

Review paper

# Discovering the health promoting potential of fermented papaya preparation- its future perspectives for the dietary management of oxidative stress during diabetes

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**Abstract:** The simplistic morphological characteristics of the *Carica papaya*, papaya or ‘pawpaw’ should not be the cause for underestimating its potential as a nutraceutical. The market for papaya has been expanding at a staggering rate, partly due to its applicability as a biofortified product, but mostly for its phytochemical properties and traditional health benefits. Recent characterization studies have showed that the entirety of papaya or using a formulation of fermented papaya promotion (FPP) displays effective free radical scavenging abilities, thought to be influenced by its phenolic, carotenoids, flavonoid or amino acid profile. Aiming at reducing the impact of free radical-induced oxidative damage in the human system, the antioxidant properties of FPP have been found to potentially target a broad spectrum of diseases ranging from neurological impairments such as senile dementia to systemic diseases, to its interference at the cellular level and support of normal biological ageing processes. FPP has thus been extensively investigated for its ability to exert cellular protective effects and reduce oxidative stress via mitigation of genetic damage, lipid peroxidation and enzymatic inactivation in diseases. Oxidative stress reduction strategies using FPP and its holistic approach in disease prevention and management, with a focus on diabetes, cancer and cognitive health, contributes unequivocally to wellness in an aging population.

**Keywords:** *Carica papaya*; fermented papaya preparation (FPP); free radical scavenging; antioxidant; oxidative stress; anti-diabetic; anti-carcinogenic

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## 1. Introduction

For several ethnic groups, fermented foods form an integral part of their dietary lifestyle. Being favored for their unique flavor, texture or health-promoting benefits, fermented foods can be prepared and consumed in a number of ways. One of the important outcomes of food fermentation

is its enrichment with essential amino acids, vitamins and minerals, for example *idli* (an Indian cake made from *Rhizopus oligosporous* fermented rice and black-gram) contains high levels of thiamine and riboflavin [1]. Similarly, *natto* (a sticky soybean dish) is popular amongst the Japanese for its stimulatory effects on high circulatory levels of vitamin K2 in normal individuals [2]. The process of fermentation coaxes microorganisms into degrading anti-nutritive compounds, making food more edible and digestible thus augmenting bioavailability of its health-protecting nutrients. *Gundruk* (fermented mustard, radish and cauliflower leaves) is eaten in Nepal for its ascorbic acid, fiber content and anti-cariogenic properties [3], while Korea's famous *kimchi* (fermented cabbage) is traditionally used to alleviate constipation, stress and regulate serum cholesterol levels [3]. Detoxification is a potential sub-process of fermentation, which can render certain foods safer to eat, as in the case of cyanogenic glucoside removal from cassava root by *Geotricum candida* and *Cornibacterium lactii* cultures [4].

Fermented papaya preparation (FPP) is one such product that has received much attention across the European, Asian and American continents under the commercial trade name of Immun'Age®. FPP is a certified natural health product [5] and has gained global recognition following its manufacture under the strictest food safety management systems (FSSC 22000 & ISO standards). The fresh ripe fruit pulp Hawaiian *Carica papaya* is used for the fabrication of FPP which is allowed to ferment in the presence of food-grade yeast for up to 12 months. The final product is granulated before being packaged and distributed. Although the general composition of FPP has been ascertained by the Japan Food Research Laboratory, recognition of the presence of novel uncharacterized oligosaccharides in FPP is suspected to be an outcome of the prolonged fermentation process. Studies scrutinizing the therapeutic qualities of papaya fruit have accredited its medicinal properties to its remarkable free radical scavenging activity [6], at the same time correlating the latter to its polyphenolic content in an attempt to explain the source of papaya's antioxidant activity [7]. This has prompted in-depth investigation of the possible polyphenolic composition of FPP. Initial fractionation of FPP by Rimbach et al. [8] brought to light the different activity patterns of high and low molecular weight fractions with respect to superoxide anion scavenging and macrophage RAW 264.7 activation. Interestingly, Fibach and Ginsburg [9] pointed out that although the overall quantity of phenols in FPP is very low when measured in a salt solution, its levels can be boosted six-fold when assayed in saliva, albumin, mucin or red blood cell suspensions, possibly owing to its ability to pass through cell membrane barriers. Chemical analysis by Japanese researchers on a fermented papaya preparation using capillary electrophoresis-time-of-flight mass spectrometry (CE-TOFMS) and liquid chromatography (LC-TOFMS) revealed several low-molecular weight phenolic acids, such as 2,5 dihydroxybenzoic acid, quinic acid, shikimic acid and m-amniophenol [10]. Disparity in terms of quantity of flavonoids in ethanolic papaya pulp extracts has been observed to vary as an obvious result of genetic differences between species, cultivation practices and microclimates in country of origin [11,12,13].

