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ORIGINAL ARTICLE

# Extracts of Mauritian *Carica papaya* (var. solo) protect SW872 and HepG2 cells against hydrogen peroxide induced oxidative stress

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**Abstract** In line with literature documenting the pluripotent activities of tropical fruits, this study evaluated the antioxidant effects of Carica papaya fruit extracts at cellular level. Investigations using cellular models of oxidative stress provided complementary evidence of the antioxidant activities of papaya fruit. At 2 mg dry weight $ml^{-1}$, extracts of seed from ripe and unripe fruit significantly reduced oxidative stress levels within human preadipocytes (SW872) and hepatocellular carcinoma cells (HepG2) exposed to hydrogen peroxide ($H_2O_2$). Maintenance of mitochondrial viability, reduction of intracellular reactive oxygen species levels and mediation of pro-inflammatory cytokine secretory levels (tumour necrosis factor-α, interleukin-6, monocyte chemoattractant protein-1) were all indicative of its cytoprotective effects against oxidative-inflammation. This work demonstrates that the Mauritian Solo papaya is an important source of natural antioxidants that could be used for the dietary modulation of oxidative stress and inflammation.

**Keywords** *Carica papaya* · Oxidative stress · Antioxidant · Inflammation · SW872 · HepG2

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## Introduction

Originating from Southern Mexico, *Carica papaya* was introduced in Mauritius in the eighteenth century and rapidly naturalized around the island. Commonly grown in back yards, it is now one of the most common fruit to be cultivated for personal consumption and commercial export. There exist several local varieties of papaya in Mauritius including: Solo, Waimanalo, Ecsotika, Wilcox, Taniung and Rodrigues. Amongst these, Solo and Waimanalo are of preferred commercial choice as they are disease resistant and produce the sweetest fruits (Mardarbokus and Ranghoo-Sanmukhiya 2012). The worldwide demand for this fruit has doubled owing to its high palatability, all-year fruiting and its traditional medicinal values (United Nations Commodity Trade Statistics database 2010). Eaten both at unripe and ripe stages, papaya fruit can be consumed raw in salads, pickled, crystallized, dehydrated or fermented into wine (Saran and Choudhary 2013). In Mauritius, establishment of a local market for pickled (unripe), dehydrated and crystallized ripe papaya pulp has gained popularity.

Traditional medicine makes use of all the different parts of *C. papaya*, including its leaves, bark, roots, latex, flowers and seeds. Each and every part of papaya has a wide range of reputed medicinal applications: antibacterial, antifungal, anthelmintic, wound healing, antisickling, abortifacient, antifertility, antitumor, hypoglycemic and hypolipidemic (Aravind et al. 2013, Vij and Prashar 2015). Although there is abundant evidence outlining its medicinal properties, only a handful of pharmacological studies have been carried out evaluating its anti-cancer effectiveness on human cancer cell lines and solid tumours. Ripened papaya fruit contains an appreciable quantity of macro-minerals such as magnesium, zinc, copper, potassium, ascorbic acid, vitamin A, folate and

vitamin B. It is also regarded as an excellent source of lycopene, which is a key intermediate for the biosynthesis of many important carotenoids like β-carotene, β-cryptoxanthine and zetaxanthine (Nwofia et al. 2012; Oloyede 2005; Wall 2006, USDA 2013). A voluminous body of literature has suggested a multiplicity of health benefits of carotenoids in humans particularly against cancers, cataract and cardiovascular diseases in part due to their powerful antioxidant and radical scavenging properties (Mayne 1996; Aravind et al. 2013).

The concept of reactive oxygen species (ROS)-dependent mitogenic and anti-apoptosis signalling pathways represents a specific vulnerability that can be selectively targeted by antioxidants. Novel bioactive components including benzyl glucosinolate have been identified in an aqueous extract of papaya and these exhibit anti-growth activities on several tumor cell lines (Otsuki et al. 2010; Li et al. 2012). The review paper of Nguyen et al. (2013) explores the anticancer activities of *C. papaya.*

The complex interaction between chronic inflammation and oxidative stress mechanisms involved during cancer growth raises the possibility of therapeutic interventions involving antioxidants could theoretically reduce the risk of base mutations and vulnerability of cells undergoing cell transformation (Lamb and Goldstein 2008; Crujeiras et al. 2013). Low grade inflammatory states and excessive secretion of inflammatory cytokines (tumour necrosis factor (TNF)-α, interleukin (IL)-6, IL-1), chemokines, and pro-inflammatory transcription factors (NF-kB) are regulated by ROS and other major mediators of cancer progression (Lamb and Goldstein 2008). The metastatic potential of chemokines and chemokine receptors and the use of pro-inflammatory cytokines as clinical predictors of several cancers have been reviewed by Gupta et al. (2012). Inflammatory responses towards pathogenic invasions form a crucial part of good health; hence a moderate suppression of inflammatory molecules such as IL-6, TNF-α and circulating monocyte chemoattractant protein (MCP)-1 is required to avoid detrimental outcomes on the good functioning of the immune system. That *Carica papaya* could target ROS and regulate inflammatory response pathways was the subject of this investigation. Overall, this study is in parallel with several other existing strategies and therapeutic approaches set up by the Ministry of Health and Quality of Life in an attempt to prevent and protect the Mauritian population against non-communicable diseases (NCDs).

## Methodology

### Preparation of fruit extracts

Mature ripe and unripe fruits were harvested from Labourdonnais Fruit Orchid (Mapou, Republic of Mauritius). Fruits were washed under running tap water and the peel and seeds separated from the pulp. Each fruit part was dried in a convection oven for 10 h (40 °C) before being pulverized in an electric blender. Aqueous extracts were prepared by macerating a known mass of dried powdered fruit in methanol (80%, v/v) for 3 consecutive days at 4 °C with constant stirring (200 rounds per min (rpm)). The resulting suspension was centrifuged at 4000 rpm for 10 min to obtain a clear supernatant, and then concentrated by flash evaporation. Aqueous extracts were stored at −80 °C until use.

