In vitro Cell Cytotoxicity of (*Carica Papaya.,L)* Seed Powder Incorporated Food Product

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Abstract

Papaya *(Carica Papaya Linn.)* is well known for its exceptional nutritional and medicinal properties through the world. The whole papaya plant including its leaves, seeds, ripe and unripe fruits and their juice are used as a traditional medicine. Now a days papaya is considered as a nutraceutical fruit, due to its multi – faceted medicinal properties. The papaya *(Carica Papaya.,L)* seed contains fatty acids, crude protein, benzyl isothiyocynate.It does not contain any heavy metals such as lead and arsenic. Papaya seed powder incorporated choco chip cookies was standardized and formulated in three variations. All the recipe variations were subjected to sensory evaluation by the panel members. The mean score of acceptability of the selected recipes with the incorporation of various level of papaya *(Carica Papaya.,L)* seed powder revealed that the variation 1 (V_1) 5 per cent had the highest level of acceptability among the other variations of the papaya *(Carica Papaya.,L)* seed powder incorporated choco chip cookies. The papaya *(Carica Papaya.,L)* seed powder cookies from variation 1 (V_1) had the highest acceptability in sensory attributes like appearance of (7.8), colour (7.5), flavour (7.8), taste(7.4), texture(7.2) and overall acceptability(7.4) among all the variations. The seed incorporated cho – co chip cookies contained anti-cancer property. It shows that it has an IC – 50 value of 92.37% µg/ml which denoted the anti cancer activity.

Keywords:*(Carica Papaya.,L),* Papaya seed, Antioxidant, Anti –cancer.

Introduction

Cancer is one of the most common causes of death worldwide (Mcguire, 2015).It refers to any one of a large number of disease characterized by the development of abnormal cells that uncontrollably and have the ability to infiltrate and destroy normal body tissue. Cancer often has ability to spread throughout the body. Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020 (Ferlay *et al.,* 2020).The estimated number of incident cases of cancer in India for the year 2022 was found to be 14,61,427 (crude rate:100.4 per 100,000). In India, one in nine people are likely to develop cancer in his/her lifetime (Krishnan *et al*., 2022).

Although there are a lot of drugs and treatments for cancer, they are costly and also have a number of side effects.Papaya (*Carica Papaya Linn*.) is well known for its exceptional nutritional and medicinal properties throughout the world.From the times immemorial,papaya is considered as a nutraceutical fruit, due to its multi – faceted medicinal properties. The prominent medicinal value of papaya include diuretic, anti – hypertension, wound healing, anti – cancer and anti fungal properties (Milind *et al*., 2011).

The papaya *(Carica Papaya.,L)* seed contained a fixed oil composed of myristic, palmitic, stearic, arachidic, behenic and unsaturated fatty acids (Puangsri *et al.*,2011), phospholipids, carpaine, benzylisothiocyanate, benzyl glucosinolate, glucopaeolin(Rossetto *et.al.*,2008).

Organo-sulfur compounds called isothiocyanate are found in papaya. In animal experiments, isothiocyanate protects against cancers of the breast, lung, colon pancreas and prostate, as well as leukemia, and they have the potential to prevent cancer in humans. Isothiocyanate have shown that they are capable of

inhibiting both the formation and development of cancer cells through multiple pathways.

Papaya seed are a good source of phytochemicals. They contain valuable phytochemicals such as phenolics, tocopherols, phytosterols and carotenoids. Phenolics, isothiyocyanates, terpenes, phytosterols, flavonoids, anthroquinones, can scavenge reactive molecules thus protecting the cellular environment against the damaging impacts of oxidative and inflammatory activities of carcinogens (Joachim, 2021). Phenolics, isothiocyanates, terpenes, phytosterols, flavonoids and anthraquinones can scavenge reactive molecules thus protecting the cellular environment against the damaging impacts of oxidative and inflammatory activities of carcinogens (Wong, Olcum and Doan, 2020).

The seed is low in free monosaccharides but sucrose is predominant sugar (75– 0% total sugars) The papaya seed oil and the oil has high fat, carbohydrate and energy values and in same revealed that seed coat possessed high crude fibre, ash and crude protein (Rajapriya, 2019).

