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Invitro anticancer effects of methanolic seed coat extract of Momordica dioica against human carcinoma cell lines

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Abstract: Cancer has been a common cause of deaths for decades, which had encouraged the use of many natural products from plants to be used as a treatment against it. *Momordica dioica* is one such important medicinal plant which has been used for the treatment of cancer, diabetes, allergies and inflammation. In this present study, the anticancer effects of the crude methanolic seed coat extract of *Momordica dioica* on human breast (MCF-7) and lung cancer (A549) cell line has been evaluated using the MTT assay and the DNA fragmentation assay of the MCF-7 cell line was performed. The methanolic seed coat extract of *Momordica dioica* exhibited 50% growth inhibition in MCF-7 cancer cell line at 50 µg/mL and A549 cancer cell line at 70 µg/mL. Further MCF-7 cell line depicted a ladder like pattern of the DNA which was observed in the DNA fragmentation assay. The crude methanolic seed coat extract of *Momordica dioica* could be a potential source of natural anti-cancer agents. This study can be extended for the isolation and characterization of the active compounds which can be used as effective anti-cancer agents.
Keywords: *Momordica dioica*, cytotoxicity, MTT, DNA fragmentation.

Introduction

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells that is the common cause of death in humans worldwide.[1-3] Natural products from fruits and vegetables have been used to impede cancer.[4,5] Certain agents such as taxanes, vincristine, vinblastine, irinotecan, topotecan etc derived from various plants have been proven to have anticancer activity.[6,7]Methanolic extracts of various plants were proven to have anti cancer effects.[8-11] Hung et al. has demonstrated that the methanolic extract of adlay seed exerted an anti proliferative effect on human lung cancer cells *in vitro* and *in vivo* that could prevent tobacco carcinogen-induced lung tumorigenesis.[12]

Momordica dioica is a perennial, dioecious climber included in the *Cucurbitaceae* family, which is commonly known as spiny gourd, teasel gourd or small bitter gourd worldwide whereas in India it is known as kankro, kartoli ,kantola, kantroli, ban karola or janglee karela. *Momordica dioica* has been known to have many medicinal properties namely anti tumorogenic, analgesic, anti diabetic, anti inflammatory and anti allergic activity.[13-16]

In view of the above, the present study was undertaken to investigate the *invitro* anti cancer activity of the methanolic seed coat extract of *Momordica dioica* using MTT assay on two human carcinoma cell lines namely, MCF-7 breast and A549 lung cancer cell lines, followed by DNA fragmentation of MCF-7 cell line which showed an high growth inhibition on addition of the seed coat extract.

Materials and Methods

Chemicals and reagents

Methanol and other reagents of high purity grade were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).The MTT assay kit was purchased from Hi-Media Laboratories, Mumbai, India and the DNA isolation kit was purchased from Genei, India.

Collection and identification of plant materials

The seeds of *Momordica dioica* were collected and authenticated from Plant Anatomy Research Centre (PARC/2011/945), Chennai.

Preparation of plant material

The seed coat of the *Momordica dioica* seeds were washed, shade dried then homogenized to coarse powder and stored. 10g of the dried material were extracted with 70% methanol solution at room temperature for 24 hours with a rotary shaker. The methanolic extract was subsequently filtered using Whatman filter paper No.1 and then evaporated to dryness followed by lyophilization.

Cell lines and culture medium

MCF-7 cells (Human breast cancer cell line), A549 (Human lung cancer cell line) and Vero (Normal African monkey kidney cell line) were purchased from NCCS, Pune, India. The cell lines were grown in DMEM (Dulbecco's Modified Eagle Medium) supplemented with high glucose , 0.1 mM Non-essential Amino acids (NEAA) , 0.1mM Sodium Pyruvate and 10% FBS. Cells were cultured at 37°C with 5% CO2 at 95% humidity.

In-vitro cytotoxic assay

The anticancer effects of the crude methanolic seed coat extracts were investigated against the MCF-7 and A459 cancer cell lines using MTT assay.[17]

Each cell line was seeded in 96- well microliter plates at a density of 1×10^6 cells/well following a 24 hour incubation. The cells were then treated with different concentrations of the methanolic seed coat extract in triplets ranging from 2 μg/mL to 100 μg/mL for 24 hours. A final concentration of MTT (5 μg/mL) was added to each well, and plates were incubated at 37°C for 4 hours to allow reduction of MTT to the insoluble formazan product by the viable cells. Cells were then lysed using DMSO. The absorbance was then read at 570 nm to measure cell viability. DMSO served as the positive control.