### Cell culture conditions

Human SW872 adipocytes (ATCC HTB-92) and HepG2 hepatocytes (ATCC HB-8065) were a kind gift from Prof. Emmanuel Bourdon (Université de La Réunion, La Réunion, France). Cells were grown in high glucose DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine added, 100 U/L streptomycin-penicillin and 0.5 μg/ml amphotericin B. Cells were maintained at 37 °C in an atmosphere of 5% $CO_2$ and 95% humidity.

### MTT assay

Cells ($10 \times 10^4$ cells/well) were grown overnight, and then treated with a papaya extract for 24 h. Cells were then exposed to extreme oxidative stress conditions for 30 min (1 mM hydrogen peroxide ($H_2O_2$). Cell viability was determined by addition of 20 μl 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/ml) to each well. After a 2 h incubation, media was removed and formazan crystals dissolved in 125 μl DMSO. Absorbance was read at 595 and 690 nm (Biotek Synergy HT, USA) and viability calculated by subtracting the absorbance of a blank (DMSO only) from that obtained at 595 nm.

### Determination of reactive oxygen species (ROS) accumulation

Accumulation of ROS in cells under $H_2O_2$-induced oxidative stress was determined using a fluorogenic dye 2′7′-dichlorofluorescein diacetate (DCF-DA). After seeding cells at a density of $10 \times 10^4$ cells/well overnight, cells were treated with papaya extracts for 24 h, then stained with DCFDA (10 μM) for a further 1 h. After incubation, cells were exposed to $H_2O_2$ (1 mM) for 1 h and fluorescence intensity measured at 485 nm (excitation) and 535 nm (emission). Fluorescence values were calculated after subtraction of a blank (medium only, no cells).

### Quantification of protein carbonyl formation

Cells ($30 \times 10^4$ cells/well) were grown for 48 h before being treated with papaya extracts overnight. Pretreated cells were exposed to 1 mM $H_2O_2$ for 30 min. To 100 µl cell culture supernatant, an equal volume of DNPH was added and allowed to incubate at room temperature for 15 min. To the mixture, 30 µl of TCA (1 g/ml) was added and kept on ice for a further 15 min. The contents were spun at maximum speed for 10 min. The resulting pellet was washed twice with ice cold acetone then dissolved in 200 µl guanidine. Absorbance was read at 375 nm. Quantification of protein carbonyl content was done according to the manufacturer's instructions using commercial kits from Biovision®.

### Quantification of inflammatory cytokines levels

Cells ($30 \times 10^4$ cells/well) were grown for 48 h then treated with papaya extracts overnight. After a 30 min exposure to 1 mM $H_2O_2$, cell culture supernatants were collected and stored at −80 °C. Quantification of pro-inflammatory (TNF-α, IL-6, MCP-1) and anti-inflammatory (IL-10) cytokines were measured according to the manufacturer's instructions using commercial ELISA kits from BioLegend®.

### Measurement of endogenous antioxidant enzyme activity

Cells were seeded at a density of $30 \times 10^4$ cells/well and allowed to grow for 48 h. Once confluent, cells were pretreated with papaya extracts for 24 h before being exposed to 1 mM $H_2O_2$ for 30 min. Media supernatant was discarded and the cell monolayer rinsed lightly with cold phosphate buffer saline (PBS) (pH 7.4). By means of a scraper, cells were collected and mixed with lysis buffer (provided by the manufacturer) then centrifuged at 10,000x *g* for 15 min at 4 °C. Supernatants were stored at −80 °C. The effect of papaya extracts on the activity of superoxide dismutase, catalase and glutathione peroxidase was measured using commercial kits from Biovision®.

### Statistical analysis

Results from at least two independent experiments carried out in triplicate were expressed as mean values or as a mean percentage (%) compared to a control, where ± represented standard deviation. Any values written within brackets () indicated a percentage change from baseline. Mean differences were determined by one-way ANOVA, followed by a Tukey's post hoc test using MedCalc® (version 11.5.1). Differences were considered significant when value of $P < 0.05$ (two-tailed). Correlations between two data sets were computed as Pearson's correlation coefficient (*r*).

## Results

### Viability of cells under oxidative stress

Panels A to D of Fig. 1 reveal the outcomes of over-exposure to high levels of hydrogen peroxide on SW872 and HepG2. Major morphological distortion and consequent cell death occurs upon prolonged exposure to oxidative stress (>1 h). Exposure of SW872 cells to $H_2O_2$ caused the cell viability to drop to 65%, representing 35% cell death. Treatment of SW872 with fruit extracts maintained viability between the range of 56.3 and 72.4% (Fig. 2a). Unripe papaya pulp could provide greater cytoprotection against $H_2O_2$-induced oxidative stress than compared to other fruit parts ($P < 0.01$). As shown in Fig. 2b, notable increases in HepG2 viability were detected at 0.1 mg dry weight (DW) $ml^{-1}$ by ripe pulp (+25.6%, $P < 0.05$), ripe peel (+27.5%, $P < 0.05$), unripe pulp (+29.1%, $P < 0.05$) and in unripe peel treated cells (+28.3%, $P < 0.05$).

