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Antioxidant and Apoptotic Activity of Papaya Peel Extracts in HepG2 Cells

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Abstract

Papaya peels (PP) are discarded after consuming the fruit. However, they contain antioxidants. Oxidative damage caused by free radicals has major implications in many chronic diseases. The objective of this study was to determine *in-vitro* antioxidant and apoptotic activity of PP extracts. Modulation of endogenous glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), cyclo-oxygenase-2 (COX-2), caspase-3 activities and DNA fragmentation by PP extracts in HepG2 cells were evaluated. Gallic acid (18.06 µg/g), caffeic acid (29.28 µg/g), *p*-coumaric acid (38.16 µg/g), ferulic acid (95.46 µg/g) and quercetin (3.17 µg/g) were the major polyphenols quantified in PP extracts. *In-vitro* antioxidant capacity of PP was determined by ferric reducing antioxidant potential (31.86 µM Fe^{+2}/g), trolox equivalent antioxidant capacity (14.56 mM trolox equivalents (TE)/g), oxygen radical scavenging activity (30.88 mM TE/g) and 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability (IC_{50}-8.33 mg/ml). Induction of oxidative stress significantly ($p \leq 0.05$) lowered SOD, CAT, GPx, GR activities and GSH levels by 3.1, 1.46, 2.87, 1.34 and 1.32 folds compared to control respectively. However, treating cells with PP extracts significantly ($p \leq 0.05$) enhanced SOD, CAT, GPx, GR activities and GSH compared to oxidative stress induced cells. Treating cells with PP extracts significantly ($p \leq 0.05$) lowered COX-2 activity, enhanced caspase-3 activity and induced DNA fragmentation, indicating that PP extracts caused cell death by apoptosis. In conclusion, anti-cancer properties of PP extracts may be due to the synergistic effect of free radical scavenging ability, induction antioxidant enzymes and by inducing apoptosis.

Keywords

Papaya Peel, Cancer, Polyphenols, Antioxidants

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1. Introduction

Reactive oxygen species (ROS) are generated in the human body during various metabolic processes. In a healthy human being generation of ROS is keeps in check by cellular antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) [1] glutathione (GSH) [2] and glutathione reductase (GR). However, an imbalance in this mechanism favoring the over production of ROS results in oxidative stress, which has major implications in the etiology of chronic diseases such as cancer, diabetes and obesity [3] [4].

Apoptosis or programmed cell death is a highly regulated process that eliminates unwanted cells. It is characterized by membrane blebbing, shrinkage of cell and nuclear volume, chromatin condensation and nuclear DNA fragmentation [5]. In a multicellular organism, apoptosis tightly controls cell number and tissue size during many physiological developmental stages. However, suppression inapoptotic mechanism may lead to uncontrolled cell proliferation that subsequently converts normal cell to cancerous cell [6]. Lack of apoptosis is one of the hallmarks of cancer. Chemotherapeutic agents targeting apoptotic pathways may be considered as a potential target for treating cancer [7]

Inflammation is body's first line of defense against external agents and pathogens. However, chronic inflammation is one of the underlying factors for development of many chronic diseases including cancer, obesity [8]. Cyclooxygenase-2 (COX-2) is an inducible enzyme that is expressed mainly in inflammatory cells including macrophages and mast cells [9] [10]. Products formed due to the action of COX-2 have key role in influencing the development of cancer [11]. COX-2 is up regulated in many cancer types including carcinomas of the colon, breast, lung, pancreas, esophagus, and head and neck [12] [13]. Hence inhibiting COX-2 is one of the potential targets for treating cancer [14].

Many epidemiological studies [15] [16] have proved the inverse association between consumption of fruits and vegetable and progression of chronic diseases such as cancer. Polyphenols are the major class of bioactive compounds found in many plant foods. They may possess antioxidant [17], anti-cancer [18] and anti-inflammatory [19] properties. Due to which the research in molecular mechanisms of their anti-cancer properties is increasing rapidly.