## 2. Oral health challenges amongst the diabetic community

The occurrence of dental caries amongst diabetics is a major health concern, especially when considering the high costs involved for the treatment and management of oral health. Consensus from epidemiological reports is that there has been a sharp increase in the prevalence of oral health complications amongst type 2 diabetics, particularly cases of dental caries, periodontitis and halitosis [14]. Given the frequency at which these disorders occur amongst the diabetic population, they are now recognized to as part of a multitude of secondary complications manifested during uncontrolled diabetes. Using animal models of diabetes, the influence of high blood glucose levels (hyperglycemia) on the development of dental caries was clearly demonstrated in alloxan-induced F344 rats [15], WBN/KObsIC rats [16] and db/db mice [17]. Collectively, the histopathological evidence strongly suggests that glycemic control by insulin treatment can greatly reduce the occurrence of dental caries and periodontitis in diabetics. The work of Campbell et al. [18] initially investigated the different types of sugars present in the saliva of diabetics. Varying quantities of lactose, sucrose, fructose,

maltose, sorbose, arbinose and galaturonic acid were reported. Many of these sugars will remain unnaturally high in the blood of those suffering from uncontrolled diabetes making elimination of biofilms difficult [19]. This could explain why diabetics are more susceptible to oral caries, bad breath and reoccurring mouth infections compared to non-diabetics (Figure 1). Hence the development of more original methods to maintain good oral health has integrated plant extracts into toothpaste, mouthwash and chewing gum formulations. The use of natural plant-based products for the dietary control and prevention of tooth decay is now favored [20]. However, despite the numerous *in-vitro* studies, only a handful of plants reach clinical testing phases due to their limited effectiveness, stability, taste and economic feasibility.

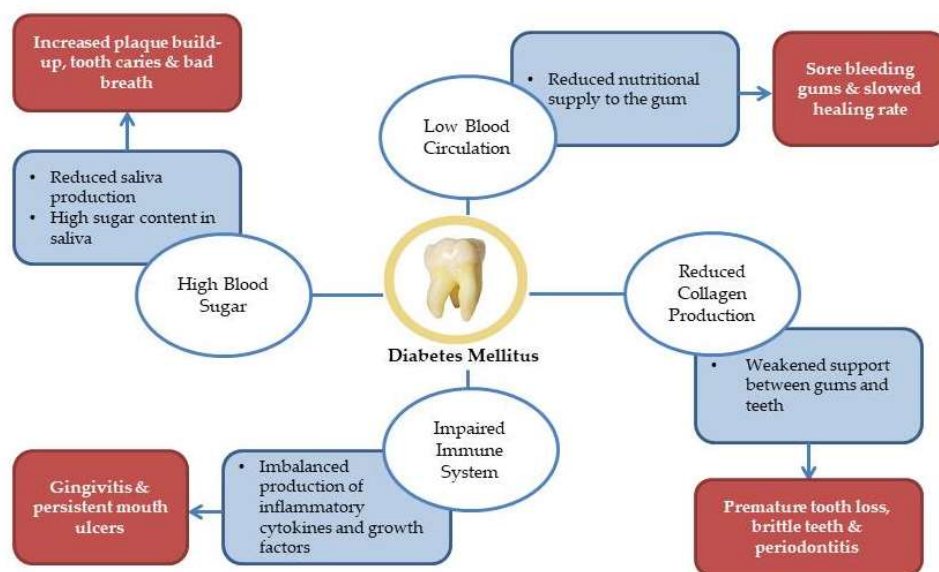


Figure 1. Common reasons as to why diabetics are prone to developing oral health issues

### Introducing *Carica papaya* based products into the domain of phytodensity

Secondary metabolites, in particularly phytopolyphenols, are now favored by researchers in the domain of phytodentistry, especially since there is a creeping tendency of microbial resistance to conventional treatments. Bacteriocidal and bacteriostatic effects of papaya fruit leaves, seeds and latex have been scrutinized in literature [21]. For example, Orhue et al. [22] indicated a strong growth inhibitory effect of ripe papaya leaves, seeds and peel extracts towards *Streptococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, the latter being a highly resistant bacterium. Radulavic et al. [23] proposes the mode of action of tannin, one of many major phytoactive compounds found in papaya extracts, was by binding to bacterial membrane polysaccharides and disrupting its structural fluidity, consequently resulting in leakage of cellular components. Tannins and several other related polyphenols are also believed to inhibit bacterial growth by targeting bacterial energy metabolism through the inactivation of proteins involved in cellular respiration [23]. There exist very few studies discussing the anti-cariogenic potential of papaya latex. Since papain can break peptide bonds between amino acids such as arginine, lysine and phenylalanine residues-it has great potential to be used in the area of oral health maintenance. Its proposed mode of action is by proteolytic degradation of bacterial cell wall [24]. Despite demonstration of its anti-cariogenic activity against *S. mutans* [25], *S. gordonii* [26] and *S. alpha* [27] to exceed that of aloe vera gel, calcium oxide, mangosteen peel and propolis- its clinical use has been rarely appreciated. However, evidence should be interpreted with caution as to whether or not these fruit extracts have the same potency *in vivo* should be clinically validated.

