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Comparative Evaluation Of Cytotoxicity Of Carica Papaya Leaf And Seed Extract - A Cell Line Study

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Abstract: Introduction Carica papaya is recognized for its ample therapeutic potential. A range of papaya components including seeds, leaves, fruit, and latex are globally employed for therapeutic purposes, serving to promote health and mitigate various ailments. The literature has also documented the noteworthy anti-inflammatory and antioxidant attributes of Carica papaya. The aim of the present study is to evaluate and compare the cytotoxicity of papaya leaf and seed extract.
Materials and Method Ethanolic extract of various concentrations, ranging from 0.2-1 mg/ml of the papaya leaf and the seed extract were tested in mouse fibroblast, McCoy cells using the MTT colourimetric assay after 48 hours of incubation.
Results C. Papaya leaves and seed extracts had a non-significant effect on cell growth in McCoy cells in a dose-dependent state after 48 hours of treatment. The viability of cells with Papaya leaf extract was significantly higher than that of papaya seed extract ($p<0.05$). The result demonstrated that the treatment of C. papaya extracts in an increasing concentration shows a slight cytotoxic in higher concentration in McCoy, mouse fibroblast cells
Conclusion Carica papaya leaf and seed extract had a non-significant effect on cell growth in McCoy cells in a dose-dependent state after 48 hours of treatment. The papaya leaf extract was significantly better than the seed extract on the basis of the percentage of viable cells in the culture. This study demonstrated the high safety of the papaya extracts to be used as drugs in therapeutic practices.

Keywords: MTT assay, Carica papaya leaf, Carica papaya seed, phytochemicals, fibroblast cell line

1. Introduction

The use of various plant species and their distinct components for the purpose of preventing and treating a variety of disease states, which has been a longstanding cultural practice, has recently been revisited. It could be hypothesized that the lower occurrence of negative effects associated with these drugs, as opposed to their synthetic counterparts, is the reason behind the extensive research in this field (1). These herbal remedies have proven to be as effective as synthetic pharmaceuticals in treating a variety of ailments. The Caricaceae family, which has four genera worldwide. There are four species in India that belong to the genus Carica Linn, with Carica papaya Linn being the most frequently farmed and well-known species. The papaya's leaves, fruits, seeds, and latex are utilised for a variety of industrial and medical processes. Numerous phytochemicals, including vitamins, enzymes, minerals, polysaccharides, proteins, lipids, oils, lectins, sterols, saponins, and flavonoids, are present in papaya extract (2–4) (3,4).

A number of in vitro investigations have been dedicated to examining the capacity of bioactive compounds contained within papaya to regulate immune-inflammatory markers. Papaya is also a promising natural source of antimicrobial agents for potential use in the treatment and prevention of bacterial infections (5,6). The safety and anti-oxidative stress potential of papaya juice have been observed to be comparable to that of the established antioxidant compound α-tocopherol. Papaya extracts are already in use as a therapeutic agent in treating various diseases such as malaria, dermatitis and psoriasis, hypertension, diabetes mellitus, hypercholesterolemia, helminthiasis and various others (7) (8–11) (12–15).

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The assurance of pharmaceutical safety constitutes an indispensable aspect in the process of developing any medication(7). The differential cytotoxic effect of seed or leaf extracts on a variety of cells may depend on differences in the fatty acid, amino acid, phenolic, and flavonoid composition of leaf or seed extracts (16). The aim of the present study is to evaluate and compare the cytotoxicity of papaya leaf and seed extracts using an MTT assay.

2. Materials And Methods

Preparation of Extract

Carica papaya seeds and leaves were procured from the Agriculture Research Farm, SHIATS, Naini, UP. The seeds and leaves were dried for 15 days at room temperature. The seeds and leaves were then ground. Separately, 500mL of ethanol was added to 2 shakers along with 300mg of the powdered seed and leaf. These beakers spent a full day in the shaker. After being cleaned, the extract was boiled for 10 minutes at 25 degrees Celsius.

Cell culture

McCoy mouse fibroblast cells were purchased from National Center for Cell Science (NCCS). The cell lines were maintained and cultured in a CO_2 incubator at 37°C using EMEM (Eagle's Minimum Essential Medium) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. To perform the cytotoxicity assay, cells were trypsinized using 0.25% trypsin-EDTA and seeded into a 96-well plate.