The antioxidants present in the papaya seed also reduce liver inflammation (Adrija, 2022).

A naturally and locally available papaya *(Carica Papaya.,L)* seed powder, which has least amount of effects but plays a role in curing diseases have been used to prevent cancer. Hence, the investigator made an attempt to incorporate the Papaya *(Carica Papaya.,L)* seed powder in the choco chip cookies in a preventive and controlling aspect.

Materials and Methods

The study was carried out in two phases. In the first phase of the study, choco chip cookies were prepared and standardized by the incorporation of papaya *(Carica Papaya.,L)* seed powder. Three variations namely V_1, V_2 and V_3 were prepared. The prepared product with different variations were subjected to sensory evaluation to find out the highly accepted level of incorporation. In the next phase of the study, the most accepted product was analysed for its cancer cell cytotoxicity.

SELECTION, PROCUREMENT AND PROCESSING OF PAPAYA *(CARICA PAPAYA.,L)* SEED POWDER MATERIALS:

Selection, procurement and processing of papaya *(Carica Papaya.,L)* Seed Powder:

In the processing of development of the papaya *(Carica Papaya.,L)* seed powder incorporated food product, papaya *(Carica Papaya.,L)* seed powder was used as a main ingredient and wheat flour, cho-co chips and butter were the other ingredients.

Treatment of Papaya *(Carica Papaya.,L)* seed powder:

The papaya *(Carica Papaya.,L)* seeds were purchased for a moderate rate from a local fruit shop and checked for insects, cleansed thoroughly with clean water for removal of dirt and dust. Then, the papaya *(Carica Papaya.,L)* seeds were spread in a dry tray for drying.

Preparation of Papaya *(Carica Papaya.,L)* seed Powder:

The papaya *(Carica Papaya.,L)* seeds were kept in the dry tray left outside in a free area for sun drying for about a duration of 2-3 days. After this, the dried *Papaya (Carica Papaya.,L)* seed were ground into a fine powder which is then ready for the incorporation.

Storage of Papaya *(Carica Papaya.,L)* Seed:

The papaya *(Carica Papaya.,L)* seed powder was stored in a glass bottle and secured with tight cap for the development of choco chip cookies and for the analysis of in vitro cell cytotoxicity.

Procurement of other ingredients

The other ingredients added to prepare the food products such as whole wheat flour, butter, jaggery, choco chips, sugar, cream, were purchased from the local market.

STANDARDISATION AND FORMULATION OF CHOCO CHIP COOKIES INCORPORATED WITH PAPAYA (*CARICA PAPAYA.,L)* SEED POWDER

Choco chip cookies were standardized and formulated as standard (S) without papaya (*Carica Papaya.,L)* seed powder and three different variations namely V_1, V_2 and V_3 with the incorporation of papaya (*Carica Papaya.,L)* seed powder.

- The standard choco chip cookies were prepared butter(7g), jaggery(10g), wheat flour(5g), choco chips(3g)without papaya seed powder.

Preparation of *Carica Papaya Linn*. Seed Powder Choco Chip Cookies:

Beat the butter for 1 minute then added sugar into the bowl

↓

Wheat flour and choco Chips were added into the bowl and beat well to form a dough

↓

Finally added Carica seed powder in the three variations namely(V1(5%), V2(10%) and V3(15%) and beaten for 2 minutes.

↓

Rolled the dough to prepare the shape of cookies

↓

Pre heated the oven at 180 degrees C for 10 minutes and then baked the dough for 10-15minutes

Allowed to cool and then stored in an air tight container

PLATE-1

Figure: 1 *Papaya (Carica Papaya.,L)* Seed Powder Incorporated Choco Chip Cookies

SENSORY EVALUATION AND ACCEPTABILITY OF PAPAYA *(CARICA PAPAYA.,L)* SEED POWDER INCORPORATED CHOCO CHIP COOKIES

The formulated Papaya *(Carica Papaya.,L)* seed powder incorporated choco chip cookies were evaluated for its acceptability against a standard by a tasting panel comprising of 20 members.The nine point hedonic rating scale was used to evaluate the sensory attributes like colour, appearance, flavours, taste, texture and overall acceptability.

