitamin E supplement group. Values shown as mean  $\pm$  SD.

Fig. 1: Effect of fermented papaya preparation (FPP) on concentration of 8-OHdG in circulating leukocytes

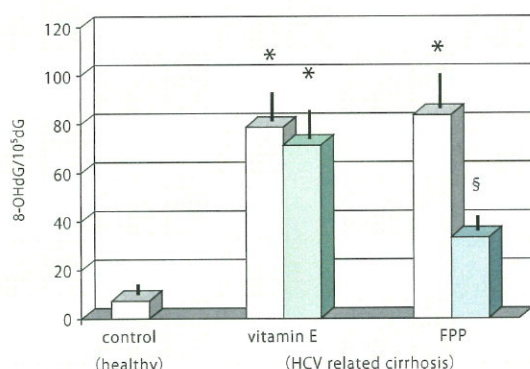
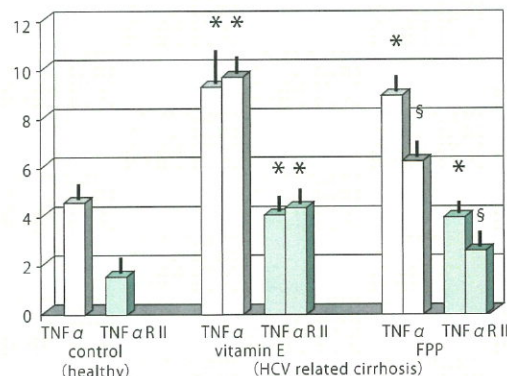


Fig. 2: Effect of fermented papaya preparation (FPP) on concentration of tumor necrosis factor (TNF)- $\alpha$  and TNF- $\alpha$  receptor type II (RII)



\* $p<0.001$  vs control § $p<0.05$  vs vitamin E group and control

# Nutraceutical Supplementation: Effect of a Fermented Papaya Preparation on Redox Status and DNA Damage in Healthy Elderly Individuals and Relationship with GSTM1 Genotype

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**【Journal】** Ann. N. Y. Acad. Sci. 1067: 400–407 (2006).

Our study group consisted of 54 elderly patients without major invalidating diseases who were randomly divided into two fully matched groups. Group A was given a certified fermented papaya preparation 9 g/day by mouth, while group B received placebo. Treatment was carried out in a cross-over manner with a 3-month supplementation followed by a 6-week washout period. Blood samples were drawn at entry and on a monthly basis to check routine parameters, redox status, and 8-OHdG in circulating leukocyte DNA. Polymorphism analysis of GSTM1 was carried out as well. 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 ( $p < 0.01$ ). There was a

weak correlation between cigarettes smoked/day and DNA adduct ( $r: 0.61, p < 0.05$ ), which also correlated with antioxidant concentrations, but only in GSTM1 (—) smokers ( $p < 0.01$ ). The fermented papaya preparation (FPP)–supplemented group showed a significant enhancement of the antioxidant protection ( $p < 0.01$  vs A) within the subgroups with GSTM1 (—) and of plasma DNA adduct, irrespective of the GSTM1 genotype. Only the GSTM1 (—) subgroup was the one that, under FPP treatment, increased lymphocyte 8-OHdG ( $p < 0.01$ ). Such preliminary data show that FPP is a promising nutraceutical for improving antioxidant-defense in elderly patients even without any overt antioxidant-deficiency state while helping explain some inconsistent results of prior interventional studies.

Fig. 1: Concentration of 8-OHdG in circulating leukocytes (only in GSTM1(—) subjects)

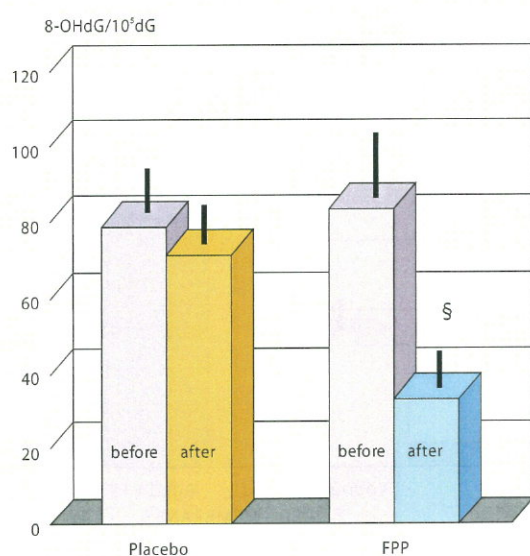
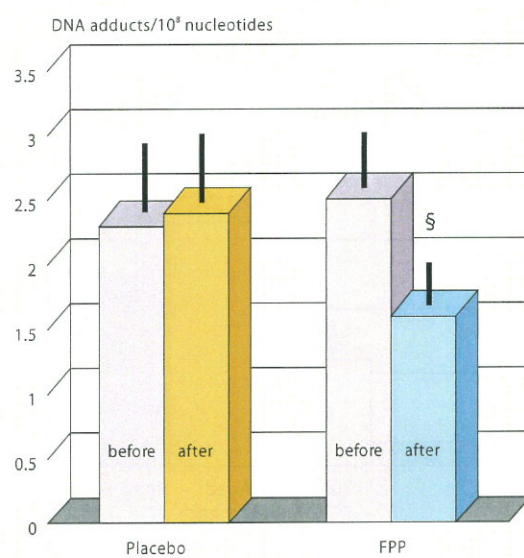


Fig. 2: Concentration of DNA adducts in all subjects



\$  $p < 0.05$  vs baseline and vs placebo

### Relationship Between Aging and Susceptibility of Erythrocytes to Oxidative Damage: In View of Nutraceutical Interventions

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【Journal】 REJUVENATION RESEARCH Volume 9, Number 2 : 227 - 230 (2006).

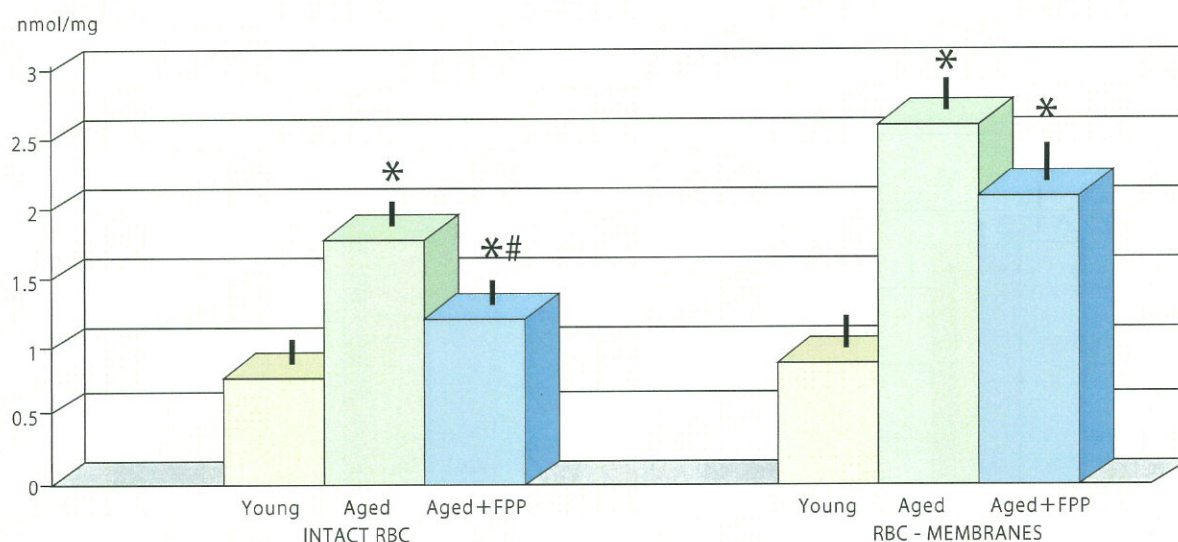
Erythrocytes and erythrocyte membranes are a feasible biological system to study in aging - related investigations, because unsaturated lipids in the cell membrane, amino acids, and DNA nucleotides represent specific target for free radical damage. Moreover, recent studies point out the role of oxidative damage to biomembranes in a number of chronic inflammatory and degenerative diseases. Indeed, despite the fact that no overt changes of membrane components have been reported in erythrocytes (RBC) with advancing age, peroxynitrite anion - related damages to platelets and RBC have been implicated in age-related neurodegenerative disease. Although there are still some conflicting results, it appears that erythrocytes from elderly individuals and aging animals are highly susceptible to oxidative stress. Although these derangements may represent an epiphenomenon of more complex epigenetic abnormalities, a tentative therapeutic intervention on the expected higher RBC vulnerability to oxidative stress might be of interest. Thus, given that susceptibility of erythrocytes to oxidative damage is altered during the aging process, the authors' aim was to assess whether this phenomenon could be beneficially influenced by a specific nutritional supply.

