Novel health promoting role for papaya fruit extracts: *Biochemical, Molecular and Clinical evidence*

:

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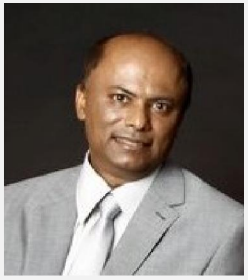
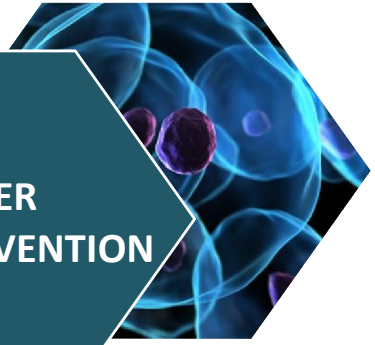


Themes under the NRC Program

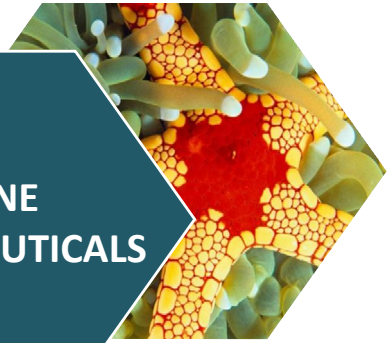


(A) CANCER CHEMOPREVENTION

Cancer chemopreventive actions of Natural products: an insight into their molecular mechanisms

(B) FUNCTIONAL FOODS & NUTRACEUTICAL RESEARCH

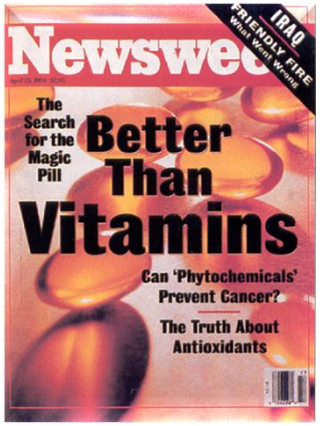
Physiological, Biochemical, Clinical, Molecular & Cellular effects of Functional foods/dietary factors

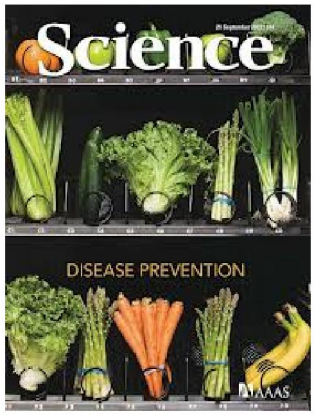


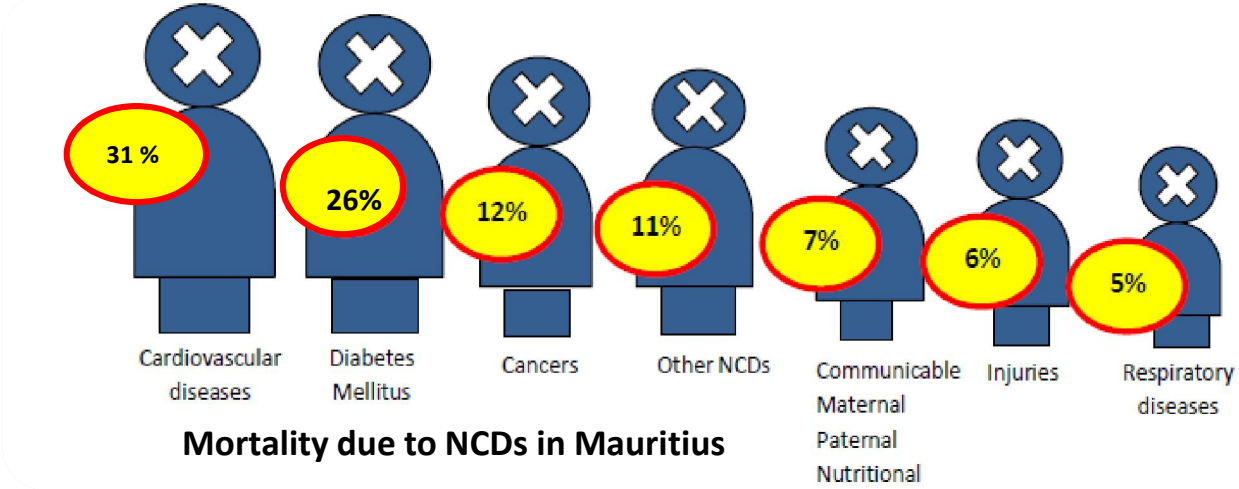
(C) MARINE PHARMACEUTICALS

Biomedical Evaluation of selected Mauritian marine macroalgae, sponges & soft corals in relation to their bioactive constituents

Antioxidant functional foods, nutraceuticals & phytochemicals



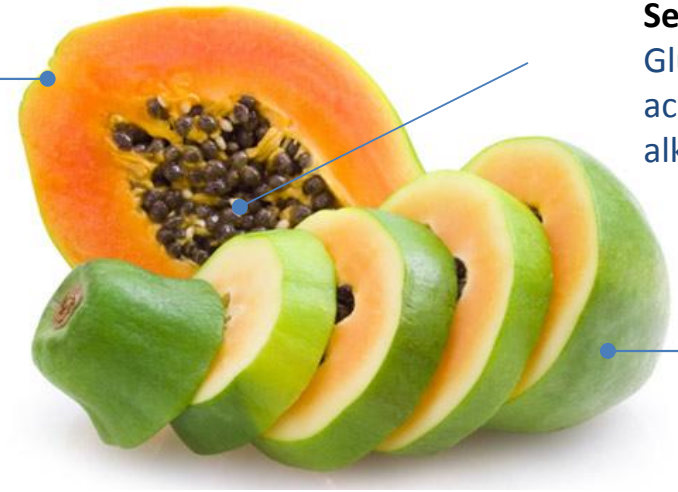




Knowledge of bioactive constituents, their clinical effects and molecular action mechanisms are relevant to maximize health benefits

Papaya: the miracle fruit?