## Examining the anti-cariogenic potential of FPP

Recognition of the positive correlation between levels of *Streptococcus* and *Lactobacillus* species and the progression of dental caries allows us to theoretically assume that a reduction of these microorganisms in the dental biofilm community is a step towards the reestablishment of oral health. This theory was the basis of a study by our group where commercialized FPP was examined for its anti-caries properties [28]. Using *in vitro* simulation models of dental plaque- bacterial growth and hydrophobicity of three opportunistic bacteria, namely *S. mutans*, *S. mitis* and *L. acidophilus*, were observed to decrease upon exposure to FPP suggesting low doses of this dietary health product may be a suitable candidate to complement good oral hygiene practices. Both fresh papaya and FPP have an elevated presence of fermentable sugars in it. According to Walsh et al [29] depending on the type of sugar and the metabolic pathway it undertakes during its fermentation, selected weak acids such as propanate, butyrate, succinate and valerate are released as a by-product. These weak acids have the ability to counteract abrupt pH changes in the dental biofilm. Such a mechanism could also apply to amino acids. Given that the amino acid profile of FPP is a highly complex one, various amino acids present in FPP such as arginine, leucine, glutamic acid and aspartic acid could have possibly exerted a buffering effect. The fine powdery consistency of FPP in combination with its high dissolvability not only facilitates its consumption, but also stimulates the secretion of copious quantities of saliva in the mouth. Walsh et al. [29] claims that saliva has a buffering effect on oral biofilms. Secretion of copious amounts of saliva in the mouth by FPP would therefore rapidly clear any large food debris and encourage the buccal pH to return to baseline. In a study by Fibach and Ginsburg [9], the authors pointed out that an individual's oxidative stress level has an influential role to play on the health status of their oral cavity. Employing two highly sensitive luminol-dependant chemiluminescence assays, the authors demonstrated that under pathological conditions FPP could easily dissolve in saliva or red blood cells to augment their antioxidant capacities- possibly by increasing the solubility and availability of polyphenols present in the FPP. This theory was also believed to be observed in a study conducted by our group [30]. Interestingly, one previous study has shown that consumption of FPP indeed leads to an increased rate of salivary secretions that is high in IgA, phase II enzymes and SOD gene expression [31]. With regards to periodontitis, a chronic buccal infection largely caused by the pathogen *Porphyromonas gingivalis*. Detection of abnormal levels of TNF- $\alpha$ , IL-6, IL-1 $\beta$  and CRP in gingival fluid and tissue indicate that this condition is characterized by chronic inflammation which is hypothesized to lead to the progressive destruction of the tissues supporting the teeth, cementum and alveolar bone. Interestingly, in an open randomized study, Russian investigators recently proved the clinically efficacy of a fermented papaya gel against periodontitis [32]. Topical administration of this gel was observed to lead to a considerable improvement of major indices of disease severity, including reduced bleeding and gingival pocket depth, and normalization of IL-10, IL-6 and IL-1 $\beta$  cytokine levels after 14 days of application. Although the exact mechanism has yet to be understood, the authors speculate that FPP can work in synergy with human granulocytes to enhance phagocytosis of *Streptococcus aureus* in gingival tissues [32].

However, the lack of studies investigating the anti-cariogenic potential of papaya renders a comparative discussion of its possible mechanisms of action difficult. Attention has rather been given to papain-containing formulations. *In vitro* testing of papain-based gels have proved them to be potentially useful agents for the chemomechanical removal of dental biofilms and caries [33,34]. The use of papain to maintain oral health has many advantages. Besides possessing anti-inflammatory properties, papain is a naturally powerful proteolytic enzyme that can degrade cross linkages between collagen fibrils located on the outer wall of bacteria, thus hindering the ability of bacteria to adhere and colonize tooth surfaces [35]. Furthermore, the activity of papain is highly selective, acting only upon carious tissue which does not express genes encoding for a plasmatic protease inhibitor: alpha 1 anti-trypsin [33]. Papaya fruit thus shows promising perspective for future studies in the area of phytodentistry.

### 3. The concept of oxidative stress as a unique therapeutic pathway by nutraceuticals for the management of type 2 diabetes

Profound interest into the etiology of free radical-induced oxidative stress in diabetes is an area claiming much attention from the scientific community. During type 2 diabetes, oxidative stress can emerge from the production of free radicals as a result of glucose auto-oxidation, protein glycosylation, low-grade inflammation and from the metabolic breakdown of free fatty acids [36]. Although the quantity of free radicals generated through normal cellular metabolism is minute, they play a vital regulatory role in many biological processes [37,38]. Environmental factors such as air contaminants, heavy metals, pesticides, vigorous exercise and exposure to infections are also potential sources of free radicals [39]. Hyperglycemia-induced oxidative stress is closely associated with impairment of antioxidant defense mechanisms, representing a central contribution to the onset, progression and pathology of diabetes and its associated health complications. Defective insulin signaling pathways, degranulation and accelerated apoptosis of pancreatic  $\beta$ -cells are also tell-tale signs of severe oxidative stress, resulting in persistent hyperglycemia states in overweight individuals [40,41,42]. Strict weight loss and exercise regimes have been proved to be highly efficient in improving insulin sensitivity,  $\beta$ -cell function and skeletal muscle oxidative capacity [43,44], thus enforcing the importance of maintaining body mass within acceptable levels- however, with current sedentary lifestyles this is an attitude with is unfortunately easier said than done.