Twelve (12) healthy elderly subjects (mean age : 68, range 62 - 75) were divided into two groups matched for age and dietary habits : (a) those given an antioxidant / NO - modulating fermented papaya preparation (FPP) 9 g / day for 4 weeks, and (b) those given the same amount of placebo (flavored sugar devoid of any antioxidant property). The following measurements were made in all subjects: blood antioxidant

status (plasma lipid hydroperoxides,  $\alpha$ -tocopherol), Fractionation of erythrocytes by age (MCV, glycohemoglobin), Oxidative challenge test (MDA, SOD activity), Erythrocyte lipid and protein analysis (phospholipids and cholesterol, RBC ghost protein) and NOs concentration. As an age - control group, a group of 8 young (mean age : 31, range 22 - 34) healthy subjects were considered as well.

No protein / lipid distribution in erythrocytes (RBC) membranes was noted among different ages and treatments. Higher RBC concentration of malondialdehyde and nitric oxide synthase were found in the elderly ( $p < 0.05$  versus "young" controls), whereas superoxide dismutase was unaltered. Such abnormalities were prevented by FPP supplementation ( $p < 0.01$ ). RBC and RBC ghosts showed an enhanced susceptibility to lipid peroxidation by using cumene hydroperoxide ( $p < 0.01$  versus young) but FPP supplementation significantly protected intact RBC ( $p < 0.05$  Fig. 1). These preliminary data suggest that nutraceuticals with antioxidant/NO-regulating properties significantly protect from RBC oxidative damage, and are potential weapons for the aging process and chronic and degenerative diseases.

Fig. 1: Effect of CumOOH oxidative stress test on RBC - MDA in intact RBC and RBC membranes : effect of FPP



\* $p < 0.01$  vs young; # $p < 0.05$  vs aged without FPP

Young : "young" controls, Aged : placebo, Aged + FPP : FPP supplementation

## Plasma Glucose Level Decreases as Collateral Effect of Fermented Papaya Preparation Use

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【Journal】 La Clinica Terapeutica 157(3): 195-198 (2006).

Fermented Papaya Preparation(FPP) is a natural healthy food that has antioxidant action, inhibitory effect on oxidative DNA damage and tissue injury, being a potent OH scavenger.

The wide use of FPP, especially by elderly people, made us note an unknown collateral effect, i.e., blood sugar level dropping signs especially in the afternoon.

The aim of the present work was to scientifically verify the possibility that individuals, who are taking the nutraceutical FPP, might have a decrease of plasma sugar levels.

For this purpose, 50 subjects, divided in two groups, were enrolled. The first group was made of 25 patients: 13 females and 12 males affected by type-2 diabetes mellitus under treatment with the oral antidiabetic drug, glybenclamide.

The control group included 25 clinically-healthy subjects: 16 females and 9 males, matching in age. All subjects were given 3 g of FPP daily, during lunch, for two months.

The results of this study confirmed the empirical experience that FPP use can induce a significant decrease in plasma sugar levels in both healthy subjects and type-2 diabetic patients.

This hypoglycaemic effect, associated with clinical signs, induced the diabetic patients to reduce the dosage of their antidiabetic oral therapy ( in one patient the therapy was really suspended ).

In accordance with these results, the FPP administration is suggested as an adjuvant drug to join the oral antidiabetic therapy in type-2 diabetes mellitus.

Fig. 1: Fasting and post-meal plasma glucose level in clinically-healthy subject

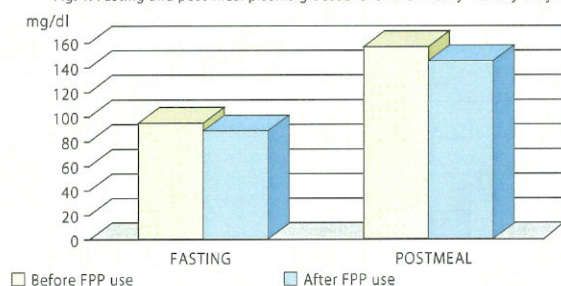
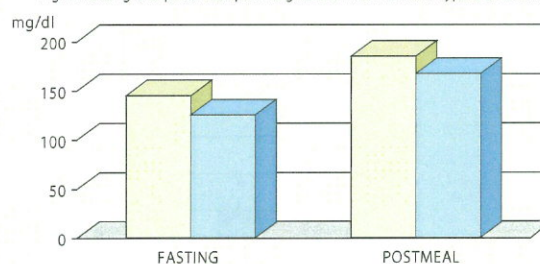


Fig. 2: Fasting and post-meal plasma glucose mean levels in type 2 diabetic patients



## Aston Martin Racing Le Mans 2007 Follow up of Oxidative Stress of 3 racers (DBR9 N° 009) during 24 Hours race

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Two drivers out of three were supplemented with FPP. The sample of urins were collected, one before race, one at middle race, one after race. TBARS, MDA, Carbonyls and protein level were analyzed.

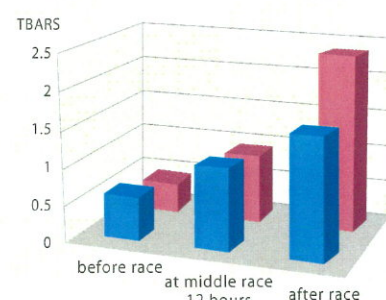
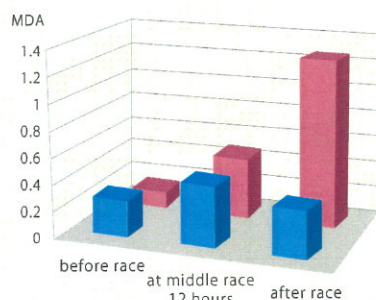
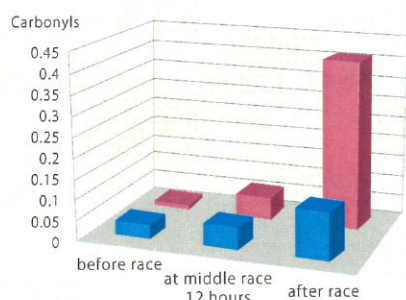
MDA, TBARS and Carbonyls levels are increased during the race for all 3 drivers, showing that 24-hour car race is an exhausting physical effort. Average increase of the 3 drivers are 3.8 fold for MDA, 3.4 fold for TBARS and 7.2 fold for Carbonyls.

The result of the two supplemented drivers shows that FPP significantly protects them against increase of oxidative stress comparatively of the non supplemented driver.

Even though the number of participants was small, only 3 drivers of the same car AMR DBR9 N°009, the difference of oxidative stress parameters level at end of race between the non supplemented driver and the two others is important.

We could also notice that even if the non supplemented driver had the lowest basic level of oxidative stress parameters, at the end he shows the highest one.

The difference was a higher oxidative attack for non supplemented driver: 2.5 fold and 2 fold higher for MDA and TBARS which means an higher attack on lipid compounds of cells (main compounds of cell's membranes), 5 fold for Carbonyls which means an higher attack on protein compounds of cells (main compound of structure compounds of cells and of enzymes). This could result to higher fatigue and more muscle's oxidative attack (stiffness, cramps). Supplemented drivers could experience less fatigue, a quicker and a better recovery during and after such an exhaustive effort. This trial confirm other previous trial on Paris-Dakar driver Philippe Gache and our last trial on a best and quicker recovery by help of FPP on high level sportsmen after surgery (CERS: European Center of Sportsmen Recovery, Cap Breton, France). Like we have already shown with previous clinical trials where oxidative stress was important, FPP is a natural non doping food supplement able to protect the body against oxidative stress for sportsmen who practice sports with exhaustive efforts. Endurance car racing is one of them.



## Nutraceutical Strategy in Aging Targeting Heat Shock Protein and Inflammatory Profile through Understanding Interleukin-6 Polymorphism

[Author] F. MAROTTA, K. KOIKE, A. LORENZETTI, Y. NAITO, F. FAYET, H. SHIMIZU, AND P. MARANDOLA

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[Journal] Ann. N.Y. Acad. Sci. 1119 : 196–202 (2007).

The aging process is paralleled by two- to four- fold increases in plasma/serum levels of inflammatory mediators, such as cytokines and acute-phase proteins. In this study we assessed the inflammatory profile and polymorphism of healthy elderly subjects and the influence of a nutraceutical supplement. Forty elderly, generally healthy subjects were recruited, divided into two matched groups, and given either a fermented papaya preparation 9 g/day by mouth or the same amount of placebo.