### ROS accumulation

Intracellular ROS levels in SW872 were found to rise 88.4% more than PBS control (11.6% vs. $H_2O_2$ control). Treatment with papaya fruit extracts caused ROS levels to vary between 32.4–110.9% in ripe extract treated cells and 34.6–101.1% in unripe extract treated cells. As described in Table 1, all extracts were found to exert strong dose-dependent ROS scavenging capacities. A descending order of ROS scavenging could be established as follows: *unripe seed* > *ripe peel* > *ripe seed* > *ripe pulp* > *unripe pulp* > *unripe peel*. As for SW872, intracellular levels of ROS abruptly increased by 93.4% in HepG2 cells upon exposure to oxidative stress (vs. 11.6% PBS control). Pretreatment of HepG2 with 2 mg DW $ml^{-1}$ fresh papaya extracts brought a 24.6% (ripe pulp), 72.3% (ripe peel) and 57.1% (ripe seed) reduction in intracellular ROS levels ($P < 0.001$ vs. $H_2O_2$ control). Similarly, unripe fruit extracts scavenged ROS by 44.9% (pulp), 69.7% (peel) and 79.1% (seed) ($P < 0.001$ vs. $H_2O_2$ control). Using $EC_{50}$ values, a ranking order could be drawn in the following descending order: *unripe seed* > *unripe peel* > *ripe peel* > *ripe seed* > *unripe pulp* > *ripe pulp* (Table 1).

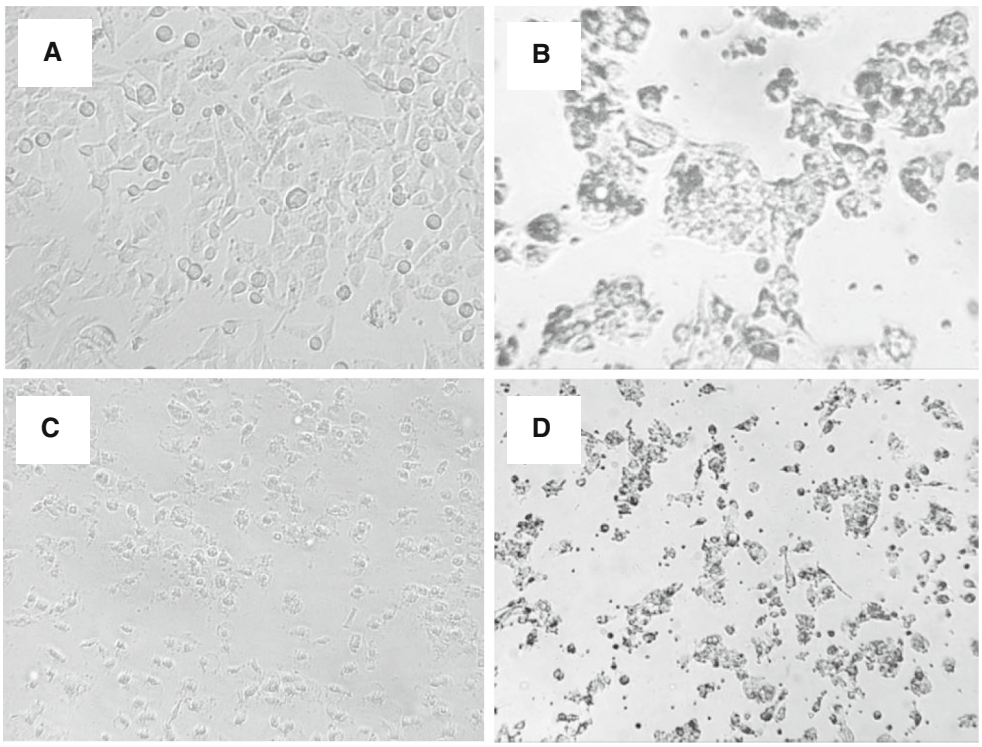


**Fig. 1** **a** SW872 and **b** HepG2 cells under normal growth conditions (magnification X100) **c** SW872 and **d** HepG2 cells upon over-exposure to severe $H_2O_2$-induced oxidative stress (magnification X40)

## Protein carbonyl production

Oxidative stress was apparent within SW872 cells as evidenced by a 25% increase in protein carbonyl levels (vs. PBS control; Fig. 3a). Pre-treatment with fruit extracts caused levels to significantly drop to between 84.4–52.9% (ripe extracts) and 102.9–71.4% (unripe extracts) (vs. 133.5% $H_2O_2$ control). Greatest reductions in protein carbonyl levels were exhibited by ripe peel (−57.6% at 20 mg DW $ml^{-1}$, $P < 0.05$ vs. $H_2O_2$ control) and ripe seed (−60.4% at 1 mg DW $ml^{-1}$, $P < 0.05$ vs. $H_2O_2$ control). Interestingly panel B of Fig. 3 shows a 63.2% increase in carbonyl production within HepG2 PBS control cells, suggesting that hepatocytes are more sensitive to oxidative stress than SW872. Papaya was observed to significantly attenuate protein oxidation at low doses, where ripe seed (−74.6%) displayed better cytoprotection than compared to its unripe counterpart (−56.9%). All reductions were significantly different from $H_2O_2$ control ($P < 0.01$).

## Inflammatory cytokine levels

### *TNF*

$H_2O_2$ caused TNF-α levels to increase abruptly by 165.3% in SW872 (265.3% vs. 100% PBS control, $P < 0.01$). Interestingly, secretory levels of TNF-α spiked from 367.1 to 643.3% by ripe peel (vs. 265.3% $H_2O_2$ control, $P < 0.01$), but this flux was independent of the effect of extract upon cell viability. Extract pre-treatment substantially decreased TNF-α levels, where greatest reductions were achieved by unripe peel and seed extracts than compared to ripe (Figs. 4a, b). These reductions correlated well with their capacity to scavenge ROS within SW872 (unripe peel: $r = 0.96$, unripe seed: $r = 0.79$). In the HepG2 cell line, secretory levels of TNF- α peaked to 140.7% in cells exposed to $H_2O_2$ only (+40.7% vs. PBS control). Consistent with observations made in SW872, greater reductions were observed by unripe extracts in HepG2. A 71.2% drop in TNF levels was recorded by unripe pulp ($P < 0.001$ vs. $H_2O_2$ control) at 2 mg DW $ml^{-1}$. While unripe peel and seed inhibited TNF secretory levels by 62.7 and 65.3% respectively ($P < 0.01$ vs. $H_2O_2$ control), a Pearson correlation analysis suggested that this anti-inflammatory effect was related to its capacity to effectively scavenge ROS within cells (unripe pulp and peel: $r = 0.99$, unripe seed: $r = 0.55$).