Carica papaya L. belongs to the family of Caricaceae. It is an herbaceous plant that grows in tropical and subtropical countries [20]. Papaya fruits contain vitamins, minerals, enzymes, proteins, carotenoids and polyphenols [21] [22]. Ripe papaya is most commonly consumed as fresh fruit whereas green papaya as vegetable usually after cooking or boiling [23]. Not only fruits, papaya peels are also a good source of polyphenols, however, they are often discarded in to the surroundings and they constitute 20% - 25% of fruit weight. There is an increasing interest in the isolation of polyphenolic compounds from byproducts obtained during fruit and vegetable processing. Hence, in this study we prepared polyphenol extracts from papaya peel (PP) with the aim of exploiting potential antioxidant and anticancer properties of PP extracts.

2. Materials and Methods

Fresh mature papaya fruits (variety Caribbean red) were obtained from a local health food store, Huntsville, AL. The fruits were washed and exocarp was peeled using potato peeler. PP were freeze dried (VirTis Genesis 35L Sp Scientific, Warminster, PA) and crushed into a fine powder using a laboratory blender prior to extraction.

2.1. Determination of Polyphenols and Flavonoids in PP Extracts

About 0.5 g of PP powder was homogenized with 20 ml of methanol: water (4:1 v/v) and sonicated for 30 min at 30˚C in an ultrasonic bath (Branson Ultrasonic Corporation IC1216-25-12). Extracts were centrifuged at 12,000× g for 15 min at 4˚C (Thermo Scientific, Sorvall legend XTR, Waltham, MA) then filtered through Whatman number 1 paper. For alkaline hydrolysis 10 mL of 4 M NaOH was added to the extracts followed by incubating in dark at room temperature for 4 h. After incubation, pH of the extracts was adjusted to 2.0 with 4 M HCl. Acidified solution was then extracted twice with 20 ml ethyl acetate. Organic phases were collected using a separating funnel. The filtrate was evaporated to dryness using rotary evaporator (Rotavapor R-215 Bushi, Zurich, Switzerland) at 40˚C under reduced pressure. Extracts were re-suspended in 10 mL of methanol: water (4:1 v/v) and stored at −80˚C until use [24]. For the cell culture studies, extracts were re-suspended in PBS.

Papaya peel extracts were filtered through 0.45 µm Whatmann filter and 20 µL of sample was injected into into HPLC (Shimadzu LC-20 AD Prominence, Maryland USA) with biobasic 18 column (Thermoelectron Corporation, 250 × 4.6, 5 µm). For mobile phases, 1% glacial acetic acid in water (solvent A) and acetonitrile: water:

glacial acetic acid (50%:49%:1%) (solvent B) was used and gradient was as follows: 0 min, 10% B; 40 min, 50% A, 45 min 10% B. The flow rate was 0.8 ml/min. Gallic acid was determined at 280 nm whereas, caffeic acid, *p*-coumaric acid, ferulic acid and quercetin were analyzed at 320 nm with reference to their respective standards.

Total polyphenolic [25] and flavonoid content [26] in PP extracts was determined and results were expressed as gallic acid equivalents and catechin equivalents respectively.

2.2. Determination of *In-Vitro* Antioxidant Activities of PP Extracts

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability was done according to [27] with slight modifications. Briefly, 210 µL of DPPH (0.1 mM) was added to 40 µL of samples and blank. The absorbance was recorded at 517 nm for 90 min at 30 min interval. Percentage of DPPH inhibition was calculated with respect to blank.

Ferric reducing antioxidant potential (FRAP) was determined according to [28] with slight modifications using $FeSO_4 \cdot 7H_2O$ standard. Reagent A: 300 mM acetate buffer, pH 3.6, reagent B: 10 mM TPTZ (2,4,6-tripyridyl-*s*-triazine) in 40 mM HCl and reagent C: 20 mM $FeCl_3 \cdot 6H_2O$ were prepared. The FRAP reagent was prepared by mixing reagents A:B:C in 10:1:1 just before use. Approximately 30 µL of FRAP reagent was added to 10 µL of standards and samples. The absorbance was recorded at 593 nm for 6 min at 1 min interval.

Trolox equivalent antioxidant capacity (TEAC) was determined according to [29]. ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical was generated by dissolving 7 mM ABTS and 2.45 mM potassium persulfate solutions and left at room temperature in dark for 12 - 16 h. Before use ABTS radical was diluted with ethanol to obtain absorbance of 0.7 ± 0.025 at 734 nm. About 290 µL of diluted $ABTS^+$ was added to 10 µL of trolox standard, and samples. Absorbance was read at 734 nm for 6 min.