Pulp
Minerals, Vitamins C, lycopene, β-carotene, β-cryptoxanthin
Caffeic acid, gallic acid, protocatechuic acids, caffeoyl hexose deoxyhexoside



Seed
Glucosinolates, oleic acid, palmitic acid, β-cryptoxanthin, tannins, alkaloids, phenols

Peel
Ferulic acid, caffeic acid, rutin, quercetin, coumaric acid, kaempferol, isohamnetin

Fermented Papaya Preparation (FPP)

Fermentation of ripe papaya pulp gives rise to novel oligosaccarides and increased amino acid levels that exert antioxidant properties

Varieties in Mauritius: Solo, Waimanalo, Ecsotika, Wilcox, Taniung and Rodrigues

- The polyphenolic profile of papaya fruit depends greatly on several factors

  e.g. *Stage of maturity, temperature, sunlight exposure, attack by insects/infections & quality of soil*

  Polyphenols work in synergy to contribute to the overall antioxidant potential of papaya

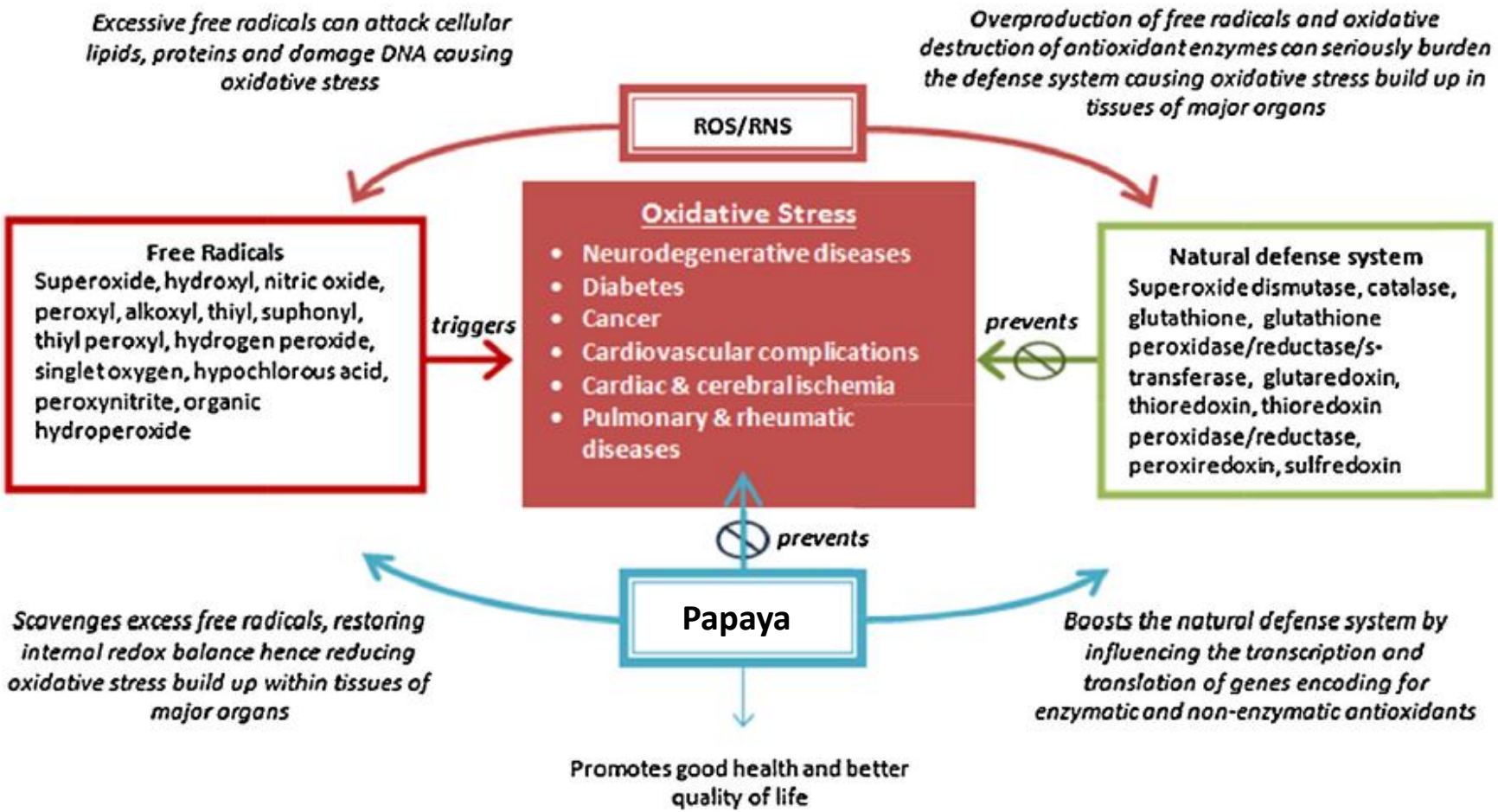
- The exact profile of FPP is the center of on-going investigations

Fermented Papaya Preparation (FPP) as a novel functional food:
What makes it so interesting to study?

General Compounds	Amino Acids
Carbohydrate	Arginine
Protein	Lysine
Fat	Histidine
Dietary fiber	Phenylalanine
Moisture	Tyrosine
Energy	Leucine
Vitamin B6	Isoleucine
Folic acid B9	Methionine
Niacin	Valine
Sodium	Cysteine
Iron	Alanine
Calcium	Glutamic acid
Potassium	Serine
Magnesium	Theronine
Copper	Aspartic acid
Zinc	Tryptophan
Glycine	Proline

- Made from ripe papaya pulp that is **fermented by yeast**
- Extensively documented for its **antioxidant** & **immune boosting properties**
- **Bio-fermentation process has rendered the nutritional composition highly complex**
- Properties due to formation of novel oligosaccarides and amino acids that are antioxidant in nature

How can papaya be beneficial for health?