Treatments were carried out in a cross-over manner with a 3-month supplementation period followed by a 6-week washout period between treatments. Ten healthy young subjects served as controls. Interleukin-6 (IL-6) promoter - 174 G/C polymorphism genotype was

determined together with blood levels for redox status, proinflammatory cytokines, high sensitivity C-reactive protein, and serum 70 kDa heat shock protein (Hsp70) concentrations. Tumor necrosis factor- $\alpha$  and IL-6 were higher in elderly subjects ( $p < 0.05$  versus young controls). The concentration of Hsp70 inversely correlated with markers of inflammation in -174 G/C-negative subjects ( $r = 0.62$ ,  $p < 0.05$ ). Nutraceutical intervention normalized the inflammatory parameters ( $p < 0.05$ ) with a rise of Hsp70 ( $p < 0.05$ ). This suggests that healthy elderly individuals may have a proinflammatory profile playing as a downregulating factor for inducible Hsp70, particularly if - 174 G/C-negative. A nutraceutical intervention seems able to beneficially modulate such a phenomenon.

Fig. 1: Effect of FPP supplementation on IL-6 and TNF- $\alpha$  in an elderly population

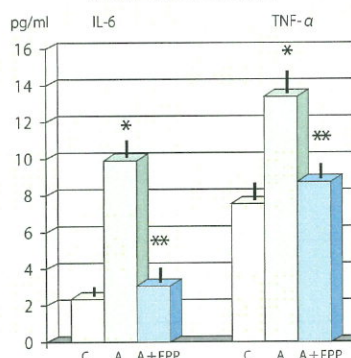
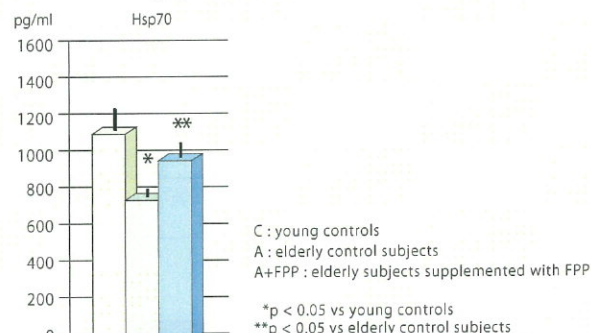


Fig. 2: Effect of FPP supplementation on Hsp70 in an elderly population



## Molecular Effects of Fermented Papaya Preparation on Oxidative Damage, MAP Kinase Activation and Modulation of the Benzo[a]pyrene Mediated Genotoxicity

[Author] Okezie I. Aruoma, Renato Colognato, Ilaria Fontana, Joanne Gartlon, Lucia Migliore, Keiko Koike, Sandra Coecke, Evelyn Lamy, Volker Mersch-Sundermann, Incoronata Laurenza, Luca Benzi, Fumihiko Yoshino, Kyo Kobayashi, Masaichi-Chang-il Lee

[Organization] Faculty of Health and Social Care, London South Bank University, UK

[Journal] BioFactors 26 (2) : 147-159 (2006).

The involvement of oxidative and nitrosative stress mechanisms in several biological and pathological processes including aging, cancer, cardiovascular and neurodegenerative diseases has continued to fuel suggestions that processes can potentially be modulated by treatment with free-radical scavengers and antioxidant. The fermented papaya preparation (FPP) was investigated for its ability to modulate oxidative DNA damage due to  $H_2O_2$  in rat pheochromocytoma (PC12) cells and protection of brain oxidative damage in hypertensive rats. Cells pre-treated with FPP (50  $\mu$ g/ml) prior to incubation with  $H_2O_2$  had significantly increased viability and sustenance of morphology and shape. The human hepatoma (HepG2) cells exposed to  $H_2O_2$  (50  $\mu$ M) showed an olive tail moment of  $10.56 \pm 1.44$  compared to  $1.37 \pm 0.29$  of the solvent control. A significant reduction ( $p \leq 0.05$ ) of DNA

damage was observed at concentrations  $\geq 10$   $\mu$ g/ml FPP, with 50  $\mu$ g/ml FPP reducing the genotoxic effect of  $H_2O_2$  by about 1.5-fold compared to only  $H_2O_2$  exposed cells. Similarly, concentrations  $\geq 50$   $\mu$ g/ml FPP significantly reduced DNA migration in co-treated cells compared with only the benzo[a]pyrene treated cells with a dose of 100  $\mu$ g/ml FPP reduced the DNA damage 2-fold. The potential of FPP to regulate the phosphorylation status of ERK 1/2, Akt, and p38 was analyzed by Western blot analysis. FPP showed the potential to modulate the  $H_2O_2$ -induced ERK, Akt and p38 activation with the reduction of p38 phosphorylation induced by 250  $\mu$ M  $H_2O_2$  being more pronounced. 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.

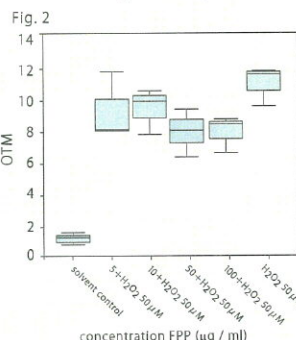
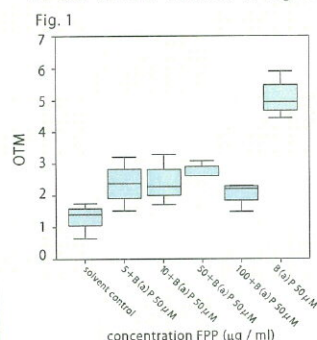


Fig. 1: Olive tail moment (OTM) of HepG2 cells exposed to FPP (5–100  $\mu$ g/ml) for 24h and Benzo[a]pyrene (50  $\mu$ M) for another 24h (solvent control = double distilled and sterilized water).

Fig. 2: Olive tail moment (OTM) of HepG2 cells exposed to FPP (5–100  $\mu$ g/ml) for 24h and  $H_2O_2$  (50  $\mu$ M) for another 30min (solvent control = double distilled and sterilized water).

## Fermented Papaya Preparation as Redox Regulator in Blood Cells of $\beta$ -Thalassemic Mice and Patients

[Author] Johnny Amer, Ada Goldfarb, Eliezer A. Rachmilewitz, Eitan Fibach

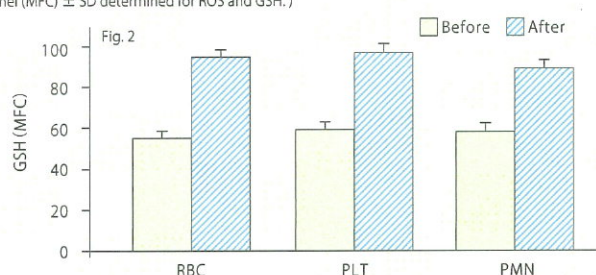
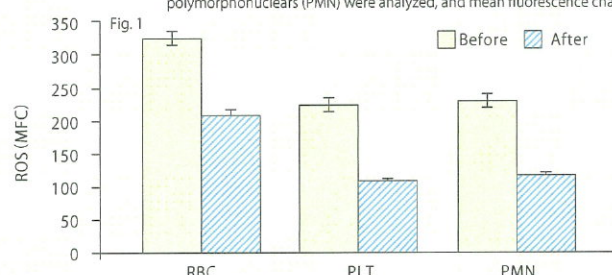
[Organization] Departments of Hematology, Hadassah - Hebrew University Medical Center, Jerusalem, Israel

[Journal] *Phytother. Res.*, 22: 820-828 (2008).

Many aspects of the pathology in  $\beta$ -hemoglobinopathies ( $\beta$ -thalassemia and sickle cell anemia) are mediated by oxidative stress. Fermented papaya preparation (FPP) was tested for its antioxidant effects. The scavenging effect was determined spectrofluorometrically in a cellfree system using 2'-7'-dichlorofluorescein-diacetate (DCF). Both spontaneous and  $H_2O_2$ -induced DCF oxidations were decreased by FPP in a dose-dependent fashion. Using flow cytometry, it was shown that in vitro treatment of blood cells from  $\beta$ -thalassemic patients with FPP increased the glutathione content of red blood cells (RBC), platelets (PLT) 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  $\beta$ -thalassemic mice (50 mg/mouse/day for 3 months) and to patients (3 g x 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), and indicates a significant decrease in reactive oxygen species (ROS) and increase in reduced glutathione (GSH). These results suggest that FPP, as a potent antioxidant, might alleviate symptoms associated with oxidative stress in severe forms of thalassemia.