Oxygen radical absorbance capacity (ORAC) was performed according to [30] [31]. Fluorescein sodium salt (60 nM) and 153 mM AAPH (2,2-azinobis(2-amidinopropane)dichloride) were freshly prepared by diluting with 75 mM phosphate buffer. About 25 µL of blank, trolox standard and samples was added to 250 µL of fluorescein and incubated at 37˚C for 10 min. After 10 min of incubation 25 µL of AAPH solution was rapidly added and fluorescence was read at λ excitation-485 nm and λ emission-520 nm for 60 min at 1 min interval using microplate reader (Biotek, Biotech instrument, Inc, Vermont, USA).

2.3. Cell Culture

Human hepatoma cell lines (HepG2) were obtained from ATCC (American Type Culture Collection, Manassas, VA). Cells were maintained in DMEM supplemented with 10% FBS, 100 µl/ml of streptomycin and penicillin at 37˚C in a humidified 5% CO_2 atmosphere. Cells were treated with different concentrations (50, 100, 250, 500 µg/ml) of PP extracts for 24 h. Following which below assays were performed.

2.3.1. Cytotoxicity and Cell Viability

The effect of PP extracts on cell cytotoxicity was determined by lactate dehydrogenase (LDH) assay kit (Roche life sciences, Indianapolis, IN) according to manufacturer's instructions. Cytotoxicity was expressed as percentage relative to control cells.

The effect of PP extracts on cell viability was determined using Abnova MTT (3-[4, 5-dimethyl thiazol-2yl]-2, 5-diphenyl tetrazoliumbromide) cell viability assay kit (Walnut, CA). Cell viability was expressed as percentage relative to control cells.

2.3.2. Caspase-3 Activity and Cellular DNA Fragmentation

The effect of PP extracts on caspase-3 activity magic red caspase 3/7 detection kit (ImmunoChemistry Technologies, Bloomington, MN) and cellular DNA fragmentation was determined (Cell death detection ELISA kit, Roche diagnostics, Indianapolis IN) according to manufacturer's instructions.

2.3.3. Cellular Antioxidant Enzyme Activities

After treating cells with PP extracts for 24 h, oxidative stress was induced by adding 500 µM H_2O_2 for another 2 h at 37˚C. Cells were then lysed in ice cold solubilization buffer and supernatant was collected by centrifuging at 10,000 g for 15 min at 4˚C. SOD, CAT, GPx, GR activities and GSH levels in cell lysates were measured using Cayman Chemicals assay kits (Ann Arbor, MI).

2.3.4. COX-2 Activity

The effect of PP extracts on COX-2 activity was determined (Cell-based ELISA assay kit, R & D systems, Minneapolis, MN) according to the manufacture instructions.

2.4. Statistical Analysis

All experiments were performed in triplicates and the data were recorded as means ± SD. One way ANOVA was performed using SAS 9.2 version. Means separation was done by t test and $p \leq 0.05$ was regarded as significant.

3. Results

3.1. Polyphenols and Flavonoid Content in PP Extracts

Total polyphenols and flavonoids in PP extracts were 4.79 and 2.38 mg/g dry weight (DW). Major polyphenols identified in PP extracts were gallic acid, caffeic acid, *p*-coumaric acid, ferulic acid and quercetin and their retention times were 4.84, 7.99, 12.04, 16.73 and 39.15 min, respectively (**Figure 1**). Quantity of polyphenols in PP were ferulic acid (95.46 ± 0.41 µg/g DW), *p*-coumaric acid (38.16 ± 0.26 µg/g DW), caffeic acid (29.28 ± 0.07 µg/g DW) and gallic acid (18.06 ± 0.83 µg/g DW). Quercetin (3.17 ± 0.18 µg/g DW) was the only flavonoid quantified in this study.

3.2. *In-Vitro* Antioxidant Activities of PP Extracts

Antioxidant activity of PP extracts was measured by DPPH, FRAP, TEAC and ORAC assays and the results are expressed in **Table 1**.