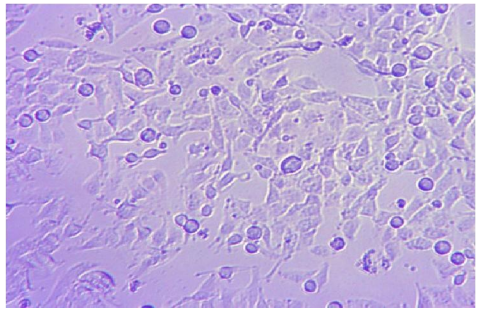
Evaluation of the effects of *Carica papaya* fruit extracts on biomarkers of oxidative stress and inflammation

AOA screening of papaya fruit

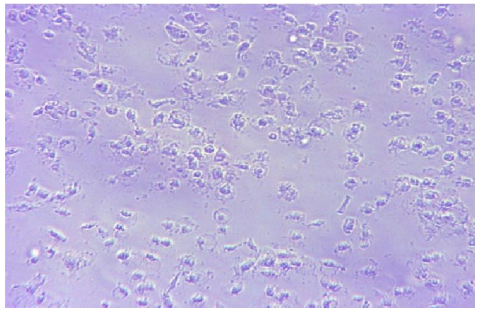
		Total Phenol Content (g GAE/100g DW)	Total Flavonoid Content (g QE/100g DW)
Ripe	Pulp	2.32	0.0077
	Peel	3.45	0.034
	Seed	2.18	0.0094
Unripe	Pulp	1.64	0.0029
	Peel	3.66	0.0517
	Seed	3.97	0.0121
	FPP	0.360	0.0066

In general, papaya peel exerted a better antioxidant activity which was related to its polyphenolic composition

	ABTS	DPPH	Superoxide Scavenging	Hydroxyl Scavenging	HOCl	FRAP	LIPID PEROXIDATION
RIPE	PEEL	PULP	PEEL	PEEL	PULP	PEEL	PULP
UNRIPE	SEED	PEEL	PEEL	PEEL	SEED	SEED	SEED

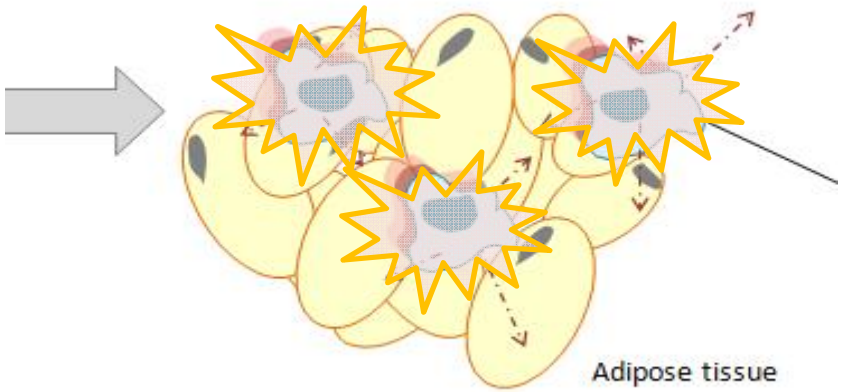


SW872 cells under normal conditions



SW872 cells under extreme oxidative stress (5mM, 1hr)

OXIDATIVE STRESS



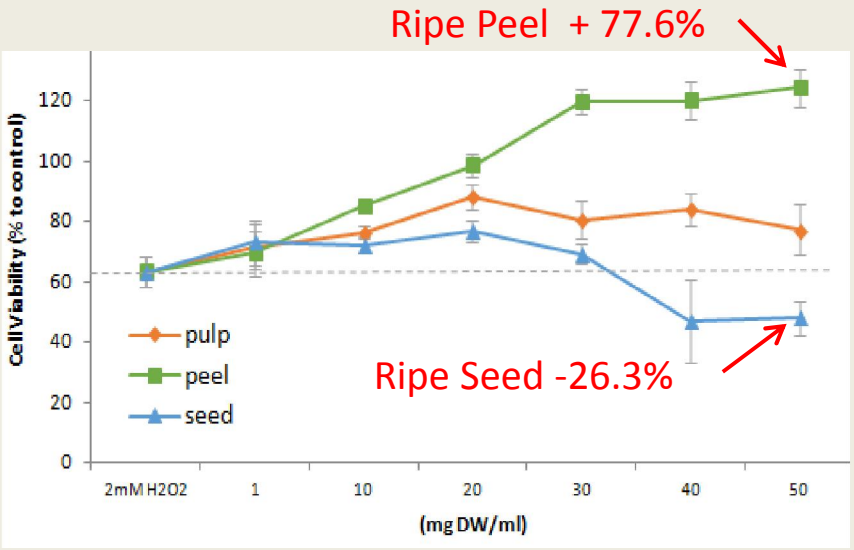
cytokines released :

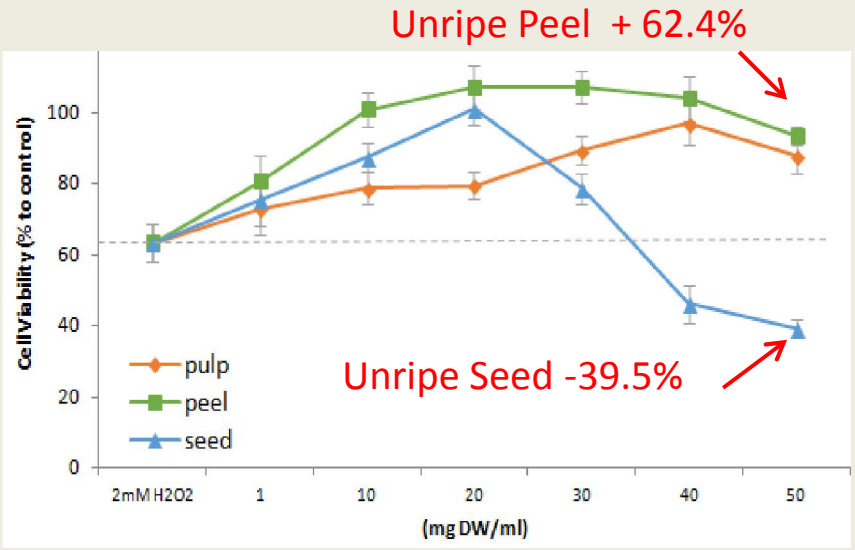
TNF-α, IL-1, IL-6, IL-8
C-reactive protein, PAI-1, MCP-1