In this study, MTT is used to assess the cell inhibition in concordance with the cell viability. The percentage of cell inhibition was calculated by the formula :

$[\, A_T - A_C / A_C \,] \times 100$

Where,

A_T is the Absorbance of the treated sample

A_C is the Absorbance of the control

DNA Fragmentation

Based on the results obtained in the MTT assay, DNA fragmentation analysis was performed in order to evaluate the mechanism of cell death in the MCF-7 cell line treated with the methanolic seed coat extract that had showed a higher percentage (50% at 50μg/ml) of cell death. The MCF-7 cells were seeded at a density of 3500 cells/well in a 6 well titre plate containing 2 mL of minimal essential medium and incubated at 37°C for 48 hours in a CO_2 incubator with 5% CO_2. The cells were then treated with a concentration of 50 μg/mL of methanolic seed coat extract and incubated for 24 hours in 5% CO_2 incubator. The media was then discarded followed with

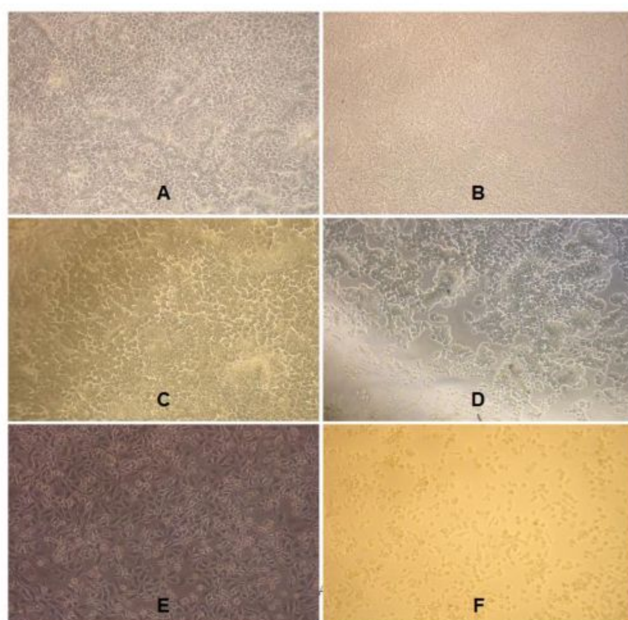
the addition of 1 mL of TPVG (trypsin, PBS, versene and glucose) and 1 mL of phosphate buffer saline (PBS) to each well. The contents of the well were collected and DNA isolation was carried out using the DNA isolation kit (Genei, India). The pattern of bands for DNA fragmentation was visualized on 1% agarose gel under UV light and photographed.

Statistical Analysis

Statistical analysis of the experimental data was performed using Microsoft Office Excel data analysis software. Data of the MTT assay were presented as the standard error and mean of three independent experiments were performed in triplicates.

Fig 1. Photomicrographs of tested cancer cell lines.

A- Normal Vero cells
B- Treated Vero cells at concentration of 100 µg/mL
C- Normal MCF-7 cancer cells



D- Treated MCF-7 cancer cells at a concentration of 50 µg/mL
E- Normal A549 cancer cells
F- Treated A549 cancer cells at a concentration of 70 µg/mL

Results

MTT Assay

MTT assay was performed to investigate the anti-proliferative activity of *Momordica dioica* methanolic seed coat extract on the growth of human breast and lung carcinoma cell lines which showed increased inhibition towards both the tested carcinoma cell lines. The vero cell lines were used as the normal cell line where Figure 1(A) depicts the untreated cells and Figure 1(B) shows the treated cells at 100 µg/mL having a 99.5% cell viability.

Figure 1(C) represents the untreated MCF-7 cells amd Figure 1(D) shows the treated MCF-7 cells where a 50% cell inhibition was observed at a concentration of 50 µg/mL The untreated A549 [Figure 1(E)] exhibited a minimal percentage of cell death as compared to the treated A549 [Figure 1(F)] with a 50% cell inhibition at a concentration of 70 µg/mL Figure 2 depicts the graphical representation of the cell growth inhibition of the tested cancer cell lines.

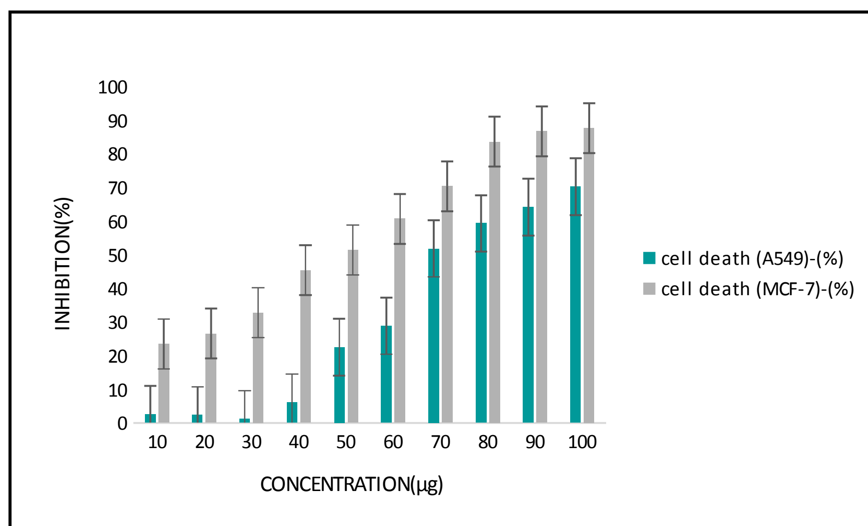


Fig 2. Effect of the crude methanolic seed coat extract of *Momordica dioica* on the proliferation of the MCF-7 and A549 human cancer cell lines. The results were expressed as an average of three replicates ± standard deviation (SD).