Oxidative stress can rapidly overwhelm the activity of endogenous antioxidant enzymes, leaving the body prone to free radical attack, hence the implication of reactive oxygen species in the pathogenesis of several complications associated with diabetes including heart disease, nephropathy, retinopathy and cancer is now widely accepted [45,46,47,48,49]. Conjointly, oxidative damage to the structure of DNA can impinge spontaneous mutations, abnormal cell growth or force premature cell death provoking the premature aging and cancer [50,49]. Common belief that oxidative stress can critically weaken the antioxidant defense system of diabetics has been the center of on-going discussions amongst the medical community and has evoked many human intervention trials focusing on the effectiveness of antioxidants to reverse the progression and occurrence of diabetes. Hence, the concept of oxidative stress may offer a unique therapeutic option for the management of type 2 diabetes by using nutraceuticals that possess a powerful free radical scavenging capacity and the ability to boost the activity of the intracellular antioxidant system. The healthful role of natural dietary antioxidants or supplements has been discredited in the past, but accumulating evidence obtained from both animal and human experimental models clearly demonstrate their efficacy to counteract the deleterious effects of oxidative stress in major organs. Compared to conventional anti-diabetes drug therapies, phytonutraceuticals possessing eminent antioxidant powers, that are locally grown, exert minimum toxicity and are cheap to process, offer a potential treatment regime that can be made accessible worldwide, not to mention- economically feasible

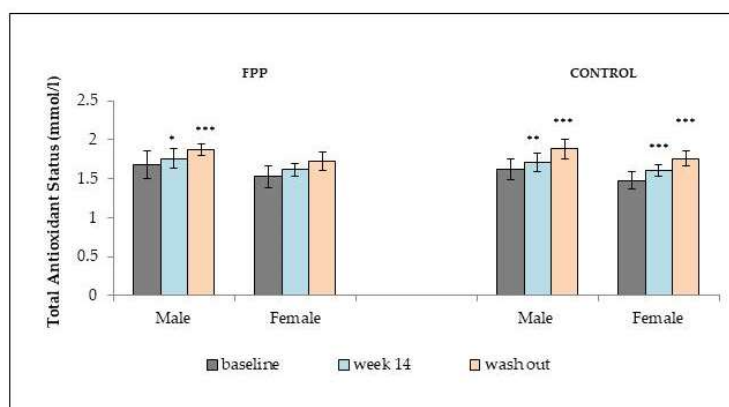
#### Interaction of FPP at the physiological and organ system levels in diabetic patients

Although an increasing number of plants are being scientifically documented for their anti-hyperglycemic, antioxidant and insulin stimulating activities [51] the lack of scientific data supporting the anti-diabetic properties of fresh *Carica papaya* is now slowly changing. The anti-hyperglycemic effect of papaya is thought to target pancreatic  $\beta$  cells by improving their sensitivity to insulin, at the same time inhibiting  $\alpha$ -amylase and  $\alpha$ -glucosidase [52], a response which bears much resemblance to a second generation sulfonylurea called glibenclamide. Indeed there exist many anti-hyperglycemic drugs that normalize plasma glucose levels, but there is a dearth of drugs that show simultaneous correction of blood glucose, lipid and antioxidant profiles.

Fermented papaya preparation is one such health product that is still relatively unrecognized for its exciting potential to be an asset for the dietary management of oxidative stress and diabetes, and deserves to be evaluated more profoundly at both molecular and clinical level. The hypoglycemic effect of FPP was initially investigated by Danese et al. [53] in an open randomized clinical trial in which 3g FPP/day for 2 months was reported to significantly reduce fasting and post-meal glucose levels in both normal and type 2 diabetic patients. These findings were further supported by Collard and Roy [54] where FPP (0.2g/kg/8 weeks) was found to also attenuate the gain in blood glucose in db/db mice. Although these findings do not directly prove the anti-diabetes activity of FPP, they are nonetheless consistent with the hypothesis that FPP may work in synergy with oral hypoglycemic drugs as adjunct therapy. While most diabetes-related clinical trials focus on single-target drugs, only a small percent of them are concerned with diabetes prevention, screening or health maintenance [55]. Diabetes care organizations such as the International Diabetes Federation and American Diabetes Association continuously argue that researchers should prioritize on finding more innovative preventive strategies that can work safely in conjunction with conventional diabetes therapies to improve their bioefficacy. In this context, a randomized clinical trial was conducted by our team with the aim of accelerating the translation of findings obtained from antioxidant assays conducted on fermented papaya preparation [28,56,57]. Results of the clinical study demonstrated that a daily supplementation of FPP for 3 months could improve the general total antioxidant status of pre-diabetic adults (Figure 2) and reduce carbonyl protein levels in plasma [30,56]. In addition, liver biomarkers AST and ALT were also affected [30] confirming the findings of Santiago et al [58] who reported the normalization of ALT and AST by FPP consumption. Elevated enzymes such as  $\gamma$ -glutamyltransferase, ALT, and to a lesser extent AST, can provide an insight into the pathology of the liver as it is one of the most susceptible organs to oxidative-related cellular damage, thus predicting the risk of developing type 2 diabetes or non-alcoholic fatty liver disease- a disease which is indeed on the rise amongst adults within the age range tested [59]. It is recommended that medical organizations should integrate a variety of biomarkers into their testing protocols, such as CRP, ALT and total antioxidant status to exercise better screening and tracking of at-risk individuals.