- Adipocytes, upon exposure to oxidative/metabolic stress, release **Monocyte Chemoattractant Protein-1 (MCP-1). MCP-1** stimulates macrophages to migrate from bone marrow and infiltrate into **adipose tissue**

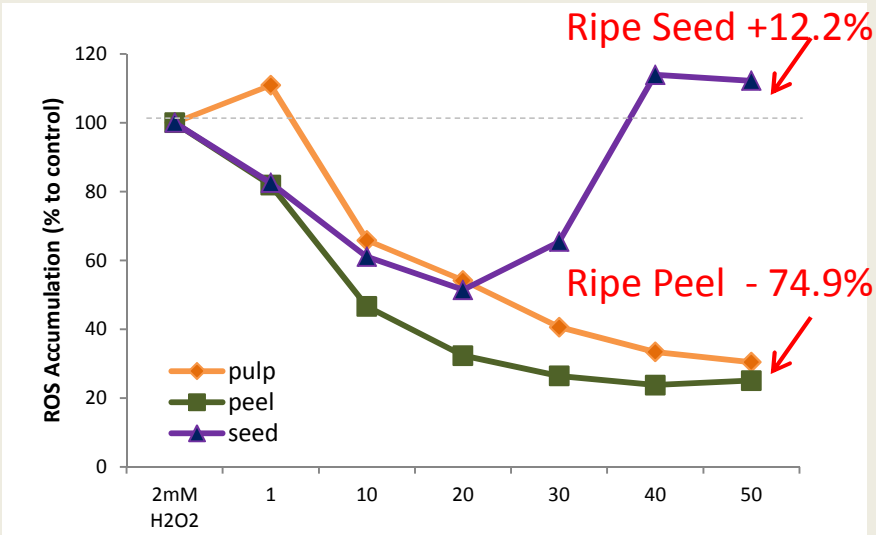
- Oxidative/metabolic stress signals will activate these macrophages can cause them to release copious amounts of **pro-inflammatory cytokines** into environment. It is these cytokines that can trigger onset of **inflammation** and **cancer** development.