ANALYSIS OF IN VITRO ANTI – CANCER ACTIVITY OF THE PAPAYA *(CARICA PAPAYA.,L)* SEED INCORPORATED CHOCO CHIP COOKIES
MTT ASSAY FOR CELL CYTOTOXICITY
PROCEDURE
Cell culture

MG63 cells (Human Osteoblast cells) were purchased from NCCS, Pune and were cultured in liquid medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 ug/ml penicillin, and 100 µg/ml streptomycin and maintained under an atmosphere of 5% CO_2 at 37^{o}C.

MTT assay

The selected variation of choco chip cookies namely BS sample was tested for *in vitro* cytotoxicity, using MG63 cells by MTT assay. Briefly, the cultured MG63 Cells were harvested by tryps inization, pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 µL) into the 96-well tissue culture plate in a DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hours at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the BS sample in a serum-free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO2 incubator for 24 h. After incubation, MTT (20 µL of 5 mg/ml) was added into each well and the cells were incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µL) were aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC50 value was calculated using Graph Pad Prism 6.0 software (USA).

Formula Cell viability % = Test OD/Control OD X 100

Analysis And Interpretation Of Data:
The collected data was consolidated, tabulated and statistically analysed. Mean and Standard deviation were used for the statistical analysis of data.

Results And Discussion

TABLE – 1

MEAN SCORE OF THE ACCEPTABILITY OF PAPAYA *(CARICA PAPAYA.,L)* SEED POWDER INCORPORATED CHOCO CHIP COOKIES

SAMPLE	MEAN ± STANDARD DEVIATION CHOCO CHIP COOKIES
Standard (S)	7.74± 0.4450
Variation 1(V_1)	7.71 ± 0.4848
Variation 2 (V_2)	6.95±0.2302
Variation 3 (V_3)	6.26± 0.2793

S- Standard, V_1- Variation 5% V_2- Variation 10%, V_3- Variation 15%

The above table -1 shows the mean score of acceptability of papaya *(Carica Papaya.,L)* seed powder.The overall acceptability papaya *(Carica Papaya.,L)* seed powder incorporated choco chip cookies revealed that the variation 1(V_1) has got the highest mean score of (7.71± 0.4848). The least accepted sample were variation 3(V_3) with a mean score (6.26± 0.2793). The mean score of the variation 1(V_1) was higher than all the variations.

IN VITRO CELL CYTOTOXICITY OF THE SELECTED PAPAYA *(CARICA PAPAYA.,L)* SEED POWDER INCORPORATED CHOCO CHIP COOKIES

TABLE-2

ANTICANCER ACTIVITY OF THE SELECTED PAPAYA *(CARICA PAPAYA.,L)* SEED POWDER INCORPORATED CHOCO CHIP COOKIES

Analysis	Result
Anti-Cancer activity	**92.37 µg/ml**

The above table – 2 depicts the (Inhibition Concentration) IC-50 papaya *(Carica Papaya.,L)* seed powder incorporated choco chip cookies. The seed incorporated choco chip cookies contain anti-cancer property. It shows it has an IC – 50 value of 92.37% µg/ml which denotes the anticancer activity.

TABLE-3
OD VALUE AT 570 nm

S. No.	Tested sample concentration (µg/ml)	OD value at 570 nm		(in triplicates)
1	Control	0.689	0.659	0.645
2	500 µg/ml	0.147	0.138	0.127
3	400 µg/ml	0.195	0.183	0.174
4	300 µg/ml	0.248	0.237	0.227
5	200 µg/ml	0.297	0.286	0.276
6	100 µg/ml	0.325	0.334	0.319
7	80 µg/ml	0.384	0.394	0.378
8	60 µg/ml	0.419	0.407	0.431
9	40 µg/ml	0.498	0.472	0.464
10	20 µg/ml	0.512	0.525	0.531
11	10 µg/ml	0.589	0.591	0.571

