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Effect of *Carica papaya* seed extracts on lymphocyte proliferation

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ABSTRACT: The present study was carried out to investigate the effect of hot and cold extracts of *Carica papaya* seeds (that contain benzyl isothiocyanate as one of its chief bioactive compound) on lymphocyte proliferation. Lymphocytes were cultured at the concentration: 500, 1000, 2000 and 3000µg/ml (PPM) for 24 and 48h. results indicated that the extracts showed no adverse toxic effect to the lymphocytes at all, the dose regimes according to the trypan blue permeability assay and visual inspection using the optimal microscope. However, there was a statistically significant increase in the number of cells treated with 1000µg and 2000µg/ml of the hot extract and 2000µg and 3000µg/ml of the cold extract in the 24hr. regime.

Key Words: Lymphocytes, *Carica papaya*, proliferation, benzyl isothiocyanate.

Introduction

The diet, particularly fruit, vegetables, nuts and seeds provide a rich source of antioxidant vitamins and other phytochemicals with antioxidant characteristics, which are important exogenous source of compounds, able to augment cellular responses to oxidative stress (1). Isothiocyanates (ITC), particularly BenzylIsothiocyanate (BITC) has been listed as one of the chemical agents capable of enhancing cellular (indirect) antioxidants (2), thereby protecting cells against a wide variety of oxidative stresses. A number of ITCs block chemical carcinogenesis in a variety of animal models by inhibiting phase II enzymes that accelerate the inactivation of carcinogens (3), thus protecting these animal models against cellular insults. Much evidence suggests that Isothiocyanates are powerful electrophiles, a property attributable to the central carbon atom of the –N=C=S group, which reacts readily with sulphur -, nitrogen – and oxygen – base nucleophiles (2).

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BITC is formed from Benzylglucosinolate by the action of the enzyme myrosinase (thioglucosidase) by crushing grinding, chewing or damaging the seeds of *carica papaya* (4,5,6,7). Literature is rife with several reports on naturally occurring and synthetic Isothiocyanates. These include, BITC induced glutathione S-transferase (GST) in rat liver epithehal RL34 cells (8); BITC induced apoptosis through a mitochondrial redox-sensitive mechanism (9). ITCs have been reported to inhibit
Rat lung, oesophagus, mammary gland, liver, small intestine, colon and bladder tunorigenensis (10,11,12,13,14). As part of our ongoing studies on BITC, we have investigated the effects of *Carica papaya* seed (CPS) extracts on proliferation in lymphocytes.

Materials and Methods

Plant material and preparation of extract.

Fresh green (unripe) fruit of *Carica papaya* (caricaceae) Linn. seeds of the solo variety (identified by Dr. Francesco Minonne, Botanical laboratory, department of Biological Sciences, University of Leece, Itally) were used in the present study. The seeds were oven dried and finely pulverized. Hot aqueous extract of the seeds was prepared by suspending 10g of the powered material in 500ml of boiling (distilled) water and left for 8 hours at room temperature. The cold extract was prepared by grinding fresh seeds with mortar and pestle. 10g of the pounded seed material was then added to 500ml of distilled water at room temperature, after which the debris was removed by centrifugation for 30 mins. Both extract suspension were filtered through a sterile Nalgene® disposable filter ware and then filter sterilized using 0.22µm filter. The final solution was stored in the refrigerator.

Cell Culture

Normal lymphocytes were isolated from human blood (15). The lymphocytes were maintained in RPMI supplemented with 10% fetal Bovine Serum (FBS), 50 units/ml Penicillin, 50µg (0.05mg)/ml Streptomycin, 50µg/ml (0.05mg/ml) of Nistadine and L-glutamine (2mM). The cell cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2. Aseptic techniques were applied throughout and the experiment was carried out in a laminar airflow cabinet.

Viability study

Viability studies were carried out at the beginning and end of the experiment with trypan blue stain. During thgis period, all cells excluded of the stain were counted within the Neubauers chamber. The data was expressed as the means ± SEM.

Statistical Analysis

Means ± SEM of value obtained for all groups were analyzed for statistically significant differences using the ANOVA. A P-value of <0.05 indicates significant difference between treatment group means.

