

Antioxidant effect of Aqueous *Carica papaya* seeds extract

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Introduction

Nature has been recognized as a rich source of medicinal compounds for hundreds to thousands of years. Today a wide range of drugs which represent the cornerstones of pharmaceutical care, are either natural products or have been derived from them. In addition to plants biologically active compounds have been isolated from a wide variety of animal sources, ranging from marine organisms, like sponges (Müller et al., 2004;; Hiort et al., 2005) and sea cucumber (Maier et al., 2001), besides microbes (Strobel, 2002). The introduction of Biotechnology in every aspect of natural products research has dramatically facilitated the potential applications by the industry. Biotechnology is also expected to play a major role in the production of natural products through biosynthesis and bioengineering which will further reduce the dependence on a large amount of plant samples and hence reduce the pressure of depleting the biogenetic resources in our tropical forests.

The aim of this research been the *in vitro* study of the biological effects of bioactive compounds from the aqueous seed extract of *Carica papaya*, using two different human cell lines, human Myeloid Leukaemia cells (HL60), which grow in suspension and human Detroit 550 cell lines, which grow attached to the surface, under normal and oxidative stress conditions. My work is focused on the expected antioxidant effect of seed extracts, and the possible influence on the production of heat shock and Bcl₂ protein *Carica papaya*, a tropical plant which grow in all tropical countries and many sub-tropical regions of the world, and it is largely used in tropical folk medicines. It contains a number of bioactive compounds (benzylisothiocyanate, alkaloids, flavinoids) which have biological activities e.g (anthelmintic (Kermanshai et al., 2001), antimicrobial, antitumor (Banerjee, 2002), and antiparasitic (Hounzangbe-Adote et al 2005)

Material and methods

Aqueous *Carica papaya* seeds extract was prepared using the traditional method. 10 grams of washed and dry seeds were taken and grinded, soaked in sterile distilled water for 24 h at room temperature (20-25°C), then filtered, sterilized by using millipore filter 0.22 µm, and stored in the freezer at -20°C ready to used. All the work with cell lines were done under a maximum sterile conditions using a microbiological safety cabinet. Apoptosis was induced for both cell lines (HL60 and Detroit 550) either by 1mM of hydrogen peroxide (H₂O₂), by 10 µg/ml puromycin (PMC), or by 10 mM of Cycloheximide. The biological activity of aqueous seeds extract of *Carica papaya* on cell lines which includes toxicity, effects on apoptosis, necrosis and proliferation, were determine using different techniques include cytotoxicity test : MTT, Light Microscopy: Hematoxylin eosin, and Hoechst 33342, Electron Microscopy: SEM and TEM spectrophotometric analysis for quantitative measurement of catalase enzyme, ELISA method for quantitative determination of cytochrome c, protein electrophoresis (SDS-PAGE) and Western blotting, using specific antibodies against HSP and Bcl₂.

Results

Our result show that *papaya* seeds extract has no toxicity on both cell lines at the concentrations used, no induction to necrosis or proliferation was found, but it exerts a decrement effect on spontaneous apoptosis especially at the concentration of 1 mg/ml. The anti apoptotic activity of extract was also studied on drug induced apoptosis using apoptotic

inducers (H₂O₂, Cycloheximide and Puromycin) that trigger apoptosis *via* different mechanism. Our results show that *papaya* seeds extract increases mitochondrial activity and decreases apoptosis but only oxidative stress induced apoptosis, thus suggesting an evident antioxidant activity. Effect of extract on catalase enzyme activity was studied due to the important role as antioxidant of this enzyme. We found that the antioxidant activity not exerted through increasing catalase activity, that conversely decreased. The antioxidant activity of seeds extract was compared with the well known antioxidant activity of vitamin C (antioxidant compound). We found that the antioxidant activity of vitamin C and *Carica papaya* seeds extract is high when added during the recovery after oxidative stress for vitamin C and during oxidative stress for *Carica papaya* seeds extract. Since proto-oncogene Bcl2 and stress protective genes i.e. heat shock protein 70 have been found to protect cells against apoptosis induced by a wide variety of stimuli, effect of *papaya* seeds extract on the expression of these gene was studied. We found that *papaya* seeds extract decreases the expression of these genes under normal and stress condition as a results of its antioxidant activity, and we found also that *papaya* seeds extract decrease the releasing of cytochrome c from mitochondria to cytosol (cytochrome c when released from mitochondria play important role in initiation of apoptosis, especially oxidative stress induced apoptosis). Finally we can conclude that *Carica papaya* seeds extract has antiapoptotic effect due to its antioxidant activity. It decreases the expression of antiapoptotic (Bcl₂) and cell protective (heat shock protein) genes, probably due to the ability of the extract to improve the internal healthy conditions of cells against free radical, therefore, reducing the need of production of these proteins

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