### *IL-6*

Treatment of adipocytes with $H_2O_2$ caused IL-6 levels to increase by only 9% ($P < 0.001$ vs. PBS control) in

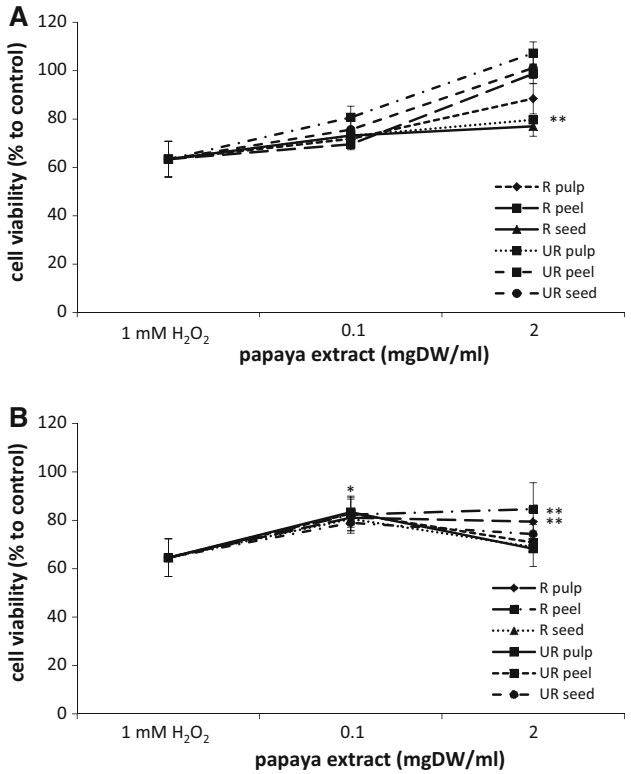


**Fig. 2** Effect of ripe and unripe papaya extracts on the viability of **a** SW872 and **b** HepG2 cells exposed to oxidative stress. Results are expressed as the mean viability compared to a control of two independent experiments (n = 3), where error bars represent ± standard deviation. *$P < 0.05$, **$P < 0.01$ versus $H_2O_2$-only control

**Table 1** Antioxidant activity of *Carica papaya* extracts

| | $EC_{50}^{a}$ (mg DW/ml) | Pulp | Peel | Seed |
|---|---|---|---|---|
| SW872 | Ripe | 20.13 | 7.60 | 19.55 |
| | Unripe | 22.95 | 24.63 | 5.19 |
| HepG2 | Ripe | 39.49 | 7.56 | 12.21 |
| | Unripe | 18.89 | 5.85 | 2.15 |

Results are expressed in terms of $EC_{50}$ values obtained for the ROS assay using SW872 and HepG2 cells exposed to 30 min $H_2O_2$

[a] $EC_{50}$: effective concentration of extract at which ROS accumulation is 50%. Values are generated from a mean of two independent experiments conducted in triplicate

SW872. Exposure to low doses of papaya diminished cytokine secretion by 21.6% (ripe pulp), 12.2% (ripe peel; $P < 0.05$ vs. $H_2O_2$ control) and 37.8% (ripe seed; $P < 0.001$ vs. $H_2O_2$ control) (Fig. 4c). A similar anti-inflammatory trend was also encountered with unripe papaya. Such reductions reflected their strong ROS scavenging capacity and ability to reduce inflammation in response to oxidative stress (ripe peel: r = 0.72, unripe peel: r = 0.77; ripe seed: r = 0.81; unripe seed: r = 0.61). However, as shown in Fig. 4d, no apparent reductions in IL-6 could be noted within HepG2 cells.

*MCP-1*

When compared to control cells, oxidative stress provoked MCP-1 levels to rise by 11.5%, The anti-inflammatory effect of solo papaya was more evident in SW872 cells pre-treated with seed extracts, where levels reached a minimum of 6.9 and 7.4% (vs. 111.5% $H_2O_2$ control) by ripe seed and unripe seed respectively (Fig. 4e). All reductions shown were found to be strongly related to their intracellular ROS scavenging capacities (ripe seed: r = 0.94; unripe seed: r = 0.99). However in HepG2, the effect was minimal. No significant dose-dependent reductions could be noted (Fig. 4f).

*IL-10*

IL-10 secretion dropped 2.1-fold (47.9%, vs. PBS control, $P < 0.001$) in SW872 upon exposure to extreme oxidative stress. In line with previous trends, pre-treatment with low doses of fruit extracts positively stimulated IL-10 secretion. IL-10 levels reached a maximum of 62.1, 72.2 and 114.9% (vs. 47.9% $H_2O_2$ control) by ripe pulp, peel and seed respectively; representing abrupt increases of 29.8% ($P < 0.01$), 50.8 and 140.1% (vs. 47.9% $H_2O_2$-only control) at 0.1 mg DW $ml^{-1}$ (Fig. 4g). In those cells treated with unripe extracts, levels also increased (+219.9% (seed) vs. +89.9% (pulp) and +62.1% (peel); $P < 0.001$ vs. $H_2O_2$ control). In HepG2, although IL-10 dropped by 19.7% in PBS control cells, seed extracts stimulated higher secretory levels at a dose of 0.1 mg DW $ml^{-1}$ (Fig. 4h).