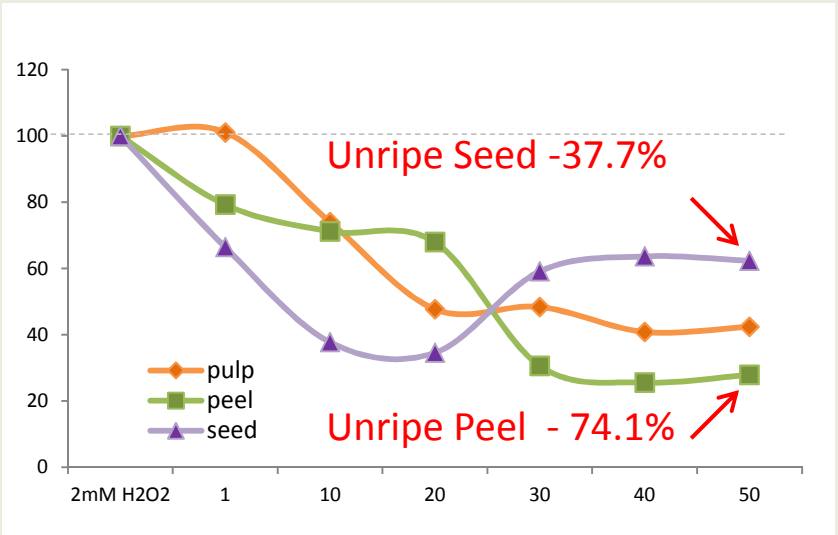
Cell Viability under oxidative stress



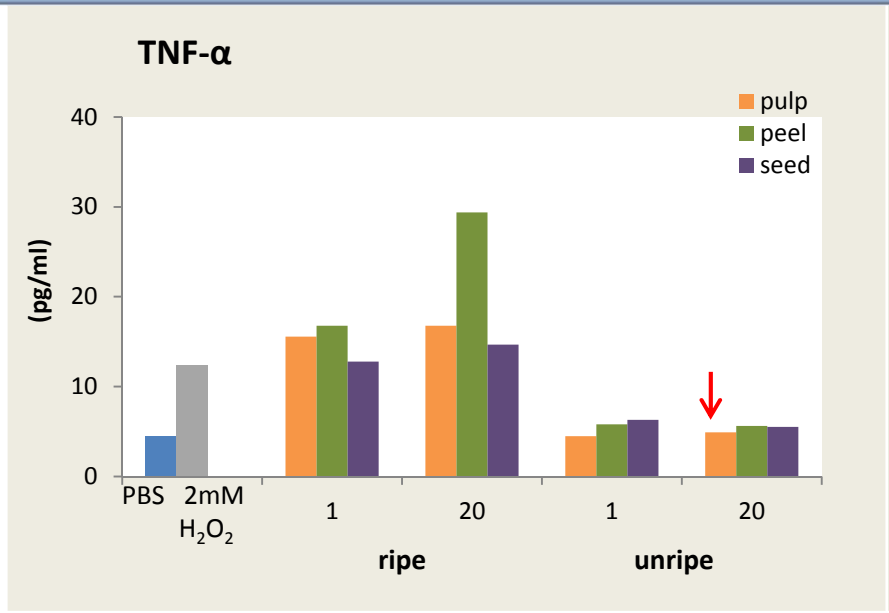


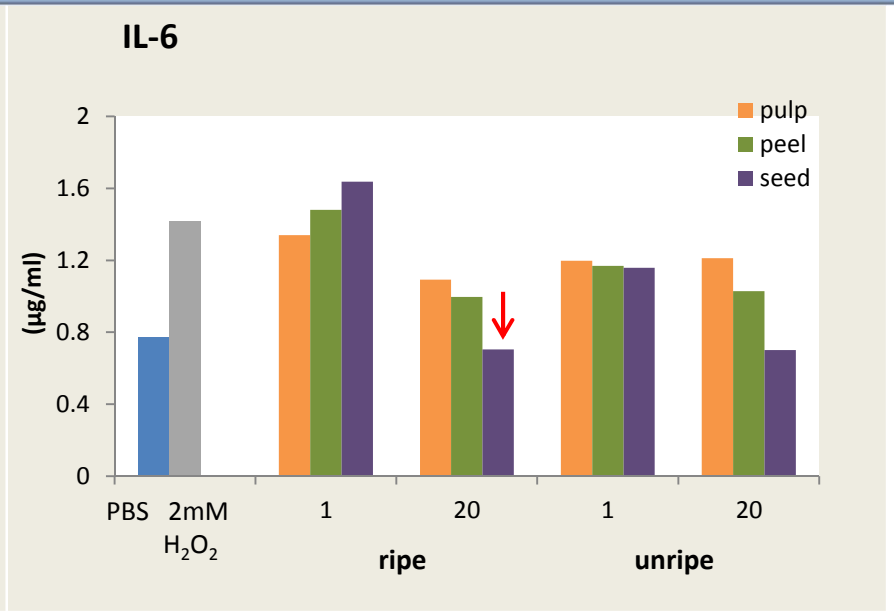
ROS Accumulation

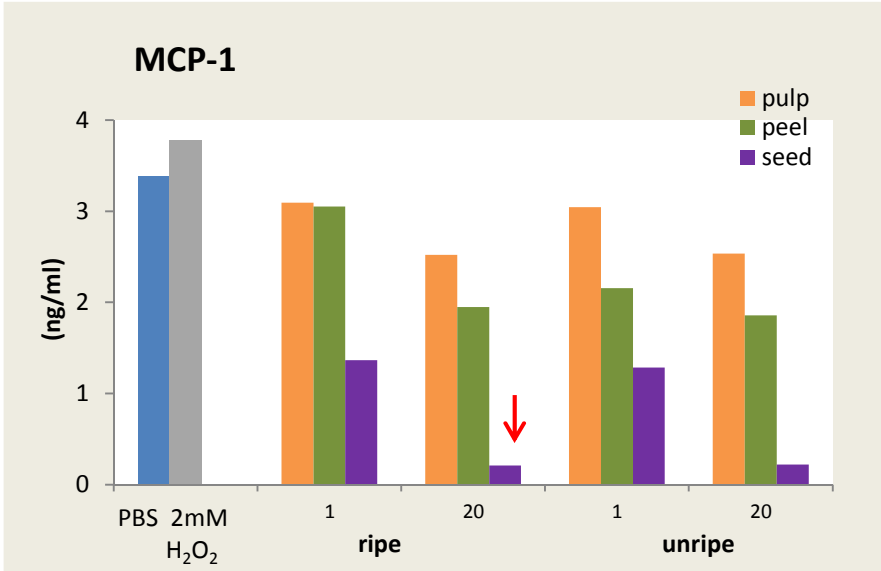




Pro-inflammatory cytokines





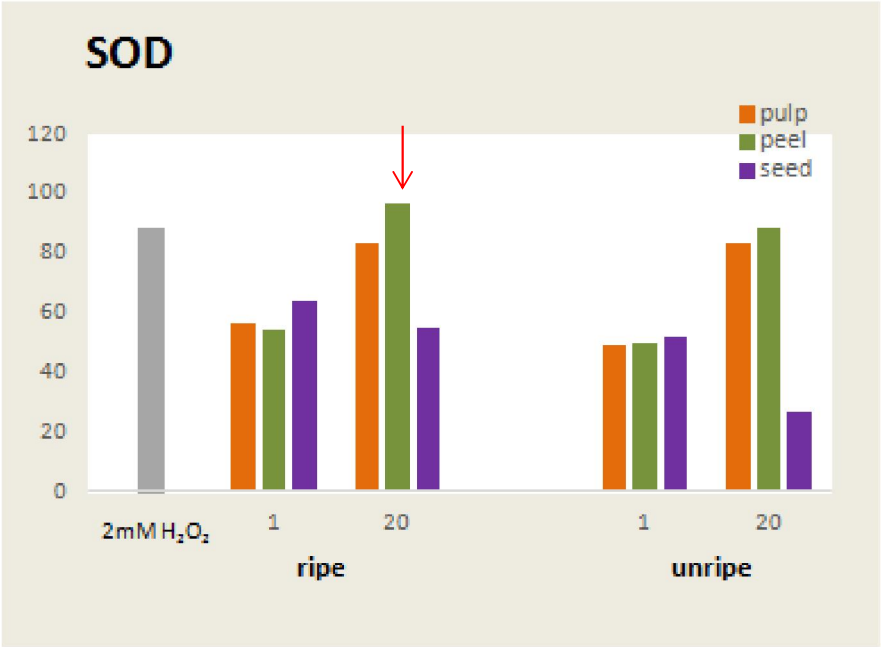


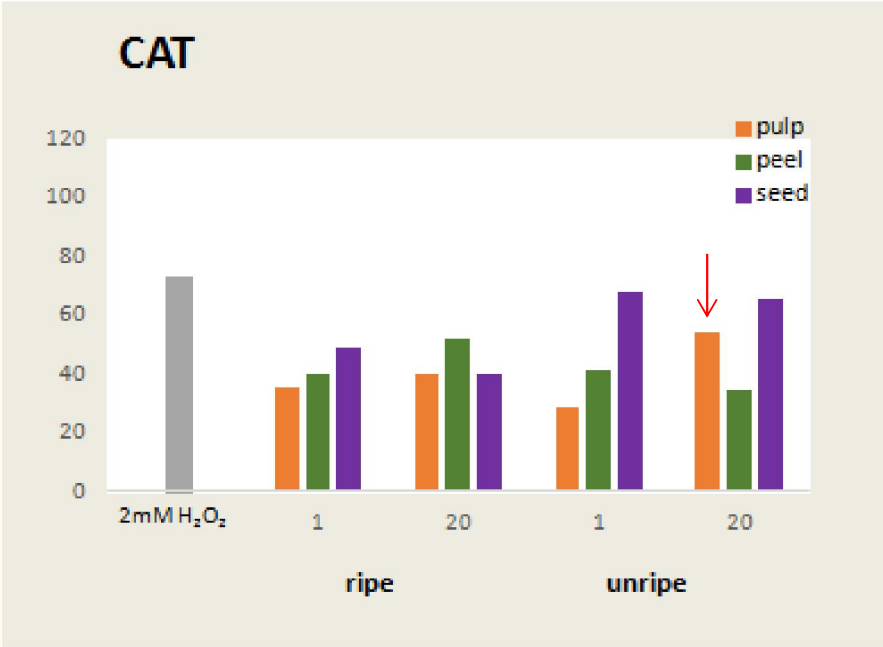
TNF-α ↓ **59.6%** by **Unripe Pulp**

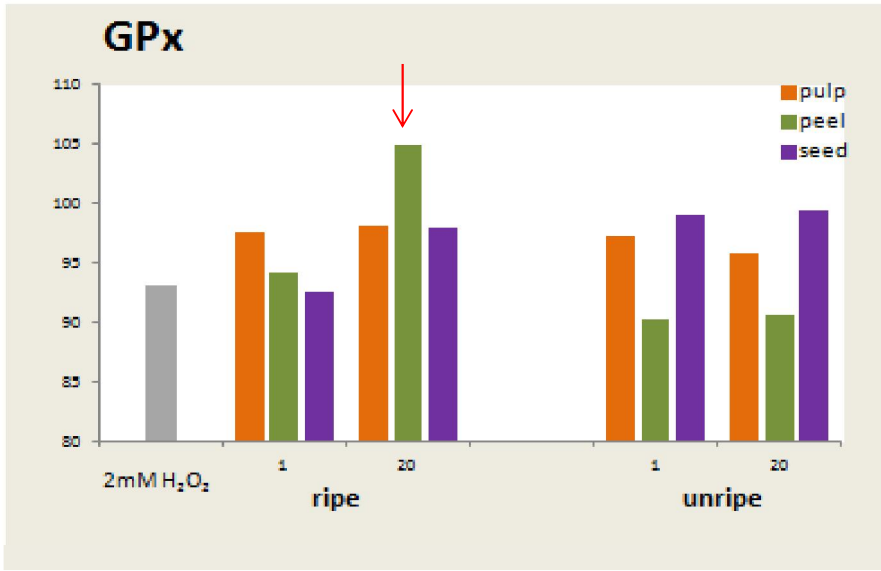
IL-6 ↓ **41.1%** by **ripe Seed**

MCP-1: ↓ **93.8%** by **Ripe Seed**

Activity of endogenous antioxidant enzymes







SOD: ↑ **9.7%** by **Ripe Peel**

CAT: ↓ **36.1%** by **Unripe Pulp**

GPX: ↑ **12.6%** by **Ripe Peel**

In general, (ripe) **peel and pulp** exerted better antioxidant enzyme-boosting effects at a dose of **20mg DW/ml**

Inhibitory effects of *Carica papaya* fruit extracts against common oral bacteria: *its implication in oral health improvement of diabetics*

Why are diabetics susceptible to dental caries & oral health complications?

High blood sugar

- Reduced saliva production (dry mouth)
- High sugar content in saliva
- Increased plaque build up in mouth

= Dental Plaque & Caries, Bad breath

Impaired immune system

- Imbalance between production of inflammatory cytokines & growth factors affects healing

= Gingivitis, Persistent mouth ulcers

Low blood supply

- Reduced nutritional supply to gums
- Slowed healing process of injured gums