3.3. Cell Culture

3.3.1. Cytotoxicity and Cell Viability

Increasing in concentration of extracts significantly ($p \leq 0.05$) increased LDH release (**Figure 2(a)**). Percentage of cytotoxicity in cells treated with 50, 100, 250 and 500 µg/ml of PP extracts was 6.82, 10.15, 16.68 and 23.87, respectively. Similarly, increasing in extract concentration lowered cell viability, however, percentage of viable cells did not differ significantly ($p > 0.05$) in cells treated with 50, 100 and 250 µg/ml (**Figure 2(b)**). In cells

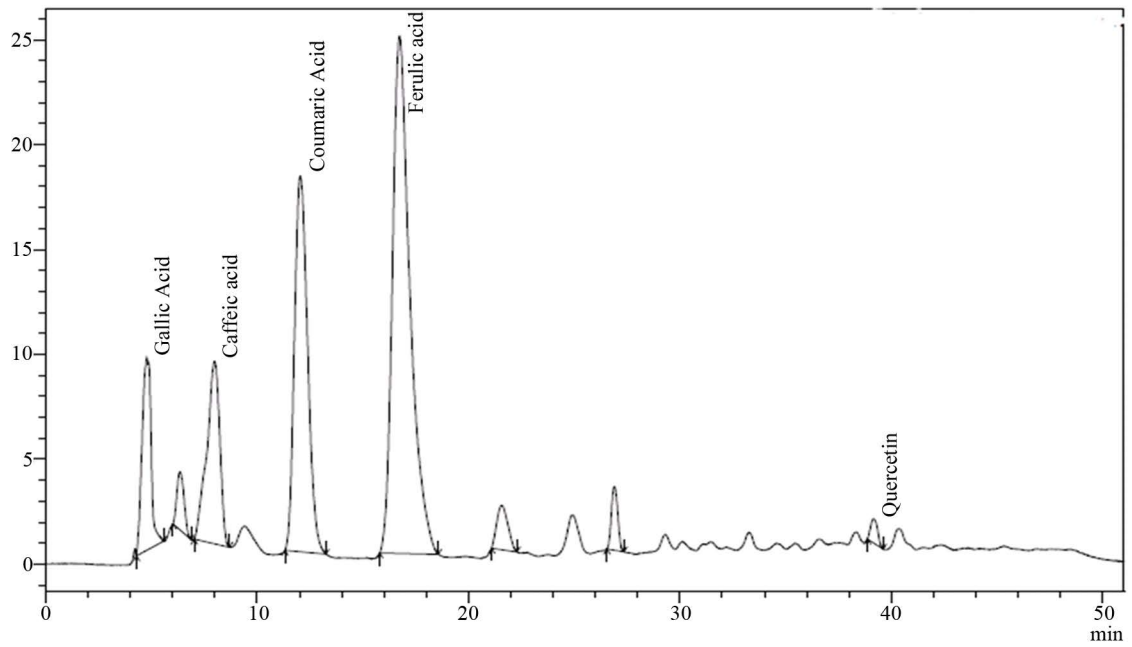
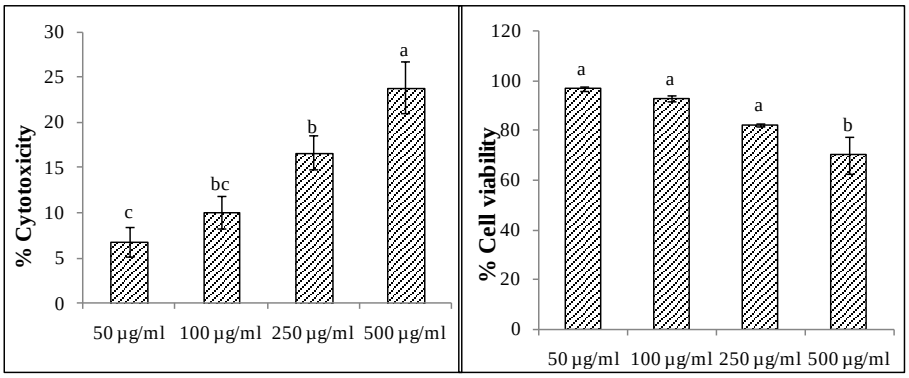


Figure 1. HPLC chromatograph of individual polyphenols and flavonoids in PP extracts.

Table 1. *In-vitro* antioxidant activities of papaya peel extracts.