**Figure 2.** Effect of the total antioxidant (TAS) status in a pre-diabetic population under the FPP (N=36) and control regimes (N=53). Data is expressed as mean TAS value (mmol/l) where error bars represent standard deviation. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 vs baseline. From [30, 56] with permission.

### Anti-inflammatory and immuno-modulatory effects of FPP in diabetic conditions



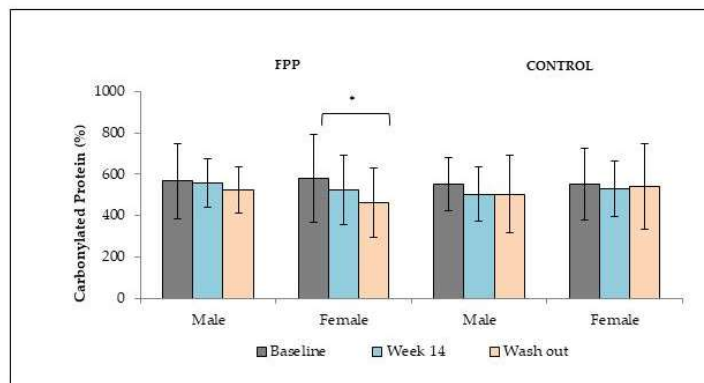
An observational study published in the American Journal of Human Genetics, Holmes et al. [60] claimed that “for every 1kg/m<sup>2</sup> gain in BMI, the risk of developing type 2 diabetes increases by 27%” supporting the notion that type 2 diabetes is a direct outcome of high BMI and increased abdominal fat mass- two major characteristics of obesity which has also been linked to sub-clinical

inflammatory states in the adipose tissue [61,62]. In attempt to understand the influence of oxidative stress on the metabolic response of adipocytes, our team used an *in vitro* cellular model to mimic the micro-environment of metabolic overload by mitochondrial oxidative stress. Using an extract of Mauritian *Carica papaya* (var. Solo), ripe and unripe seed extracts were found to significantly reduce oxidative stress levels within human pre-adipocytes (SW-872). Maintenance of mitochondrial viability, reduction of intracellular reactive oxygen species levels and mediation of pro-inflammatory cytokine secretory levels (TNF- $\alpha$ , IL-6, MCP-1) were all further confirmations of its cytoprotective effects against oxidative-inflammation [63]. Our investigation provided complementary support to the concept that papaya fruit is an important source of natural antioxidants and indeed has the potential to be used in the dietary modulation of oxidative stress and inflammation. Several studies have also successfully demonstrated FPP to be a valuable immune modulator. For example: the synergistic interaction of FPP with IFN- $\gamma$  has been reported to regulate the secretion of TNF- $\alpha$ , one of the central regulatory cytokines in macrophage anti-microbial activity and induce the production of nitric oxide [64,65,8]. Even though excessive nitric oxide may be detrimental to our body, it is also an indispensable vasodilator and important agent against tumorigenic cells especially since the process of wound healing is greatly slowed in diabetic individuals. A constant state of hyperglycemia coupled with a deficient immune system, narrowed blood vessels and low nitric oxide availability heightens the risk of developing gangrene, sepsis or ulcerations- major causes of limb amputations amongst diabetics [66].

Papain isolated from the latex of unripe papaya pulp is documented to be one of the earliest substances used in wound care and chronic skin ulcer therapy for its anti-bacterial and fibrinolytic properties [66,67]. Collard and Roy [54] found that FPP could also accelerate wound healing in db/db mice through the elevation of nitric oxide levels, IL-6, TNF- $\alpha$  and circulating CD38 at the wound site. Interestingly, observations from a study by our group showed that ripe papaya peel extract triggered an unexpected surge in TNF- $\alpha$  within SW-872 cells [63]. This trend was also reported by Rimbach et al. [8] where high molecular weight glucans triggered excessive TNF- $\alpha$  secretion in RAW 269.7 macrophages. This could be explained by the fact that both papaya pulp and FPP are indispensable sources of D-glucans, since FPP is made from yeast fermentation of fruit pulp where the major structural cell wall constituent of yeast is also (1,3) D-glucan. The exact immune-modulatory role of FPP is still under investigation and cannot be explained solely through the use of ELISA techniques; therefore further investigations are highly warranted in order to appreciate its true role.