Fig. 1, Fig. 2: The effect of FPP in thalassemic patients on ROS and reduced GSH (Nine patients with  $\beta$ -thalassemia were treated with FPP per os (3 g x 3 times a day) for a 3-month period. Blood samples were drawn and their RBC, platelets (PLT) and polymorphonuclears (PMN) were analyzed, and mean fluorescence channel (MFC)  $\pm$  SD determined for ROS and GSH.)



## Assessment of the Effect of Fermented Papaya Preparation on Oxidative Damage in Spontaneously Hypertensive Rat Brain Using Electron Spin Resonance (ESR) Imaging and L-band ESR Spectroscopy

[Author] Fumihiko Yoshino, Masaichi-Chang-il Lee, Kyo Kobayashi, Yuki Hayashi, Okezie I. Aruoma

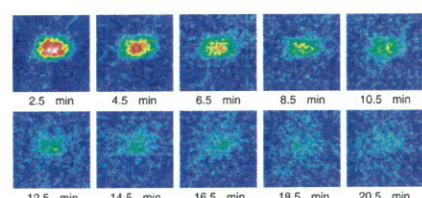
[Organization] Department of Pharmaceutical and Biomedical Sciences, Touro College of Pharmacy, New York, NY, USA

[Journal] *Journal of Functional Foods* 1: 375-380 (2009).

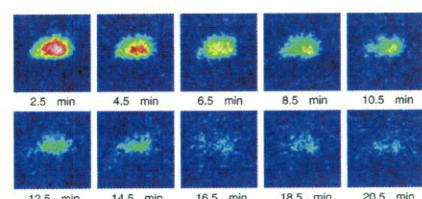
Oxidative and nitrosative stress mechanisms are widely implicated in the biological and pathological processes involved in aging, cardiovascular and neurodegenerative diseases. Although this has continued to fuel suggestions of the benefits of antioxidant functional foods, in vivo methods for assessing the integrity of this remain limited. A novel electron spin resonance (ESR) technique for evaluating oxidative stress and location of its damage in the brain of spontaneously hypertensive rats (SHR) has been described [Lee, M.-C., et al (2004). Assessment of oxidative stress in the SHR brain using electron spin resonance (ESR) imaging and in vivo L-band ESR. Hypertension Research, 27: 485-492].

The reconstructed 2D ESR images of the distribution of a blood brain

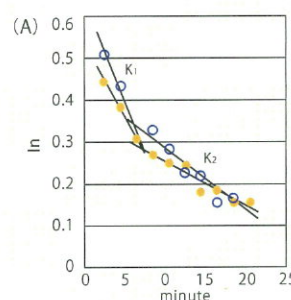
barrier-permeable nitroxyl spin probe, 3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (MC-PROXYL) was used to investigate the ability of fermented papaya preparation (FPP) to modulate oxidative stress of SHR brain. Supplementation (5-7 months) with FPP (50mg/rat/day) significantly increased the decay of the ESR images of the MC-PROXYL, suggesting that FPP may have up-regulated the redox defence activity in the SHR brain. Herein is an in vivo noninvasive technique for the study of oxidative stress and its modulation by dietary factors (that may be intended for applications as neuroprotectants in chronic degenerative disease involving loss of brain function).



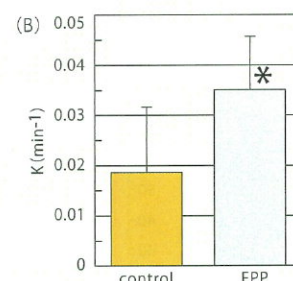
Typical 2D ESR images (y-z plane) of MC-PROXYL distribution in isolated brain of SHR. As indicated by the attached color scale, ESR images were reproduced in 16 colors and signals lower than 10% of the maximal signal intensity detected in all slices were regarded as noise.



Effects of long supplementation (5-7 months) with FPP on 2D ESR images (y-z plane) of MC-PROXYL distribution in isolated brain of SHR. The ESR images are reproduced in 16 colors with signals lower than 10% of the maximal signal intensity detected in all slices are regarded as noise.



(A) Typical L-band ESR signal decay of MC-PROXYL in the isolated SHR brain after i.v. injection of MC-PROXYL (●), and the effects of long supplementation (5-7 months) with FPP (○). The logarithmic signal intensity of the second peak of the ESR spectrum of MC-PROXYL was plotted against time. Linearity was observed in phase I and phase II of the corresponding semi-logarithmic plots.



(B) The logarithmic signal intensity of the second peak of the ESR spectrum of the MC-PROXYL was plotted against time. Each  $K_1$  indicates the decay rate constant ( $\text{min}^{-1}$ ) for the control and effects of long supplementation (7 months) with FPP. Each column represents the mean  $\pm$  SEM ( $n = 3-6$ ). \* $p < 0.05$  vs corresponding value for controls

### Amelioration of Oxidative Stress in Red Blood Cells from Patients with $\beta$ -thalassemia Major and Intermedia and E- $\beta$ -thalassemia Following Administration of a Fermented Papaya Preparation

【Author】 Eitan Fibach, Ee-Shien Tan, Saumya Jamuar, Ivy Ng, Johnny Amer and Eliezer A. Rachmilewitz

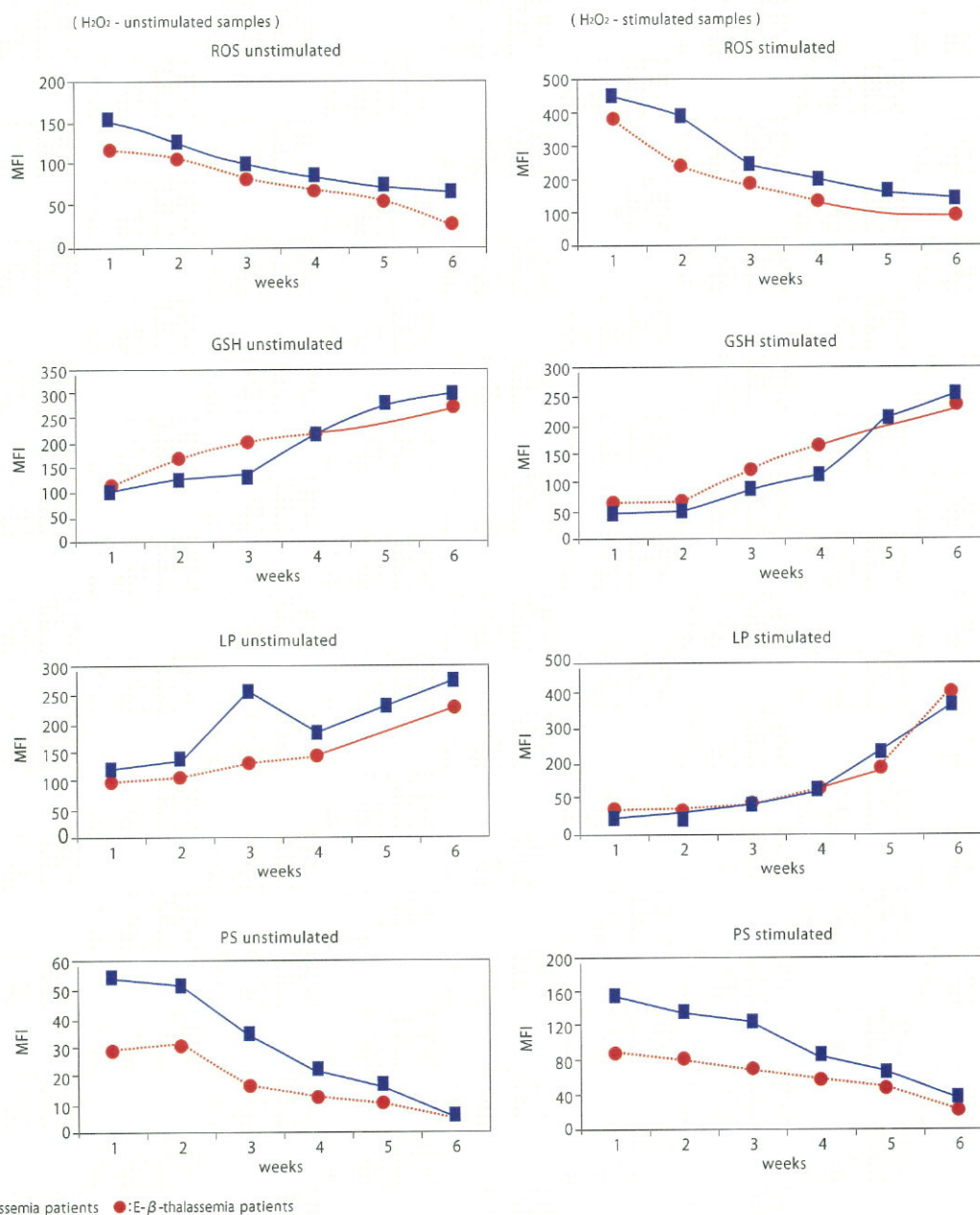
【Organization】 Department of Hematology, Hadassah - Hebrew University Medical Center, Jerusalem, Israel

【Journal】 *Phytother Res.*, 24 : 1334-1338 (2010).