Cell viability assay

In this study, the cytotoxicity of C. papaya extract (Concentration 0.2 – 1 mg/ml) from leaves and seeds was tested in mouse fibroblast, McCoy cells using the MTT colourimetric assay. Cells were seeded into a 96-well plate at a density of 1 X 10^4 cells per well and incubated overnight for cell attachment. The compound was then diluted in dimethyl sulfoxide (DMSO)-EMEM media mixture to obtain concentrations ranging from 0.2 – 1 mg/ml, and added to the wells for 48 hours of incubation in a CO_2 incubator. After treatment, the supernatant in the plate was removed, and the wells were washed with Phosphate Buffered Saline BS buffer. MTT solution was then added to each well and incubated for 1 hour. The formazan crystals were solubilized with DMSO, and the absorbance was measured at 590 nm. The percent cell viability was calculated using a control, and a graph was plotted using GraphPad Prism software.

Statistical Analysis

Statistical analysis was done using the Independent Sample T-test in SPSS Software. Results obtained were expressed as Mean + SE. Significant differences in treatments were accepted at $P < 0.05$.

3. Result

In our study, we evaluated the cytotoxicity of C. Papaya extract (Concentrations of 0.2 – 1 mg/ml) from leaves and seeds against McCoy mouse fibroblast cell lines using MTT colourimetric assay. The results, as shown in Fig 1, indicated that C. Papaya leaves and seed extracts had a non-significant effect on cell growth in McCoy cells in a dose-dependent state after 48 hours of treatment. The viability of cells with Papaya leaf extract was significantly higher than that of papaya seed extract ($p=0.00$). The result demonstrated that the treatment of C. papaya extracts in an increasing concentration shows a slight cytotoxic in higher concentration in McCoy, mouse fibroblast cells (Table 1).

4. Discussion

In dental practices, adherence to biocompatibility standards is a fundamental prerequisite concerning the materials employed in dental treatments. Such adherence requires the materials to fulfil specific criteria to ensure their safe usage within body tissues. These criteria mandate that the material utilized does not pose harm to the pulp and soft tissue and does not contain elements that may incite a widespread response upon diffusion or absorption into the circulatory system. Additionally, the material must lack carcinogenic potential, thereby posing no malignant risk to the treated subject (17). Many plants, although exerting therapeutic benefits, and having been used traditionally for diseases since ancient times, are potentially cytotoxic (18,19). Toxicity evaluation of C. papaya leaves becomes more important as they are not only consumed widely as food but also prepared and used as a traditional medicine.

In the present study, it was reported that both the papaya leaf and seed extract did not exhibit significant cytotoxicity at concentrations varying between 0.2-1 mg/ml at 48 hours incubation period. The percentage of viable cells was significantly higher in papaya leaf extract compared to the seed extract at all therapeutic

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concentrations. This is in agreement with the investigation conducted on the acute toxicity of Carica papaya leaf extract which elicited no substantial harmful consequences at dosages up to 2 g/kg of body weight. This dose is equated to 14 times the amount utilized in conventional therapeutic treatment. Additionally, the notion was put forth that the administration of Carica papaya leaf extract orally, for a duration of 14 days, at concentrations lower than 2 g/kg body weight, did not yield any consequential toxicity or negative effects (20),(21). The variation in the cytotoxicity can be attributed to the variation in the amounts and types of phytochemicals available in the papaya leaf and seed extracts.

The safety profiles of Carica papaya extract may vary depending on the method of extraction employed, specifically either ethanol or aqueous extraction, due to the presence of distinct chemical constituents in each extract. The therapeutic efficacy of papaya seeds and leaves can be attributed to the presence of numerous densely-packed phytochemicals including flavonoids, phytosterols, carotenoids, alkaloids, phenolic compounds, and cyanogenic compounds such as benzyl glucosinolate (22). The advantageous effects of papaya seeds and leaves have been linked to the mitigation of various ailments such as diabetes mellitus, hepatic and renal complications, fertility, hyperglycemia, amoebic dysentery, and more recently, antitumor activities (23)(23,24).
The high therapeutic value of papaya leaf and seed extracts and their minimal cytotoxicity to the fibroblast cell line demonstrated in the present study enables us to understand the high safety index of the extract to be used as a drug in treating several diseases. Furthermore, animal and cell line studies using various extraction media and the effect of individual compounds of the extract can be analyzed to enable further understanding of the therapeutic and cytotoxicity effect of the papaya leaf and seed extracts.

5. Conclusion

Carica papaya leaf and seed extract had a non-significant effect on cell growth in McCoy cells in a dose-dependent state after 48 hours of treatment. The papaya leaf extract was significantly better than the seed extract on the basis of the percentage of viable cells in the culture after 48 hours. This study demonstrated the high safety of the papaya extracts to be used as drugs in therapeutic practices.