Antioxidant potential	PP extracts
DPPH (IC_{50} mg/ml)	8.33
FRAP (μM $FeSO_4$/g DW)	31.86 ± 0.1
TEAC (mM TE/g DW)	14.56 ± 0.74
ORAC (mM TE/g DW)	30.88 ± 2.54

Values are means ± SD. Abbreviations: PP—papaya peel, DPPH-2,2 diphenyl 1-picryl hydrazyl, FRAP—ferric reducing antioxidant potential, TEAC—trolox equivalents antioxidant capacity, ORAC—oxygen radical absorbance capacity, TE—trolox equivalents, DW—dry weight.



(a) (b)

Figure 2. Papaya peel extracts on (a) Cytotoxicity, (b) Cell viability in HepG2 values are means ± SD. Bars with different data labels differ significantly at $p \leq 0.05$.

treated with 50, 100, 250 and 500 µg/ml of PP extracts, 97%, 93%, 89% and 70% of cells were viable respectively.

3.3.2. Caspase-3 Activity and Cellular DNA Fragmentation

Dose dependent increase in caspase-3 activity was observed in cells treated with PP. Caspase-3 activity in cells treated with 500 µg/ml peel extracts was 1.3, 1.2 and 1.1 folds higher compared to cells treated with 50, 100 ad 250 µg/ml peel extracts respectively (**Table 2**). Cellular DNA fragmentation was significantly higher ($p \leq 0.05$) in cells treated with 500 µg/ml PP extracts, however, it did not differ significantly ($p > 0.05$) among cells treated with 50, 100 and 250 µg/ml (**Table 2**).

3.3.3. Cellular Antioxidant Enzyme Activities

SOD, CAT, GPx, GR activities and GSH levels in H_2O_2 treated cells was 3.1, 1.46, 2.87, 1.34 and 1.32 folds lower ($p \leq 0.05$) compared to control. However, treating cells with PP extracts significantly ($p \leq 0.05$) enhanced antioxidant enzyme activities (**Table 3**).

Dose dependent increase in SOD activity was observed in cells treated with PP extracts. SOD activity in cells treated with 50, 100, 250 and 500 µg/ml was 5.95, 6.04, 6.5 and 7.34 fold higher compared to H_2O_2 treated cells. CAT activity was significantly ($p \leq 0.05$) higher in cells treated with 100 µg/ml PP extracts, however, did not differ significantly ($p > 0.05$) among the cells treated with 50, 250 and 500 µg/ml peel extracts. CAT activity in cells treated with 100 µg/ml was 3.02 folds higher compared to H_2O_2 treated.

GSH levels in cells treated with papaya peel extracts was significantly ($p \leq 0.05$) higher compared to control and H_2O_2 treated cells, however, did not differ significantly ($p > 0.05$) in cells treated with different concentrations of PP extracts. GPx activity in cells treated with 50 µg/ml did not differ significantly ($p > 0.05$) from control and H_2O_2 treated cells, however, increasing concentration of extracts, significantly ($p \leq 0.05$) increased GPx activity. GPx activity in cells treated with 500 µg/ml was 5.05 folds higher compared to H_2O_2 treated cells. GR activity was significantly ($p \leq 0.05$) higher in cells treated with 250 µg/ml followed by 100 µg/ml. GR activity in cells treated with 50 and 500 µg/ml did not differ significantly from H_2O_2 treated cells. GR activity in cells

Table 2. Caspase-3 activity and DNA fragmentation in cells treated with papaya peel extracts.

Papaya peel extracts	Caspase-3 activity*	Cellular DNA fragmentation (Enrichment factor)
50 µg/ml	0.97 ± 0.02^{d}	1.01 ± 0.06^{z}
100 µg/ml	1.06 ± 0.03^{c}	1.06 ± 0.03^{yz}
250 µg/ml	1.18 ± 0102^{b}	1.12 ± 0.03^{xy}
500 µg/ml	1.27 ± 0.04^{a}	1.34 ± 0.10^{x}

Values are means ± SD. Columns and rows within an assay with different data labels differ significantly at $p \leq 0.05$. *Folds respect to control.

Table 3. Superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione levels in cells treated with papaya peel extracts.