In  $\beta$ -hemoglobinopathies, such as  $\beta$ -thalassemia (thal) and sickle cell anemia, the primary defects are mutations in the  $\beta$ -globin gene. However, many aspects of the pathophysiology are mediated by oxidative stress. Fermented papaya preparation (FPP), a natural health food product obtained by biofermentation of carica papaya, has been shown to limit oxidative stress both in vitro and in vivo. We studied the effect of FPP on two groups of  $\beta$ -thal patients:  $\beta$ -thal, major and intermedia, (in Israel) and E- $\beta$ -thal (in Singapore). The results indicated that in both groups FPP treatment increased the

content of reduced glutathione (GSH) in red blood cells (RBC), and decreased their reactive oxygen species (ROS) generation, membrane lipid peroxidation, and externalization of phosphatidylserine (PS), indicating amelioration of their oxidative status, without a significant change in the hematological parameters. Since the turnover of the erythron is relatively slow, it is possible that longer duration of treatment, probably with the addition of an iron chelator, is required in order to achieve the latter goals.

Fig. 1: Changes in parameters of oxidative stress following administration of FPP to patients with  $\beta$ -thalassemia



## Redox Balance Signalling in Occupational Stress: Modification by Nutraceutical Intervention

【Author】 F. Marotta, Y. Naito, F. Padriani, X. Xuwei, S. Jain, V. Soresi, L. Zhou, R. Catanzaro, K. Zhong, A. Polimeni, D.H. Chui,

【Organization】 ReGenera research group, Milan, Italy

【Journal】 Journal of Biological Regulators & Homeostatic Agents, 25(2): 221-229 (2011).

There is increasing evidence that psychosocial stress can be viewed as a system-wide derangement of cellular homeostasis, with heightened oxidative stress and triggered proinflammatory mechanisms. The aim of this study was twofold: a) to replicate findings that psychological stress increases oxidative damage and b) to determine whether a fermented papaya preparation known to exert a significant protective antioxidant properties could buffer such increases in nuclear DNA damage while also inducing epigenetic protective mechanisms. Twenty-eight sedentary men and women (age range: 28-52), who reported living a stressful lifestyle but with an overall positive attitude, were recruited for this study. Chronic diseases as well as severe burnout and use of drugs for anxiety constituted exclusion criteria. Subjects were supplemented for 1 month with 9 g/day (4.5g twice a day) of a certified fermented papaya preparation. All subjects were given a stress and sleep quality questionnaire together with a diet and

life style assessment. Blood was collected at 2 and 4 week, erythrocyte and leukocyte were separated to assess redox balance and heme oxygenase-1 (HO-1) gene expression while bilirubin oxidized metabolites (BOMs) were tested in the urine. Stressed individuals showed a significant abnormality of redox status with increased MDA of erythrocyte and increased level of 8-OHdG in leukocyte and BOMs excretion ( $p < 0.05$ ). Nutraceutical supplementation brought about a normalization of such values already at the 2 week observation ( $p < 0.05$ ) together with a significant upregulation of HO-1 ( $p < 0.01$ ). Taken together, the results of this study confirm that stressful occupational life per se, without any overt psychiatric illness, may be associated to increased oxidative stress. Supplementation with functional food affecting redox regulation may be part of the therapeutic armamentarium to be considered in this clinical setting.

Fig. 1: 8-OHdG level in leukocytes in stressed subject : effect of FPP

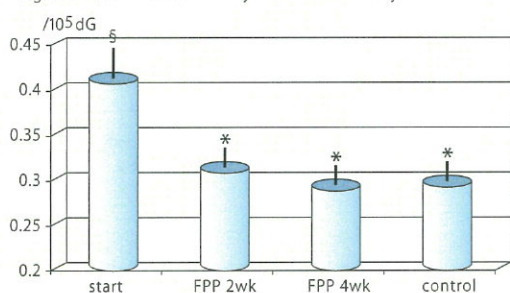


Fig. 2: Urinary bilirubin metabolites excretion (relative values) in stressed individuals : effect of FPP

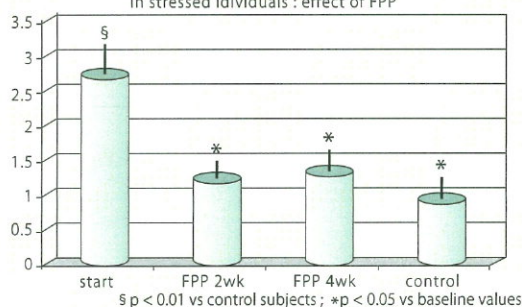


Fig. 3: HO-1 / GAPDH mRNA(AU)

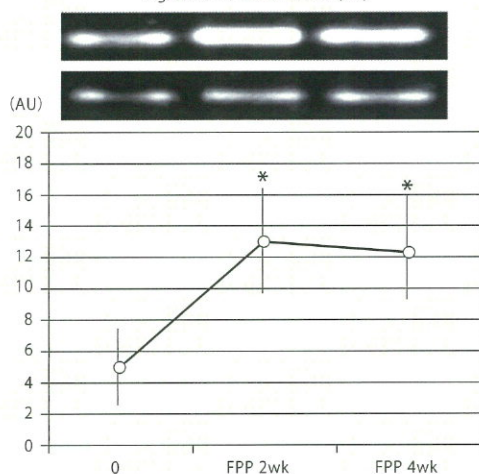


Fig. 4: HO-1 / CD14 mRNA(AU)

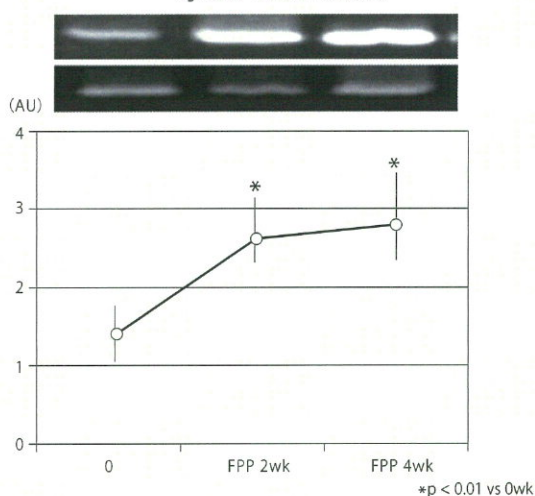


Table 1: Erythrocyte concentration of redox status and MDA : effect of supplementation

	SOD U/g Hb	GPX μmol NADPH /min/g Hb	MDA μmol/g Hb
Healthy control			
start	122.6 ± 21.2	40.4 ± 20.1	0.33 ± 0.15
2 wk	119.8 ± 25.2	38.8 ± 9.9	0.38 ± 0.08
4 wk	127.3 ± 24.3	34.9 ± 12.4	0.27 ± 0.10
Stressed subjects with FPP			
start	166.9 ± 13.7 §	25.5 ± 16.4 §	0.67 ± 0.23 §
2 wk	159.7 ± 11.7 §	22.9 ± 16.6 §	0.39 ± 0.17*
4 wk	129.9 ± 16.9*	44.7 ± 18.5*	0.32 ± 0.14*

§ p < 0.01 vs control subjects; \* p < 0.05 vs baseline values

## Decreased Hemolysis Following Administration of Antioxidant — Fermented Papaya Preparation (FPP) to a Patient with PNH

**[Author]** Hussam Ghoti, Hanna Rosenbaum, Eitan Fibach, Eliezer A. Rachmilewitz  
**[Organization]** Department of Hematology, Edit Wolfson Medical Center, Holon, Israel  
**[Journal]** Ann Hematol 89: 429-430 (2010).