= Sore, Bleeding gums

Reduced collagen production

- Weakened support between gums & teeth

= Periodontitis, Brittle teeth, Premature loss of teeth

Simulatory models of dental plaque & caries formation
(mimics hydrophobic nature of teeth & gum surface)

Inhibition of Growth
(Islam B *et al.*, 2008)

Adherence to Glass
(Islam B *et al.*, 2008)

Hydrophobicity to Hexadecane
(Ooshima T *et al.*, 2000)

Acid Production
(Ooshima T *et al.*, 2000)

	S.mutans	*S. mitis*
Inhibition of Growth	Ripe Seed ↓ 58.5%	Ripe Seed↓ 87.7%
Reduction of Biofilm formation	Ripe Seed ↓ 71.7%	Unripe Pulp↓ 88.7%
Inhibition of Acid Production	Ripe Seed↓ 11.8%	Ripe Peel↓ 35.9%
Reduced Hydrophobicity	Ripe Pulp↓ 131.9%	Ripe Pulp↓ 112.8%

Protective effect of FPP on N-methyl N-nitrosourea-induced hepatocarcinogensis in balb/c mice

Diets high in nitrosatable foods cause cancer

- high levels of nitrates used in food preservation are carcinogenic

- 300 carcinogenic nitrogenous compounds identified in several commercial foods:

Cigarette smoke, Beer & wine,
Cheese, luncheon & sausage meats,
Canned foods, Chinese-style salted fish,
Soy sauce, Pickled vegetables, fish sauce

- Examples of foods <u>naturally</u> high in nitrates:

Fish, oysters, mussels, crab, lobster, Chinese cabbage, some leafy vegetables

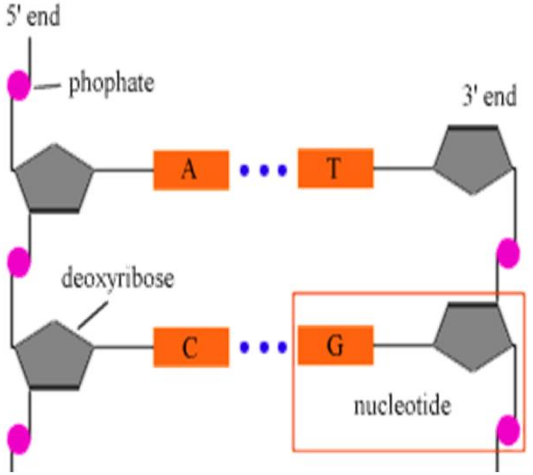
A possible reason why Asians have high rates of stomach & mouth cancers?