### **Attenuating type 2 diabetes dysfunctionality & associated diseases using the anti-oxidant properties of FPP**

Oxidative stress and inflammation mechanisms play pivotal roles in the pathophysiology of cancer and diabetes [68,69]. Chronic oxidative stress can increase the susceptibility of erythrocytes to undergo hemolysis, which is a likely result of free radical attack to erythrocytes membrane proteins and lipids. The ability of FPP to counteract oxidative stress in human erythrocytes was proven through a randomized supplementation study, where a dose of 6g FPP/day for a period of 14 week clinically reduced the rate of haemolysis and accumulation of protein carbonyls (*in-vivo* indices of oxidative stress) in the blood plasma of pre-diabetic adults [56] (Figure 3). This finding also compliments that of Raffaelli et al [70] in which the authors improved platelet function, by enhancing Na<sup>+</sup>/K<sup>+</sup>-ATPase activity and membrane fluidity, and ameliorated the antioxidant system functionality, through an increase in total antioxidant capacity, SOD activity and a parallel decrease in conjugated diene levels in patients with type 2 diabetes. Moreover, through a multitude of *in-vitro* assays, our group has also demonstrated that FPP exhibits potent free radical scavenging potentials that are consistent with those ascribed to FPP in literature [56]. Such positive outcomes strongly suggest FPP to be a therapeutic functional food that can improve the integrity and quality of blood products in pre-diabetics and diabetics.



**Figure 3.** The effect of fermented papaya preparation (FPP) on carbonyl protein accumulation as a percentage compared to control (1% NaCl) in a pre-diabetic population under the FPP (N=41) and control regimes (N=58); error bars represent standard deviation. \*P<0.05 vs baseline. From [56] with permission.

At genomic level, the complex interaction between chronic inflammation and oxidative stress mechanisms involved in type 2 diabetes (T2DM), therapeutic interventions involving antioxidants could theoretically reduce the risk of base mutations and vulnerability of cells to undergo cell transformation during diabetes. The intriguing electron spin resonance data of Aruoma et al. [71] and Yoshino et al. [72] provides ample evidence that FPP is one such antioxidant. Originally the antioxidant activity of FPP was ascribed to its hydroxyl scavenging and iron chelating properties, but this theory has been further extended to its modulatory effects of mitogen activated protein kinases (MAPKs) [71]. Later through several human interventional supplementation studies also confirm that FPP greatly influences transcriptional modification of key antioxidant enzymes and DNA repair genes (glutathione-s-transferase, superoxide dismutase, catalase, glutathione reductase, hoGG-1, heme oxygenase-1) [73,64]

The individual components of FPP and its interaction with the metabolic activity of diabetic patients further advocates its potency as an anti-diabetic drug. Findings by Nieto Calvache et al. [74] showed a mixture of soluble and insoluble dietary fibers along with carotenoids, ascorbic acid and phenolic compounds providing evidential support to the characterization of FPP as stated by [28]. Furthermore, the intestinal bioavailability of the polyphenols in a dietary fibre concentrate was capped at 65% similar to the pharmacokinetic properties of other diabetic drugs available on the market [75]. Polyphenols have been heavily investigated for their roles in glucose metabolism and buffering against insulin resistance features. While on a broader scale, components of the *Carica papaya* have been found to decrease serum glucose, triglycerides and transaminases in STX-induced diabetic rats [76] and positively influence vascular functions and reduce insulin resistance in human subjects [77], plants of similar characteristics have been listed as potential herbal therapies in diabetes patients [78]. Studies by Martini et al. [79] have shown the ability of polyphenols in upregulating the transcriptional activity of paraoxonase I (PON1), potentially via its protective effects against oxidative stress-induced inactivation, hence altering the pathophysiological processes of diabetes. Other intricate mechanisms have associated polyphenols to improved insulin sensitivity via AMPK activation and modulation of energy sensors [80]; downregulation of miRNA-335 expression to improve insulin signalling and lipid metabolism via disinhibition of genes such as *InsR*, *Irs1*, *Sirt1*, *Prkaa1*, *Ppargc1a*, *Ppara*, *Lpl*, *Foxo1* and *Gsk3b* [81]; and modification of the circadian circuitry via *Cry1* and *Bmal1* genes to enhance insulin sensitivity and regulate glucose homeostasis respectively [82].