A 36-year old woman was diagnosed in 1996 with PNH complaining of generalized weakness and recurrent episodes of nocturnal hemoglobinuria. The diagnosis was confirmed by Ham's test and flow cytometry. The patient received folic acid, iron supplement, and danazol without significant change in her average Hb level which was ~7.5 gm/dl with LDH ~2500 u/lit. Before receiving FPP, she had anemia (Hb = 7.4 gr/dl, MCV 95 fl), leukopenia (leukocyte (WBC)=1500/mcl), lactate dehydrogenase (LDH) up to 5300 u/l, indirect bilirubinemia, and elevated plasma Hb (Fig. 1). Serum haptoglobin was undetectable, serum ferritin and transferrin saturation were low, 7 ng/ml and 5%, respectively. A recent

bone marrow disclosed erythroid hyperplasia. FPP supplied as sachets, containing 3 g powder, by Osato Research Institute, Gifu, Japan, was given three times daily for 4 months. Following FPP therapy, Hb level increased by 1 gr/dl, WBC to 3,200 per microliter, and all the hemolytic parameters have been significantly improved (Fig. 1). Malondialdehyde levels, a product of lipid membrane peroxidation, were significantly decreased—which may reduce its mutagenic and leukemogenic effect. The patient was symptomatically improved, with less fatigue and better performance.

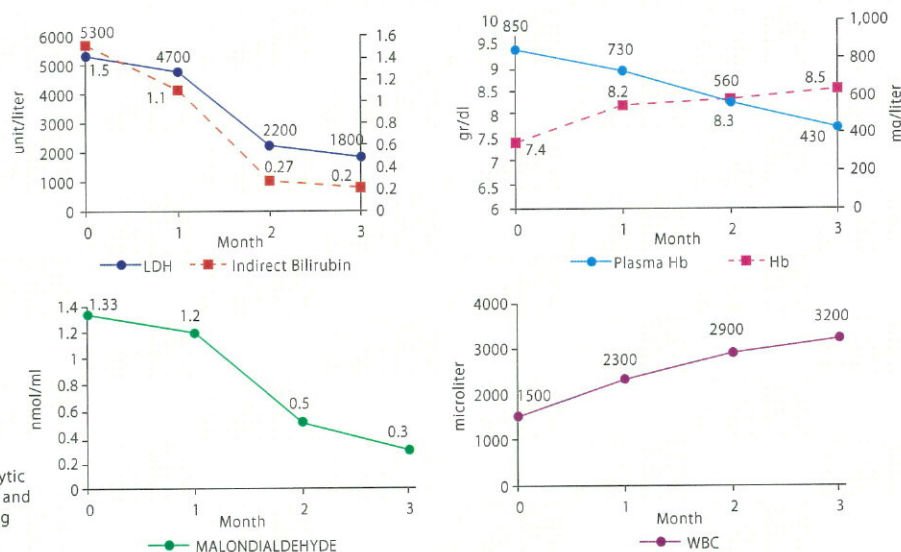


Fig. 1: Changes in Hb levels, WBC, and in hemolytic and oxidative stress parameters induced and maintained for 3 months following during administration of FPP

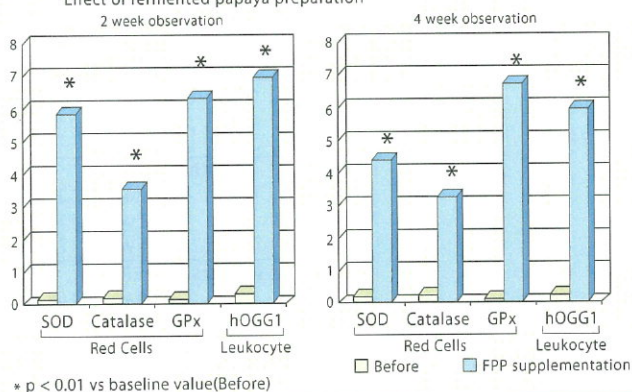
## Regulating Redox Balance Gene Expression in Healthy Individuals by Nutraceuticals: A Pilot Study

**[Author]** Francesco Marotta, Keiko Koike, Aldo Lorenzetti, Shalini Jain, Paola Signorelli, Yussef Metugriachuk, Pierre Mantello, Nicola Locorotondo  
**[Organization]** ReGenera Research Group, Milano, Italy  
**[Journal]** Rejuvenation Research Volume13, Number 2-3: 175 - 178 (2010).

We tested the effect of a fermented papaya preparation (FPP ; ORI, Gifu, Japan) on redox balance gene expression in 11 healthy nonsmoker, teetotaler individuals subjected to a detailed dietary and lifestyle questionnaire who refrained from any multivitamin supplement or fortified food. Redox status was assessed by erythrocyte and plasma parameters together with related leukocyte mRNA (GPx, SOD, Catalase, hOGG1) before/after 6 g of FPP supplementation. At either the 2 and 4 weeks observation points, the plasma level of the tested redox parameter did not change after FPP supplementation, besides a nonsignificant decrease of MDA. On the other hand, starting at the 2-week observation point, FPP brought about a significant upregulation of all gene expression investigated ( $p < 0.05$ ) (Fig. 1), which remained stable at a later testing time. These data applied irrespective of GSTM-1 and hOGG1 genotype profiles. Although posttranscriptional / translation protein modifications do occur and larger and longer studies are awaited, these preliminary data suggest that a transcriptomic modification

of key redox and DNA repair genes may offer further insights when attempting to interrelate "nutragenomics" to clinical phenomena.

Fig. 1: Semi-quantification of antioxidant and DNA repair enzyme mRNA: Effect of fermented papaya preparation



## Improved Function of Diabetic Wound-Site Macrophages and Accelerated Wound Closure in Response to Oral Supplementation of a Fermented Papaya Preparation

[Author] Eric Collard and Sashwati Roy

[Organization] Comprehensive Wound Center, Department of Surgery, The Ohio State University Medical Center, Columbus, Ohio, USA

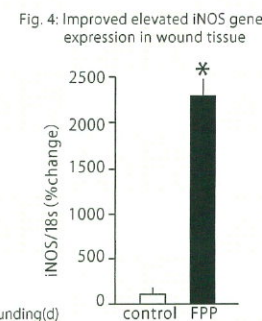
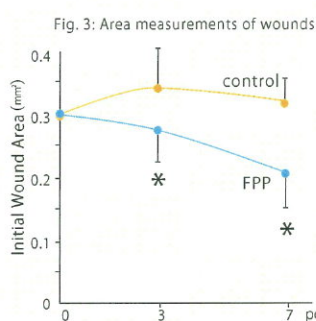
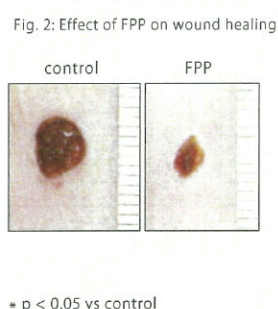
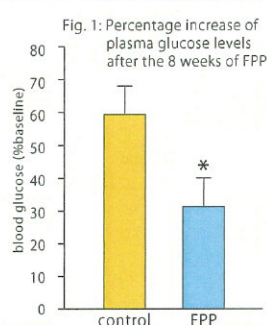
[Journal] Antioxidants & Redox Signaling. Volume 13(5) : 599-606 (2010).

Carica papaya Linn is widely known as a medicinal fruit. We sought to study a standardized fermented papaya preparation (FPP) for its effects on wound healing in adult obese diabetic (db / db) mice. FPP blunted the gain in blood glucose and improved lipid profile after 8 weeks of oral supplementation. However, FPP did not influence 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. Reactive oxygen species support numerous aspects of wound healing. 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 proangiogenic response. This work provides the first evidence that diabetic- wound outcomes may benefit from FPP supplementation by specifically influencing the response of

wound-site macrophages and the subsequent angiogenic response. Given that FPP has a long track-record of safe human consumption, testing of the beneficial effects of FPP on diabetic wound- related outcomes in a clinical setting is warranted.

Eight weeks of oral FPP supplementation to diabetic(db / db) mice results in:

1. Attenuation in the percentage gain in blood glucose without any effects on body weight gain.
2. Improved blood lipid profile including lower LDL, TGL and Tchol levels and an increase in HDL levels.
3. Significant increase in the rate of wound closure and wound angiogenesis.
4. Increase in wound closure rate was associated with an augmented nitric oxide and superoxide production by wound macrophages suggesting an improved macrophage function.
5. The iNOS and VEGF gene expression were markedly upregulated in the wounds FPP supplemented db / db mice.



Commentary: 2010

## Applications and Bioefficacy of the Functional Food Supplement Fermented Papaya Preparation

[Author] Okezie I. Aruoma, Yuki Hayashi, Francesco Marotta, Pierre Mantello, Eliezer Rachmilewitz, Luc Montagnier

[Organization] Department of Pharmaceutical and Biomedical Sciences, Touro College of Pharmacy, New York, NY, USA

[Journal] Toxicology 278 (1) : 6-16 (2010).

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  $\beta$ -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.