Example: **N-methyl-N-nitrosurea** (MNU)

- MNU causes several cancers in animal models including monkeys, targets the <u>liver</u> in mice

Methyl group transferred to nucleobases

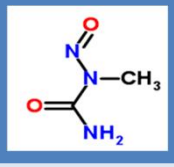


Leads to **AT:GC** mutations

Study Design

Balb/c Mice

administered orally twice/day for 12-weeks

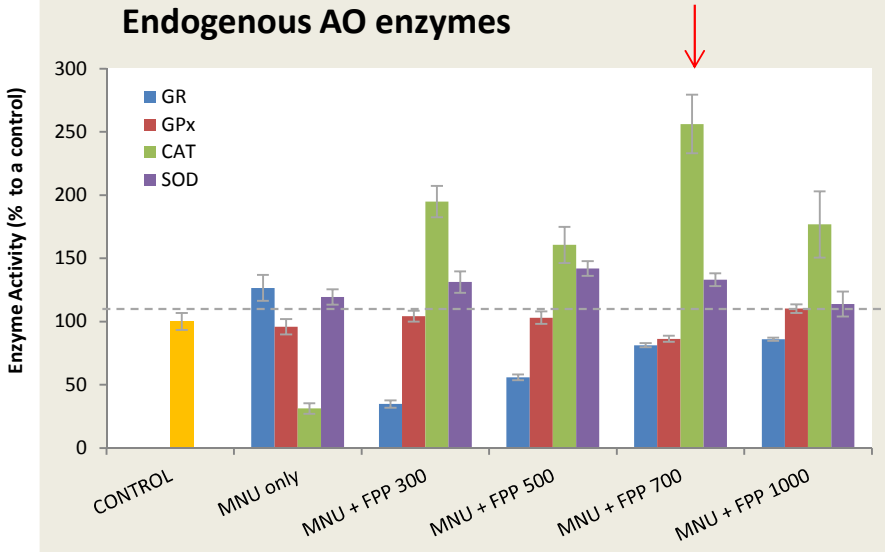
FPP	MNU	CONTROL
		
Dose administered (mg/kg BW)		
300 500 700 1000	50	PBS

HEMATOLOGICAL ASSAYS & BIOMARKER ANALYSIS

HISTOLOGICAL STUDY OF LIVER CELLS

DNA ISOLATION & RAMAN ANALYSIS

Antioxidant status

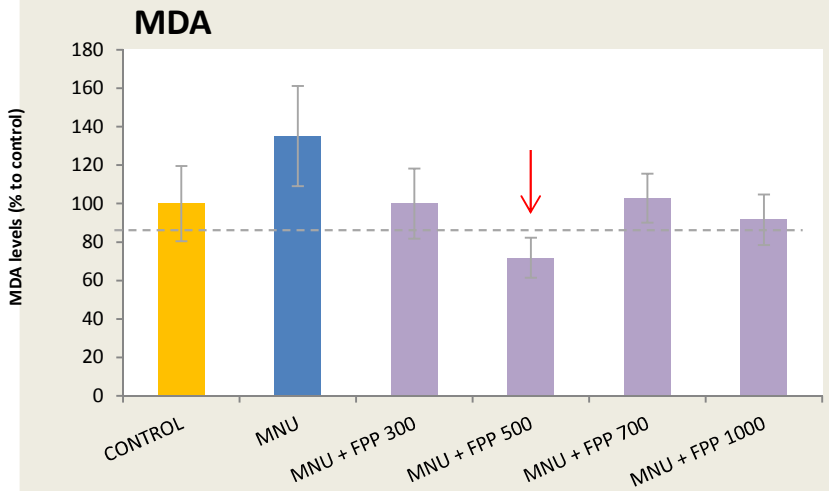


A dose of 700 mg FPP/kg was optimum for:

- Boosting the antioxidant status (↑65.3%)
- Increase CAT activity (↑87.8%)

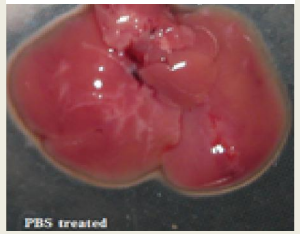
A dose of 500mg FPP/kg was optimum for:

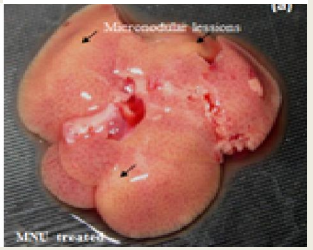
- Increase SOD activity (↑18.8%)
- Increase GPX activity (↑ 7.8%)
- Decrease MDA lipid peroxidation (↓46.9%)



PBS treated

MNU treated





- exposure to MNU induced liver tumor formation in Balb/c, FPP supplementation could reduce these effects of MNU

Clinical effects of a short-term supplementation of FPP on biomarkers of oxidative stress in a multi ethnical pre-diabetic population

127 subjects recruited

FPP (n=49)

Control (n=78

2 sachets (6 grams) / day before mealtimes for 14 weeks	2-3 glass of water/day before mealtimes for 14 weeks
"wash-out" period of 2 weeks	
44 completed study 5 discontinued	57 completed study 21 discontinued

Inclusion Criteria for Participation:

- blood glucose range 110-126 mg/dL
- age range 35 – 65 years
- non-smoker (or stopped since 6 months)
- daily alcoholic intake < 2 drinks per day
- post-menopausal women not on hormone replacement treatment
- non-hypertensive
- not taking anti-hypertension/anti-diabetes drugs