## Activity of endogenous antioxidant enzymes

*Superoxide dismutase (SOD) activity*

Enzyme activity in SW872 cells dipped by 36.1, 38.7 and 27.7% ($P < 0.001$ vs. $H_2O_2$ control) by ripe pulp, peel and seed respectively, but recovered when treated with doses of 2 mg DW $ml^{-1}$ For example: ripe pulp: +48.1% ($P < 0.05$ vs. $H_2O_2$ control) and ripe peel: +79% ($P < 0.001$ vs. $H_2O_2$ control). A similar dose-dependent boost in SOD activity was brought about by their unripe counterparts (unripe pulp: +69.3% $P < 0.001$, unripe peel: 78.4% $P < 0.001$) (Table 2A). As indicated in Table 2, seed extracts has an opposing effect on SOD activity. Seed caused a 1.6- (ripe) and 3.5-fold (unripe) inhibition of SOD in SW872. In comparison, SOD activity ranged between 71.4–98.2% (ripe extract treated cells), and 89–110% (unripe extract treated cells) (vs. 90.5% $H_2O_2$ control) in HepG2. Dose-dependent increments were noted by fruit extracts at 2 mg DW $ml^{-1}$, where SOD activity was significantly improved by unripe peel (at 2 mg DW $ml^{-1}$: +21.9%, $P < 0.01$ vs. $H_2O_2$ control) (Table 2B).

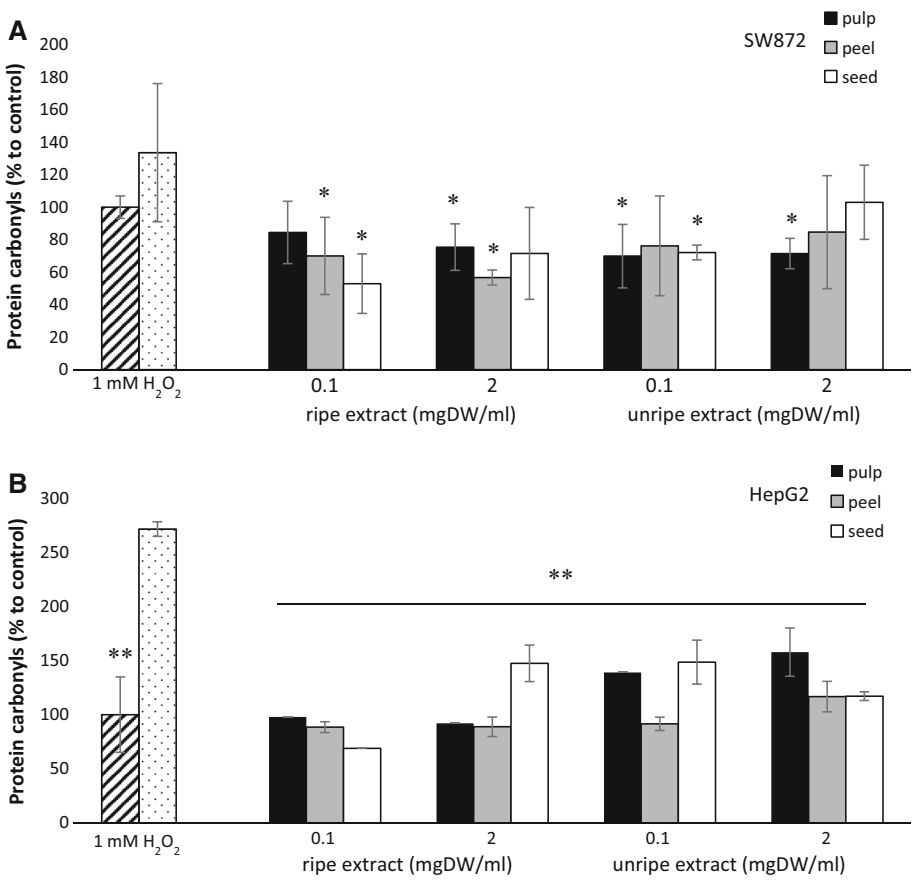


**Fig. 3** Effect of ripe and unripe papaya extracts on protein carbonyl accumulation in **a** SW872 and **b** HepG2 cells exposed to oxidative stress. Results are expressed as the mean viability compared to a control of two independent experiments (n = 3), where error bars represent ± standard deviation. *$P < 0.05$, **$P < 0.01$ versus $H_2O_2$-only control

### Catalase (CAT) activity

For this assay, since activity remained low between 30.4–49.1% and 28.2–67.8% (vs. 73.5% $H_2O_2$ control) in ripe and unripe extract treated cells (Table 2A), solo papaya did not influence this enzyme. A Pearson correlation test suggested that CAT activity in unripe pulp and peel treated cells was dependent upon their effect on cell viability (unripe pulp: r = 0.82; unripe peel: r = 0.99). A similar observation was also noted in HepG2 cells where pretreatment with papaya could not restore the activity of CAT in cells under oxidative stress (ripe extract treated cells: 2.7–138.5%; unripe extract treated cells: 21.7–60.3% vs. 73.5% $H_2O_2$ control, Table 2B). Here also, a positive correlation between the activity of CAT and cell viability could explain the inhibitory effect for unripe peel only (r = 0.84).

### Glutathione peroxidase (GPx) activity

In contrary to the effects of solo papaya extracts on CAT, the activity of endogenous GPx was found to remain relatively stable. Although no significant increases were detected, the overall activity of GPx was slightly superior to that of the $H_2O_2$ control (90.2–104.9% vs. 93.1% % $H_2O_2$ control, Table 2A). A Pearson correlation analysis indicated that enzyme activity was dependant on viability for peel extracts (r = 0.99) and ripe seed (r = 0.98) only. Exposure of HepG2 to short term oxidative stress negatively influenced its GPx activity, as levels dropped drastically by 47.6% (52.4% vs. PBS control), pre-treatment with papaya could restore GPx activity in a dose-dependent manner. Recovery from oxidative suppression was more evident at 2 mg DW $ml^{-1}$, for example: +75.5% (ripe pulp) and +223% (unripe peel) ($P < 0.001$ vs. $H_2O_2$ control). Interestingly, ripe seed, unripe pulp and unripe peel extracts caused GPx activity to exceed that of PBS control, suggesting an enzyme boosting-effect (Table 2B).