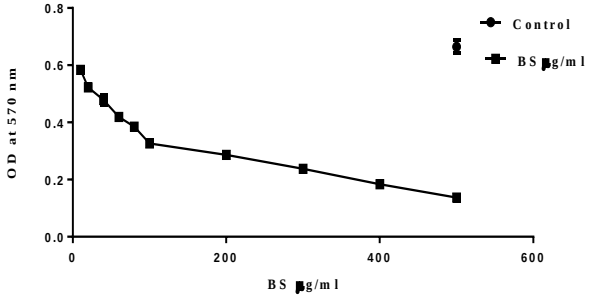
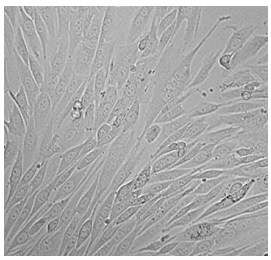


TABLE-4
IN VITRO CANCER CELL VIABILITY (%) UNDER DIFFERENT CONCENTRATIONS OF THE CHOCO CHIP COOKIES

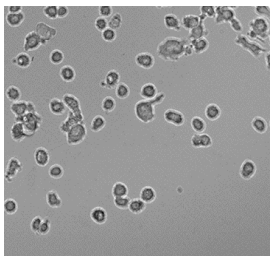
S. No	Tested sample concentration (µg/ml)	Cell viability (%) (in triplicates)			Mean Value (%)
1.	Control	100	100	100	100
2.	500 µg/ml	21.3353	20.9408	19.6899	20.655337
3.	400 µg/ml	28.3019	27.7693	26.9767	27.682659
4.	300 µg/ml	35.9942	35.9636	35.1938	35.717191
5.	200 µg/ml	43.106	43.3991	42.7907	43.098579
6.	100 µg/ml	47.1698	50.6829	49.4574	49.103343
7.	80 µg/ml	55.7329	59.7876	58.6047	58.041718
8.	60 µg/ml	60.8128	61.7602	66.8217	63.131573
9.	40 µg/ml	72.2787	71.6237	71.938	71.946774
10.	20 µg/ml	74.3106	79.6662	82.3256	78.767446
11.	10 µg/ml	85.4862	89.6813	88.5271	87.898226

The above figure - 9 shows the cell viability of the selected papaya (*Carica Papaya.,L*) seed powder incorporated choco chip cookies. It is clear that a concentration of 500µg/ml of the test sample has the highest anti-cancer activity.

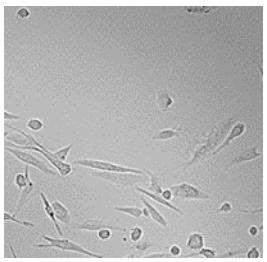
IMAGES OF CONTROL CELLS AND *CARICCA PAPAYA., L* SEED POWDER INCORPORATED CHOCO CHIP COOKIES TREATED CELLS



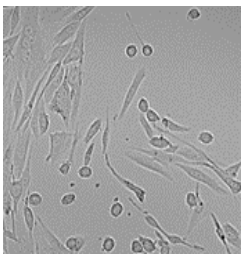
Control cells



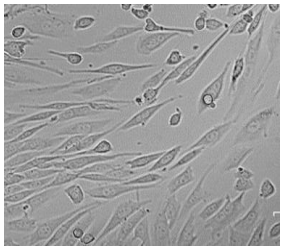
BS 500 µg/ml



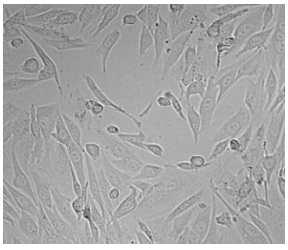
BS 300 µg/ml B



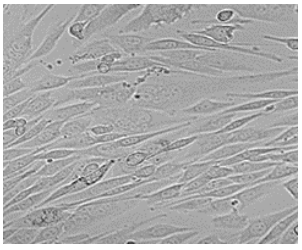
S 100 µg/ml



BS 80 µg/ml



BS 40 µg/ml



BS 10 µg/ml

Conclusion

The present study proved that the papaya *(Carica Papaya.,L)*seed powder incorporated choco chip cookies were palatable with anticancer effect and can be used for preventing cancer. As it proved a good in vitro cell cytotoxicity, it can be tried out with human trials after ethical clearance to kill the cancer cells which is a big challenge to the entire world.

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