## Is There a Potential Application of a Fermented Nutraceutical in Acute Respiratory Illnesses? An In-Vivo Placebo- Controlled, Cross-Over Clinical Study in Different Age Groups of Healthy Subjects

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 v ol. 26 no. 2 : 283 - 292 (2012).

The role of oxidants in viral diseases is fairly complex because it includes metabolic regulation both of host metabolism and viral replication. However, a role for reactive oxygen species (ROS) and reactive nitrogen species (RNS) as mediators of virus-induced lung damage is supported by studies and antioxidants can thus be expected to act at many different levels. The aim of the present pilot study was to test an antioxidant nutraceutical approach on some relevant immunological parameters known to be affected in common seasonal respiratory tract infection. The study population consisted of 90 sedentary healthy patients, previously selected as being GSTM1-positive, divided into three groups: A) 20-40 years; B) 41-65 years; C) over 65 years. Each patients was administered a life style and dietary questionnaire. Subjects were supplemented for 6 weeks with either 9g/day (4.5g twice a day sublingually) of a fermented papaya preparation (Osato Research Institute, Gifu, Japan) or placebo. After a further month period of wash out, subjects were treated again in a crossover manner. Parameters checked were as follows: routine blood tests with WBC formula, saliva

flow rate and secretory IgA and lysozyme production and redox gene expression of Phase II enzyme and SOD from upper airways cells (from nasal lavage). Salivary secretion rate showed an age-related decline and was significantly increased by FPP supplementation only in the youngest age-group ( $p < 0.05$ ). Subjects treated with FPP showed a significantly higher level of IgA and lysozyme production., irrespective of age group while their baseline production was significantly lower in the oldest age-group as compared to the youngest one (C vs A,  $p < 0.05$ ). FPP treatment brought about a significant upregulation of all Phase II enzyme and SOD gene expression tested in nasal lavage cells. In conclusion, FPP supplementation during 6weeks resulted in higher salivary IgA and increase in Phase II and SOD enzyme expression, i.e. the most important antioxidant in the respiratory tract. The biological significance of these effects i.e., whether it will help reducing the whole respiratory oxidative stress in the human airway and hopefully, the incidence and/or severity of URTI remains to be demonstrated in longer clinical trials.

Fig. 1: Salivary Flow Rate : Effect of FPP in different age groups

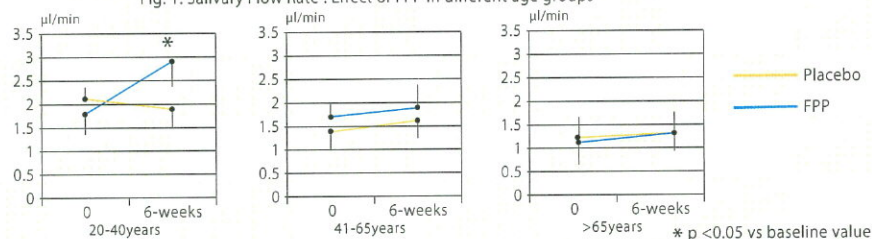


Fig. 2: Salivary IgA Secretion : Effect of FPP in different age groups

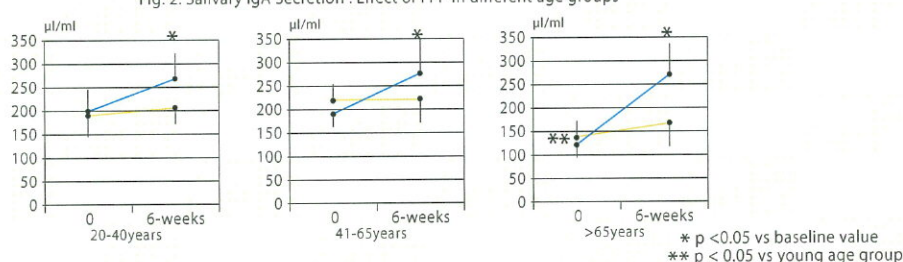


Fig. 3: Salivary Lysozyme Secretion : Effect of FPP in different age groups

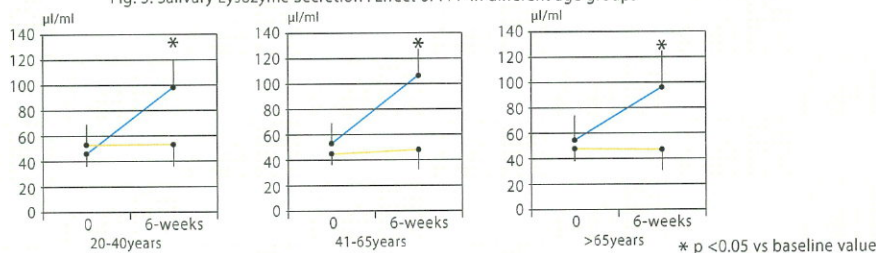
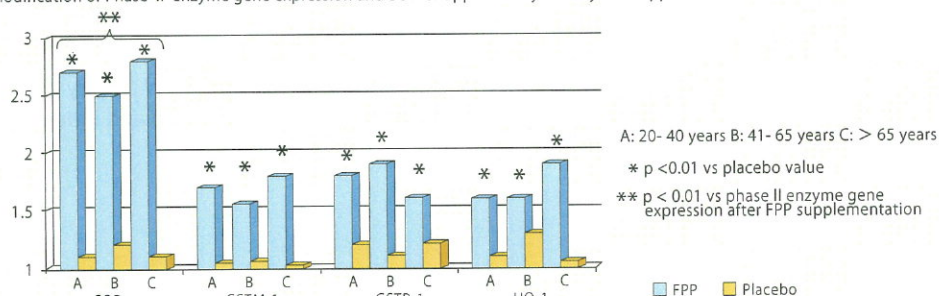


Fig. 4: Epigenomic modification of Phase II enzyme gene expression and SOD of upper airway cells by FPP supplementation



## Effect of a Fermented Nutraceutical on Thioredoxin Level and TNF- $\alpha$ Signalling in Cirrhotic Patients

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【Journal】 Journal of Biological Regulators & Homeostatic Agents vol.25 no.1 : 37-45(2011).

The aim of this study is to gain further insights into the possible nutraceutical effect on redox balance via thioredoxin(Trx) modulation and on the intrinsic susceptibility of monocytes to generate an inflammatory response. The study group consisted of thirty-two patients with compensated ChildA- C, HCV-related cirrhosis. The patients were supplemented for 6 months with 6 g / day of a certified fermented papaya preparation (FPP).

Fifteen unsupplemented, age/ gender-matched healthy subjects served as controls. The patients filled in a detailed diet-life style questionnaire, and blood samples were collected to test routine biochemistry, Trx, redox status(GSH, GSSG, GSH/GSSG ratio, 4-HNE and  $\alpha$ -tocopherol). Moreover, isolated monocytes were tested for ex-vivo LPS-stimulated TNF- $\alpha$  production and TNF- $\alpha$  mRNA. As compared to control, patients with liver cirrhosis showed a significantly higher serum level of Trx. FPP supplementation brought about a significant reduction of Trx with

levels comparable to the ones of healthy controls. Ten patients Child C (31.2%) showed borderline low levels of  $\alpha$ -tocopherol while all cirrhotic patients, as a whole, showed a significantly abnormal redox balance. Supplementation with FPP did not modify  $\alpha$ -tocopherol depletion but significantly improved redox balance parameters. Patients with liver cirrhosis showed a significantly upregulated TNF- $\alpha$  production in a time-dependent manner and this effect was more pronounced in more advanced stages of the disease and showed a significant correlation with  $\alpha$ -tocopherol level. Supplementation with FPP significantly, although partially, downregulated TNF- $\alpha$  production from monocytes. Taken altogether, it would appear that the typical oxidative-inflammatory biochemical milieu of these patients is mirrored by a significant TNF- $\alpha$  upregulation at a monocyte level while a targeted nutraceutical might be a potentially amenable intervention to be part of validated scheduled treatments.