## Discussion

This study made use of two cell lines derived from human lipocarcinoma (SW872) and hepatocarcinoma (HepG2). These cells being of cancerous origin were easily manipulated to display a degree of oxidative stress and were thus regarded as a dual model of oxidative stress and cancer allowing us to explore the antioxidant and anti-inflammatory properties of *Carica papaya*.

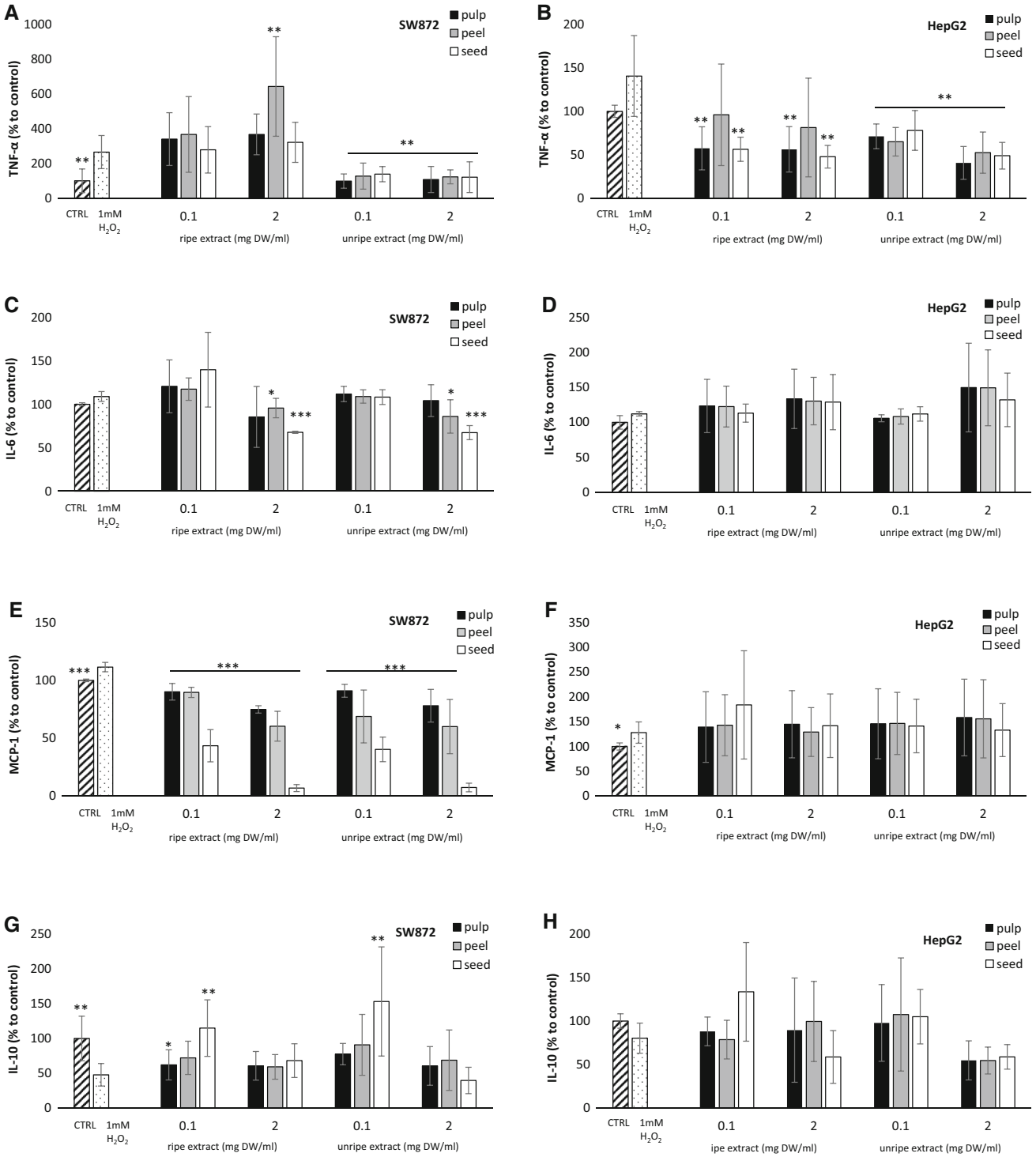


**Fig. 4** Effect of ripe and unripe papaya extracts on inflammatory cytokine levels in SW872 and HepG2 cells exposed to oxidative stress. Results are expressed as the mean viability compared to a control of two independent experiments (n = 3), where error bars represent ± standard deviation. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus $H_2O_2$-only control

In the present study, papaya pulp was found to exert minimal cytotoxicity. Since the cell viability of both SW782 and HepG2 increased compared to the control, it is casually suggested that *Carica papaya* extracts could neutralize harmful ROS liberated from $H_2O_2$. However, papaya seed exhibited anti-proliferative effects in HepG2. Two compounds present in papaya seed studied for their growth inhibitory activities are benzyl isothiocyanate