	SOD (U/ mg protein)	CAT (nmol/min/mg protein)	GSH (µM GSH)	GPx (nmol/min/mg protein)	GR (nmol/min/mg protein)
Control	1.36 ± 0.01^{d}	90.17 ± 1.23^{c}	10.35 ± 0.97^{b}	9.95 ± 0.45^{cd}	31.32 ± 0.13^{ab}
H_2O_2	0.44 ± 0.01^{e}	61.84 ± 1.17^{d}	7.85 ± 0.02^{c}	3.46 ± 0.35^{e}	23.44 ± 0.22^{c}
50 µg/ml PP	2.62 ± 0.02^{c}	172.88 ± 1.43^{b}	16.84 ± 0.02^{a}	7.39 ± 0.36^{de}	23.68 ± 1.09^{c}
100 µg/ml PP	2.66 ± 0.06^{c}	186.94 ± 1.44^{a}	17.72 ± 0.66^{a}	13.55 ± 1.33^{bc}	29.16 ± 2.24^{b}
250 µg/ml PP	2.87 ± 0.02^{b}	169.56 ± 1.12^{b}	17.78 ± 0.06^{a}	14.34 ± 1.05^{ab}	33.43 ± 1.53^{a}
500 µg/ml PP	3.23 ± 0.01^{a}	161.43 ± 7.43^{b}	16.63 ± 0.19^{a}	17.49 ± 1.58^{a}	24.89 ± 1.29^{c}

Values are means ± SD. Means with superscript without a common letter in a column differ significantly at $p \leq 0.05$. PP—papaya peel extracts.

treated with 250 µg/ml was 1.42 folds higher compared to H_2O_2 treated cells. Overall, peel extracts were effective in inducing in-vitro antioxidants activity in oxidative stress induced cells.

3.3.3. COX-2 Activity

COX-2 activity significantly was lowered ($p \leq 0.05$) by treating cells with PP extracts. COX-2 activity in cells treated with 50, 100, 250 and 500 µg/ml was 3.4, 2.7, 1.93 and 1.9 fold lower compared to control cells (**Figure 3**).

4. Discussions

In plants, polyphenols play major role in many physiological functions such as protecting from pathogens, predators, UV radiations, abiotic stress and oxidative stress [32] [33]. Due which majority of polyphenols are found in the exocarps [34]. Few polyphenols identified in maradol papaya exocarps were gallic acid, caffeic acid, ferulic acid, quercetin, myricetin and kaempferol [35]. Ferulic acid (277.49 - 186.63 mg/100 g DW), *p*-coumaric acid (229.58 - 135.64 mg/100 g DW) and caffeic acid (175.50 - 112.88 mg/100 g DW) in mardol papaya exocarps [22]. Differences in polyphenolic content reported in this study might be due to varietal difference or stage of maturity as polyphenolic content decreases as fruit ripens [36].

There are limited studies on quantifying the antioxidant potential of PP extracts, however, *in-vitro* antioxidant potential of PP extracts may be due to the presence of polyphenols. Many earlier studies [37]-[39] have quantified the antioxidant potential of polyphenols. The mechanism of action of polyphenols as antioxidants is complex. Due to which multiple antioxidant assays that differ in reaction mechanisms and experimental conditions were performed in this study to obtain comprehensive understanding of the antioxidant potential of PP polyphenols. The violet colored DPPH radical is reduced by polyphenols to form colorless DPPH by hydrogen or electron transfer [27]. FRAP of polyphenols involves the reduction of ferric to ferrous by electron transfer under acidic conditions [28]. TEAC of the polyphenols is to reduce green colored $ABTS^{+}$ to colorless ABTS by hydrogen or electron transfer [29]. ORAC was performed to determine the peroxyl radical scavenging ability of antioxidants by hydrogen atom transfer under acidic conditions [30] [31].