Fig. 1 : Ex-vivo LPS-stimulation test of TNF- $\alpha$  production from monocytes and PCR-electrophoresis : nutraceutical modulation.  
Data were obtained at 3 months observation

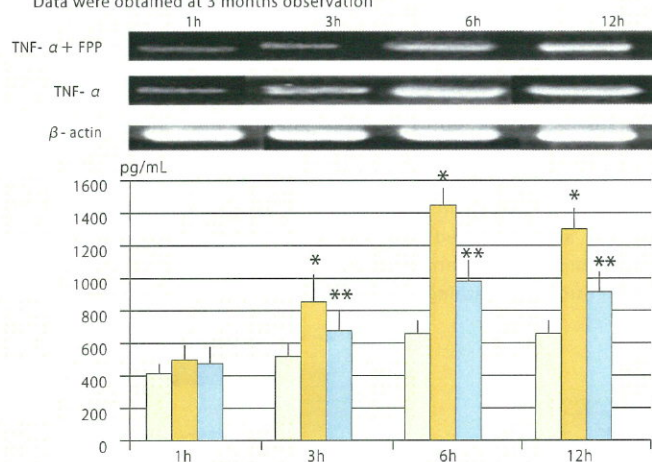
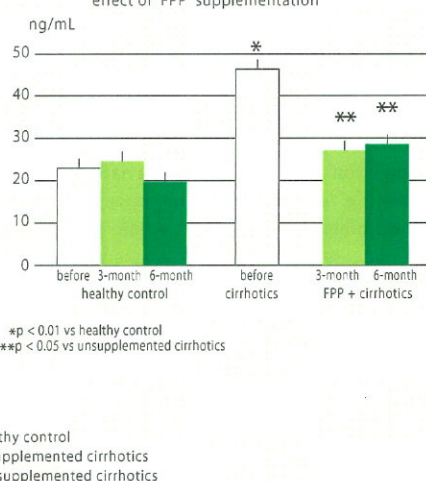


Fig. 2 : Serum level of thioredoxin in cirrhotics : effect of FPP supplementation



\*p < 0.01 vs healthy control  
\*\*p < 0.05 vs unsupplemented cirrhotics

## Effects of a Short Term Supplementation of a Fermented Papaya Preparation on Biomarkers of Diabetes Mellitus in a Randomized Mauritian Population

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【Journal】 Preventive Medicine 54 : S90-S97 (2012).

Clinical evidence and cellular models have shown an inverse relationship between the intakes of plant and fruit based diets and oxidative stress, suggesting the suitability of natural antioxidants in the management of diabetes mellitus and its complications.

A randomized controlled clinical trial was conducted at the Cardiac Centre, SSRN Hospital, Pamplemousses, (Mauritius) to determine the effect of a short term supplementation of a fermented papaya preparation (FPP) on biomarkers of diabetes and antioxidant status in a multi-ethnic neo-diabetic population from November 2010 to March 2011.

Supplementation of 6 g FPP / day for a period of 14 weeks could improve the general health status of several organs targeted by

oxidative stress during diabetes. When comparing experimental to control groups with independent samples t-test, C-reactive protein levels significantly decreased ( $p = 0.018$ ), LDL / HDL ratio was considerably changed ( $p = 0.042$ ), and uric acid levels were significantly improved ( $p = 0.001$ ).

ANOVA results also validated the same findings with significant differences in C-reactive protein, LDL / HDL ratio, uric acid and in serum ferritin levels.

FPP may present a novel, economically feasible nutraceutical supplement for the management of diabetes and for those at risk for cardiovascular disease, neurological disease and other conditions worsened by overt inflammation and oxidative stress.

Table 1 : Effect of a FPP on a neo-diabetic Mauritian population under the FPP and control regimens during the intervention period November 2010 to March 2011  
Significance testing as Independent samples t-test amongst the pre and post supplementation groups when comparing experimental and control groups

Biomarker	FPP regime		Control regime	
	F-value	Significance	F-value	Significance
LDL/HDL ratio	4.393	0.042*	0.285	0.596
CRP	6.078	0.018*	7.414	0.190
Uric acid	12.550	0.001***	0.910	0.345

\*p<0.05  
\*\*\*p<0.001

## Fermented Papaya Preparation RESEARCH FORUM - Potential Benefits of FPP in Humans Health Following Exposure to Radiation -

The Osato Research Institute (O.R.I.) has been focusing on the effect of radiation to the human body during airline travels and addressing the question as the possible protection that fermented papaya preparation could have towards radiation-induced biological damage through basic research and clinical trials. During a typical 13- hour flight from Tokyo to New York for example, passengers and the airline crew are likely to be exposed to  $100 \mu\text{Sv}$  of natural cosmic radiation which is equivalent to approximately  $7.7 \mu\text{Sv} / \text{hour}$  (Figure 1). However, it is safe to travel by airplane and the risk of radiation exposure during flights has not been proven scientifically. Nevertheless, a healthy person on a long flight with adequate antioxidant and immune function integrity can have increased protection against the aviation radiation exposure. In the case of compromised health status where the immune system might be weakened, oxidative DNA damage due to the radiation exposure might take longer

to repair due to compromised repair mechanisms.

Since the occurrence of the FUKUSHIMA Nuclear Power Plant accident, due to the greatest earthquake and tsunami in that has ever been experienced in Japan, the effect that radioactivity has on body has been drawing a lot of attention around the world. Many people remained anxious about the hazards presented by the potential exposure. Therefore, we decided to transmit an emergency message regarding the effect of radiation to the human body and the effectiveness of FPP against the damage. The action pathway of radiation to the human body can visualized in two ways: one is direct action and the other one is an indirect action (Figure 2). The direct action is DNA breakage. DNA has essential information to make body. The damaged DNA would cause apoptosis and mutation of cells and increase a risk of diseases (Figure 3). The indirect action is generation of radical oxygen in the human body. We are influenced by radiation not only through environment exposure but also through breathing air

and eating food. The DNA base damage mediated by radical oxygen would disturb normal cell growth and cause a functional decline of the body. FPP adjust the functions of anti-oxidant systems and immune systems and many papers on the clinical trials have been presented. FPP can prevent DNA damage and inflammation as well as reduce the risk for developing diseases and helps the quick recovery of damaged tissues in the human body. It is most notable that FPP consumption reduces 8-OHdG levels and this is an important biomarker outcome for DNA damage, and controls DNA adduct, which may impact the progression towards the development of cancer. FPP has potential to reduce the risk of developing disease due to oxidative DNA damage.

Research at ORI will continue to define the potential defense function of FPP against radiation induced physiological changes as in chronic human diseases.

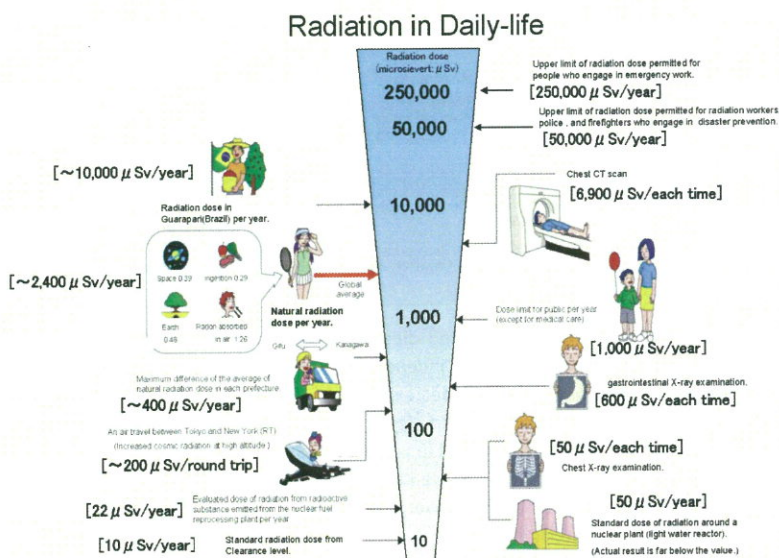


Figure 1.: Reported by the Ministry of Education, Culture, Sports, Science and Technology - JAPAN  
The maximum reading of the environmental radioactivity level in Tokyo was  $0.8 \mu\text{Sv}/\text{h}$  (3.15. 2011)

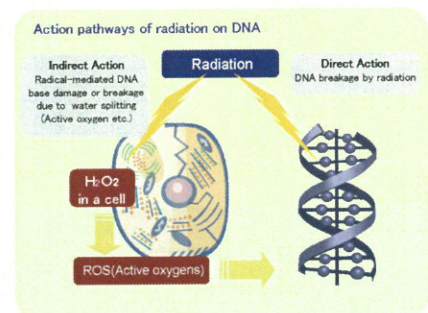


Figure 2 : Action Pathways of radiation to the human body on DNA

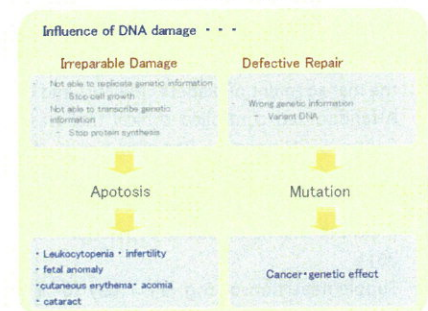


Figure 3 : Influence of DNA damage