Biomarkers of Oxidative Stress & Type 2 Diabetes Mellitus tested for …

Blood Sugar Profile	▪ Fasting blood glucose ▪ Glycated hemoglobin, HbA1c
Lipid Profile	▪ Total cholesterol ▪ HDL, LDL ▪ Total triglycerides
Immune Defense System	▪ Total antioxidant status, TAS
Liver Functioning	▪ Aspartate aminotransferase, AST ▪ Alanine transaminase, ALT
Kidney Functioning	▪ Microalbumin ▪ Urea ▪ Creatinine
Systemic Inflammation	▪ C-reactive protein, CRP ▪ Uric acid
Iron Accumulation	▪ Ferritin

Blood & urine samples were analyzed using a fully automated clinical chemistry analyzer AU480 Beckman Coulter Inc. ®

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Preventive Medicine 54 (2012) S90–S97

Contents lists available at SciVerse ScienceDirect

Preventive Medicine

journal homepage: www.elsevier.com/locate/ypmed

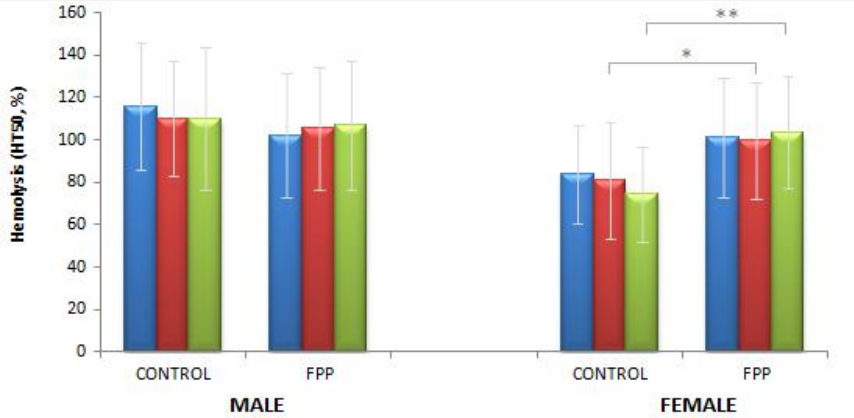
Effects of a short term supplementation of a fermented papaya preparation on biomarkers of diabetes mellitus in a randomized Mauritian population

Jhoti Somanah [a], Okezie I. Aruoma [b,*], Teeluck K. Gunness [c], Sudhir Kowelssur [d], Venkatesh Dambala [e], Fatima Murad [f], Kreshna Googoolye [g], Diana Daus [h], Joseph Indelicato [h], Emmanuel Bourdon [i], Theeshan Bahorun [a,*]

Biomarkers	Male (at week 14)		Female (at week 14)	
Arterial blood pressure	↓	1%	↓	3.1%
LDL cholesterol	↓	6.5%	↓	5.3%
Uric acid	↓	1.9%	↑	7.3%
C-reactive protein	↓	13.3%	↑	55.3%
Total Antioxidant Status	↑	4.9%	↑	5.7%
AST & ALT	↑	3.6%	↓	12.6%
Microalbumin to Urinary Creatinine ratio	↓	21.8%	↑	84.1%

baseline | week 14 | wash out

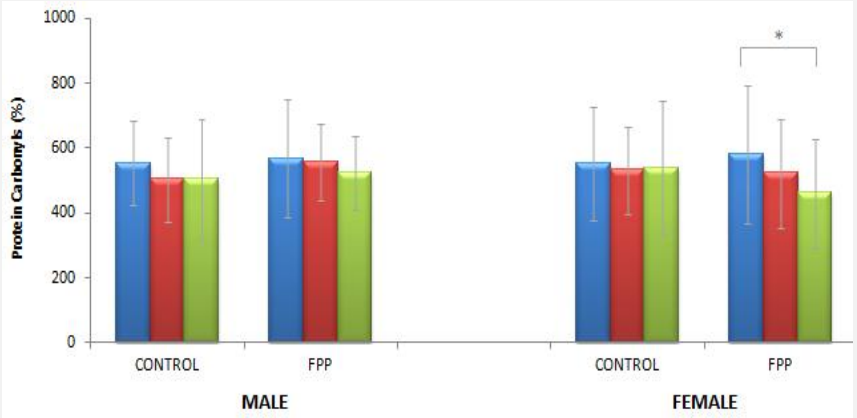
Rate of Hemolysis



Compared to the control group, FPP increased the antioxidant status and reduced the susceptibility of human red blood cells to undergo hemolysis:

	Week 14	Wash-out
males	↓ 4.4%	↓2.8%
females	↓23.3%	↓39.1%

Protein Carbonyl Formation



Compared to baseline values, FPP consumption reduced the formation of protein carbonyls by :

	Week 14	Wash-out
males	↓ 1.9%	↓ 5.8%
females	↓ 9.7%	↓ 11.9%

Food and Chemical Toxicology 65 (2014) 12–17

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Relationship between fermented papaya preparation supplementation, erythrocyte integrity and antioxidant status in pre-diabetics ☆

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Summary

Papaya fruit extracts:

- very good free radical scavenging activities in both cell-free and human cell systems (RBCs, SW872), attributed to its polyphenolic content
- boost antioxidant status through the up-regulation of endogenous antioxidant enzymes (SOD, GPx)
- down-regulate the over-secretion of pro-inflammatory cytokines (TNF, IL-6, MCP-1)
- Papaya seed has potential to be studied as anti-cancer agent

Papaya fruit extracts:

- ✓ FPP is a very good free radical scavenger.
- ✓ Consuming 2 sachets per day can reduce oxidative stress in major organs and in red blood cells
- ✓ Consuming FPP on a daily basis can greatly reduce the risk of type 2 diabetes
- ✓ FPP causes no side effects

Biopharmaceutical unit: structure and Organisation

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Functional Foods & Diabetes

Cancer Biology and Chemoprevention

Marine Pharmaceuticals

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- Ministry of Health and Quality of Life
- Apollo Bramwell Hospital
- Mauritius Research Council
- University of Mauritius
- Tertiary Education Commission
- Bio Santé Ltd
- Université de la Réunion
- CYROI (Réunion)
- Touro College of Pharmacy (USA)
- Osato Research International (Japan)
- CSJM Kanpur University (India)
- Banarasi Hindu University (India)