FPP exhibits enormous potential towards a more holistic approach in the treatment of diabetes-associated diseases. Combination therapy using metformin and ascorbic acid has been effective in the reduction of depressive behaviors by decreasing corticosterone levels via AMPK pathways in the hypothalamic-pituitary-adrenal axis and inducing a decrease in pro-inflammatory cytokines such as TNF- $\alpha$  and IL-6 which are linked to neurological disorders [83]. Ascorbic acid exhibits protective effects in terms of the development of macular edema associated to diabetic retinopathy by reducing the apoptotic-induced loss of pericytes and mitigating endothelial dysfunction [84].

#### **4. Connecting the dots between the anti-cancer and anti-diabetic effects precipitated by FPP**

Despite large investments made in the area of cancer prevention, escalating prevalence of cancer amongst diabetics clearly indicates that the success rate of present clinical therapies is low. One prominent explanation is that preclinical research on anti-cancer drugs are flawed, in the sense that they overlook treating the fundamental cause of cancer: oxidative stress. Recognition between prolonged oxidative-inflammatory insults during diabetes as the etiology of cancer has sparked our interest into searching for natural but innovative anti-cancer agents. Understanding of how the diabetes micro-environment can predispose one to the onset of cancer has been reviewed by our group in Aruoma et al. [40].

Whilst modest amounts of ROS do trigger pancreatic  $\beta$ -cell degranulation, tumor cell proliferation, invasion and survival [85,86], the effects of free radicals cannot be entirely regarded as a detrimental phenomenon as they also form an integral component of basic cell regulation and signaling pathways [38,87]. Levels of ROS that are above cellular tolerance can suppress tumor progression-forming the basis of most chemotherapeutic and radiotherapeutic agents, a paradox that presents a great challenge for the development of anti-cancer therapies exploiting ROS-induced oxidative stress [88]. The concept of ROS and cytokine dependent signaling pathways represents a specific vulnerability that can be selectively targeted by antioxidants. Novel bioactive components including benzyl glucosinolate have been identified in papaya which exhibit anti-growth activities on several tumor cell lines [89,90]. The review paper of Nguyen et al. [91] explores the anticancer activities of papaya.

In light of the previous sections which lengthily discuss the pertinence of Mauritian Solo papaya and FPP to modulate biomarkers of oxidative stress and inflammation within cell-based models, the eventual goal of our group was to shed light on the anti-cancer propensity of the papaya based product- FPP. Common combinational therapies include surgery, chemotherapy, radiation and immunosuppressant drugs which are deemed effective, but highly aggressive leading to the experience of unpleasant side-effects such as acute headaches, vomiting, nausea and occasional bouts of unconsciousness. As a result of high levels of ionizing radiation, severe oxidative stress of can lead to the structural damage of the skin, spermatogia and hematopoietic stem cells amongst others [92,93,94]. Data published in 1995 by a group of Russian researchers were amongst the first to notice a positive effect upon regular oral consumption of FPP in children undergoing radiotherapy [95], in terms of the alleviation of the side effects associated with aggressive radiotherapy. Referring to published findings of our group in the *Journal Life Sciences* [96], our group used an N-methyl-N-nitrosourea (MNU)-injected balb/c mice model to explore the modulatory effect of FPP against MNU-induced hepatocellular carcinoma. Amongst all doses tested, mice of the 500 mg FPP/kg BW group were found to benefit the most of this treatment. Reduced shedding of hair, improved alertness and a gain in both weight and appetite were noted. Moreover, from a haematological point-of-view, compared to the control group where a subsequent drop of nearly 31% haemoglobin level, fractions of whole blood such as hemoglobin concentrations, leukocyte and platelet counts were found to normalize indicating the counter-occurrence of MNU-induced hemolysis, undoubtedly caused by excessive free radical attack on vulnerable erythrocytes and phase II detoxifying/antioxidant enzymes. Also, that platelet count in MNU control mice remained exceptionally high was indicative of the formation of metastatic lesions within the liver. This was visually confirmed by the appearance

of red, swollen and inflamed growths on the abdominal area of treated mice. Circulating malondialdehyde (MDA), a toxic product of lipid peroxidation, is considered to be indirect tumor promoter and co-carcinogenic agent. In our study, reduced circulating MDA levels (Figure 4) and simultaneous augmentations in enzymatic SOD (+20%), CAT (+81%) and GPx (+66%) release in FPP-supplemented mice, support similar trends reported in previously in literature [95,6,96,73], however no profound molecular studies have been conducted on FPP to explain how it achieves these patterns.