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Table 1: Cytotoxicity of Paya leaf and seed extract using MTT Assay

Concentration	Papaya Seed Extract		Papaya leaf Extract	
	Average Optical Density	Percentage of Viable Cells	Average Optical Density	Percentage of Viable Cells
0mg/ml	0.954	100±0.00	0.86867	100±0.00
0.2mg/ml	0.92933	$97.57±0.25_{a}$	0.85847	$98.94±0.24*_{a}$
0.4mg/ml	0.86833	$90.97±0.17_{ab}$	0.82033	$94.66±0.29*_{ab}$
0.6mg/ml	0.797	$83.11±0.49_{abc}$	0.768	$88.58±0.47*_{abc}$
0.8mg/ml	0.662	$68.94±0.55_{abcd}$	0.69867	$80.90±0.45*_{abcd}$
1mg/ml	0.57567	$60.73±0.38_{abcde}$	0.627	$72.72±0.58*_{abcde}$

Inference: Average Optical Density and percentage of viable cells at 48 hours using papaya leaf and seed extract. Subscript ‘a’- significant difference between control and 0.2mg/ml, ‘b’- significant difference between 0.2mg/ml and 0.4mg/ml, ‘c’- significant difference between 0.4mg/ml and 0.6mg/ml, ‘d’ -significant difference

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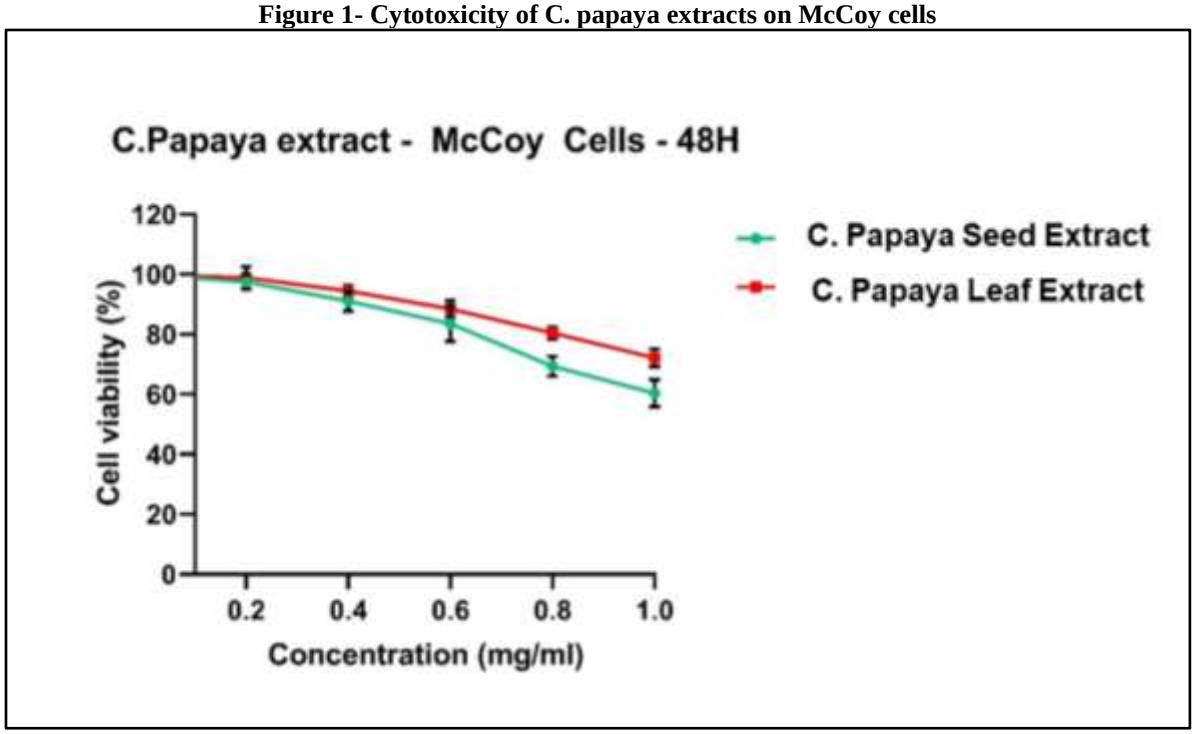
between 0.6mg/ml and 0.8mg/ml, 'e'- significant difference between 0.8mg/ml and 1mg/ml, *- Significant difference between papaya leaf and seed extract. $p<0.05$ is considered significant.

Figure 1- Cytotoxicity of C. papaya extracts on McCoy cells



Inference 1- MTT assay was evaluated to measure the cytotoxicity for C. Papaya extracts (Leaves and seeds) in an increasing concentration of 0.2 – 1 mg/ml treated for 48 hrs in mouse fibroblast McCoy cells. All the results are expressed as mean ± SD and the percent cell viability of all the groups (C. papaya extract) was compared to that of controls. Outcomes were statistically analyzed using GraphPad Prism software.

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