**Table 2** Influence of *Carica papaya* extracts on activity of endogenous enzymes in (A) SW872 and (B) HepG2 cells challenged with oxidative stress

| | Pulp (mg DW/ml) | | Peel (mg DW/ml) | | Seed (mg DW/ml) | |
|---|---|---|---|---|---|---|
| | 0.1 | 2 | 0.1 | 2 | 0.1 | 2 |
| (A) | | | | | | |
| SW872 | | | | | | |
| SOD | | | | | | |
| Ripe | 56.48 ± 19.46 (−36.1%)*** | 83.62 ± 10.18 (−5.4%) | 54.19 ± 96.98 (−38.7%)*** | 96.98 ± 8.77 (+9.7%) | 63.9 ± 16.18 (−27.7%)** | 55.13 ± 15.67 (−37.6%)*** |
| Unripe | 49.0 ± 22.63 (−44.5%)*** | 82.98 ± 8.98 (−6.1%) | 49.71 ± 19.60 (−43.7%)*** | 88.7 ± 7.68 (+0.4%) | 52.04 ± 21.28 (−41.1%)*** | 25.55 ± 21.3 (−70%)*** |
| CAT | | | | | | |
| Ripe | 34.99 ± 18.46 (−52.4%)** | 36.49 ± 25.81 (−50.3%)** | 40.06 ± 6.26 (−45.5%)*** | 42.26 ± 7.3 (−42.5%)*** | 49.13 ± 14.0 (−33.1%)** | 30.44 ± 11.65 (−58.6%)*** |
| Unripe | 28.17 ± 20.97 (−61.7%)*** | 46.98 ± 14.93 (−36.1%)** | 41.69 ± 20.45 (−43.3%)** | 43.81 ± 40.4 (−40.4%)** | 67.84 ± 14.32 (-7.7%) | 34.32 ± 16.16 (−53.3%) |
| GPx | | | | | | |
| Ripe | 97.50 ± 4.89 (+4.7%) | 98.16 ± 4.39 (+5.4%) | 94.19 ± 6.94 (+1.1%) | 104.85 ± 13.55 (+12.6%) | 92.49 ± 9.23 (−0.7%) | 98 ± 3.78 (+5.2%) |
| Unripe | 97.28 ± 1.94 (+4.5%) | 95.74 ± 5.57 (+2.8%) | 90.22 ± 3.78 (−3.1%) | 90.68 ± 9.39 (−2.6%) | 98.92 ± 7.16 (+6.2%) | 99.34 ± 5.78 (+6.7%) |
| (B) | | | | | | |
| HepG2 | | | | | | |
| SOD | | | | | | |
| Ripe | 71.43 ± 1.90 (−21.1%)*** | 89.74 ± 1.68 (−0.8%) | 94.87 ± 1.27 (+4.9%) | 94.51 ± 2.20 (+4.5%) | 98.17 ± 0.63 (+8.5%)** | 97.07 ± 4.16 (+7.3%) |
| Unripe | 89.01 ± 2.20 (−1.6%) | 92.67 ± 1.68 (+2.4%) | 99.27 ± 1.68 (+9.7%)** | 110.26 ± 5.19 (+21.9%)** | 97.80 ± 1.90 (+8.1%)** | 93.41 ± 4.40 (+3.2%) |
| CAT | | | | | | |
| Ripe | 58.38 ± 11.8 (−38.5%)* | 22.72 ± 8.11 (−76.1%)*** | 49.89 ± 15.83 (−47.4%)* | 28.09 ± 17.43 (−70.4%)** | 138.51 ± 0.55 (+45.9%)** | 27.82 ± 15.22 (−70.7%)** |
| Unripe | 29.99 ± 4.06 (−68.4%)*** | 21.66 ± 10.54 (−77.2%)*** | 55.8 ± 7.54 (−41.2%)** | 31.24 ± 14.76 (−67.1%)** | 60.28 ± 5.24 (−36.5%)** | 51.77 ± 19.1 (−45.4%) |
| GPx | | | | | | |
| Ripe | 37.4 ± 12.52 (−28.6%) | 91.87 ± 18.63 (+75.5%) | 60.49 ± 5.07 (+15.5%) | 51.22 ± 32.27 (−2.2%) | 86.99 ± 18.63 (+66.1%) | 116.26 ± 39.20 (+122%) |
| Unripe | 105.2 ± 49.43 (+100.9%) | 230.08 ± 39.2 (+339.4%)** | 37.40 ± 19.86 (−28.6%) | 169.11 ± 14.08 (+223%)*** | 26.5 ± 17.29 (−49.4%) | 22.76 ± 18.63 (−56.5%) |

Result are expressed as mean percentages (%) compared to a PBS control, where ± represents standard deviation. Percentage changes from a $H_2O_2$ control are indicated in brackets ()

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus $H_2O_2$ control

(BITC) and its corresponding glucosinolate: benzyl gucosinolate (reviewed in Nguyen et al., 2013). Findings of Huang et al. (2012) and Kim et al. (2011) bring forward evidence that BITC acts as a "signal deamplifier" causing cell cycle arrest at G2/M phase via a cyclin A mediated pathway, providing a plausible explanation as to why cancer colony formation hastily declines upon exposure to papaya seed. Papaya is a rich source of phenolic acids such as ferullic acid, *p*-coumaric acid and hydroxycinnamic acid, which may contribute to its therapeutic and antioxidant properties. Interference of the expression of cell cycle genes such as CEP2 (centrosome activity) and SMC-1L1 (a S-phase checkpoint protein) has been the subject of ongoing research for Janicke et al. (2011), where ferullic acid

reportedly influenced up to 517 genes involved in the cell cycle, partly explaining why cancerous cells abruptly stop growing upon exposure to high concentrations of papaya seed.

Results of the ROS assay showed that intracellular ROS formation was considerably lowered in the presence of papaya fruit extracts, especially peel and seed where a dose of 2 mg DW $ml^{-1}$ proved to be more protective against $H_2O_2$. Using the same method, Pathak et al. (2014) reported an approximate 3.5-fold reduction in ROS levels within human epithelial kidney Hek-293 cells treated with a flavonoid rich fraction of papaya seed. A cold water extract of unripe papaya peel could also protect human SY5Y neuronal cells against $H_2O_2$-induced oxidative stress (Guizani et al. 2011). Low doses of ripe or unripe pulp extracts were found to raise ROS levels non-significantly-suggesting that a dose of 0.1 mg DW $ml^{-1}$ insufficiently scavenges ROS. Along the same line, a pro-oxidant behavior of unripe pulp was reported by Oloyede et al. (2012) which the authors attributed to its ascorbic acid content.