Chemoprevention is the utilization of chemically active compounds to reverse, suppress or prevent the progression of cancer [40]. Due to the toxicity issues associated with current chemotherapeutic drugs there is an

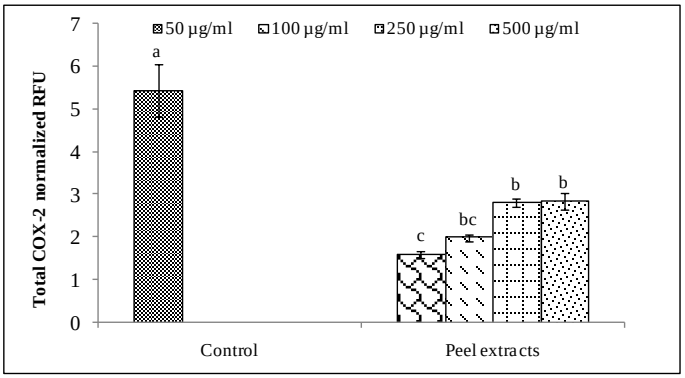


Figure 3. COX-2 activity in HepG2 cells treated with treated with papaya peel extracts Values are means ± SD. Bars with different data labels differ significantly at $p \leq 0.05$.

increasing interest in dietary phytochemicals. The preventive mechanisms of tumor promotion by phytochemcials may by regulation of cell cycle [41], increased antioxidant [41], anti-inflammatory activity [19], lowering cell proliferation and inducing apoptosis [42] [43].

Induction of cytotoxicity by PP extracts was determined by measuring LDH release. LDH is present in most of cells and any damage to the cell membrane causes its release in to the cell culture media [44]. Cell viability assay is dependent on the conversion of yellow colored MTT to purple color formazan crystal by mitochondrial dehydrogenases in the living cells [45]. Dose dependent increase in LDH release by PP extracts correlated with lowered cell viability. Inducing cytotoxicity and lowering cell viability by PP extracts may be due to the presence of polyphenols. Earlier studies [46] [47] have also determined lowered cell viability by polyphenols.

Cancer cell do not undergo apoptosis. Induction of apoptosis is considered as one of the hallmark of cancer [48]. In the cascade of apoptosis, caspase-3 is the effector protein. Activated caspase-3 finally initiates DNA fragmentation [49]. Increasing concentration of PP extracts increased caspase-3 activity, indicating that PP extracts lowered cell viability by initiating apoptosis. This is also supported by increased DNA fragmentation in cells treated with PP extracts. Apoptotic activity of PP extracts may be due to the presence of polyphenols. Some earlier studies [50] [51] have also demonstrated apoptotic properties of polyphenols.

In a healthy human being free radicals are neutralized by various endogenous antioxidants. SOD destroys highly reactive superoxide anions to oxygen and less reactive hydrogen peroxide. Peroxides are further neutralized by CAT or GPx in to water and oxygen [52]-[54]. Collectively, these antioxidant enzymes provide first line of defense to the cells against oxidative stress. H_2O_2 is the most abundantly generated ROS in aerobic organisms. H_2O_2 can be readily transported through lipid bilayer. It initiates Fenton reaction by reacting with metal ions to form extremely toxic hydroxyl radicals and cause oxidative stress [55]. Hence H_2O_2 was used in this study to induce oxidative stress.

Reduction in endogenous antioxidant enzyme activities in H_2O_2 induced cells might be due to the over production of free radicals. An earlier [56] have also reported the reduction in cellular antioxidant enzyme activities by inducing oxidative stress. Induction of SOD, CAT, GPx, GSH and GR activities in cells treated with PP extracts indicates the antioxidant activity of the extracts. Protective effect of PP extracts against oxidative stress may be due to the presence of polyphenols. Some possible mechanisms of antioxidant activity of PP extracts are: by scavenging free radicals or by chelating metal ions thus inhibiting Fenton reactions or by enhancing the activity of genes involved in the expression of antioxidant enzymes. SOD, CAT, GPx, GSH and GSH/GSSG activities were enhanced by phenolic acids such as gallic acid, ferulic acid and p-coumaric acid in rats [57].

COX-2 inhibitory effect of PP extracts may be due to i) lowered generation of free radicals in treatment groups as oxidation induce inflammation [58] or ii) Inhibition of COX-2 activity by phytochemicals in the extracts [59]. In this regard, molecular mechanisms by which extracts lowered COX-2 activity needs to be evaluated.

5. Conclusion

In conclusion, PP exhibited *in-vitro* antioxidant activities and also offered protection to cells from oxidative

damage. Moreover, PP also act as anti-cancer agent by: inducing antioxidant enzymes, lowering cancer cell viability, inducing apoptosis and by lowering COX-2 activity.

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