Results

Cytotoxic effect

No adverse cytotoxic effect was observed in the cells at all the dose regime tested. However, at the 24h regime, the mortality rate was much lower in the cells treated with the extracts of CPS than the control cells. At the 48h regime however, in both extracts there was a striking increase in mortality at 500µg/ml.

It is interesting to note that the cold extract at both 24 and 48h, the mortality rate in the cells treated with 2000µg/ml was 0% (Fig. 3 and 4).

Proliferative effect

At the 24h regime, the rate of proliferation was statistically significant in cells treated with 1000µg/ml and 2000µg/ml of the hot extract and 2000µg/ml and 3000µg/ml of the cold extract when compared with the control ($P<0.05$). At the 48h regime however, there was no statistically significant increase in growth in the cells at all dose regimen tested with both extracts.

Discussion

Cell proliferation is a complex process which is under the control of cellular signal transduction pathways. The primary cellular target of ITCs is still unknown; however, there is clear evidence that the intracellular GSH level, regulating the redox state of the cell, may play an important role in the initiation of the cellular responses to numerous compounds (16). This agrees with Lindsay et al., (1), who reported that any alteration in the redox state of an organelle can act as a powerful trigger for signal transduction, gene transcription and post-translational modification of proteins.

It has been reported that the increase of reactive oxygen species (ROS) following various external stimuli at low concentration may function as cellular signaling intermediators and be associated with cellular proliferation (17). There is now growing evidence that ROS at low concentration play some physiological roles during activation and proliferation of lymphocytes (18,19). It has also been reported that at least 1h of exposure to BITC was sufficient to evoke the elevation of GST activity. These data suggest that ITCs may induce GST and or other phase II detoxification enzymes through the stress signaling pathway involving oxidative stress and JNK or p38 cascade, similar to other stimuli, including hydrogen peroxide (16), UV light (20), Osmotic stress (21), the DNA-damaging agent (22), inflammatory cytokine (23), and lipid peroxidation products (16).

In both extracts, the effect was most significant at 24h (Fig. 1) suggesting that beyond this time regiment, a plateau is reached. Hence, while the rate of proliferation was statistically significant in cells treated with 1000µg/ml and 2000µg/ml of the hot extract and 2000µg/ml and 3000µg/ml of the cold extract at the 24h regime (Fig. 1), there was no statistically significant increase at the 48h regime (Fig. 2). At all the dose regimes, BITC has been shown to possess no adverse toxicity to cells. However, with the cold extract at the 24h and48h regime, mortality rate in the cells treated with 200µg/ml was 0% (Figs. 3 and 4). It could therefore be suggested from the study that the potent effect of BITC was best demonstrated with the cold extract at the 2000µg/ml dose regime.

Even though our study agrees with the suggestion of Zhang et al., (24) that there might be a relationship between lymphocyte proliferation and ROS production and the report of Iwata et al (25) that thiol (-SH) is involved in the regulation of lymphocyte proliferation, we suggest that since BITC in common with other phase Ii enzymes raise tissue GSH levels, this alteration in the redox state of the cell could act as a trigger for signal transduction, favouring proliferation.

Conclusion

It is still premature to specify precisely, the mode of action of CPS extract because, the cancer preventive or promoting potential, threshold, and target organ of ITCs have to be distinguished in detail, further studies on intracellular oxidative stress and the subsequent events induced by the ITCs are necessary to provide supporting information. However, we report the effect of CPS extracts on lymphocyte proliferation.