Protein carbonyls, quantified through 2,4-dinitrophenylhydrazine (DNPH) tagging, are an immediate biomarker of oxidative damage. Our results demonstrate a clear reversal of $H_2O_2$-induced oxidative damage in human adipocytes and hepatocytes upon treatment with ripe *Carica papaya* peel and seed. They also coincide with findings of Tham et al. (2012) who found an ethanolic extract of papaya leaves to effectively lower protein carbonyls in rats exposed to sub-cytotoxic levels of lead acetate. Using the same cell line SW872, Ramful et al. (2010) showed that a flavedo (peel) extract of Tangelo 1A (a citrus fruit locally cultivated in Mauritius) had the ability to inhibit $H_2O_2$-induced protein carbonyl formation by 17%. For that study, the authors attribute its antioxidant activity to the interdependent effects of its major flavonoids: neohesperidin, hesperidin, naringenin and naringin towards ROS.

Recognition that the pathological conditions of cancer are accompanied by a weakened immune system is thought to be brought about by the involvement of free radicals in tissue inflammation (Balistreri et al. 2010; Kern 2006). Peroxyl and hydroxyl radicals released upon $H_2O_2$ metabolism can directly attack cellular macrostructures rich in lipids disrupting membrane fluidity, perplexing ligand-ligand interactions and eliciting uncontrolled inflammatory responses. This explains to a degree why cytokine levels were abnormally high in SW872 and HepG2 upon exposure to severe $H_2O_2$. Although HepG2 was less responsive to *C. papaya,* the marked reduction of pro-inflammatory TNF (59.6%), IL-6 (38%) and MCP-1 (93%) levels in SW872 in response to papaya pre-treatment demonstrates its anti-inflammatory potential. This trend agrees with that published by Pathak et al. (2014) who reported a benzene fraction of papaya seed to induce a 67 and 59.6% decrease in TNF-α and IL-6 in human pancreatic cells HPDE. In our study, papaya pulp caused TNF-α and IL-6 levels to unexpectedly exceed that of the $H_2O_2$-only control. These intriguing results could be explained based on the findings of Rimbach et al. (2000) where they mention that papaya pulp is an indispensible source of D-glucans and high molecular weight glucans which have been reported to trigger TNF-α secretion in RAW 269.7 macrophages. It is possible that SW872 may have incorporated glucans present in papaya pulp, thus triggering excessive TNF-α secretion. However, this assumption would need to be clarified by further investigation. Enhanced production of cytokine IL-10 provides additional evidence of the anti-inflammatory properties of papaya, especially that of the seed (SW872: ripe +140% unripe +219%; HepG2: ripe +66.2%, unripe +30.6%). Data published by Otsuki et al. (2010) are in support of these results, in which authors also report a comparable rise in IL-4 and IL-2 in peripheral blood mononuclear cells co-cultured with a papaya extract. Moreover, evidence reviewed by Pan et al. (2009) suggests that secondary metabolites present in seed, for instance: benzyl isothiocyanate, β-carotene and lycopene, can exert anti-inflammatory responses through the inhibition of MCP-1 and COX-2 expression and down regulation of NF-kB transcription factor and IFN-γ secretion. Moreover, the modulation of macrophage oxidative burst by papaya cannot be overlooked.

Protection of the body against redox imbalances is achieved through the action of several radical neutralizing enzymes (Bouayed and Bohn, 2012; Halliwell, 2005), the activities of which can be strongly depressed by chemical stress-inducers like hydrogen peroxide ($H_2O_2$), carbontetrachloride ($CCl_4$), gamma radiation, acrylamide and benzo(a)pyrene. However, alongside an improved antioxidant capacity of cells, several studies, including ours, have found a simultaneous restoration of endogenous SOD, CAT, GPx or GR enzyme activity in response to pretreatment with polyphenol-rich fractions of *Carica papaya* (Patak et al. 2014; Guizani et al. 2011; Osman and Hamza 2013). Secondary metabolites identified in papaya such as quercetin, catechin and p-hydroxybenzoic acids and phenolic acids like ferulic acid, p-coumaric acid and caffeic acids (Riviera-Pastera et al. 2010) are collectively thought to positively influence the activity of such antioxidant enzymes. Also the presence of both macro (e.g. magnesium) and oligo (e.g. zinc) elements in papaya fruit acting as enzyme co-factors may encourage a boost in activity (Nwofia et al. 2012; Wall 2006). Possibility of polyphenols participating synergistically with SOD, CAT and GPX as a free radical scavenger is also very likely. However, the puzzling suppression of CAT by papaya seed could also be interpreted as an anti-proliferative (pro-apoptotic?)

mechanism against SW782 and HepG2 which deserves confirmation using immunoblotting techniques.

## Conclusion

Despite our findings, the exact immune-modulatory role of solo papaya is not yet fully understood and cannot be explained solely through the use of ELISA techniques; therefore further investigations are highly warranted in order to gain a better insight of the molecular pathways undertaken in the cancer microenvironment. Overall, our results conclude that a notable protection against oxidative stress was offered by seed and peel extracts. Their capacity to maintain cell viability, inhibit protein oxidation and restore SOD activity by actively neutralizing the build-up of intracellular ROS suggests that waste products of papaya fruit exhibit an important regulatory role against oxidative stress and inflammation which deserves to be further explored by those in search of economical and innovative methods to curb the progression of oxidative-inflammation.

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