DNA Fragmentation

DNA Fragmentation was performed to study the effect of the seed coat extract on the induction of apoptosis inMCF-7 cells was analysed. Agarose gel electrophoresis of DNA isolated from MCF-7 cells treated with 50 µg/mL concentration of the methanolic extract showed a ladder pattern [Figure 3, Lane A], indicating cell death due to DNA fragmentation. In case of untreated cells, the intact chromosomal DNA [Figure 3, Lane C] was observed. These interpretations confirm that the cell death caused by the crude methanolic extract occurred through apoptosis.

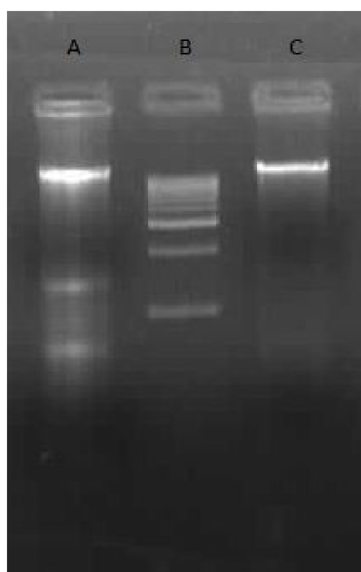


Fig 3. Agarose gel depicting DNA fragmentation of MCF-7 cells showing the treated cells (A), marker – 1 kb ladder (B) and the control – untreated cells (C).

Discussion

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide] assay is a colorimetric assay for assessing cell viability. NAD(P)H-dependent cellular oxidoreductase enzymes reflect the number of viable cells present in the assay. These enzymes are capable of reducing the tetrazolium dye MTT to its insoluble formazan, which has a purple color.[18,19]

In the present study, MTT assay was conducted to investigate the anticancer effects of the methanolic seed coat extracts of *Momordica dioica* against human carcinoma cell lines. At a concentration of 100 µg/mL, the methanolic seed coat extract of *Momordica dioica* caused increased (50% cell inhibition at a concentration of 50 µg/mL) cell growth inhibition in both the treated cancer cell lines.

The A549 human lung cancer cell line showed 50% cell inhibition when treated with 70 µg/mL of the *Momordica dioica* methanolic seed coat extract. Comparable work has been reported where the IC_{50} values of methanolic extract of *Artocarpus heterophyllus* to be 35.26 µg/mL against A549 cell line by MTT assay.[20]

Similarly Sumathy et al. observed that the methanolic fruit extract of *Pedalium murex* exhibited cytotoxic effects against A549 cancer cells at concentration of about 1000 µg/mL.[21]

The MCF 7 human breast cancer cell line showed a 50% cell inhibition at a concentration of 50 µg/mL of the seed coat extract that clearly represents a better anti-cancer potential as compared to the A 549 cell line. The results obtained were in concordance with the anti prolifertive activity of the alcoholic extracts of *Ganoderma lucidum* against the MCF-7 cells where a 70% inhibition was observed at a concentration of 500 µg/mL.[22] Previous studies have also reported that the ethanol fractions of *Ononis hirta* (aerial parts) and *Inula viscosa* (flowers) showed anti cancer activity against MCF-7 cells with IC_{50} of 27.96 and 15.78 µg/ml respectively.[23]

The DNA fragmentation was carried out for the MCF-7 cell line treated with methanolic seed coat extract at 50µg/ml concentration which showed the induction of apoptosis in MCF-7 cell line. A ladder pattern indicated fragmentation of the DNA in [Figure 3, Lane A]. These results were similar with the previous study where the *Bombyx batryticatus* extracts on tumor cell growth appears to be mediated through induction of apoptosis which was demonstrated by characteristic morphological changes, and internucleosomal DNA fragmentation.[24] Similarly, Campbell et.al also reported a study where the aqueous extract of chinese medicinal herbs showed an significant activity (50% growth inhibition) on MCF-7 cell line with an IC_{50} values ranging from 10 µg/mL to 1 mg/mL.[25]

Conclusion

In view of the findings obtained in this present study, it is clear that the methanolic seed coat extract of Momordica dioica can be further analyzed for the detection of potent anti cancer compounds.

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