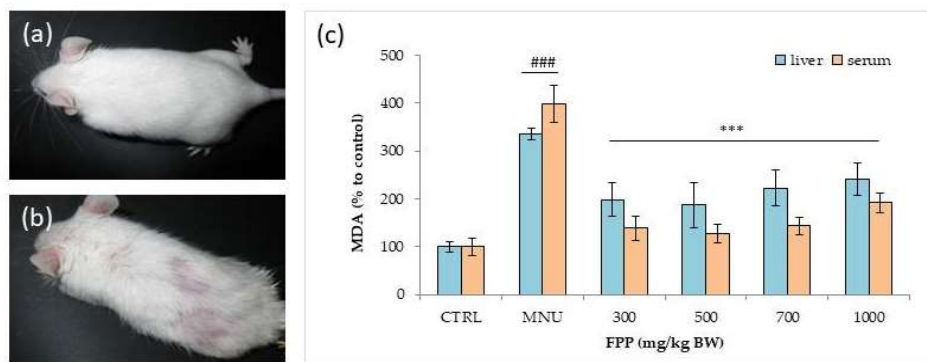


Figure 4. (a) Physical appearance of balb/c mice from PBS control group and (b) N-methyl-N-nitrosourea (MNU) control group. (c) Malondialdehyde (MDA) levels in fermented papaya preparation (FPP)-supplemented balb/c mice treated with or without MNU. Data is presented as the mean of 5 replicates where error bars represent  $\pm$  standard deviation. ###P<0.001 vs. PBS control; \*\*\*P<0.001 vs. MNU control. From [96] with permission.

Since genotoxins like MNU, benzo(a)pyrene, Fe-NTA and H<sub>2</sub>O<sub>2</sub> are documented to attack DNA and distort its stability through two basic pathways either by reaction with a DNA nucleophile or electrophile or by reaction with the pi ( $\pi$ ) or C-H bonds located within nucleotides, as evidenced by increased peak intensities at 1190, 1254, 1322, 1405, 1152 and 1463 cm<sup>-1</sup> [97], our group utilized Raman laser spectroscopy to detect any structural alterations inflicted by MNU on DNA that were reversed by FPP. Reduction in intensity of peaks at regions nucleotide bases or to the phosphodiester backbone by FPP [96, 98], provided us with sufficient evidence that FPP can indeed protect DNA through radical scavenging as proposed in an earlier study by Rimbach et al. [8] and Aruoma et al. [99] (Figure 5). Molecular data suggests that FPP reduces the extent of DNA damage by enhancing the activation of ERK, p35 and Akt. Such protein kinases are activated in response to DNA damage, providing a cellular signal to DNA repair enzymes (e.g. hOGG1), survival proteins (e.g. bcl-2), cell cycle control factors (e.g. cyclin D1) and several transcription factors [73,100]. FPP is also thought to divert hydroxyl radicals away from the  $\pi$  bonds of C5-C6 pyrimidines and N8-N7 or C4-C8 bonds of purines- thus protecting the vulnerable areas of DNA from any major structural alterations [101,96,72].

Results of our study [96] have clearly demonstrated that FPP could simultaneously boost the recovery of the immune defense system, hinder DNA damage, reduce symptoms of ill health associated to aggressive carcinoma and increase the longevity of balb/c mice undoubtedly proved that liver cancer can be managed without any harsh medical intervention. Till date the hepatoprotective effects of FPP have not been explored using this animal model or MNU as a tumorgen, these findings are therefore of great importance to the field of phytochemotherapy.

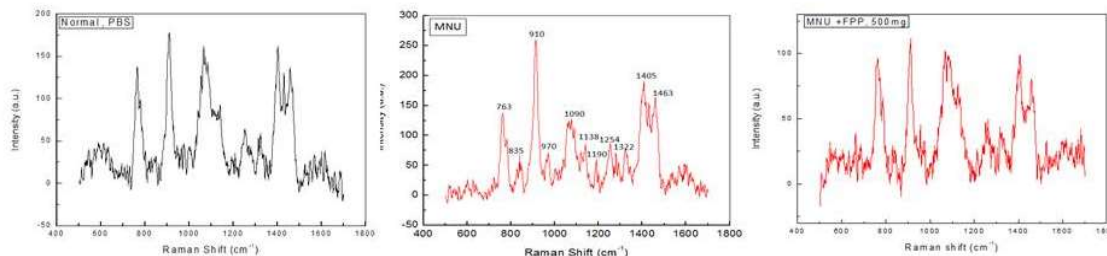


Figure 5. Comparison of Raman laser spectra of liver DNA in the region of 400 – 1700cm<sup>-1</sup> for all experimental groups: (A) PBS control (b) MNU control (C) MNU + 500 mg FPP/kg BW. Data is representative of 8 replicates. [Parameters: resolution cm<sup>-1</sup>, step of 100 nm, laser power 5 mW, exciting source 514.5nm argon ion laser] From [96] with permission.

## 5. Conclusion

No adverse effects have been noted in literature regarding the consumption of FPP deeming it safe for both adults and children. The former was recently confirmed in a study by Mankowski et al [102] who conducted a placebo-controlled clinical trial using a cohort of elderly adults aged 70 years and above. Extrapolation of these observations and those reported by our group and other investigators thus appraise FPP to be a feasible and remarkable phytonutraceutical which can be used to prevent or manage diseases governed by chronic oxidative stress, especially diabetes and cancer.

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**Conflicts of Interest:** Okezie I. Aruoma is actively involved in biomedical research involving fermented papaya preparation for the Osato Research Institute, Gifu, Japan

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