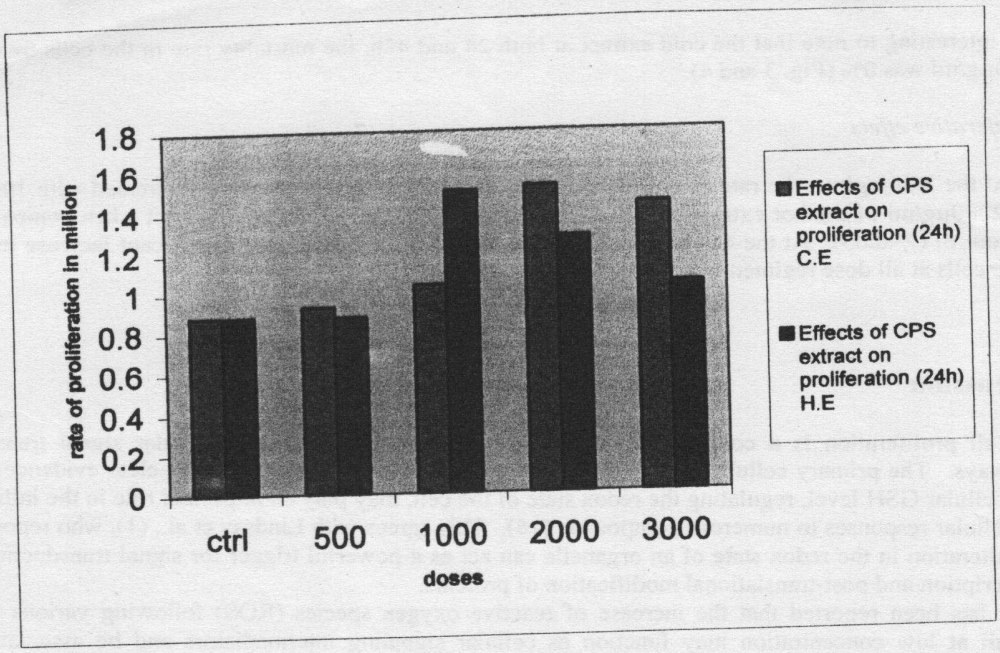

Fig. 1: Effects of CPS extract on proliferation of lymphocytes (24h).

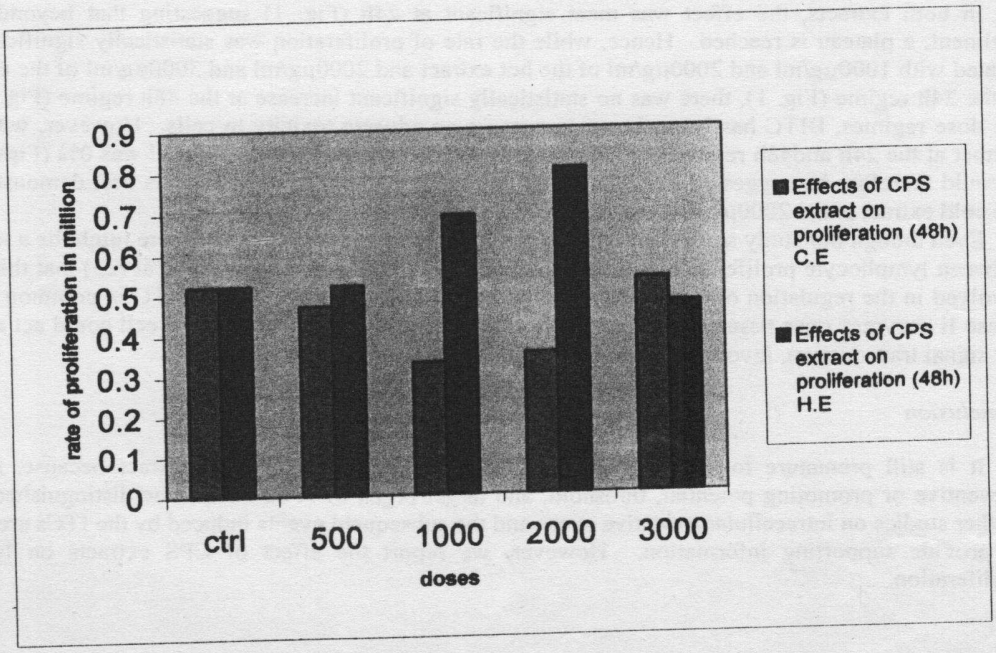

Fig. 2: Effects of CPS extract on proliferation of lymphocytes (48h).

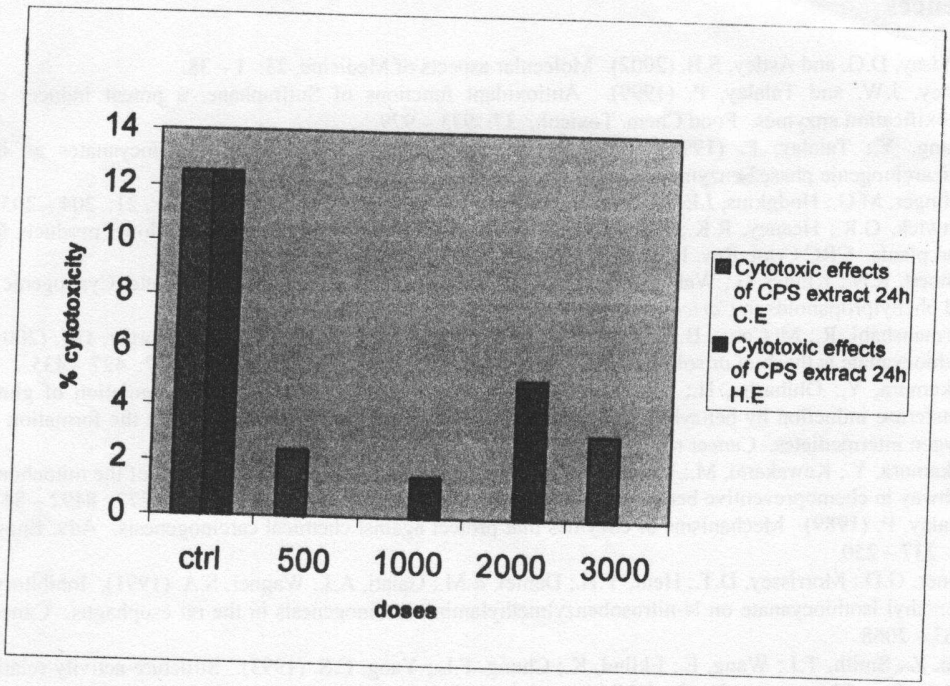

Fig. 3: Cytotoxic effects of CPS extract on lymphocytes (24h).

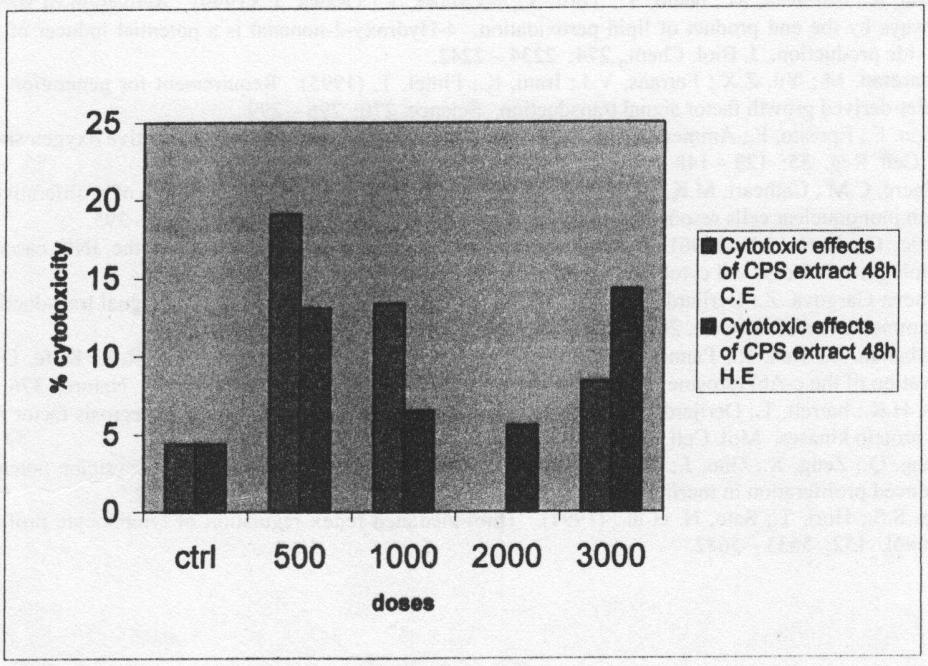

Fig. 4: Cytotoxic effects of CPS extract on lymphocytes (48h).

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